UNIVERSITÀ DI PARMA

UNIVERSITA' DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN

SCIENZE DEGLI ALIMENTI

CICLO XXXI

Technologies for the improvement of animal welfare in relation to the quality of milk

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Anni 2015/2018

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Preface

Ensuring the integrity of farm animals, their protection, nutrition, care and freedom to express their nature are topics that involve the whole society, produces conflicts of interest and ethical dilemmas. Attitudes towards animal welfare can be considered the result of two forces; one arises from a cognitive judgment that leads to building conceptions on animals and their use; a second is mainly characterized as an emotional and affective response and includes personal empathy towards animals. Science based information is needed to produce and communicate knowledge that allows conscious choices by consumers and helps the sense of responsibility and transparency of producers.

Introduction

There is a recognized need to reconcile productivity, sustainability and social values regarding the use of animals for producing food. The growing interest of consumer in knowing how animals are kept is pushing the market towards more sustainable livestock products.

In the next few years animal welfare and biosafety will have to be considered essential components of an integrated production quality system of food of animal origin, which guarantees the consumer products from environmentally sustainable farms and where the animals are bred according to criteria that respect fundamental needs.

A survey conducted at European level (Eurobarometer, 2016) found that the vast majority of European citizens consider animal welfare to be very important and would like to see significantly improved animal husbandry standards. The eurobarometer survey shows that:

- 94% of citizens think that protecting the welfare of farm animals is important;
- 82% of citizens think that farm animals should be better protected than they are now;
- 9 out of 10 respondents believe that the EU should do more to promote awareness of animal welfare.

Worries about animal welfare are often associated with expectations about quality of animal products. There is a widespread perception that unhappy and sick cows produce bad milk; however, a direct relation between animal welfare and food quality is not easily demonstrable because “food quality” and “animal welfare” are, both, very complex concepts.

For decades, legislation has represented the way to protect animal welfare. More recently, however, given the increasing demand from consumers for quality of food products and a more ethical food production, animal welfare is emerging as an area with potential added value for producers, retailers and other operators in the food supply; thus the leading role of the market in driving development of animal friendly methods of production has received big attention and the public awareness is beginning to influence directly farm practices.

So far, one of the main obstacles on the way to a characterization of the products in relation to the animal welfare has been the difficulty of measuring it by feasible and objective methods based on scientific data. Over time, many protocols have been proposed for the assessment of welfare. An important European project (Welfare Quality®) also highlighted a significant use of animal welfare as an element to differentiate products. Another European research project, EconWelfare, highlighted that distribution companies and the food industry are interested in going beyond the minimum levels required by law.

However, the potential conflict between the welfare standards and those of economic profitability is a complex issue. In most cases, when farmers are requested to reach higher animal welfare, they have to invest in facilities or reduce income (e.g. when stocking density must be

reduced or space allowance must be increased). To understand and cope with this challenge, many other aspects must be taken into account and all economic benefits that high standards of animal welfare can bring should be considered.

Health status is an important part of animal welfare and there are clear economic advantages in management systems that improve livestock health; there are well-known direct and indirect economic benefits through more healthy animals. Economic losses due to lameness and mastitis have been estimate in different dairy production systems and different geographical areas

Other aspects of animal welfare have positive effects on economic efficiency, but their evaluation is more difficult.

A number of internal and external factors limit the full expression of the high genetic potential of many individual animals currently farmed in developed countries. Nutritive stress, environmental stress, cognitive stress and diseases cause physiological and behavioural responses that increases the costs for the body in addition to those for maintenance and production and reduce efficiency of transformation of feed to food.

On the other hand, it was suggested that the increased efficiency obtained by genetic selection for productive traits had reduced the animals' ability to respond to stress; Oltenacu and Broom (2010) found a competitive relationship between the genetic selection for dairy production and adaptability due to limited physiological resources, resulting in poorer adaptability by selection for milk yields; however the relationship between the individual variability in biological efficiency and the ability to respond to stressors is not clear.

Individuals differ in stress reactivity and coping style; these differences have been associated with different states of the immune system in humans, laboratory animals and birds. These associations are based on the interactions between the immune system, nervous system and hormonal system. Coping style influence also the reproductive efficiency; genetic selection for productive traits is suspected to be partially responsible for the reduction of fertility and signs of oestrus in dairy cows. It is known that females prioritize the fulfilling of their current needs at the expenses of future needs (i.e. milk synthesis instead of future progeny) so that metabolic stress during lactation may negatively affect the reproductive performances. The link between the neuroendocrine axis and the immune system suggest that also positive feelings/emotions could affect productive and reproductive efficiency.

Objectives

Objectives of the PhD thesis project were:

-improving knowledge on relationship between quality of milk and welfare of cows in order to better characterize food in relation to the animal welfare as an important attribute of sustainability of animal productions;

-providing science-based suggestion on how to preserve milk quality while improving quality of animal lives by using innovative technologies.

Chapter 1- Technologies for the improvement of animal welfare

Introduction

The study of animal welfare is a recent science; the researcher who dealt with this topic had to refine methods, agree on terminology and define shared principles. Animal welfare science is constantly evolving however, some principles are consolidated:

-animal welfare is a multifactorial attribute that belongs to the individual and as such must be assessed by a multicriteria approach and objective indicators measurable on the animal;

-measuring animal welfare must provide information on the quality of the life of an animal; therefore, indicators of animal welfare must be sensitive not only to the physical health, but also to an animal response in relation to its experience of the living conditions;

-indicators must scientifically reliable and feasible in practice.

According to a widely accepted definition of welfare in terms of attempts to cope with environment, "Welfare should refer to a characteristic of the individual animal rather than something given to the animal by man" (Broom, 1991), however, under farming conditions, many factors controlled by humans can influence the level of animal welfare (genetics, nutrition, housing, social interactions, care). More and more often modern technologies are used to improve and assess the animal welfare at farm level.

Dairy cattle farming is distinguished by some features that highlight the importance of welfare of individual animal:

- high value of the individual cow that has a higher productive life expectancy than most other bred species;

-lactating cows engaged in a high productive effort, especially in some specific phases of the production cycle (peripartum) may become more susceptible to hot stress, metabolic problems (ketosis, acidosis, hypocalcaemia), infectious diseases (mastitis, metritis) and multifactorial diseases such as lameness;

- high interest in the fertility of the individual cow; there may be conflict at the level of the organism between the reproductive and productive demands; it is well-known that as long the cow is in a state of negative energy balance, the fertilizations are doomed to fail.

All this makes it more interesting than in other types of farming to invest in the health and welfare of the individual animals; this is probably one of the reasons why dairy cattle breeding is characterized by a high level of technological and biotechnological innovation.

In dairy farms, technologies, sensors and software are being widely used to assist farmers in controlling animal health and behaviour, measuring performance and making decisions.

The most widespread monitoring systems are those for automatic detection of time of oestrus. Many of these systems are based on the control of the activity of the cows. For decades, dedicated software have been developed to provide the user with information to detect the optimal period for insemination; more recently, software have been developed to better exploit the use of data on abnormal motor activities, for example sudden or prolonged declines or inactivity, as indicators to timely alert about the development of diseases that would otherwise be diagnosed only when marked by clinical signs.

More recently, systems that associate motion activity with other indicators such as ruminal activity have been proposed on the market. These systems are becoming increasingly useful for the diagnosis of health and management problems; in fact, the variations of rumination are very early indicators of different pathologies and when associated with other information such as the production, the reproductive status and the days of giving birth can become also very specific.

In other systems the detection of the oestrus occurs by in-line analysis of the progesterone level in milk; progesterone level in milk indicates the presence of ovulation or pregnancy or infertility; these systems allow also the measurement of other metabolites and enzymes useful indicators of metabolic diseases (e.g. beta-hydroxybutyrate), udder health (lactate dehydrogenase) and nutritional balance (urea).

These are examples of the so-called precision livestock farming (PLF)**.**

Precision livestock farming systems allow the continuous automatic monitoring of animal behaviour and health and an automated management; these systems are based on sensors that detect and transmit individual data to a computer; dedicated software elaborate these data, obtaining indications to detect oestrum and pathologies; the system alerts the farmer in real time, usually sending an alert to his smartphone, so that he can timely put in place the necessary interventions.

There are currently different types of sensors and data transmission technologies to collect data and produce warnings in animal farm. The miniaturization and reduction of production costs of these technologies allow real time monitoring of many environmental (e.g. temperature and radiation) and biological parameters (respiration rate, body temperature, position of the animal, muscular and cardiac activity). Data are often managed by a single microcontroller available at affordable prices. The mathematical models for data processing and the data presentation interface are also included in the software loaded in the microcontroller.

At individual and farm level, the main benefits of existing livestock precision farming systems are the early detection of health and behaviour problems and the precise management of animals. Early warning system detecting sudden or unforeseen changes in rumination, activity,

body condition, body temperature, progesterone in milk, milk temperature, and milk conductivity allow early timely corrective actions.

The current PLF systems allow putting in relation animal-based variables (performances, animal welfare indicators) with management variables (feeding, comfort, health and reproduction management).

Objectives

Aim of this chapter is to analyse the technologies currently available and their impact on animal welfare.

Livestock precision farming can provide real-time decision support systems helping producers to detect health and welfare disorders at an early stage and to improve management procedures.

Automation of barn operations and PLF are key factors to improve husbandry and increase robustness, having the potential to increase milk yield and optimize process inputs meanwhile helping animal welfare.

Results

The numerous applications of the PLF have been studied by several authors (Berckmans, 2014) that reviewed:

-systems for electronic identification (Samad et al., 2010);

-systems for the real-time evaluation of body weight of animals (Kollis et al., 2007),

-use sensors for the assessment of animal welfare and animal behaviour (Shao and Xin, 2008));

- use of sensors for the diagnosis and monitoring of pathologies (Maatje et al., 1997; Eradus and Jansen, 1999);

Milk yield

Recording the daily milk yield is a basic prerequisite to manage the herd's productivity and hence profitability. Recording helps deciding which cows to breed or which ones to cull, calculating the herd's feed rations and identifying health issues. Milk recording can be performed using either milk meters, recording jars or milk flow indicators.

There are many different milk meter manufacturers; only meters that are tested and approved by the International Committee for Animal Recording (known as ICAR) may be applied in Dairy Herd Improvement programs.

Activity

Activity is an important measure for monitoring behaviour and health status of cow. Computer models are used to interpret individual cow behaviour measured by the sensors and to create individual behaviour patterns. Disease will create changes in the expected activity of cows. When animal behaviour differs from its expected behaviour the farmer will get an alert.

Rumination

Rumination behaviour is an indicator of wellbeing; abnormal rumination behaviour has been linked to detection of anxiety (Bristow and Holmes, 2007) and distress (Schirmann et al 2011).

Brown Swiss cows showed a higher number of chewing cycles a shorter duration of rumination in 24 h when compared with Holstein Friesian and Swiss Fleckvieh (Braun et al., 2015); however, the number of chewing cycles per regurgitated cud resulted not different between breeds. Thus, genetics could have e certain effect on rumination which needs to be better understood:

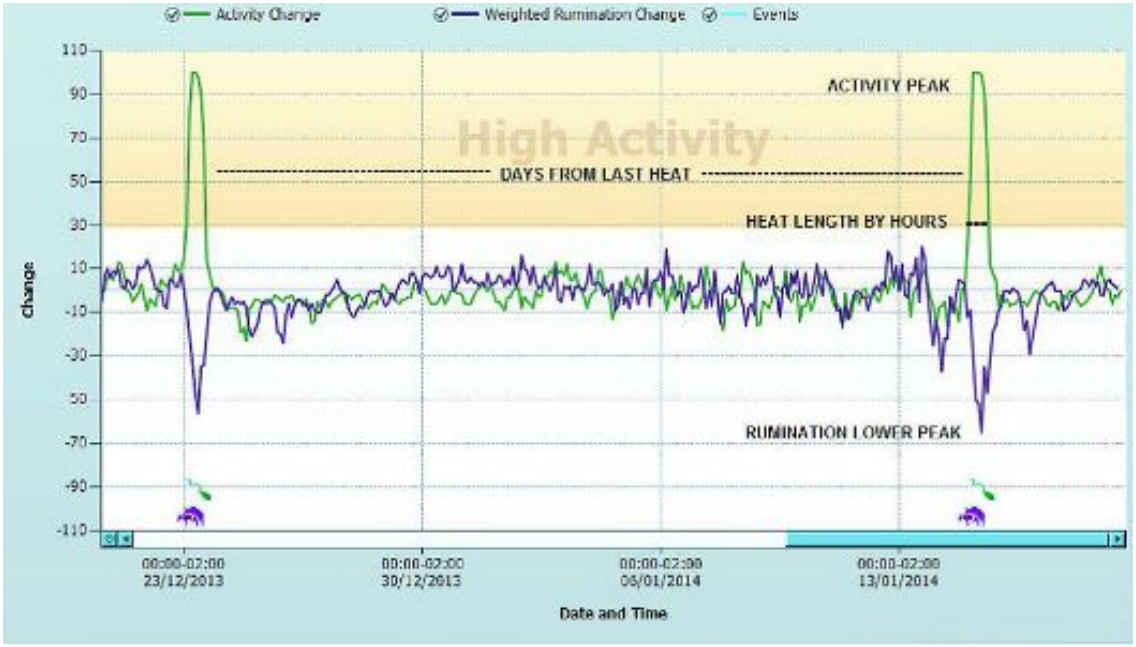
The best-known factor affecting rumination is the physical structure of the feed.

All these factors contribute to the baseline parameters of different herds and of individuals in the herds. However, the most useful information to manage a dairy herd is the deviation of rumination from baseline values; on-farm systems have become available to monitor rumination; expected changes in rumination time for a variety of management routines and biological processes have been reported based on accumulated on-farm observations with a monitoring system that functions on sound created while chewing. Reported deviations in rumination include: calving, -255 min/d; oestrus, -75 min/d; hoof trimming, -39 min/d; heat stress, -20 to -70 min/d; and mastitis, -63 min/d (SCR, 2013).

Activity plus Rumination

In recent years, commercial systems for monitoring rumination have become available at farm level. These systems have been studied at experimental and farm level for many years providing data reasonably correlated with those collected by visual observation. Currently, the most successful use of these system is in combination with activity to detect oestrus; changes in rumination as well as feeding times around oestrus has been demonstrated useful aid for early oestrus detection. Pahl et al. (2015) found that rumination was reduced for about 30 hours around oestrus but the primary drop occurred at 6:00 am on d -1 and noon on d 0. Drops in rumination time can be caused by a number of internal and external factors, but drops around oestrus in combination with peaks of activity produce very distinctive graph (figure1.1).

Figure 1.1 A sample report from a system for oestrus detection based on changes in rumination and activity



Latest systems are wearable by the cow as ear or neck tag; applications are flexible; at the start level the available reports regard reproduction parameters: heat report; cows with irregular heat report; suspected for abortion report; anoestrous cows report. At advanced level, more reports are available: health reports; fresh cow reports; distress report; distress alerts and hot stress reports.

Stangaferro et al., 2016 evaluated the performance of one these systems to identify cows with metabolic and digestive disorders, including displaced abomasum, ketosis, and digestive disorders; the considered system uses an alert (health index score, HIS) that combines rumination time and movement activity; the sensitivity of the HIS was 98% for displaced abomasum; 91% for ketosis; 89% for indigestion; and 93% for all metabolic and digestive disorders combined. Days from the first HIS alert and clinical diagnosis were -3, -1.6, -0.5, and -2.1 (-2.5, -1.6) for displaced abomasum, ketosis, indigestion, and all metabolic and digestive disorders, respectively. The overall sensitivity and timing of the system alerts for cows with metabolic and digestive disorders indicated that the systems that combine rumination and activity could be a useful tool for identifying cows with metabolic and digestive disorders.

Van Hertem et al., 2013 validated a mathematical model to detect clinical lameness based on data from sensors that measured neck activity and ruminating time; they reported a sensitivity of 0.89 and a specificity of 0.85, with a correct classification rate of 0.86.

Body Temperature

Body temperature can be measured with a ruminal bolus, which incorporates also a pH sensor. The bolus allows a continuous measurement of pH and temperature of rumen. The bolus can be inserted into the rumen by a livestock balling gun.

The body temperature can also be measured by an electronic ear tag; for example, FeverTags is a system to monitor body temperature every 15 minutes in calves; if the temperature is raised above 39.7°C for a period of six hours the tag will flash. The tags act as a visual sign to alert the farmer.

The body temperature can also be measured by the temperature of milk or by implanted telemetric temperature probes.

Body Condition Score

Body condition score (BCS) estimated energy reserves of a cow by the visual appreciation of the degree of coverage of the bone structure.

An optimal body condition score in the beginning and end of lactation is critical to optimize milk production; minimize fertility disorders and minimize health disorders.

At calving, a BCS ≤ 3.25 means too little energy provided in late lactation and dry period, with risk of low milk production and poor reproduction.

At calving, ≥ 3.75 means too much energy in late lactation and dry period, with risk of metabolic disorders.

At peak lactation, BCS of high producing cows may drop below 2.75, but that must be regained later to avoid reproductive problems.

At dry off, BCS ≥ 3.75 can be due to too much energy in the diet, but also to prolonged calving intervals; high BCS at dry off can leads to problems with calving and reproduction in next lactation, like uterine infection, retained placenta.

Manual scoring of body conditions is hard work and subjectivity can bias the results; automatic scoring allows a very frequent (daily) scoring.

