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Predictive Modelling for Dairy Cow Welfare Assessment

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Riccardo Moretti, 30/10/2017

Abstract

Animal welfare is a rather complex concept. It includes various different facets that make it difficult for scientists to produce an ultimate definition. Leaving out ethical discussions, science aims at finding key concepts that could work as objective indicators for the welfare status of farmed animals. Among the commonly accepted indicators, disease presence in dairy farms is of main importance. The negative impact of diseases on animal welfare and productive performances is straightforward. Recently, however, great attention has been given to the environmental conditions where the animals are reared. Specifically, the heat stress issue has been addressed in different papers available in scientific literature.

In this PhD thesis, rumination fills a key role in animal welfare definition. This physiological process, which differentiates and characterises ruminants from the rest of the mammals, has been thoroughly studied. Mechanisms involved in rumination are well known, as well as the diseases that strike the rumen. However, rumination is still poorly considered in the general context of welfare in dairy cows.

In the papers produced during this PhD project and presented in Part II (some of them already published on international journals), rumination relationships with the main factors influencing animal welfare were studied. In the first paper, heat stress effects on rumination time were described. The paper shows that rumination decreases when discomfort caused by heat stress increases. In the second paper, rumination was hypothesised as a predictor for various diseases groups, commonly affecting dairy farms. Other results are presented as drafts of papers that will be submitted to international journals as soon as ready. The first draft explores the heritability of rumination and its genetic correlation with milk production traits. Only few studies can be found in literature about rumination heritability, and none of them evaluated the same genetic relationships we investigated. Lastly, in the second draft a first rough attempt to build a predictive model for diseases prediction was made. Fine tuning is still needed to improve the obtained results. Minor results are presented as abstracts (oral presentations at two international meetings) in the last section of Part II.

Riassunto

Il benessere animale è un concetto alquanto complicato e comprende svariate sfaccettature che rendono la sua definizione un tema tuttora discusso dagli scienziati. Tralasciando discussioni di carattere etico, la scienza punta ad individuare dei concetti chiave che possano fungere da indicatori oggettivi per la valutazione dello stato di benessere di un animale allevato dall'uomo. Fra gli indicatori più comunemente accettati troviamo la presenza di patologie nell'allevamento, il cui effetto negativo sul benessere è ben chiaro; recentemente, però, grande attenzione è stata posta anche sulle condizioni ambientali in cui gli animali vengono allevati e, nello specifico, la questione dello stress da caldo è stata affrontata in vari lavori disponibili in letteratura scientifica.

In questa tesi di dottorato, il ruolo chiave nella definizione del benessere viene ricoperto dalla ruminazione: questo processo fisiologico, che differenzia e caratterizza i ruminanti dal resto dei mammiferi, è stato largamente studiato per quanto riguarda funzionamento, utilità e patologie, ma viene ancora poco considerato nel quadro generale dello stato di salute delle bovine da latte.

Nei lavori prodotti e presentati nella Parte II (in parte già in fase di pubblicazione), la ruminazione viene studiata in relazione ai principali fattori che influenzano il benessere animale: nel primo articolo, sono stati studiati gli effetti dello stress da caldo sulla ruminazione stessa, ed è stato dimostrato come, all'aumentare del disagio prodotto da questo effetto negativo, la ruminazione giornaliera ne risenta. Nel secondo articolo, invece, la ruminazione è stata proposta come predittore della comparsa di alcuni gruppi di patologie che comunemente affliggono le stalle da latte. Altri risultati sono presentati come bozze di lavori, i quali verranno sottomessi a riviste internazionali non appena terminati. La prima bozza esplora l'ereditabilità del tratto ruminazione e la correlazione genetica con altri tratti produttivi. Pochi studi in letteratura trattano l'argomento, e nessuno di essi analizza queste correlazioni. Infine, nella seconda bozza, un primo tentativo di creare un modello predittivo per varie patologie è stato provato. Altri risultati minori sono presentati come abtract alla fine della Parte II.