The BCS camera takes a 3D image of the cow's lower back every time they pass under the camera. The software then calculates the body condition score of each cow and sends it to the computer.

Body Weight

The bodyweight of cow can be measured by a weight scale system formed by a weight platform on top of load cells. The cows walk over a weight sensor in the ground, and the load cell calculates an average weight. Some system also measures the weight distribution providing indicators of lameness.

Milk Analysis

Milk analysis is a good tool to detect metabolic diseases. Very useful parameters in milk composition are beta-hydroxybutyrate (BHB), acetone, fat and protein as these provide the best indicators of metabolic disorders.

Fat to protein ratio. Fat to protein ratio in milk is a very sensitive indicator of metabolic disorders. The fat to protein ratio is a good indicator of energy balance and ketosis. A fat to protein ratio comprised between 1 and 1.5 is optimal and indicates a positive energy balance. With a ratio above this value, the cow is in a negative energy and there is a risk of ketosis. A fat to protein ratio lower than 1 indicates a risk of acidosis.

Beta-hydroxybutyrate. BHB in milk is a direct measure of ketosis.

Milk acetone. Acetone in milk is also a useful indicator of energy balance, being the most abundant ketone body present in milk and highly correlated to blood ketone levels. The range of milk acetone is 0 to 2 mM. A high level of acetone in milk indicates that cows are in a negative energy balance.

Lactate dehydrogenase (LDH). LDH is an enzyme that occurs in nearly all livings cells, and the concentration rises during infection.

Lactose. Mastitis causes a decrease of milk lactose content. However, a lactose measurement in itself is not reliable enough to distinguish cows with mastitis from healthy cows.

Progesterone. The milk content of hormone progesterone provides a lot of information about the reproductive stage of the cow. The progesterone level in the milk can be used to determine whether or not a cow: is in oestrus; is not cycling (anoestrus); is pregnant; needs to be treated for ovarian cysts.

Pregnancy can also be diagnosed in the milk by determining the PAG-levels (pregnancy-associated glycoproteins). PAG are produced by the placenta, starting from 29 days after the (successful) insemination.

Urea. Urea is formed in the liver and kidney from ammonia (NH3), which is in turn produced by degradation of crude proteins in the rumen; the excess of ammonia that is not used by the microorganisms passes through the ruminal wall into the bloodstream, reaching the liver, where it is converted into urea; urea is then excreted from the body in urine and being water-soluble, diffuses readily into body tissue. Urea also readily diffuses from blood into milk, making urea a normal constituent of milk, as part of the non-protein nitrogen normally found in milk. Increased liver urea synthesis can result from excess dietary degradable or undegradable protein or a relative deficiency of fermentable carbohydrate required by the rumen microbes to capture ammonia; thus, measuring the amount milk urea nitrogen helps evaluating how efficiently the dietary protein is used. Milk urea values can vary significantly between herds and even within cows. This should be taken into account when interpreting individual milk urea values of cows.

Furthermore, target values differ in pasture-based farms. Milk urea values are typically higher in grazing cattle, without necessarily any detrimental effects on their health.

In-line analysis.

Given the daily variations in milk components (especially the fat content), one individual analysis per month –as is often the case in Dairy Herd Improvement programs- is not enough to timely detect metabolic diseases on a herd level. Automatic in-line monitoring provides a more accurate estimate of the long-term milk composition, even though the reliability of the commercial sensor technologies is inferior compared to laboratory analysis.

Some system on the market

Herd Navigator (DeLaval) is an on-farm system designed to detect lactate dehydrogenase (mastitis), beta-hydroxybutyrate (ketosis) and progesterone (heat, pregnancy, infertility). The fully automated system is available for both conventional milking parlours and milking robots. For cost-effectiveness, the cows are not sampled every milking, but the systems itself decides when the cow should be sampled based on her history and lactation stage.

MQC (Lely) unit provides an indication of the somatic cell count, fat, protein, lactose.

Afilab (Afimilk) is a near-infrared spectroscopy (NIR) sensor for inline measurements of the protein, fat and lactose content, together with the presence of blood, in individual cow's milk. During milking, the sensor measures these components per 200 mL of milk that passes through the apparatus and reports the average of multiple measurements.

Real-time location

Understanding how the cows use the dairy barn during the day can provide essential information to the farmer on cow health, productivity and welfare. Real-time location systems are now available to allow the farmers to track the movements of individual cows without the need for a human observer.

There are currently some systems able to detect exactly where are all the cows in a free-stall barn; some of them combine position and activity monitoring.

Nedap Cow Positioning®. Is a tool able to locate individual cows in the barn; the neck tag provides information on cow's activity and health, but also provides their location. It is always combined with Nedap Heat Detection, Health Monitoring (Eating monitoring) and ISO identification for feeding, separating and milk measurement.

GEA CowView®. This neck collar localizes each cow in real time, provides information about oestrous activity and gives warning about diseases. This system uses the animal-specific movement profile, normal behaviour patterns and also the behaviour of animal groups; if lying, eating or daily habits change, a warning for a smartphone, tablet or PC will be generate.

Chapter 2- Technologies for the improvement of animal welfare: predicting calving for an optimal start of a healthy dairy industry

Introduction

Calving is a major event of the lifecycle of dairy cows; it is an intrinsically risky process, physically challenging and painful and can have negative effect on health, welfare and performances of both dam and calf. Difficulties at calving (dystocia) reduce dairy productivity. Calves born after dystocia are more likely to develop disease. After dystocia, the dams are less likely to get pregnant again and are more likely to be culled in the next lactation; dystocia also increase the risk of infection; for all these reasons, dystocia poses a threat to the cows and hence to the productivity of the farms; thus, prediction of calving is central to a cost effective and healthy dairy industry.

Individual monitoring of cows around the time of calving allows early detection of difficulties or health problems and facilitates timely human intervention; it was observed that insufficient monitoring lengthened the duration of the second stage labour (Gundelach et al., 2009). The latter, together with presentation, position and posture of the calf is a key risk factor for perinatal mortality (Gundelach et al., 2009); therefore, careful management of calving helps by minimizing pain and distress during this critical event and prevent consequences of dystocia. Dystocia has been reported as one of the major causes of bovine perinatal mortality (approximately 35% at international level) (Mee, 2013). Mee et al. (2013) found that calving management was the most important area of concern in herds with high perinatal mortality. Moreover, a prompt presence of caretakers at calving and the knowledge of the precise time of birth assure the provision of colostrum to calves within the first 6 hours of life; this helps the intake of an adequate level of colostral immunoglobulins that are essential for the survival of new-borns. Experienced stockmen use qualitative criteria based on physical and behavioural changes to realize when a cow is close to delivery. However, direct visual observation is time consuming and the continuous presence of an observer during stage two of calving has been associated with an increased number of calving problems and cases of assisted delivery (Dufty, 1981).

Automated systems are becoming widely used for milking, feeding and detecting oestrus in dairy cows while automatic systems for monitoring calving are still scarcely used, even if various methods have been developed to predict the calving time; some of them automatically measure physiological indicators such as body temperature (Aoki et al., 2005; Burfeind et al., 2011), blood level of oestrone-sulphate and 17 beta-oestradiol (Shah et al., 2007), blood level of

progesterone (Matsas et al., 1992; Streyl et al., 2011) and electrolytes in mammary secretion (Bleul et al., 2006); other systems monitoring physical indicators such as the relaxation of pelvic ligaments, physical separation of the vulva lips (Palombi et al., 2013) and abdominal contractions have been proposed. According to Saint-Dizier and Chastant-Maillard (2015) who reviewed literature on methods to predict calving, measuring relaxation of pelvic ligament and assays for circulating progesterone and oestradiol-17β are both accurate and sensitive methods to predict calving within 24 h from delivery. Moreover, the measurement of incremental daily decrease in vaginal temperature and the combination of pelvic ligament relaxation and teat filling estimates are reliable signs to accurately predict calving within the 12–24 h from parturition. Recently, Rutten et al. (2017) showed that a combination of data from sensors detecting cumulative activity, rumination activity, feeding activity and body temperature improved the accuracy of prediction of the time of calving process initiation, compared to a prediction based only on the date of the insemination.

Adin et al. (2009) and Soriani et al. (2012) found decrease in rumination time on the day of calving.

However, the prediction of the specific hour in which calving starts is still difficult. Systems based on behavioural indicators seem the most promising because significant changes of behaviours can be observed also within the day of calving. Main changes in behaviour before calving have been reported in the scientific literature starting from several days before delivery until the calving time: searching for isolation (Proudfoot et al., 2014); tail movements (Bueno et al., 1981); aimless walking (Owens et al., 1984/1985), turning head toward abdomen (Jensen, 2012), reduction of lying time (Rice et al., 2017), sniffing the ground (Owens et al., 1984/1985) and frequent change in the posture (Huzzey et al., 2005; Miedema et al., 2011b).

These variations in behaviour, associated with hormonal changes and uterine contractions, are considered expressions of an overall set that is usually termed restlessness (Owens et al., 1984/1985). Previous evidence (Owens et al., 1984/1985; Huzzey et al., 2005; Jensen, 2012; Miedema et al., 2011b; Proudfoot et al., 2014; Rice et al., 2017) suggests that monitoring these behaviours can provide important information on the progress of the calving process.

Video cameras or accelerometers recording behaviour of cows can be integrated in systems using image analysis (Cangar et al., 2008) or locomotive activity to alert the farmer when calving is approaching; however, alerting systems require input of benchmark information about behaviours and changes in behaviours which can be predictive of the time of calving.

An observational study was carried out to contribute to knowledge regarding the development of calving prediction systems by identifying specific behaviours to be associated with an imminent delivery.

Materials and Methods

The study was carried out at a dairy farm located in Cremona (Italy). The routine procedures and grouping strategies commonly used in the farm were applied during the study: cows were moved from the dry cow free stall to a dedicated calving pen 3 weeks before their expected calving date. The calving barn was a straw bedded pen sizing 16.60 m x 9 m; it was adjacent to the external paddock of the barn of the lactating cows.

Cows were fed ad libitum once a day, approximately from 7:00 to 7:30 h; feeding operations lasted few minutes; bedding maintenance was done when cleaning or adding straw were needed, but at least after each new calving and within a week from the last bedding; it was generally made between 7:30 and 8:00 and lasted 10-15 minutes. Animal behaviour was video recorded continuously by a video monitoring system located at a corner of the barn in a way that gave the best possible view of the whole barn. The equipment was a video surveillance system made by SIRZOO (Si.re.com. srl, S. Martino in Rio, Reggio Emilia, Italy) containing a fixed 3 megapixel camera (3MPX Mobotix M12, Mobotix, Langmeil, Germany), twilight-controlled external infrared illuminators and a digital recorder. The system was connected by Wi-Fi connection system and GSM, gprs, hdspa and hyperlan radio 5.6 Ghz to the research office building, from where it was possible to remotely manage the system and record video by a PC recording system equipped with the Nuuo Platform.

The recordings of the pre-calving behaviour of eight Italian Friesian cows that calved between May and July, going into their second or greater lactation are discussed in this chapter.

Before analysing video recordings, a provisional ethogram was drawn up by listing all categories of behaviour that we could expect to observe; then, one data recording, randomly chosen, was analysed by ad libitum sampling technique; the results of this sample analysis were used to correct the provisional ethogram removing the behaviour never observed in the sample analysis. The ethogram reported in Table 2.1 was used to observe the behaviour of the last 24 h before delivery; delivery time was defined when the calf was fully expelled. The observation was recorded by instantaneous sampling recording rule every 5 min (Martin and Bateson, 2007).

Instantaneous recording method is not suitable to detect events or rare behaviours; thus, it was expected that urination and defecation events would not be detected accurately; when observed, they were nevertheless recorded, but not statistically analysed.

Table 2.1. Ethogram of cows housed in the calving barn

Behaviour	**Description**
Lying inactive	Lying on sternum or side without performing any other described behaviour, head can be rested or raised[1]
Standing inactive	Body supported by four legs, without performing any other described behaviour [2]
Walking	Moving around the barn, not sniffing the ground
Head towards abdomen lying	Head lifted and orientated towards abdomen on stretched neck[1], lying on sternum or side
Head towards abdomen Standing	Head lifted and orientated towards abdomen on stretched neck[1], standing
Eating	Head is placed in feeding trough or over feeding trough while the cow is chewing
Ruminating lying	Jaws move in the act of chewing in a continuous manner, lying on sternum or side
Ruminating standing	Jaws move in the act of chewing in a continuous manner, standing
Drinking	Muzzle is placed in the drinking bowl[1]
Sniffing the ground	Stretching the neck toward the ground, swinging the head
Allogrooming lying	Stretching the neck towards another cow or a calf, lying on sternum or side
Allogrooming standing	Stretching the neck towards another cow or a calf
Selfgrooming lying	Biting or scratching itself using hoofs or leaning against the structures of the barn, lying
Selfgrooming standing	Biting or scratching itself using hoofs or leaning against the structures of the barn, standing
Urination	Release of urine
Defecation	Release of solid waste

[1] From Jensen (2012); [2] modified from Proudfoot et al. (2013);

Total scans spent standing were calculated by summing up scans spent standing inactive, walking, head towards abdomen standing, eating, ruminating standing, drinking, sniffing the ground, allogrooming standing, selfgrooming standing, urination, defecation. Total scans spent lying were calculated by summing up scans spent lying inactive, head towards abdomen lying, ruminating lying, allogrooming lying, selfgrooming standing.

The number of changes from a lying to a standing posture and vice versa (posture changes), was estimated from the information about lying and standing: if a cow was found to be in a posture different from the posture recorded 5 minutes before, then a change in the posture was counted.

Scan spent by performing different behaviours and number of posture changes for each cow occurring within the same hour relative to delivery were summed up and divided by 12 in order to have results expressed as proportions of total scans.

Four cows calved during night, between 00.00 and 05.00 h and four cows calved during day, between 09.00 and 19.00.

A mixed model was used to assess whether and at what time any of the observed behaviours varied significantly; the hour relative to delivery was considered as a fixed factor (24 levels); also, the time of calving (2 levels: night, day) and its interaction with the distance from calving were put in the model as fixed factors. The animal within time of calving was considered as a random factor (8 levels).

When possible, in behavioural studies, animals in the same pen should not be considered as independent experimental unit because of the potential confounding effect of the social interactions. However, a large number of scientific papers report statistical analysis of behavioural data that consider the individual as an experimental unit because this approach can be beneficial to answer specific research questions (Huzzey et al., 2005; Rice et al., 2017). In the current study, preliminary observations and specific rearing conditions suggested that, regarding investigated variables, individual cows could be considered sufficiently independent: the calving barn was sufficiently large to avoid competition for space, food or water; moreover, few positive interactions were expected.

The observed cows calved at 8 different dates so that the date of calving was confounded with the animal factor; it was not possible to consider the calving date as source of variability in the statistical model.

The model was analysed using the MIXED procedure of the statistical analysis software SAS/STAT (2008).

Results

Descriptive statistics are reported in table 2.2. Table 2.2 shows also estimated durations calculated on the basis of hourly proportion of records.

Table 2.2 Descriptive statistics of hourly proportions of records and estimated durations

Behaviour	**Cows, n**	**Sample points, n/hour**	**Mean of hourly proportion**	**Standard deviation of hourly proportion**	**Duration[2], min/24 h**	**Duration[2], min/12 h**
Lying inactive	8	12	0.326	0.332	544	222
Standing inactive[1]	8	12	0.378	0.255	470	290
Walking	8	12	0.026	0.048	38	14
Head towards abdomen[1]	8	12	0.065	0.090	94	40
Eating	8	12	0.007	0.030	10	8
Ruminating[1]	8	12	0.081	0.138	117	50

Drinking	8	12	0.014	0.034	21	7
Sniffing the ground	8	12	0.045	0.007	65	36
Allogrooming	8	12	0.002	0.020	3	2
Selfgrooming[1]	8	12	0.003	0.053	37	17
Total Lying	8	12	0.451	0.369	791	332
Total Standing	8	12	0.549	0.369	650	387

[1] standing + lying;
[2] time was calculated summing up minutes estimated at each hour on the basis of the proportion of records (proportion x 60)

In our conditions, assistance at calving didn't affect behaviour at any hour relative to delivery.

Eating, ruminating, drinking, sniffing the ground, allogrooming and self-grooming were not affected by the hour relative to calving nor by time of calving.

Table 2.3 reports results of the analysis of variance regarding those behaviours affected at least by one of the considered factors ($P \leq 0.05$)

Table 2.3 Effects of hour relative to delivery and time of delivery.

	Hour Relative to Calving	**Time of Calving**	**Assistance at calving**	**Number of cows in the calving barn**	**Hour Relative to Calving * Time of Calving**
Behaviour					
Lying inactive	n.s.	n.s.	n.s.	n.s.	P<0.05
Standing inactive	n.s.	n.s.	n.s.	n.s.	P<0.05
Walking	n.s.	P<0.005	n.s.	n.s.	P<0.05
Head towards abdomen	n.s.	n.s.	n.s.	n.s.	P<0.05
Posture					
Lying	n.s.	n.s.	n.s.	n.s.	P<0.05
Standing	n.s.	n.s.	n.s.	n.s.	P<0.05
Posture change	P<0.0001	n.s.	n.s.	n.s.	P<0.05

Figures 2.1 and 2.2 show the patterns of lying and standing inactive during the 24 h before calving by time of calving.

Figure 2.1. Least square means and 95% confidence limits (cl) of lying inactive behaviour for cows that calved at night and by day; on x axis: hour relative to delivery; on y axis: proportion of sample points in an hour (n/12).

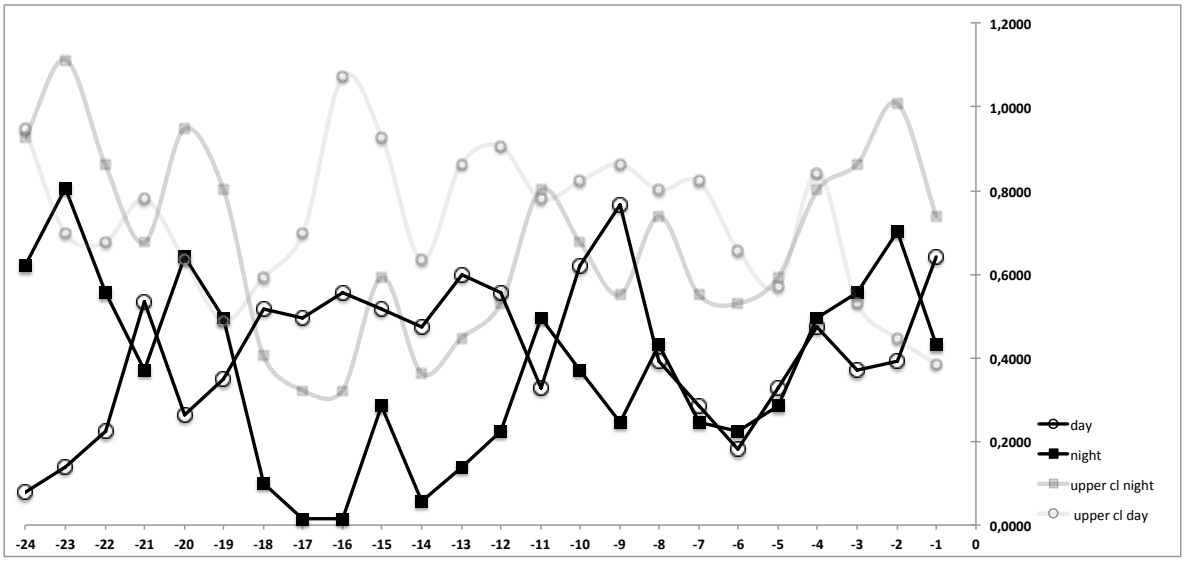


Figure 2.2 Least square means and 95% confidence limits (cl) of standing inactive behaviour for cows that calved at night and by day; on x axis: hour relative to delivery; on y axis: proportion of sample points in an hour (n/12).

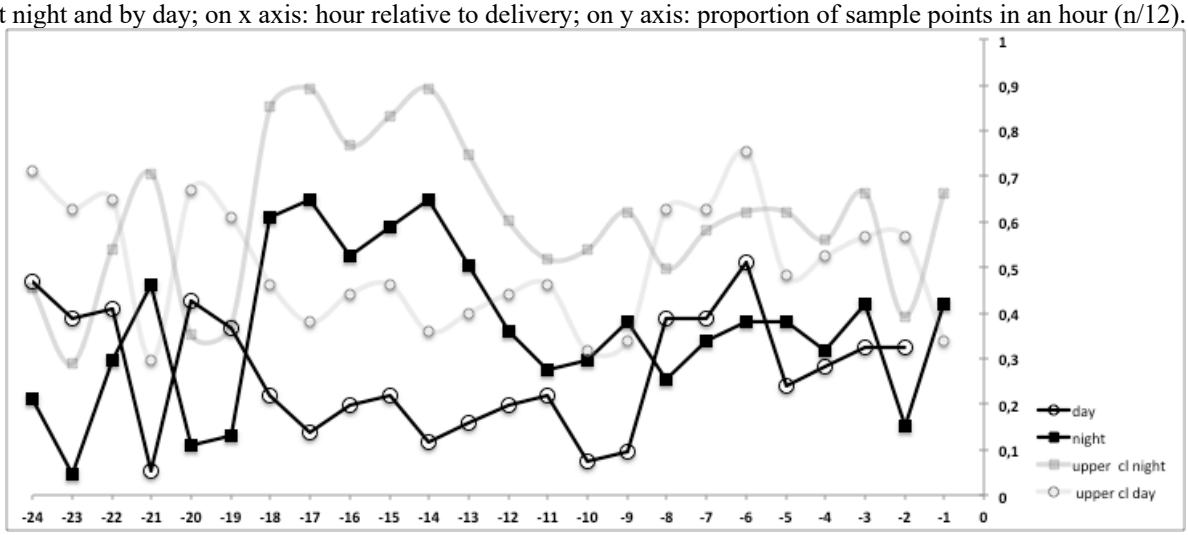
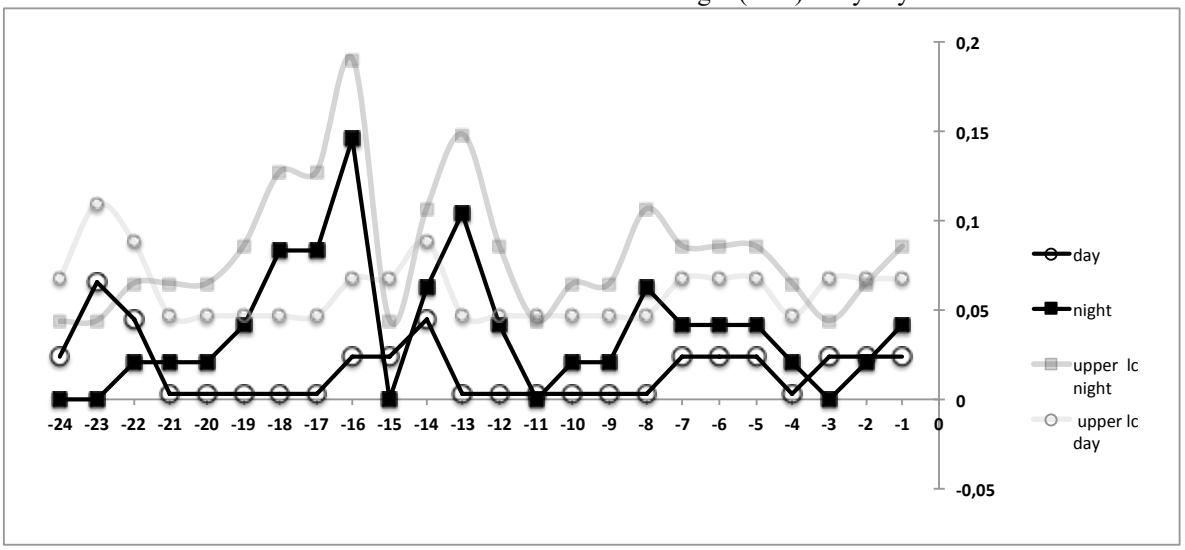


Figure 2.3 shows pattern of walking

Figure 2.3. Least square means (**LSM**) and 95% confidence limits (cl) of walking for cows that calved at night and by day; on x axis: hour relative to delivery; on y axis: proportion of sample points in an hour (n/12). Different letters indicate differences between LSM for cows that calved at night (bold) or by day.



The proportions of record at which cows were observed with head towards abdomen are reported in Figure 2.4.

Figure 2.4. Least square means and 95% upper confidence limits for head toward abdomen of cows that calved at night and by day; on x axis: hour relative to delivery; on y axis: proportion of sample points in an hour (n/12).

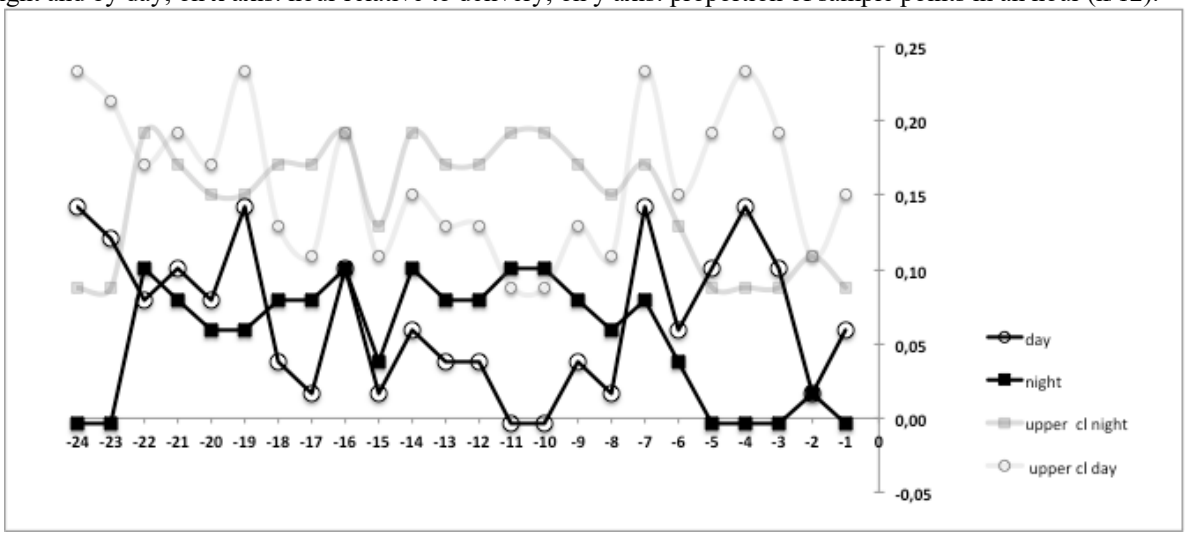


Figure 2.5 and 2.6 shows proportion of records spent totally in lying and standing posture.

Figure 2.5. Least square means and upper confidence limits of lying behaviour for cows that calved at night and by day; on x axis: hour relative to delivery; on y axis: proportion of sample points in an hour (n/12).

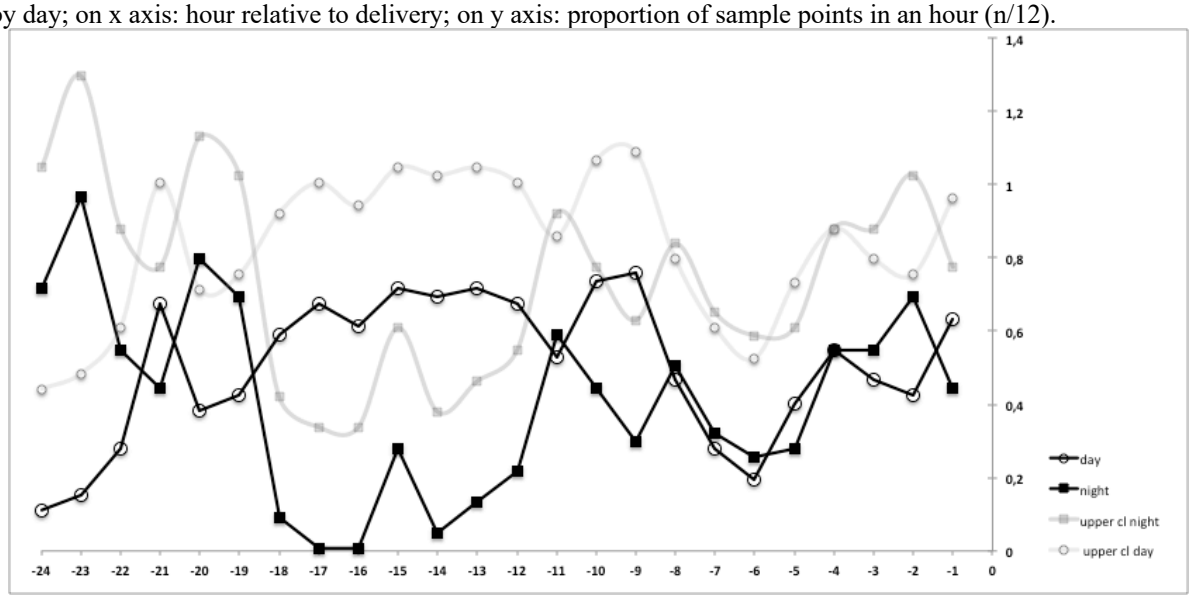
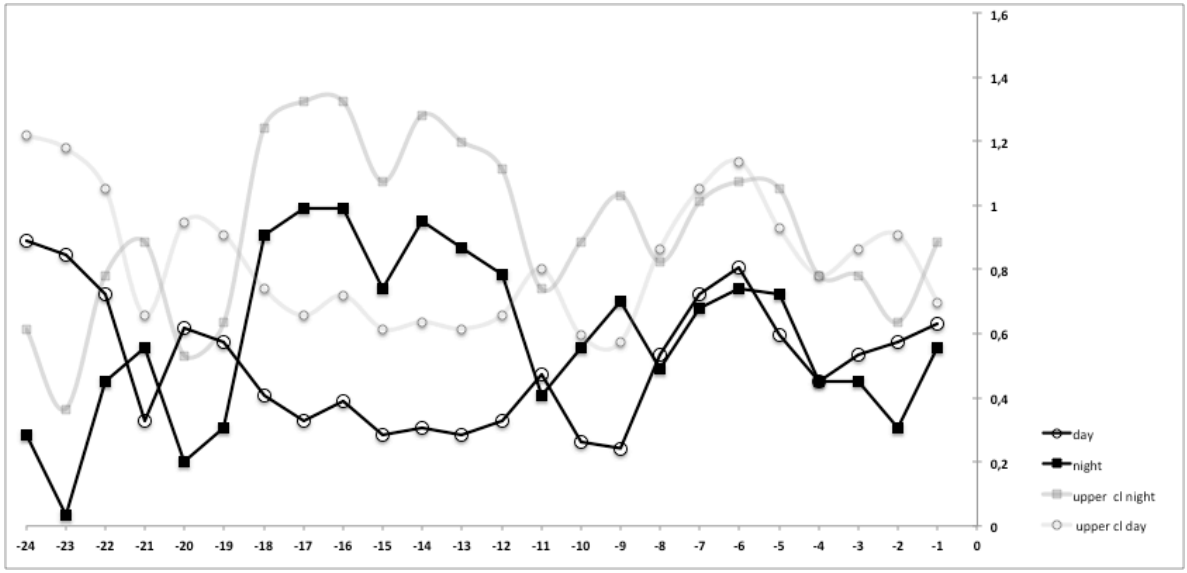
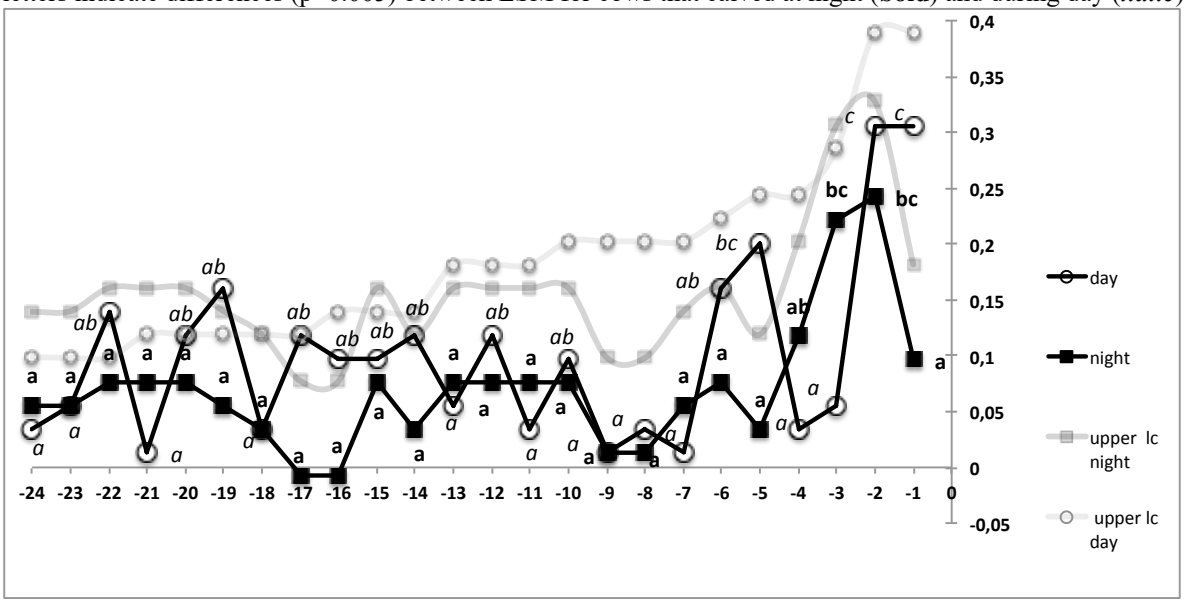


Figure 2.6. Least square means and upper 95% confidence limits of standing behaviour for cows that calved at night and by day; on x axis: hour relative to delivery; on y axis: proportion of sample points in an hour (n/12).



The proportion of posture changes was significantly affected by the hour relative to delivery even if a certain degree of interaction with the time of delivery must be considered (figure 2.7).

Figure 2.7. Least square means and upper 95% confidence limits of posture changes for cows that calved at night and by day; on x axis: hour relative to delivery; on y axis: proportion of sample points in an hour (n/12). Different letters indicate differences (p<0.005) between LSM for cows that calved at night (**bold**) and during day (*italic*)



Cows that calved at night differed (P< 0.05) from those that calved during the day only at -17, -16, -3 and -1 h relative to delivery; for both groups, two hours before delivery the proportion of records at which posture changes were observed was higher than each proportion calculated from -24 to -6 hours relative to delivery (P < 0.005). On average, posture changes counted at 2 h relative delivery were 3.6 times the average the previous hours relative to delivery.