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“Believe you can

and you’re halfway there”

Theodore Roosevelt

PART I

1. INTRODUCTION

1.1 ANIMAL WELFARE

Dictionaries define animal welfare as the "protection of the health and well-being of animals". This is, however, a semplicistic definition of something that is, in reality, a complex concept to explain. This is due, for example, to the fact that animal welfare is composed by highly multidimensional factors (Fraser, 1995) and, therefore, its assessment is quite complicated. Furthermore, differences in cultural heritages, traditions and religious faiths make it difficult to agree on some aspects of animal welfare (Szűcs et al., 2012). Scientifically speaking, but still in a broad and quite generic sense, animal welfare could be defined as the state of an animal regarding its attempt to cope with the environment it lives in (Broom, 1986). The most accepted practical definition, which outlines the main welfare issues related to animal farming, are the "Five Freedoms". The rough concept of these freedoms originated in December 1965 from a report of the UK Technical Committee. This Committee was charged to study the welfare status of animals reared under intensive husbandry systems in response to the book "Animal Machines" published in 1964 by Ruth Harrison (Jun 1920 – Jun 2000). In her book, the British animal welfare activist described the harsh condition suffered by animals reared in intensive farms. This report (known as the Brambell Report, named after Professor Francis W. R. Brambell, Feb 1901 – Jun 1970, which led the investigation) stated that farmed animals should have the freedom "to stand up, lie down, turn around, groom themselves and stretch their limbs". Later on, the British Government established in July 1979 the Farm Animal Welfare Council, which updated the Brambell Report to account for recent attention to behaviour and, by the end of that year, officially listed the "Five Freedoms" as they are known today:

1. Freedom from thirst, hunger and malnutrition;
2. Freedom from discomfort;
3. Freedom from pain, injury and disease;
4. Freedom to express normal behaviour;
5. Freedom from fear and distress.

The Five Freedoms address both the physical fitness and the mental suffering. Although absolute attainment of all Five Freedoms is unrealistic, they should be used as practical guidelines to assess the strengths and weaknesses of any husbandry system. Since the complete absence of any stress in impossible to achieve, aim of these freedoms is to prevent suffering, which may occur when an animal fails to cope with stress (Webster, 2001).

In the last years of the XXth century, animal welfare has become a major research area in animal husbandry. Various scientific studies based on animal welfare assessment were funded because of both the ethical concerns over the quality of life of animals and the increasing interest of many countries' agricultural policy on quality rather than quantity (Thornton, 2010). Furthermore, the public opinion started looking to such research for guidance regarding these concerns (Fraser et al., 1997). So far, no absolute nor ultimate criteria underlying animal welfare standards have been defined. Thus, assessment of animal well-being is outlined by guidelines only. An example is the Scientific Opinion by the European Food Safety Authority (EFSA), titled "Guidance on risk assessment for animal welfare" (published in January 2012) which tried to provide some methodological guidance to address this topic.

Animal welfare standards are conventionally divided into resource-based and animal-based standards, according to the criteria underlying them. Resource-based standards describe the environment in which the animal lives (that is determined by the resources of the owners of the animals themselves). On the other hand, animal-based standards describe the actual state of the analysed animal. As the resource-based assessment can fail to properly answer questions about some aspects of an animal welfare (one example is the behavioural, or "psychological", state: distress, fear or anxiety), there has been a rising interest in developing animal-based methods, more reliable in assessing welfare of farmed animals (Webster, 2009). To fulfil this target, there is a strong need to define objective parameters, tied to single animal and herd healthiness (e.g., presence of various diseases in a farm). Unfortunately, these parameters are difficult to record because of their quantity and complexity.

Diseases are one of the key factors affecting the efficiency of farms (Heikkilä et al., 2012). Mastitis, for example, is considered the most costly disease in dairy farming (Seegers et al., 2003), but the economic impact of other diseases is not ignorable. Although disease prevention is a fundamental element in livestock production system (Schwabenbauer, 2012), it is a goal often difficult to pursue. The early detection of the abovementioned diseases is, therefore, important (de Mol et al., 1999; de Mol et al., 2013), and even more useful would be to find some indicators able to predict their onset before the clinical symptoms appear.

1.1.1 HEAT STRESS

In addition to health status' parameters, environmental parameters also play a key role in animal welfare. Scientists define "thermo-neutral zone" that situation of thermal equilibrium between the animal and the environment where it lives (Nardone et al., 2010). In lactating dairy cows, the "thermo-neutral zone" is defined in the range between 5°C and 26°C (Berman et al., 1985; Roenfeldt, 1998). That said, heat stress defines all the changes in animal physiology towards a disorder status (e.g., production declines and its composition changes) whenever the temperature exceeds this thermal zone, trespassing either the upper or lower limit (Johnson, 1980). Heat stress (i.e., the non-physiological status of an animal that is subject to high temperatures and/or humidity) is an important threat to cattle breeding, especially in the Mediterranean basin, which is supposed to undergo a gradual increase in temperature and humidity in the coming years up to 2050 (Segnalini et al., 2013). Moreover, the current trend in the dairy industry is towards fewer but larger farms, rearing a great number of animals in the same structure (Winsten et al., 2010). Overcrowding, higher temperatures, and humidity can indeed result in harsh conditions for dairy cows. Responses of dairy cows to heat stress are copious: for example, raised respiration rates (Omar et al., 1996), and panting and sweating (Blazquez et al., 1994). However, these responses are not always enough, and animals fail to cope with their environment. This is especially true in high-yielding cow that are continuously subject to high metabolic stress due to the high milk production (Bernabucci et al., 2014). In these animals, associations between heat stress, milk yield and reproductive performances have recently been studied by scientists (Kadzere et al., 2002).

The various effects of length, severity, and interactions with the lactation phase are still unknow. An overall evaluation of heat stress could be assessed by body temperature measuring, which is highly susceptible to hot environmental temperatures (Araki et al., 1984). One of the most used indexes to quantify heat stress is the Temperature-Humidity Index (THI, Hahn et al., 2003). This index combines the ambient temperature and the relative humidity into a single parameter. Other environmental variables (e.g., solar radiation or wind) are not included in THI equation. THI is known to be inversely related to productive and reproductive performances in dairy cows (Bouraoui et al., 2002; Biffani et al., 2016), but it is still less clear its relationship with rumination. THI could be an important welfare index, and could be used to automatically activate cooling systems in barns, but its relationships with rumination and other physiological and pathological events must yet be further explored.

1.2 RUMINATION

Differently from monogastrics (e.g., human, horse, pig, rabbit…) the ruminants' digestive tract consists of a complex four-compartment stomach. It includes the rumen, the reticulum, the omasum, and the abomasum. The abomasum (also called the true stomach) is comparable to the stomach of the non-ruminant, and it is the only compartment with a glandular lining. It secretes hydrochloric acid and digestive enzymes, needed for the breakdown of feeds. It normally lies on the floor of the abdomen, but, due to the presence of high quantity of gasses, can sometimes move upwards, this phenomenon being called abomasum displacement. The omasum is a globe-shaped structure (also called the "manyplies") that contains leaves of tissue, stratified like pages in a book. It absorbs water and other substances from digestive contents. The reticulum is a pouch-like structure in the forward area of the body cavity, and the structure of the internal wall resemble a honeycomb. A small fold of tissue lies between the reticulum and the rumen, but the two are not actually separate compartments. Heavy or dense feed and metal objects eaten by the cow drop into this compartment and this is frequently the cause of severe lacerations of the tissues. Lastly, the rumen (which is placed on the left side of the animal) is the largest of the four compartments and is divided into several sacs. The rumen main function is to act as a fermentation vat. The rich symbiotic microbial population in the rumen (between 10^8 and 10^{10} microorganisms per gram of rumen content. Wilson and Briggs, 1955) digests or ferments feed eaten by the animal. Specifically, the microbial community composed by bacteria, protozoa, and fungi break down cellulose and lignin, aiding their digestion and producing volatile fatty acids, essential amino acids, and proteins, which are then absorbed into the rumen. This production supplies about 60-80% of the cow's energy. Conditions within the rumen favor the growth of microbes. The pH normally ranges between 6.5 and 7.2, with a temperature ranging from 37.8 to 40°C. Changes in pH levels compromise cow healt (Plaizier et al., 2008), affecting, for example, feed intake, milk production, and causing different severe diseases (e.g., diarrhea, laminitis and inflammation). To prevent these changes, large amounts of saliva, which act as a buffer for the ruminal pH (Beauchemin, 1991), are produced each day.