Discussion

Durations calculated on the basis of hourly proportion of records as reported in Table 2 are an oversimplification pretending that each sample point at which a cow was observed to perform a

specific behaviour indicated a bout of 5 minutes for that behaviour; these estimates were very useful to compare current observation with those from literature but must be considered with caution, especially regarding behaviour usually performed in very short bouts such as allogroomimg and sniffing the ground.

In our conditions**,** rumination, eating, drinking, allogrooming, selfgrooming, and sniffing the ground were not affected neither by the hour relative to delivery nor by time of calving.

Regarding rumination, we expected an underestimation, as the method of observation did not always allow us to identify the movement of the jaw; when cow could be seen only from the back, the still photogram didn't help to understand if the cow was chewing/ruminating or not. Our values for ruminating behaviour were in fact lower than those reported by Schirmann et al. 2016 and other authors that measured rumination times by sensors the day of calving. Schirmann et al. 2016 found that cows decreased time spent ruminating by 15% in the 24 h before delivery and reported that the decrease in rumination began about 4 h before expulsion of the calf. Also, in the current study a decrease of rumination was observed: a negative correlation between average proportion of records spent ruminating with hour relative delivery (R=-0,41; P=0,471) indicates a tendency to decrease during the day of calving. The mean of the estimated time spent ruminating at 4 h before delivery was 50% of the average of all previous periods. Moreover, during the last hour before delivery rumination dropped at 23% of the average of all hourly periods from -24 h to -5 h relative to delivery. However, the high variability of observations (hourly proportions of records ranging from 0 to 2) from -24 h to -5 h together with the small size sample didn't allow detecting this effect size as statistically significant. The continuous recording rule would have improved the observation, but direct observation of rumination behaviour is always challenging. However, validated system for automatic monitoring of rumination time are now available for use on commercial farms; these systems are very useful also for research purposes and should be always used.

Drinking was slightly higher than that reported by Jensen et al. (2012).

Eating was lower than expected and found by other authors by continuous recording rule (Jensen et al., 2012; Miedema et al., 2011b). We can suppose that counting for eating when a cow's head was placed in feeding trough or over feeding trough while the cow was chewing had excluded those part of meals when cows head was above or close the feeding trough, but the chewing was not attributable with certainty.

As expected, allogrooming was rarely observed. Also, selfgrooming was little observed.

Sniffing the ground was observed for 4.5 % of the sampled time that is much more than what reported by Miedema et al. (2011a) regarding a similar explorative behaviour, licking the ground; in the current study sniffing the ground didn't show any tendency during the 24 h of observation

while Miedema et al. (2011a) found a significant increase of time spent licking the ground during the final six hours before delivery.

For other behaviours the interactions between hour to delivery and time of calving suggest that circadian rhythm and managing routine mask or reduce the effect the approaching to delivery.

Estimates for walking were very similar to those observed by Miedema et al. (2011b) and Houwing et al. (1990).

Comparing the last 12 hours before calving, head towards abdomen was less observed than in the study by Jensen et al. (2012) who found a decrease of this behaviour at 2 hours before calving.

The animals that calved at night and those which calved during the day showed a rather complementary pattern of the lying behaviour, both inactive and active. It is worth to highlight that this complementarity does not occur at 6 hours before delivery, when both groups have a drop of lying down to perfectly overlapping values.

Consistently, at 6 hours before delivery, both groups showed an increase in standing behaviour.

Changes of posture showed a dramatic increase at two hours before delivery in both groups. This is related to increased restlessness that is associated with uterine contractions and has been often identified by an increase in the frequency of transitions from lying to standing; such changes of posture are frequently measured as lying bouts or standing bouts (Barrier et al., 2012).

According numerous authors, instantaneous sampling with an interval length of 10-15 min is a suitable recording method to estimate the proportion of time spent lying by cattle (Haley et al., 2000; Mitlöhner et al., 2001; Schenck, 2010; Mattachini et al., 2013; Chen et al., 2017). Therefore, we assumed that the number of posture changes we estimated from recording behaviour by instantaneous sampling every 5 min was a good estimator of the actual number of transitions in posture and therefore of the number of lying bouts.

Continuous recording rule is considered the gold standard for measuring the frequency and the length a behaviour performance; however, collecting data at fixed time intervals (instantaneous sampling) is a labour-saving alternative method to improve efficiency of data collection. Chen et al. (2016) compared instantaneous sampling and continuous observation of dairy cattle; they found that the amount of time spent lying was accurately captured using sample intervals $\leq$ 30 min. Concerning the number of lying bouts they found that sampling interval of 5 min produced data strongly related to those obtained by continuous observation but with a certain degree of underestimation; moreover, the study showed that a sample interval $\leq$ 3 min accurately estimated the number of lying bouts. Having used a sample interval of 5 min, a slight underestimation of posture changes was possible in our study.

The average of the number of posture changes totally recorded across the 24 hours before calving in the current study was 26.75 (standard deviation: 8.97 times/day). Similar frequencies of

standing bouts and lying bouts have been reported in literature (Huzzey et al., 2005; Miedema et al., 2011b); this figure is about twice the average values of lying bouts recorded by a previous study (Blackie et al., 2006) in the control periods (first, sixth and twelfth weeks of lactation). According to Miedema et al. (2011b), the average lying bouts observed in pre-calving control period (-1 ÷ -10 days relative to calving, median = -3) was 16.4 ± 4.8 times/day.

The peak of posture changes at two hours before calving is partially in agreement with results from Jensen (2012) that video-recorded cows housed in individual pens. The latter found the increase in frequency of lying bouts starting at -4 hours relative to calving and hourly average lying bouts at - 2 hours relative to calving being 2.2 times the average of frequency observed from -12 hours relative to calving. Similarly, in our study, frequency of posture changes at -2 hours was 2.8 times the average from -12 h; also, Miedema et al. (2011b) reported an increase in frequency of lying bouts starting at approximately 4 hours and 13 minutes before calving. Comparing assisted and unassisted calvings, the same authors (Miedema et al., 2011a) reported that lying frequency increased from -6 h relative to calving in unassisted animals, but only during the final 2 hours before calving in assisted animals.

Conclusions

Taking into account the results obtained in this study and others we can conclude that the increase in the frequency of posture changes may be a very useful indicator in identifying cows that are close to delivery. This type of data can be collected automatically via accelerometers, attached to the animal's leg or worn around the neck, making their use very effective.

Systems currently used for oestrus detection could be implemented also for calving prediction (Borchers et al., 2017). Further studies are needed to define the benchmark increase in lying bouts frequency to be used in alerting systems.

Moreover, the higher number of posture changes observed in 24 hours before calving confirms the need to pay great attention to the comfort of the calving barns.

Chapter 3- Technologies for the improvement of animal welfare: measuring quality of colostrum for an optimal start of a healthy dairy industry

Introduction

It is important that the calves, future heifers and cows, start their life in the best way.

Colostrum is the first and most important line of defence for a newborn calf.

The calf, at birth, has an immature immune system that reacts slowly to infections and therefore makes it more exposed to pathogens. Moreover, at birth the calf is free of the maternal antibodies; during gestation antibodies cannot cross the syndesmochorial placenta that is impermeable to the large molecules; thus, passive transfer of immunity is essential for the short- and long-term health of dairy calves.

The first immune protection is given by the immunoglobulins (Ig) that the calf ingests in the first hours of life through the colostrum produced by the mother. A portion of these immunoglobulins, once absorbed through the small intestine, provides protection against systemic diseases. Another part of immunoglobulin acts directly in the intestine where it can neutralize pathogens and prevent the development of diarrhoea. With the colostrum, the calf also ingests the maternal lymphocytes that, through the intestinal mucosa, reach the different tissues and stimulate the development of immune system.

Colostrum also contains transferrin and lactoferrin that reduce bacterial growth by binding to the iron that would otherwise be used by bacteria. Colostrum also has a considerable importance for its caloric intake: the fat content of colostrum allows the calf to warm up and maintain a body temperature sufficient to survive. Often the mortality in the first twenty-four hours of life of newborn calves is linked to the immaturity of the body thermoregulation system. Colostrum contains different types of immunoglobulins: G, A and M. The content of immunoglobulins of type G (IgG) is the commonly considered to evaluate the quality of the colostrum, due to their greater presence, while IgA and IgM are more present in milk, ensuring local intestinal protection. A good level of passive immunity is obtained by administering good quality colostrum, in the adequate quantity, and within a few hours after birth. The quality of the colostrum produced varies from cow to cow and depends on the calving parity, the season and the time between the delivery and the first milking (Maunsell et al., 2014). Because of the wide variability, a key factor to ensure a good passive immunity transfer is the use of tools to evaluate the quality of the colostrum: colostrometer or refractometer.

The colostrometer is a simple tool consisting of a densimeter characterized by a graduated scale, coloured to indicate the thresholds of relative density or specific gravity (SG) that correspond to different degree of colostrum quality (Figure 3.1).

Figure 3.1 Colostrometer

The colostrometers on the market are provided with a special 250 ml cylinder which is filled with colostrum and in which the densimeter is subsequently immersed. Observing in correspondence of which colour of the graduated scale the colostrometer meets the superior surface of the colostrum sample, allows immediate estimation of the colostrum quality. If the colostrometer floats at the green level, the sample is very dense, meaning of excellent quality.

The use of the colostrometer allows to estimate the concentration of IgG; the GS is in fact related to the total solids content that are largely represented by proteins; most of the colostrum proteins are composed of globulins and above all IgG (Mechor et al., 1992). Since the temperature influences the density, the measurement should be carried out at a colostrum temperature of 20 ° C. Alternatively, the data obtained from the measurement must be adjusted according to the temperature of the colostrum (Mechor et al., 1992).

The correspondence between different areas of the graduated scale and SG value is established on the basis of the following relation measured by Fleenor and Stott (1980) between SG and colostrum globulins:

SG <1035 = globulins <21.8 g / l = red zone = insufficient quality

1035≤ SG≤1046 = 21.8 g / l ≤ globulins ≤49.82 g / l = light green area = moderate quality

SG> 1046 = globulins> 49.82 g / l = bright green area = excellent quality.

The refractometer measures the refraction of light in a liquid solution that is affected by the total solids content present in the liquid (Figure 3.2).

Figure 3.2 optical refractometer

A drop of the sample is placed on the prism; the amount of refracted light is measured. The result is immediately read on the scale which is expressed as a refractometric index. The refractometric index can be transformed into Brix (Morrill et al., 2012) which expresses the percentage concentration of the soluble solid content in a sample. In practice, it is considered that a value of 22% Brix is equivalent to the IgG concentration of 50g / L, the minimum level for a quality colostrum (Bielmann et al., 2010).

Also, digital refractometers are available on the market (Figure 3.3)

Figure 3.3. Digital rifractometer

To maximize the passive immunity transfer, the cow should be milked early after calving; in fact, at every hour since the birth, the IgG in colostrum decrease by 3.7% (Morin et al., 2010); for these reasons it is advisable to milk and provide the colostrum as soon as possible after calving rather than wait for the routine milking time. The calf should take colostrum within 6 hours of birth, since the intestinal permeability to the IgG decreases with the time (Godden, 2008).

Actually, the effective timing of administration of the first colostrum varies considerably between farms and within farms depending on the time of birth and the organization of work. In some farms the calves born at certain times of the day can have the first administration well over 6 hours after birth.

Generally, the passive transfer of the colostral immunoglobulin to the newborn calf is considered good if the calf serum concentration of IgG reaches a level between 10-15 mg / ml and optimal if the concentration is greater than 15 mg / ml. Serum concentration in the range of 5-10 mg / ml indicates a partial failure of passive immunity transfer, while a serum concentration value of less than 5 mg / ml is indicative of a complete failure of passive immunity transfer (Furman-Fratczak et al., 2011). Passive transfer of colostral immunoglobulins to calves can be influenced not only by birth / colostrum time interval, volume of colostrum administered, colostrum quality, but also by metabolic disorders of the calf (metabolic and respiratory acidosis) following prolonged calving, which probably, reduce the ability to ingest an adequate amount of colostrum (Weaver et al., 2000). Insufficient intake of IgG via colostrum predisposes to the "Failed passive colostral transfer syndrome" (FTP): increased susceptibility to pathogens, slow growth, lower production and lower number of lactations.

Measurement of the serum IgG concentration in the calf can be determined in the laboratory by Elisa, RID (Radial Immuno Diffusion), electrophoresis of the serum proteins or indirectly by measuring the total serum proteins. At farm level an evaluation can be done by the refractometer.

Although, the importance of giving good quality colostrum timely it is well-known, few farmers are equipped to make an immediate measurement of colostrum quality at farm level.

This chapter reports the results from a field study carried out to evaluate the feasibility of quality assessment at farm level and the average values and variability of quality of colostrum at two use-case farms.

Material and methods

The colostrum of the first milking of 98 cows was collected over 12 months in two farms in the Po Valley; the quality of the colostrum was estimated by measuring the relative density with a colostrometer; 86 samples were analysed for the total protein content and whey protein electrophoresis; 74 samples were subjected to refractometric analysis.

Protein content of colostrum samples was analysed by spectrophotometer for infrared analysis with Fourier transform (Milkoscan FT2, Foss Italy). The determination of the IgG content was made on colostrum serum obtained by treating 40-50 ml of colostrum with a dose of rennet ranging between 200 and 300 µl to obtain coagulation and separation of the whey; coagulation was helped by keeping the samples in a thermostatic bath at 37° C; once formed, the coagulum was finely broken and the sample was again thermostatically bathed at 37° C to facilitate the expulsion of the whey; the serum was then recovered by filtration and analysed for the protein content using an automatic analyser for clinical chemistry (ILAB, Aries, Instrumentation Laboratory, USA).

The day after birth the blood of 53 calves was sampled to determine the IgG serum as an indicator of the efficacy of colostrum administration.

Serum proteins of colostrum and blood were separated by an automatic system for agarose gel electrophoresis (Hydrasis, Sebia Italia), using a standard kit for blood serum proteins (Hydragel30, Sebia Italia). The gels were read by densitometry and interpreted by a dedicated software (Phoresis, Sebia Italia).

The feasibility of using rapid tests to assess the level of passive immunity transfer was also considered. The market investigation led to the conclusion that the tests of this type are few and their use is still not widespread. We tested the cost and practicality of using a semi-quantitative rapid test to determine the concentration of circulating IgG in serum and / or whole blood of calves using a competitive lateral flow immunological reaction. Using whole blood samples, we can distinguish three cut-off intervals and the respective evaluations of passive immunity transfer:

<5mg / ml → transfer failed

between 5 and 12 mg / ml → risk of partial failure

12 mg / ml → probable good transfer

> 12 mg / ml → certain excellent transfer

The test was applied to 10 calves born at the farm B; the test was fairly simple to be performed, but it was necessary to contact the supplier to update the methods of execution with respect to what is indicated in the enclosed instructions. The time taken to perform the test was on average 15 minutes. The cost of the single test was about € 9. One of the samples indicated a safe passive immunity transfer failure, 4 indicated a good transfer, only 5 samples indicated a safe passive transfer. The sample that resulted in a failed transfer actually belonged to a calf given insufficient amount of colostrum at the first feed after birth. The test was also tested in two external companies out of a total of 15 calves that had been provided with excellent colostrum. In all cases the test resulted in an excellent transfer of passive immunity.

Results

Table 3.1 reports the mean values of specific gravity (SG) of colostrum administrated to 45 of 53 calves monitored for hematic content of IgG.

Table 3.1 Mean of specific gravity (SG) of colostrum given to calves tested for hematic content of IgG.

Farms	Parity	n	SG (sd)
Farm A	Primiparous	14	1051.64 (13.08)
	Multiparous	16	1059.00 (13.43)
	Total	30	1055.57 (13.56)
Farm B	Primiparous	3	1042.67 (17.50)
	Multiparous	12	1059.79 (8.45)
	Total	15	1056.37 (12.25)
Total		45	1055.83 (13.00)

Table 3.2 Frequency of specific gravity (SG) of colostrum administrated to calves tested for hematic IgG

Farms	SG class	Total
Farm A	Excellent	26
	Medium	0
	Insufficient	4
	Total	30
Farm B	Excellent	12
	Medium	0
	Insufficient	3
	Total	15

For 33 of the 53 monitored calves it was also possible to determine the refractive index of colostrum taken at the first administration (Tables 3.3 and 3.4).

Table 3.3 Means of refractive index and Brix

Farms	Parity		Refractive index (ds)	% Brix (ds)
Farm A	Primiparous	9	1.3398 (0.0020)	21.94 (6.55)
	Multiparous	15	1.3399 (0.0020)	22.56 (6.55)
	Total	24	1.3399 (0.0020)	22.33 (6.42)
Farm B	Primiparous	2	1.3405(0.0000)	24.50 (0.00)
	Multiparous	7	1.3401 (0.0014)	23.01 (4.38)
	Total	9	1.3402 (0.0012)	23.34 (3.85)
Total		33	1.3400 (0.0018)	22.61 (5.79)

Table 3.4 Frequency distribution of Brix class of colostrum given to calves

Farms	Brix	Frequency
Farm A	<22%	13
	≥22%	11
Farm B	<22%	3
	≥22%	6

Mean values for IgG in the serum of calves are reported in Table 3.5

Table 3.5. Mean values for IgG in the serum of calves

Farms	Birth- colostrum lag administration (hours)	Parity	N	Average intake of colostrum at first administration, (ds)	Average serum IgG, g/l, (ds)
Farm A	> 6	Primiparous	6	1.00 (0.00)	9.81 (3.68)
		Multiparous	4	1.25 (0.50)	12.58 (12.56)
		Total	10	1.10 (0.32)	10.92 (7.88)
	< 6	Primiparous	13	1.08 (0.28)	9.07 (5.44)
		Multiparous	15	1.20 (0.41)	11.80 (5.44)
		Total	28	1.14 (0.36)	10.53 (5.51)
		Total	38	1.13 (0.34)	10.63 (6.11)
Farm B	> 6	Primiparous	3	2.00 (0.00)	10.14 (0.48)
		Multiparous	2	2.00 (0.00)	18.42 (13.28)
		Total	5	2.00 (0.00)	13.45 (8.05)
	< 6	Primiparous	0	-	-
		Multiparous	10	2.10 (0.32)	17.44 (4.95)
		Total	10	2.10 (0.32)	17.44 (4.95)
		Total	15	2.07 (0.26)	16.01 (6.24)
Total			53	1.40 (0.53)	12.08 (6.54)

The overall mean value indicates a good level of passive immunity in agreement with the excellent average quality of the colostrum provided. The mean value for farm A would have indicated an acceptable immune transfer, but the high variability suggested a probable high proportion of failed passive immunity transfers; indeed, looking at the frequency distribution in the different reference classes (Table 3.6) there is a 47% partial failure in farm A.

Table 3.6 Frequency distribution of passive immunity transfer.

Farm	Transfer*	mean serum IgG g/L (ds)	Mean birth-first colostrum administration interval, h:mm (ds)	Average number of meals before blood sampling (ds)	Number of calves
Farm A	Failed	2.89(1.26)	4:28(1:25)	1.20 (0.45)	5 (13.16%)
	Partially failed	7.75(1.50)	5:54(4:19)	1.56 (0.51)	18 (47.37%)
	Good	13.52 (1.13)	4:59 (2:19)	1.63 (0.52)	8 (21.05%)
	Excellent	20.28 (5.17)	5:25 (4:22)	1.86 (0.38)	7 (18.42%)
	Total	10.63 (6.11)	5:26 (3:38)	1.58 (0.50)	38 (100%)
Farm B	Failed	-	-	-	0
	Partially failed	9.33 (0.43)	7:15 (0:21)	1.50 (0.71)	2 (14.29%)
	Good	11.03 (0.81)	5:45 (1:39)	2.00 (1.15)	4 (28.57%)
	Excellent	20.18 (5.02)	3:09 (2:22)	2.38 (0.52)	8 (57.14%)
	Total	16.01 (6.24)	4:28 (2:32)	2.14 (0.77)	14 (100.0%)
	Total	12.08 (6.54)	5:10 (3:22)	1.73 (0,63)	52

IgG<5g/l =failed; 5g/l ≤ IgG <10 g/l = partially failed; 10 g/l ≤ IgG ≤ 15 g/l = good; IgG>15 g/l = excellent. (Furman-Fratczak et al., 2011):

The serum IgG content in calves serum was poorly correlated with the birth-first administration interval, while a moderate significant correlation was found with the amount of IgG ingested with the first administration of colostrum, estimated on the basis of SG (R=0.40; P = 0.0206). The amount of IgG taken is due to the combination of quantity and quality of the colostrum; the results indicate that, in our conditions, the quantity of given colostrum explained a greater share of variability of passive immunity transfer; the correlation coefficient between IgG in blood and the amount of colostrum administered at the first feed is 0.40 (P = 0.0154).

Conclusions

The field study on colostrum reached two objectives:

- to evaluate the feasibility of quality assessment at farm level;

- have a knowledge of the average quality values of colostrum and their variability. The information collected will be useful to prepare infographic and other educational material to promote better management of colostrum at farm level.

Chapter 4- Relationship between animal welfare and milk quality: literature reviews

Introduction

This Chapter aims at contributing to meet an urgent need in the area of animal source food: characterizing food in relation to the animal welfare as an important attribute of sustainability of animal productions.

There is a strong demand of information based on scientific evidence making it possible to define the products in a transparent and measurable way. An increasing part of consumers have concerns about welfare of animals used for food production; the main reasons of these concerns are that husbandry has become more and more intensive and genetic selection for production traits could have side effects on animal reactivity. Consumers' ability of processing information about quality is limited because some attributes of food quality cannot be evaluated by visual perception or by experience. Considering the consumers 'ability to distinguish it, foods traits have been classified into search, experience or credence attributes. An attribute is considered as a search attribute if consumers are able to identify its quality before purchase through either inspection or research; a credence attribute is classified as one for which quality could not be assessed even after the product was purchased and consumed; animal welfare is often a credence attribute. One of the main obstacles on this way has been the difficulty of measuring it by feasible and objective methods based on scientific data; now, there is a general agreement on the fact that very effective direct measurements are available and must be used; moreover, new technologies are available to measure and improve animal welfare.

Even there are growing evidences that a higher animal welfare could be economically convenient for farmers, one of the problems that must be considered is that improving animal welfare can increase cost of products; thus, one question is if the people will accept higher prices for more friendly animal products.

The Eurobarometer survey (2016) showed that more than half of all Europeans are prepared to pay more for products sourced from animal welfare-friendly production systems (59%); more than a third of respondents (35%) are prepared to pay up to 5% more, while only a small minority (3%) are ready to pay more than 20%. However, respondents want to receive more information on the conditions under which farm animals are treated.

De Graaf et al. (2016) explored the possible introduction of a Welfare Assurance Scheme (WAS) for dairy cattle in Flanders (Belgium) with added value for the animals, consumers and farmers. They considered research questions regarding: a credible, implementable and cost-efficient welfare

assessment; an existing or potential consumer market for animal-friendly milk; a positive relationship between animal welfare and farm performance. Their conclusions are that the integrated herd welfare scores from an existing welfare assessment protocol (the Welfare Quality® protocol) do not accurately reflected dairy cattle welfare in a way that matches well with the opinions of trained users of this protocol. Therefore, an alternative welfare assessment protocol was developed, which is transparent, simple, multidimensional, discriminative, and corresponds with expert opinion. A highly heterogeneous consumer market for animal-friendly milk was identified in Flanders, for which product differentiation is a promising strategy. Dairy cattle health, access to pasture and perceived advantages of such milk are promising selling propositions. For a sample of 41 dairy farms in Flanders, de Graaf et al. (2016) found no clear link between farm performance and animal welfare, meaning that efforts on one criterion not necessarily jeopardize the other. Their overall conclusion is that the introduction of a WAS in Flanders is possible, but that the implementation strategy is vital to achieve a balance between credibility, feasibility and costs.

Pala and Atakisi (2012) showed that sensory perception of different level of animal welfare by tasting yogurt was strengthened when sensory input was supported with information provided on animal welfare; they suggest that friendly animal products should be advertised and labelled clearly to increase consumer sensory acceptance and willingness.

We can assume a higher willingness to pay for animal welfare if the animal welfare attributes become search attribute and can be demonstrated associated with higher quality of products.

Environmental stressors, such as those due to climate changes, housing and interaction with humans have effect on welfare of cows and udder physiology and milk composition. Moreover, the stress enhances the likelihood of infection; classic explanation for this fact is that activation of the sympathetic nervous system under stress leads to the release of neuroendocrine mediators, which may impair innate and adaptive immunity; a new discipline, microbial endocrinology, is giving an important role to the bacteria within the ruminant digestive tract to better understanding the mechanisms by which stress influences the pathogenesis of infectious disease; Freestone and Lyte (2010) showed that enteric pathogens have evolved systems for directly sensing stress hormones by demonstrating that exposure of enteric pathogens to physiological concentrations of stress hormones can result in increases in growth and changes in expression of virulence factors such as adhesins and toxins. These findings are promising a new approach to better understand how stress influences ruminant physiology and products.

The following paragraphs will analyse how some stress and management factors that have consequences on welfare of lactating dairy cows can also affect the milk quality,

Sickness and metabolic diseases

Mastitis

The best demonstrated link of milk quality with health of cows is the effect of mastitis on composition and physical characteristics of milk and milk products (Barbano et al 1997; Barbano et al 2006).

Milk produced during mastitis is characterized by a higher somatic cell count (SCC); mastitis induces increased proteolysis, lipolysis, and free fatty acids content (Table.1).

Effect on the animal physiology	Effects on milk characteristics	Negative consequences on quality of milk and dairy products
Accumulation of leukocytes produced by the cow's immune system at the infection site (udder)	Increased cell somatic count; increased activity of non-plasmin proteases (somatic cell protease)	Reduction of shelf life of fluid milk due to accumulation of small peptides developing bitter and astringent off-flavours (Ma et al., 2000) Increase in rennet coagulation time and reduction in curd firming rate (Politis and Ng-Kway-Hang, 1988).
Increased tight junctions permeability	Increased plasmin activity	Reduction of shelf life of fluid milk due to accumulation of small peptides developing bitter and astringent off-flavours Increase in rennet coagulation time and reduction in curd firming rate (Ismail and Nielesen, 2010)
	Reduced de novo synthesis in the udder Increased leakage of blood components into the udder	Lower concentration of calcium, lactose, casein and fat Higher concentration of sodium, chlorine and serum protein (Delamaire and Guinard-Flament, 2006)
Increased susceptibility of milk fat substrate to lipase activity	Increased lipolysis	Reduction of shelf life of milk and yogurt due to increased free fatty acids content leading to rancid off-flavours (Fernandes et al., 2007).

Table 1. Demonstrated effects of mastitis on milk and dairy products

The increased proteolysis is, partially, due to increased activity of plasmin. Since 1995, Ballou et al., through an investigation into the bulk milk of 200 farms ranked on the basis of the SCC, showed that the level of plasmin (PL) in milk is higher for farms with higher SCC. Plasmin is a protease, part of a complex protease-protease inhibitor system that exists in milk in its inactive zymogen form, plasminogen that can be converted into active PL by plasminogen activators (Grufferty and Fox, 1988). The proteolysis induced by PL can have both positive or negative effects on the texture and the flavour of dairy products (Ismail and Nielsen, 2010) depending on the degree of hydrolysis and the type of product; proteolysis in cheese during ripening results in texture modifications, pH increase through NH3 formation and the production of flavour compound (Fox et al., 1993); thus, a certain degree of protein's hydrolysis contributes to develop the consistency and flavour you want; in pasteurized or sterilized milk, excessive proteolysis is undesirable because reduce the shelf life and may lead to develop bitter off-flavours (Ma et al., 2000) due to the accumulation of small peptides.

The variations of the content of plasmin and plasminogen in milk are, partially, related to changes in the tight junctions (TJ) permeability; TJ are the intercellular junctions of the secretory cells of the mammary gland. In healthy udders, during lactation, the mammary epithelial TJ limits permeability and exchanges between milk and blood; when a pathogenic microorganism penetrates in the teat canal irritates and invades the mammary tissue, causing an inflammatory response with partial or complete breaking of TJ and increase of permeability of epithelium. In such conditions, de novo synthesis of milk components in the udder is reduced and the influx of the blood components into the milk is increased; there is an increased transfer of plasminogen from blood plasma to milk where it is activated to plasmin. The same thing happens in situations of stress and at the end of lactation.

Moreover, when the TJ become leaky, calcium, lactose, casein and fat contents are reduced in milk, while concentrations of sodium, chlorine and serum proteins increase (Kitchen 1981; Stelwagen, 1999; Delamaire and Guinard-Flament, 2006).

However, during mastitis, also a contribution of non-plasmin proteolytic activity from somatic cells, especially phagocytic leukocytes (polymorphonucleocytes and macrophages) occur. These cells contain active proteases; when milk SCC is maintained elevated (1 million cells/ml or more) their contribution becomes significant.

When SCC is high also lipolysis is high and affects negatively taste and technological properties of milk. Lipases catalyse hydrolytic release of free fatty acids from triglycerides causing a flavour defect in fluid milk described as “rancid” (Shipe et al 1978).

Increased lipolysis in yogurt (Fernandes et al., 2007) and lower yield in cheeses (Politis and Ng-Kwai-Hang 1988) have been also reported as consequences of mastitis.

Ketosis

The changes of milk composition during ketosis in dairy cows have been investigated by many decades providing consolidated knowledge about the effect of ketosis on fat; fat percentage and fat:protein ratio are used as indicator of risk of ketosis. However, the changes of mineral components, fatty acids content; acidity and coagulation properties have been very little or no studied at all.

Contaminants

Concerns grow over potential contamination of milk with antimicrobials and antimicrobial-resistance (AMR). Antimicrobials in the veterinary sector are a fundamental means for controlling infectious diseases. Their introduction has contributed to the improvement of animal welfare and is an important way to guarantee the safety of food of animal origin, however, currently concern for AMR phenomenon (i.e. the ability of a micro-organism to resist the action of an antibiotic) calls into question the conventional and often excessive use of antimicrobials. Antimicrobial resistance is a natural biological phenomenon that occurs due to the emergence and propagation of bacterial resistance factors

to antibiotics, but it was triggered and amplified by the selective pressure exerted on the microbial populations through the use of these drugs.

Improving the welfare and living conditions of farmed animals reduces the need for treatment.

The relationship between a good level of animal welfare and a good immune response is documented and the fundamental role of stress in this relationship is known; preventive action must therefore limit stress by avoiding overcrowding and under and over nutrition, providing adequate quantities and quality of water, comfort and environmental hygiene.

Overcrowding and low hygiene reduce the effectiveness of the immune response and can also have a direct effect on increasing the infectious pressure.

In the case of dairy cattle, the main aspects of animal welfare to be considered in order to prevent pathologies and in particular infectious diseases are: proper management of calving, providing colostrum of calves, proper litter maintenance, microclimate (proper natural ventilation inside in the barns, cooling systems),

Precision livestock farming promises to be help: clinical signs of disease could be detected earlier through regular or continuous analysis of sensor data. This in turn enables early countermeasures, such as separation of diseased animals and targeting the use of antibiotics to only a limited and small number.

The Istituto Zooprofilattico della Lombardia e dell'Emilia Romagna recently reported that percentage of positivity regarding inhibitor substances residues decreased from 1997 to 2017 (https://www.izsler.it/pls/izs_bs/v3_s2ew_consultazione.mostra_pagina?id_pagina=530) passing from 0.727% to 0.141; this means that positivity regarded only 130 samples of the more than 97,000 analysed in 2017, compared with the 1450 cases of positivity on almost 200,000 samples analysed on 1997. This result was partly explained by the reduced use of drugs (especially those used to cure mastitis) and partly by the gradual improvement of procedures for the correct execution of the treatments, the correct registration of the treated animals, and the respect of the suspension times and the execution of the controls on the single treated animals.

Oxidative stress

Oxidative stress is the result of an imbalance between prooxidants processes and antioxidants processes.

Tissues of animals under oxidative stress show an excessive production of free radicals; free radicals are compounds with an unbalanced electronic structure that gives them a great reactivity towards organic constituents and cellular structures; for this characteristic, free radicals are involved in a large number of redox reactions and are formed continuously, along the energy metabolism, contributing to the adaptation of an organism to the environment; however an excess of free radicals is harmful, can alter cellular structures and reduce the effectiveness of the immune system cells.

Diseases, bacterial, viral infections and any situation of stress, such as improper handling of animals, extreme cold and heat stress, excessive dietary levels, unbalanced diets stimulate the production of free radicals.

In cows, oxidative stress is frequent during the first three weeks after calving; the intensive metabolism observed during this period is accompanied by an increase in the amount of reactive oxygen species. While the effects of oxidative stress on the characteristics of the meat is well known, less is known about the relationship between oxidative status of the body (evaluated by the oxidative status of blood) and oxidative status of the milk; it is not yet clear to what extent the direct effect of oxidative stress is reflected in the milk in terms of greater content in oxidized compounds or lower content of antioxidant molecules. However, Andrei et al. (2011) reported a value of glutathione peroxidase higher in mastitic milk than in normal milk. Moreover, was clearly demonstrated that grazing affects oxidative status of milk; Pizzoferrato et al., (2007) proposed a parameter (DAP or degree of antioxidant protection) able to distinguish milk and cheese from grazing and zero-grazing animals; they found a discrimination between goat's milk and cheese produced from different feeding systems and concluded that cholesterol was highly protected against oxidative reactions when the herbage was the only feed or was dominant in the goat diet but they also suggest that grazing allows goats to select their favourite herbage and plays a key role in improving animal welfare and milk composition.

Grazing

Grazing provides a "natural" diet and freedom of movement although cattle at pasture are not free of all welfare problems (Hemsworth et al., 1995; Petherick, 2005).

The EFSA Panel on Animal Health and Welfare reviewed the effects of access to pasture on health (EFSA, 2009) and highlighted that a number of epidemiological or experimental studies in different countries found that lactating cows without access to pasture suffer from a higher incidence of a variety of health problems including mastitis; the most commonly reported welfare problem associated with restricted grazing is lameness. It was reported that a lack of access to pasture in winter was a significant risk factor for a high incidence of digital dermatitis, and that providing access to a dry-lot was not sufficient to overcome this (Rodriguez-Lainz et al., 1996; Wells et al., 1999). A survey of 86 dairy farms in the Netherlands (Somers et al., 2003) reported that all types of hoof disorders were more prevalent in cows in zero-grazing systems than among cows with some access to pasture; the prevalence of severe cases of sole haemorrhage was twice as high with zero-grazing compared to other housing systems. When cows had some seasonal access to pasture, hoof disorders (but not digital dermatitis) were more prevalent during the period of indoor housing compared to the end of the period of summer access to pasture. A survey of 37 farms in the UK found a higher prevalence of lameness

in cows that could not graze compared to cows that have some ability to graze (prevalence of 39% versus 15%) as well as a higher frequency of swollen knees (Haskell et al., 2006).

Hernandez-Mendo et al., 2007 reported that even a short period of access to pasture can reduce lameness. Cows in indoor housing are more likely to be standing in manure and on concrete, and eating more grain than cows at pasture, all of which increase the likelihood of lameness.

Grazing determines also an improvement of the nutritional characteristics of milk in comparison with indoor feeding. Two main factors have been well documented by literature:

a) the fresh forage composition together with the plant secondary metabolites, influence the rumen fatty acid metabolism, leading to an increase of omega 3 and conjugated linoleic acid concentration in milk. Puppel et al., 2017 showed that the DAP values were lower in milk from indoor feeding treatment than in milk from cow fed with pasture.

b) the sunlight exposure determines a higher concentration of Vitamin D in milk, 2007; Mogensen et al., 2012). Vitamin D is the main regulator of calcium homeostasis, and is important in skeletal development and bone mineralization, as well as for cell proliferation and the immune system (Horts et al., 1994). UV radiation with wavelengths between 290 and 315 nm can penetrate the epidermis and photochemically activate the synthesis of D3 vitamin. In pasture-grazed dairy cows with no supplementation of vitamin D there was a seasonal variation of vitamin D3 in the milk with the maximum level being reached during the summer (Kurmann and Indyk, 1994).

Comfort, housing and freedom of movement

Poor comfort has been associated with increased rates of lameness in intensively managed dairy cattle; the discomfort and pain associated with lameness affect resting, walking and feeding behaviour.

There is a general assumption that high standards of cow comfort and the management of clean cows improve milk quality. Some studies have made associations between clean housing, clean cows, satisfactory beds and lower bulk tank somatic cell counts and lower incidence of mastitis (Barkema et al., 1998; Barkema et al., 1999; Ward et al. 2002).

Summer et al. (2014) observed a highest proportion of milk samples with poor lactodynamografic profiles in tied stall barns than in free stall barns.

Hot stress

Effect of hot stress on welfare of dairy cows and milk composition has been by reviewed Summer et al. (2018). Heat stress has considerable effects on cattle welfare and production. In hot and humid climate conditions, dairy cows produce less milk with lower milk quality characteristics, especially those related to cheese-making. (Malacarne et al., 2003)

Several authors observed reduction of milk protein percentage. Bernabucci et al. (2002) showed that the reduction of milk protein content observed in the summer was due to the reduction in

the casein content (αs-casein and β-casein) and suggested that these changes might explain the alteration in cheesemaking properties of milk commonly observed during summer.

Nutritive stress

One of the most critical moments for the welfare of dairy cattle is the postpartum. The negative energy balance, typical of the early stage of lactation, causes changes in milk fat that become particularly rich in polyunsaturated fatty acids resulting from the mobilization of body reserves; the oxidation of these fats can cause unwanted taste.

Conclusions

Environmental stressors, such as those due to climate changes, housing and interaction with humans have effect on welfare of cows and udder physiology.

There are evidences of relationships between specific welfare issues of dairy cows and quality of milk; the best demonstrated links are the effects of mastitis and oxidative stress on composition, shelf life, sensory and technological characteristics of milk and milk product.

Effect metabolic diseases on gross composition of milk is also well-known, but little is known about the effects on other milk characteristics which are of great importance: fatty acids content; mineral content; acidity and coagulation properties.

Further interdisciplinary efforts should be made: welfare of dairy cows should be evaluated at farm level by a widely agreed protocol using outcome-based animal indicators; how and how much the animal welfare, measured in such way, affects the quality of milk should be studied by very large surveys and carefully designed experiments

Chapter 5- Effects of postpartum metabolic diseases on bovine milk quality: the ketosis

Introduction

Ketosis, definition, indicators and diagnosis

Ketosis is a metabolic condition that is established as a consequence of a negative energy/protein balance that leads to excessive liver production of ketone bodies; acetone, acetoacetate and ß-hydroxybutyrate (BHB) increase in blood beyond the physiological until they leak into urine and milk. Often, other metabolic changes occur during ketosis: free fatty acids and triacylglycerol increase in plasma (Baird, 1977; Shultz, 1971); glycogen decreases, while lipids increase in liver; and this can lead to severe liver damage. Blood glucose concentrations can decrease from 50-60 to 25 mg/100 ml (Littledike et al., 1981).

Shaw (1956) reported that ketosis was described "as early as 1849 according to Udall"; Shaw also stated that several cases reported before 1874 as "mania puerperalis" presented symptoms typical of ketosis and were treated by chloral hydrate. According Shaw, "Sjollema and Van Der Zande were the first to present data showing abnormally high levels of acetone bodies in the blood and urine of cows exhibiting symptoms of ketosis"

Clinical signs of ketosis are rapid loss of body weight, lack of appetite, dry faeces, rapid decrease in milk production; less common signs are nervous disturbances as pica, abnormal licking, incoordination and abnormal gait, bellowing and aggression.

Often, ketosis occurs in a sub clinical form: level of serum ketones is higher than normal, but clinical signs are not evident.

Most cases of ketosis occur before 6 or 8 weeks postpartum (Littledike et al., 1981) when the high energy requirements combined with low dry matter intake make dairy cows highly susceptible to the metabolic diseases. A negative energy balance initiates few weeks before calving; during the last 2-4 weeks of pregnancy there is an increase of energy requirements due to fetal development and the needs of colostrum synthesis; in the same period, dry matter intake (DMI) capacity decreases and is not sufficient to meet nutritional requirements for maintenance, growth, production, reproduction. (Grant and Albright, 1995; Herdt, 2000).

If nutritional imbalances are of short duration and are not too severe, cows are able to compensate through the mobilization of body fat. Mobilization release non-esterifies fatty acids (NEFA) that increase in blood; NEFA are converted to ketone bodies in the liver and supplying an alternative fuel source for tissues allowing glucose to be conserved for milk production.

An excessive accumulation of ketone bodies in the blood can lead to anorexia and worsen the illness.

Among ketones, BHB is more stable than acetone; for this reason, BHB is commonly used as indicator to test ketosis at 5-7 days of lactation.

Different cut-off values for BHB in blood (Ospina et al., 2010) and in milk (Whitaker, 1997; Oetzel, 2004) have been reported by scientific literature. Concentrations between 1,000 μM (10.4 mg/dL) and 1,400 μM (14.4 mg/dL) in blood are commonly used. Also different monitoring strategies have been proposed to detect subclinical ketosis, however 5- 60 days in milk (DIM) is considered an optimal sample time for checking BHB level in milk A sample of 12 cows is considered good to provide an alarm risk of ketosis at herd level; if more than 10% of sampled animals have a BHB concentration in excess of the threshold, it should be considered that there is a herd-level problem (10% with 1.4 mmol/l indicates risk of ketosis).

Milk level of NEFA measured from 2 to 14 DIM is considered a good indicator of a prepartum negative energy balance. The most commonly used cut point is a concentration of blood NEFA ≥ 0.400 mEq/l (Oetzel, 2004).

The F /P ratio is a good indicator of risk of ketosis because it increases (Koeck et al. 2014; Vanholder et al., 2015) in milk with abnormal ketonemia

According Duffield et al. (1997), 1.33 can be considered as the upper margin for healthy cows; for other authors an F /P ratio 1.5 is the threshold indicating a risk level for subclinical ketosis (Richardt 2004, Gantner 2015).

Incidence and prevalence

The incidence of a disease in a herd is the number of new cases that occurred during the risk period divided by the number of cows who completed the risk period. Determining the incidence of SCK requires repeated testing of cows throughout this risk period (early lactation). Testing must occur twice or more weekly in order to accurately assess the incidence of SCK. This is necessary because the median time for the resolution of SCK is about 5 days (McArt et al., 2011). If testing occurs only once a week, a cow could potentially develop and resolve her SCK between test intervals (McArt et al., 2012).

Shultz (1968) reported an average incidence of 4% in USA and 2% in UK; cases seemed to increase in winter. Hibbit (1979) reported that in some herds, in the UK, up to 33% of cows resulted positive at milk or urine ketones test.

Incidence rates of ketosis between about 40% and 60% were reported by authors (Emery et al., 1964; Simensen et al., 1990; Duffield et al., 1998) that tested blood BHBA once a week in early lactation. McArt et al., 2012 tested 1,717 cows in 4 large commercial herds and reported an average cumulative SCK incidence rate of 43.2% for. The SCK incidence ranged from 26.4% to 55.7% by herd.

They also found that new cases of SCK occurred very soon after calving; peak incidence was at 5 days in milk.

Prevalence measures the current status of a group at a given point in time. Repeated testing of individual cows is not necessary for determining prevalence of a disease; testing to evaluate prevalence of SCK is usually done for a subset of the early lactation cows within a herd. Herds can be tested repeatedly for SCK and the results pooled into a cumulative prevalence; this increases the reliability of the estimate of the herd's prevalence of SCK. For practical reasons, almost all herd-level evaluations for SCK are conducted as prevalence testing instead of as incidence.

McArt et al., 2012a reported a peak of the prevalence of SCK occurred at 5 DIM. At 5 DIM, 28.9% of cows were positive for SCK.

Higher ketosis prevalence was observed in multiparous cows compared to first-parity cows (Gantner et al. 2016) but Ospina et al. (2010) reported 20% of primiparous and 15% of multiparous with BHB≥ 12 mg/dl.

Ketosis and behaviour

Cows prefer eating and resting together as a group, but they form a dominance hierarchy that may influence their behavioural pattern; overcrowding limits their ability to access the desired resources (lying areas, feed, or water) at the times they would prefer (Munksgaard et al., 2005). Stocking density at the feed bunk affect the feeding time, in subordinate cows (DeVries et al., 2004; Huzzey et al., 2006); subordinate cows spend more time standing waiting to feed rather than competing for a spot at the feed bunk.

Cows with SCK may lie down for longer periods of time to conserve energy (Hart, 1988) needed for milk production.

Cows with subclinical ketosis had lower rumination times than healthy cows within the first few days after partum (Soriani et al., 2012; Soriani et al., 2013) and founded negative association of rumination time and blood BHB concentration.

Consequences of ketosis: effects on health and animal welfare

Subclinical ketosis increases the risk of other pathologies (Duffield et al. 2009). The risk of having a displacement of abomasum increases by 3 to 8 times in cattle with ketosis already starting with 1.2 mmol/I of BHB. Every increase of 100 μmol/ of BHB at first test, increases 1.1 the risk of displacement.

The risk of placental retention increases by two times. (LeBlanc, 2004). Duffield reported that a level of 1.2 mol/l of BHB in the first week after delivery, the risk of this disease increases by 3.4 folds, probably for the interference with the immune system, especially cell-mediated. For the same reason there is a correlation between ketosis and mastitis. Some studies reported that cattle with

subclinical ketosis have a 3-fold risk to leave the farm in the first 30 days of lactation compared to the healthy cows.

Effect on milk production

A number of studies investigated the effect of ketosis on milk yield; however very different methods have been used so that it is difficult to compare the results.

Dohoo and Martin (1984) found that the loss of production associated with a positive milk ketone test was 1.0 to 1.4 kg/day of milk for lactation.

Detilleux et al. (1994) estimated a reduction of milk production of 44.3 kg over a period of 17 days comparing cows treated or not treated for ketosis.

Antanaitis et al. (2015) estimated a difference of 5.6 kg before the emergence of clinical symptoms.

Gantner et al. 2016 observed a decrease in milk yield of 4.21, 2.73, 2.78, 2.83 and 3.72 kg day−1 in 1, 2, 3 and 4+ parity cows respectively within 35 days after the detection of subclinical ketosis.

Duffield et al. (2009) found that BHB>1.4 mmol/l during first week of lactation resulted in a loss of, 1.8 kg (-5.5%) of milk at the first DHIA control.

According Chapinal et al. (2012): BHB>1.4 mmol/l during first week results in a loss of 2,4 kg (-6.9%) at the first DHIA control.

According McArt et al. (2012) cows affected by subclinical ketosis produced 1,2 kg less (3.4%) in the first 30 days of lactation.

Each increase of 100 µmol/l of blood causes a reduction of milk of 0.5 l.

However, the effect on milk yield lost milk depends also from the onset of the disease. Cows positive to the test from 3 to 7 DIM produced 2 kg less milk in the first 30 days after calving compared to those positive from 8 to 16 days.

Effects of ketosis on milk quality

Effect of ketosis on gross composition of milk is well-known; in fact, fat content and fat: protein ratio are used as indicator of subclinical ketosis; while little is known about the effect on other milk characteristics which are of great importance: fatty acids content; acidity; rheological properties.

Fat percentage. Generally, fat percentage increases (Miettenen, 1994; Miettenen and Setälä, 1993; Vanholder et al., 2015) due to the higher availability of fatty acids for milk fat synthesis in the udder. However, Palich et al., 1984 reported a lower content of fat for ketonemic cows.

Protein percentage. When a ketosis occurs, protein percentage generally decreases (Miettenen, 1994; Miettenen and Setala, 1993; Vanholder et al., 2015) due to the lower availability of glucose at mammary level.

Casein and casein index. Palich et al., 1984 found that milk of cows affected by ketosis (ketone bodies in blood > 10, average=18.88, sd=1,39 mg/%) had lower content of casein and casein index: 2.73 *vs* 1.90 and 81.12 *vs* 74.44 respectively.

Such difference of protein content can lead to a reduction of cheese yield of 2,54 kg /100 kg milk (8,87% *vs* 6.33%), according the predictive equation proposed by Formaggioni et al., 2015.

Fatty acids. Palich et al., 1984 found a different distribution of short, medium and long chain fatty acids in milk from ketotic cows; percentage of short chain fatty acid was lower in cows with ketosis than in healthy cows (12,23 *vs* 17,88); also, medium chain fatty acids percentage was lower than in healthy cows whether long chain fatty acids percentage was higher.

Also, proportions of saturated and non-saturated fatty acids were different between healthy and ketotic cows: percentage of saturated fatty acids were 59.31% and 76.04% in ketotic and normal cows; unsaturated fatty acids were 35.90% and 25.94%, respectively.

Melendez et al., 2016 showed results partially in disagreement with Palich et al., 1984; they founded that most of short and medium chain fatty acids and 18:1 were higher in cows with BHBA > 0.7 mmol/L than in cows with BHBA < 0.7 mmol/L; they also founded a higher concentration of CLA and all isomers of 18:1 in milk of cows with BHBA < 0.7 mmol/L than in milk of cows with BHBA > 0.7 mmol/L.

Minerals. Palich et al., 1984 found that contents of Calcium, Magnesium and Phosphorus were lower in milk of ketotic cows; sodium and potassium resulted slightly higher; content of Chlor was not different between groups

Coagulation properties. Palich et al., 1984 reported a reduced rennet coagulation time for milk of ketotic cows compared with milk of healthy cows (4,22 min, sd=4.10 min, *vs* 6.99 min, sd=4.99 min).

Role of innovative technologies in reducing risk and effect of ketosis

Digitalization and PLF promise to be a help in reducing future incidence of ketosis allowing early detection of behaviour and milk abnormalities by:

-continuous monitoring of locomotion activity

-continuous monitoring of ruminal activity

-in-line measurement of milk indicators (fat:protein; urea; BHB)

-precise feeding.

Objectives

The changes of milk composition during ketosis in dairy cows have been investigated for many decades providing consolidated knowledge about the effect of ketosis on fat; fat percentage and fat. However, the changes of fatty acids content; acidity and coagulation properties have been very little or no studied at all.

The observations reported in this chapter was designed to explore the variability of these parameters in relation to metabolic disorders, especially subclinical and clinical ketosis; the purpose of this pilot study was to provide preliminary information necessary to design a larger survey.

The pilot study was carried out at a farm equipped with a system for continuous monitoring of motion and rumination activities of individual cows. Measures of motion and rumination activities and their relationship with milk parameters were analysed as honest indicators of animal welfare, potential indicators of subclinical disorders and potential early predictors of an imminent metabolic disorders, having in mind three specific scientific objectives:

- contribute to the knowledge on how precision livestock farming may improve animal welfare;
- contribute to the knowledge about the relationship between animal welfare and milk quality;
- using information about rumination and activity parameters to better design future larger studies; one of the reasons why little is known about the quality of milk from ketotic cows is that it is difficult to design a milk sampling from sick cows if metabolic disorders are diagnosed post hoc by analysing the milk itself; if the motor and rumination behaviours were able to give early warnings about impending disease events, the sampling could be done in a more targeted way on the milk of the animals at risk.

Materials and methods

Animals and management

The study was carried out from March 2017 to January 2018 at the dairy farm of CREA located in Lodi (Italy). Two different periods sampling were characterised; first period (1st March 2017-31st May 2017) covered a period mainly characterised by springtime weather conditions; the second period started 31rst August and finished 23rd January with autumn and winter conditions. The routinely procedures and grouping strategies commonly used in the farm were applied during the study. The animal management in this study agrees with the current Italian law for the experimental use of farm animals (Decreto Legislativo 4 marzo 2014, n. 26).

Cows were housed in a freestall barn holding a herd of 58 Italian Friesian lactating cows; resting area was equipped with cubicles. Cows were milked twice daily (0400 and 1600 h) in a 4 + 4 herringbone milking parlour (DeLaval) fitted with automatic milk meters (Alpro, Delaval).

Milk yield and quality were monthly assessed by the recording system of Italian National Breeders Association (AIA).

Cows were fed a total mixed ration (TMR) prepared and distributed once daily, at 0730 ± 0030 h, for ad libitum consumption, monitoring refusals to be limited within 5 % of the supply. Diets were target for lactating cows producing 55 kg/d at 100 DIM. Diets were formulated using CPM Dairy Ration Analyzer v3.07a

The composition and nutritive values of the TMRs used during the experimental periods were reported in table 5.1 Fresh potable water was available ad libitum.

Table 5.1. Total mixed rations used during the study

		First period	Second period	
Item (kg as fed)	**Unit**	21/2/17	22/9/17	28/11/17

Corn silage	kg as fed	27.0	27.0	19.0
Meadow hay	kg as fed	5.5	7.0	4.5
Haysilage	kg as fed	2.0	-	3.0
Commercial concentrate	kg as fed	12.4	12.4	12.4
Sugar-beet molasses	kg as fed	1.0	1.0	1.0
Commercial mineral-vitamin pre-mix	kg as fed	0.7	0.7	0.7
Rumen-protected fats (Megalac)	kg as fed	0.30	0.3	0.3
Water	kg as fed	4,0	4.0	9.0
Dry matter intake (DMI)	kg/d	26.8	27.1	26.2
Forage	% DM	53.35		52.43
Crude protein	% DM	15.7	15.5	15.9
Rumen degradable protein	% DM	9.6	9.4	9.4
Neutral detergent fiber (NDF)	% DM	31.98	32.26	31.2
Neutral detergent fiber from forage	% DM	26.3	26.6	25.4
peNdf	%	25.6	26.2	24.7
Lignin	% DM	2.6	2.6	2.6
Starch	% DM	25.4	25.0	27.0
Ether extract	% DM	4.8	4.8	4.8
Ca	% DM	0.90	0.90	0.90
P	% DM	0.41	0.41	0.41
Mg	% DM	0.34	0.34	0.34
K	% DM	1.57	1.57	1.57
S	% DM	0.42	0.42	0.42
Na	% DM	0.39	0.39	0.39
Cl	% DM	0.57	0.57	0.57
Metabolizable energy	mcal/ kg	2.74	2.73	2.76
Net Energy for lactation	mcal/ kg	1.76	1.76	1.78
Metabolizable protein	g/d	3028	3073	2979
Predicted MUN	mg%	13	13	13

Measurements and analyses

Forty lactating dairy cows were involved in the measurements, 12 of which were primiparous; the frequency distribution among different class of parity was reported in table Table 5.2.

Table 5.2 Frequency distribution within lactation parity

Parity	Frequency
1	12
2	15
3	8
4	2
5	1
6	1
7	1
Total	40

Milk yield and composition

Daily milk yield was automatically recorded at each milking by the Alpro system (Alpro, Delaval).

Samples of milk were taken during a sampling session carried out once a week; at each sampling session cows that were between 5 and 78 days in milk (DIM) were sampled; consecutive samples within each cow were classified according 5 classes of DIM (DIM Class, table 5.3)

Table 5.3 Days in milk classes

DIM class	Range
1	5-13
2	14-28
3	29-43
4	44-58
5	59-78

Milk samples were analysed for lactose, fat, protein, casein, total microbial count, somatic cells count, pH, titratable acidity, urea, rennet coagulation time (r), curd firming time (k20) curd firmness (a30).

Lactose, fat, crude protein, casein and urea were determined by means of the infrared analysis (Biggs 1978) with Milko-Scan FT 6000 (Foss Electric, DK- 3400 Hillerød, Denmark). From those values the casein number was calculated as: casein x 100 / crude protein and the fat to protein ratio was calculated as: fat / crud protein

The values of pH and titratable acidity were assessed by reading with specific probe of Chrison and by titration of 50 mL of milk with 0.25 N sodium hydroxide according to the Soxhlet-Henkel method (Anon. 1963)

Freezing point was determined by a cryoscopy according to Horvet (1921)

Total bacterial count (TBC) was measured using the flow cytometry method with BactoScan FC (Foss Electric, DK-3400 Hillerød, Denmark) (Grappin et al. 1985)

Somatic cell count (SCC) was made using the fluoro-opto-electronic method (Schmidt-Madsen 1975) with Fossomatic (Foss Electric, DK-3400 Hillerød, Denmark).

The rennet coagulation properties (RCP) were assessed according to McMahon and Brown (1982) with Formagraph (Foss Electric, DK-3400 Hillerød, Denmark). A 0.2 ml (1 : 100) rennet solution (1 : 19 000; Chr. Hansen, I-20094 Corsico MI, Italy) was added to milk samples (10 ml). Milk clotting time (r), curd firming time (k20) and curd firmness (a30), were measured at 35°C. Milk clotting time is the time from the addition of rennet to the onset of gelation. Curd firming time is the time from the onset of gelation till the signal attains a width of 20 mm. Curd firmness is the width of the signal 30 min after the addition of rennet.

According to their rennet coagulation parameters, the milk samples were classified in lactodynamographic types identified by capital letter as reported in table 1. reported by Malacarne et al., 2014 and according the original researches of Annibaldi et al. (1977) and Pecorari et al. (1984); on this basis, samples were then grouped in 4 classes (Optimal, Suboptimal, Poor and Non-coagulating).

Rumination time and activity data

Movement and rumination activities were measured using the HR-Tag rumination monitoring system (SCR Engineers Ltd.). The system consisted of data loggers, stationary readers, and software for processing the electronic data (Data Flow Software, SCR Engineers Ltd.). A neck collar positioned the logger on the left side of the neck. The logger contained using MEMS (Micro Electro-Mechanical Systems) that were able to record rumination and activity.

The system summarizes and reports data in 2-h intervals. Two variables automatically reported by the system have been considered in this study:

Total daily rumination time (RumDay) is the sum of the 2-h interval values recorded from 2400 to 2400 h of the following day.

Total daily activity index is the sum of the 2-h interval values recorded from 2400 to 2400 h of the following day (ActDay),

Calculations and Statistical analysis

The risk of metabolic disorders was estimated on the basis of the fat-to- protein ratio (F/P ratio), according the following rule Gantner (2015):

F/P < 1.0 acidosis risk;

1.0 ≤F/P ≤1.5 normal conditions and an indicates acidosis risk;

F/P >1.5 ketosis risk.

On the basis of this criterion, cows were classified as belonging of 4 groups: healthy cows; acidosis, contained cows that had at least one sample indicating acidosis risk; ketosis contained cows that had at least one sample indicating ketosis risk; cheto-acidosis, contained cows that gave milk samples indicating acidosis risk and samples indicating ketosis risk.

A screening of the whole dataset was done computing descriptive statistics by PROC MEANS, PROC FREQUENCY and PROC UNIVARIATE of SAS (SAS Institute Inc. 2009).

To compare milk quality, rumination and activity parameters of cows classified according the F/P ratio the MIXED PROC of SAS (SAS Institute Inc. 2009) was performed according a model

$\gamma_{ijkl}=\mu+\alpha_i+\beta_j+\gamma_k+\partial_l+\pi_m+\varepsilon_{ijklmn}$

where:

y = dependent variable;

μ = mean effect;

α_i = group effect (i = 4 levels);

β_j = DIM class effect (k= 5 levels);

γ_k= period effect (k= 2 levels);

∂_l =parity effect (l = 2 levels);

$\pi_{(i,k,l)m}$= random effect of each cow nested within the group, season and parity (j = 1 to 40)

ε_{ijklm}= residual error. Group, DIM class, season and parirty order were considered as fixed factors.

To study covariations among studied variables, simple correlation coefficient (Spearman) was calculated (CORR PROC of SAS, SAS Institute Inc. 2009) using means for cow for each variable.

Moreover, regressions of milk parameters with RumDay, ActDay, Rum7Days, Act7Days, dsRum7Days, dsAct7Days were compared among groups; for this comparison an analysis of covariance approach was chosen; parameters of rumination and activity were used as continuous independent variable and groups of cows as classification variable (Littel et al., 1996).

Results

Descriptive statistics

Descriptive statistics of variable analysed in the study were reported in table 5.5

Table 5.5. Descriptive statistics of variable analysed in the study

Variable	N	Mean	SD	Min	Max
Milk produced the day before milk sampling (MY), kg/day	39	38.90	8.23	14.30	58.67
Average milk yield of 7 days before milking sampling (MY7d), kg/day	40	614.08	51.46	496.26	737.45
Protein,%	40	3.23	0.21	2.64	3.61
Casein, %	40	2.49	0.18	1.96	2.90
Somatic cell count (SCC), x1000	40	237.1	315.6	9.3	1681.0
Acidity, SH_50	40	3.47	0.26	2.86	4.22
Freezing point	40	-0.5220	0.0043	-0.5310	-0.5095
*MYmonth, kg/day	39	42.82	7.99	26.92	63.33
*FatMonth, %	39	4.44	0.89	2.85	7.74
*CaseinMonth%	39	2.45	0.17	2.05	2.80
*UreaMonth, mg/100ml	39	22.80	6.40	12.30	36.33
*SCCMonth, x1000	39	425.5	965.8	10	5630
*SH50Month	40	3.47	0.26	2.86	4.22
*Acetone, mmoli/l	39	0.0316	0.0388	0	0.1600
*BHB, mmoli/l	39	0.0510	0.0370	0	0.2000
*Saturated fatty acids	39	2.941	0.548	2.244	5.036
*Polyunsaturated fatty acids	39	0.166	0.032	0.111	0.269
Total rumination time of the day before milk sampling (RumDay), min/day	40	626.44	53.24	494.60	742.00
Average rumination time of the 7 days before milk sampling (Rum7days), min/day	40	614.07	51.46	496.26	737.45
Standard deviation of rumination time of the 7 days before milk sampling (DsRum7days), min/day	40	59.56	28.48	26.43	181.93
Total activity of the day before milk sampling (ActDay)	40	601.09	109.93	395.00	890.00
Average activity of the 7 days before milk sampling (Act7days)	40	594.63	101.47	402.41	803.14
Standard deviation activity of the 7 days before milk sampling (dsAct7days)	40	68.93	35.508	18.92	147.02

* monthly assessed by the recording system of Italian National Breeders Association (AIA).

Analysis of variance

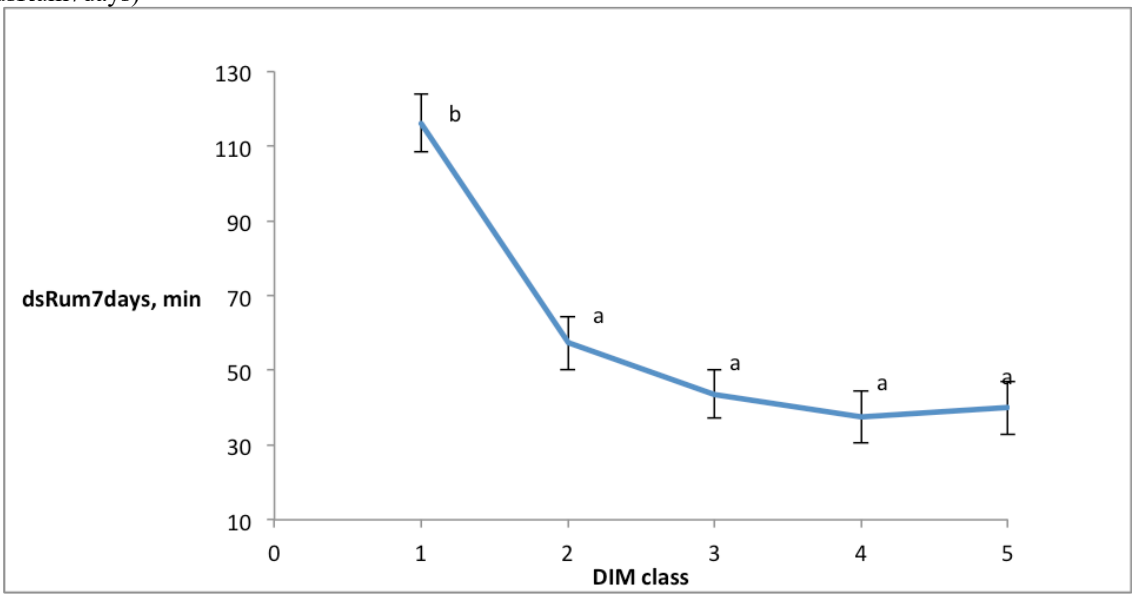
Rumination and activity

Total rumination time the day before sampling was not affected by group and other considered factors.

Average daily rumination of DIM of 7 days before sampling was affected by class of DIM; when sample was taken between 5 and 13 DIM (class 1), Rum7days was lower (554.13 min/day) than for all others DIM class (625.52 min/day on average).

Standard deviation of 7 daily rumination times before the day of sampling was heavily influenced by DIM class (Figure 5.1)

Figure 5.1. Least squares means (LSM) for standard deviation of rumination time of the 7 days before milk sampling (dsRum7days)



Activity index of the day before milk sampling was higher (P= 0.0486) during first period of sampling (647.96 ± 25.95) than during second period (546.17 ± 28.37).

Also, the average daily activity of the 7 days before sampling was higher (P = 0.0113) during the first than the second period (638.02 ± 23.53 *vs* 546.33 ± 25.76).

Variability of daily activity of 7 days before sampling was lower (P=0,0085) during first period than second (45.17 ± 7.62 *vs* 76.21 ±7.99) and was affected by DIM class (0.0240); when sample was taken between 5 and 13 DIM (class 1), dsAct7days was higher (91.46 ± 11.44) than at all others DIM class.

Milk yield

As expected, milk yield of the day before sampling was affected by parity (P <0.0001) and DIM class (P <0.0001) but was also different between periods (P=0.0243); it was higher in the first (39.47 ±1.42 kg/d) than in the second period (34.65 ± 1.58 kg/d). It was not different among groups.

Analysis of variance of daily milk yielded on 7 days before milk sampling showed the same results: multiparous produced 11.2 ±1.8 kg/d (P<0.0001) more than primiparous; it increased with DIM class (P<0.0001) and was higher(P=0.0208) in the first (39.04 ±1.29 kg/d) than in the second period (34.48 ± 1.40 kg/d).

Milk somatic cells and bacterial count

Table 5.6 reports LSM for logSCC.

Table 5.6. Least squared means for Log SCC, d different superscripts indicate significant differences in columns (P<0.05)

Group	Log SCC
acidosis	4.6417 ^{ab}
ketoacidosis	4.7753^{ab}
ketosis	5.0559^{b}
healthy	4.6811^{a}

Bacterial count was not affected by the considered factors.

Milk composition parameters

Table 5.6 shows the effect of metabolic disorders as detected by fat/ protein ratio on fat, protein and casein.

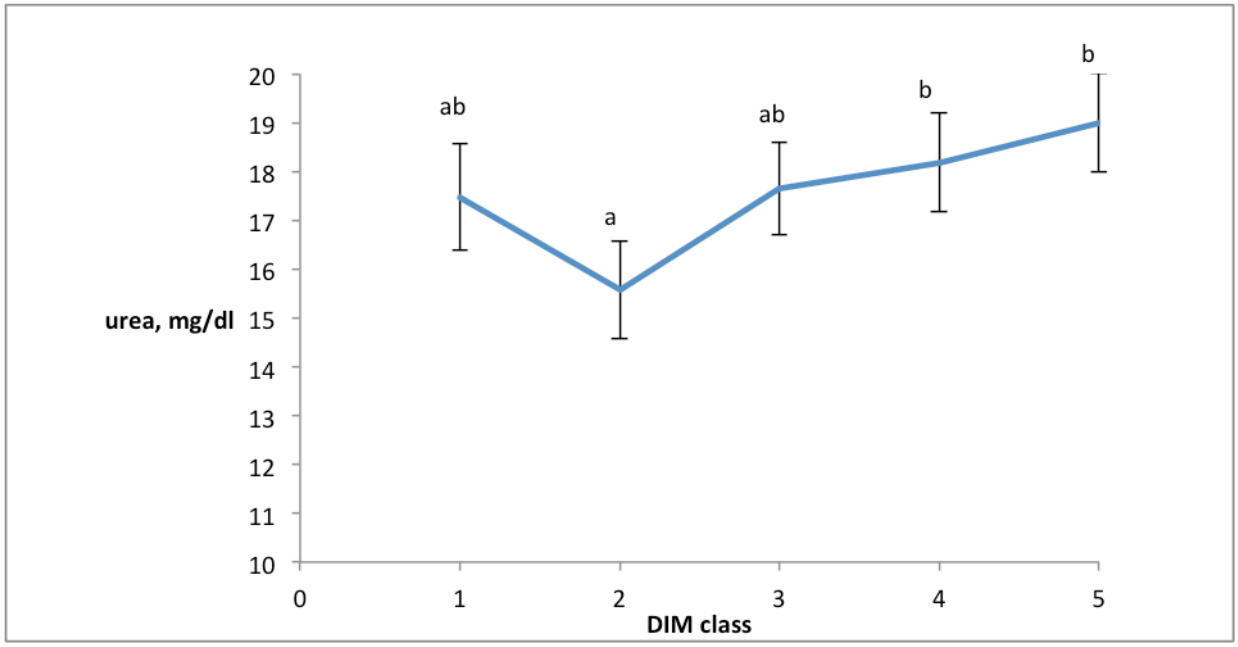
Table 5.6. Least squared means for fat, protein and casein; different superscripts indicate significant differences in columns (P<0.05)

	Fat, %		Protein, %		Lactose, %		Casein, %	
Group	LSM	es	LSM	es	LSM	es	LSM	es
acidosis	3.57^{a}	0.31	3.33^{b}	0.09	5.03 ^{ab}	0.90	2.57^{ab}	0.08
ketoacidosis	3.98^{a}	0.23	3.31^{b}	0.07	6.26 ^{b}	0.69	2.55^{ab}	0.06
ketosis	4.79^{b}	0.17	3.13^{a}	0.05	4.67 ^{a}	0.50	2.41^{a}	0.05
healthy	4.00^{a}	0.16	3.31^{b}	0.05	4.85 ^{ab}	0.48	2.57^{b}	0.04

Caseina number (casein % of total milk protein), was not significantly influenced by any of the considered factors.

Milk urea percentage was not affected by the group, but was higher in the first period than in the second period and was affected by DIM class (Figure 5.2).

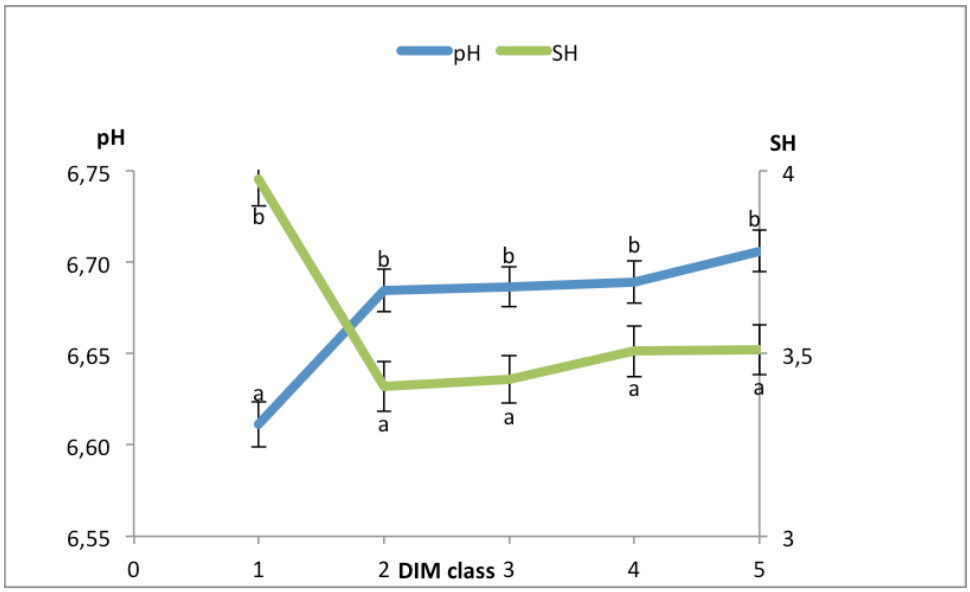
Figure 5.2. Least squares means (LSM) for urea



Acidity

pH was not affected by the group, but was higher (P=0.0319) for multiparous than primiparous (6.69 ± 0.01 *vs* 6.66 ± 0.01) and was affected by DIM class as well as SH (Figure 5.3).

Figure 5.2. Least squares means (LSM) for pH andì SH

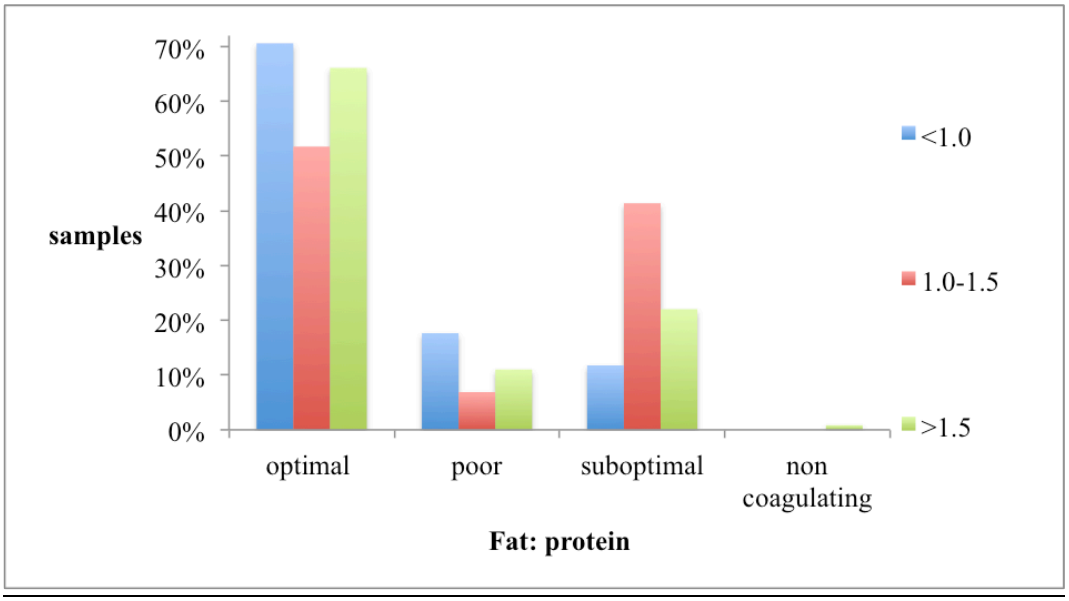


Milk cogulation parameters

Figure 5.3 and 5.4 show the proportions of samples belonging to the 4 lactodynamographic profiles.

Figure 5.3 Percentages of samples belonging to 4 different 4 lactodynamographic profiles within fat:protein ratio class

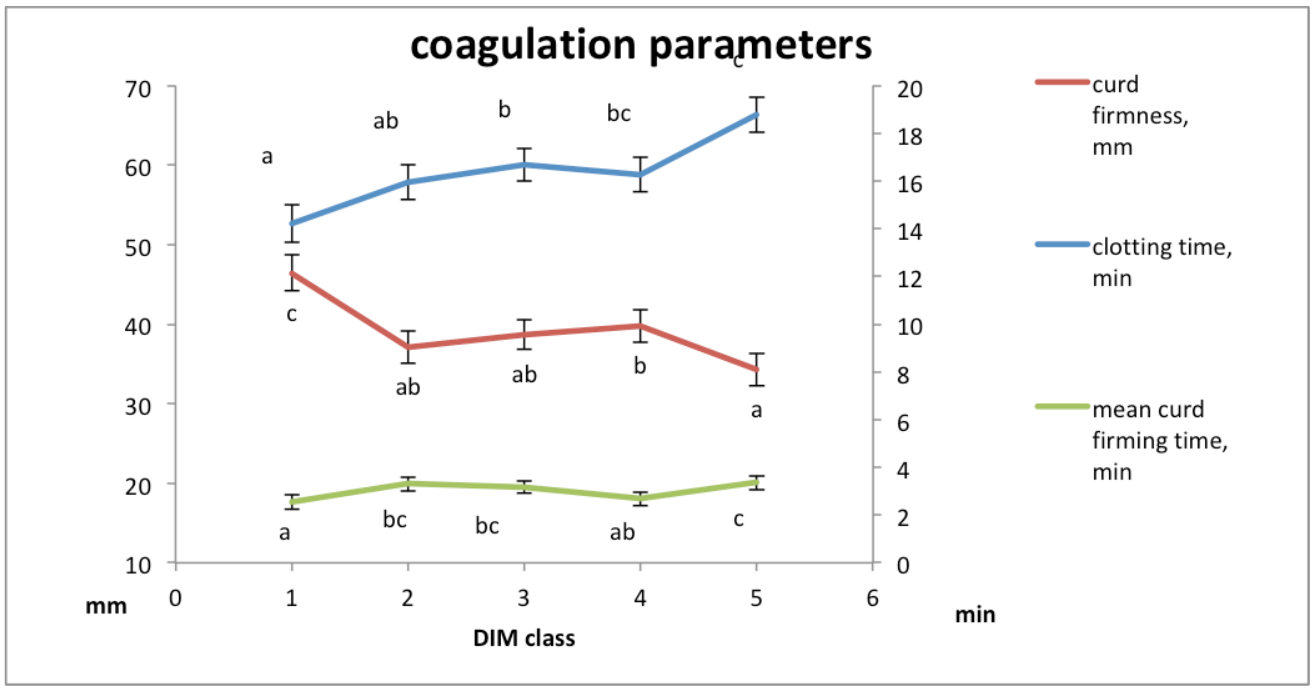
Figure 5.4 Percentages of samples belonging to fat:protein ratio class within the 4 lactodynamographic profile



Milk clotting time and curd firming time were not different among groups of cows classified on the the basis of fat: protein ratio, but were influenced by DIM class (Figure 5.5).

Curd firmness was tendentially (P=0.0508) lower for cows with ketosis risk than healthy cows (35.99 ± 1.94 *vs* 41.30 ± 1.78).

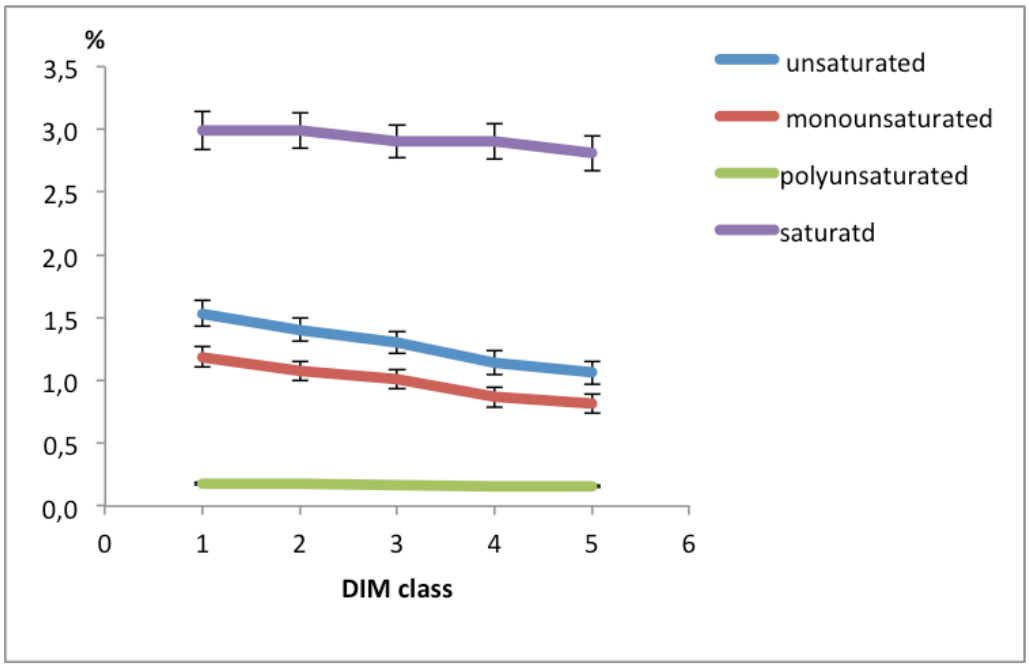
Figure 5.5 Least square means for coagulation parameters



Fatty acid profile and ketone bodies at monthly test

Percentage of saturated, unsaturated, monounsaturated polyunsaturated fatty acids were affeted by DIM class and percetage of polyunsaturated fatty acids were higher during first perion than second period (0.1869 ± 0.0074 %vs 0.1509 ±0.0081%).

Figure 5.6 Least square means for fatty acids groups



Acetone and BHB were not significantly influenced by any of the considered factors.

Correlations

Correlations of milk parameters with rumination and activity measures are reported in table 5.6

Table 5.6. Spearman correlation coefficients and statistical significance (R and P was reported when P < 0.1)

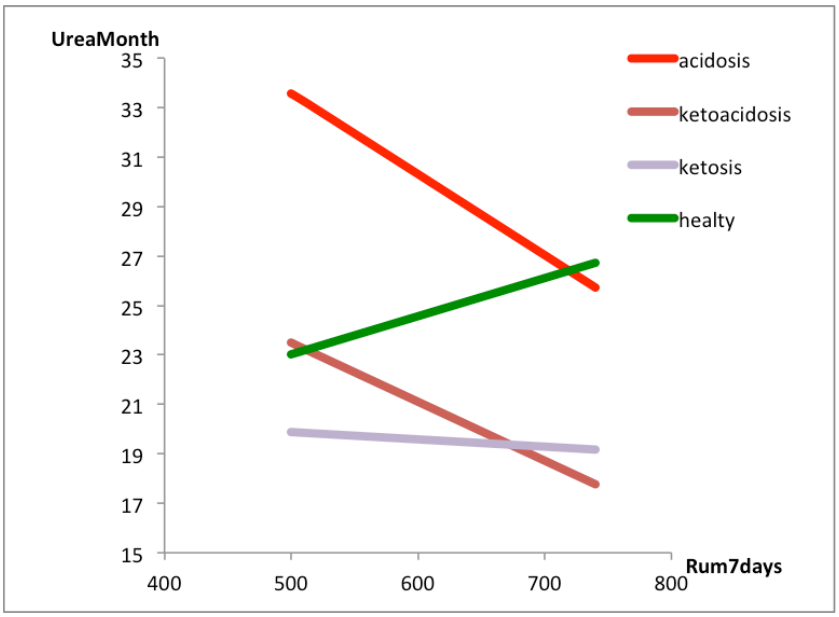
Variable	N	RumDay	Rum7days	DsRum7days	AttDay	Att7days	dsAtt7days
MY, kg/day							-0.41
	39	ns	ns	ns	ns	ns	0.0085
Fat, %			-0.29	0.36	-0.31	-0.30	0.46
	40	ns	0.0643	0.0205	0.0534	0.0622	0.0025
Protein, %				0.31			0.30
	40	ns	ns	0.0483	ns	ns	0.0530
Fat/protein				0.37			0.32
	40	ns	ns	0.0178	ns	ns	0.0449
SCC							
	40	ns	ns	ns	ns	ns	ns
Urea, mg/100ml		-0.33		-0.56			-0.58
	40	0.0358	ns	0.0002	ns	ns	<0.0001
Freezing point depression							0.32
	40	ns	ns	ns	ns	ns	0.0406
ProteinMonth							0.30
	39	ns	ns	ns	ns	ns	0.0672
UreaMonth, mg/100ml				-0.63			-0.55
	39	ns	ns	<0.0001	ns	ns	0.0003
Acetone							0.30
	39	ns	ns	ns	ns	ns	0.0625
Bhb							0.33
	39	ns	ns	ns	ns	ns	0.0398
Unsaturated fatty acids			-0.31				
	39	ns	0.0579	ns	ns	ns	ns
Mono-unsaturated fatty acids			-0.27	0.32			
	39	ns	0.0926	0.0484	ns	ns	ns
Poly unsatuated fatty acids			-0.33				

39	ns	0.0038	ns	ns	ns	ns

Unequal slopes

Figure 5.4 shows how is different the relationship between urea and rumination for cows belonging to the four groups indentified on the basis of fat:protein ratio in milk.

Figure 5.4. Regression between urea and rumination time of 4 groups



Discussion

In our conditions, milk yielded one day and 7 day before milking was not affected by metabolic subclinical diseases detected by fat: protein ratio.

As expected, DIM and parity had major effect on milk yield variability.

Days in milk were also a great source of variability for many other variables: percentage of urea, acidity, coagulation parameters and fatty acid profile.

The difference in log SCC between healthy cows and cows with ketosis risk confirm the associations between subclinical ketosis and clinical mastitis or a high SCC reported by some studies (Raboisson et al., 2014); however, the result is valuable because, Raboisson et al. 2014 stressed the scarcity of data on the relationship between subclinical ketosis and SCC. High NEFA or BHBA concentrations were reported to induce immune dysfunction (LeBlanc et al., 2011; LeBlanc, 2012).

Low percentage of casein in cows with ketosisis risk confirm results of Palich et al. 1984.

Period of sampling affected milk yield and percentage of urea and polyunsaturated fatty acids.

Analysis of variance of rumination and activity parameters dind't provide statistically significant evidence of differences among groups, showing majors effects of DIM and period.

However, simple correations higligted positive associations between milk fat:protein ratio and variability of rumination and activity.

It seems interesting to better explore the association between rumination and polyunsaturated fatty acids percentage in milk.

Covariations between couples of variables could be also studied with an approach as that we used here to understand if the association between rumination and milk urea was the same for all groups.

In our conditions, days in milk and period of sampling were the major sources of variability for many of the studied variables; nevertheless, from the data emerge information useful to further deepen the relationships between ketosis and rumination and activity and the effects that this relationship can have on the quality of milk.

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Abbreviations

antimicrobial-resistance (AMR).

beta-hydroxybutyric acid (BHBA),

beta-hydroxybutyrate BHB

body condition score (BCS)

days in milk (DIM)

degree of antioxidant protection (DAP)

Dairy Herd Information Association (DHIA)

dry matter intake (DMI)

failed passive colostral transfer syndrome (FTP)

immunoglobulins (Ig)

International Committee for Animal Recording (known as ICAR)

Italian National Breeders Association/ Associazione Italiana Allevatori (AIA)

Lactate dehydrogenase (LDH)

Least square means (LSM)

micro electro-mechanical systems (MEMS)

near-infrared spectroscopy (NIR)

non-esterifies fatty acids (NEFA)

pregnancy- associated glycoproteins (PAG)

plasmin (PL)

precision ubclivestock farming (Plf)

radial immuno diffusion (RID)

subclical ketosis (SCK)

specific gravity

somatic cell count (SCC);

sample period (SP)

tight junctions (TJ)

total mixed ration (TMR)

ultraviolet (UV)

Welfare Assurance Scheme (WAS)