Rumination is defined as the regurgitation of ingesta, followed by remastication and reswallowing (Erina et al., 2013). It provides for effective mechanical breakdown of roughage and thereby increases substrate surface area to fermentative microbes. Regurgitation starts with a contraction that allows a bolus of ingesta to enter the esophagus, in conjunction with relaxation of the distal esophageal sphincter. The bolus is then carried into the mouth by reverse peristalsis, where it is remasticated and reswallowed. Rumination occurs predominantly when the animal is resting, and, on average, cows spend about 8 hours per day ruminating. Different studies have shown that non-physiological conditions such as acute stress (Herskin et al., 2004), anxiety (Bristow and Holmes, 2007), and diseases (Stangaferro et al., 2016a; b; c) affect rumination time by significantly decreasing it. Rumination could be, therefore, a valuable indicator of the welfare status of dairy cows. However, excluding the physiological point of view, which has been studied for decades, other aspects (e.g., heritability) of rumination are still not fully understood.

1.2.1 HERITABILITY

Although rumination process have been studied thoroughly from both the physiological and the pathological point of view, its heritability is still almost completely uninvestigated. Heritability is a statistic, widely used in animal breeding and in human population genetics, that estimates the degree of variation in a phenotypic trait (in a specific population) that is due to genetic variation between individuals in that population (Wray and Visscher, 2008). Heritability is a population parameter and, therefore, it depends on population-specific factors. This means that the heritability of the same trait could vary between different populations of the same animal breed. Nevertheless, it can be noticed that heritabilities are often similar across populations (Visscher et al., 2006). Heritability could be estimated on two different levels. When considering all the genetic contributions to the phenotypic variance (i.e., including additive, dominant, and epistatic components), the statistic is called “broad-sense” heritability (denoted by an upper case H^2), and is defined as:

$$H^2 = \frac{Var(G)}{Var(P)}$$

where Var(G) is the variance of the genotype, and Var(P) is the phenotypic variance, that is the sum of the genotypic variance, the environmental variance, and twice their covariance. However, in animal breeding, it is more commonly used the “narrow-sense” heritability (denoted by a lower case h^2), in which only the additive variance part of the whole genetic variance is used. This variance represents the genetic component passed from parents to their offspring (i.e., the component responsible for the resemblance between parents and offspring; Hill et al., 2008), thus this is a key variable for selection and breeding. “Narrow-sense” heritability is defined as:

$$h^2 = \frac{Var(A)}{Var(P)}$$

where Var(A) is the variance of the additive portion of the genotype, and Var(P) is as defined in the previous equation (Kempthorne, 1957).

To be a valuable selection criterion, rumination should have a moderate-to-high heritability and should be genetically correlated to other meaningful factors. Ranging from 0 to 1 (given its definition as a ratio), a heritability value of 0 means that no additive variance is available in the population, and values close to 0 describe a low heritable trait (fitness traits, for example, often have a low heritability; Visscher et al., 2008). On the other hand, a heritability value of 1 means that all of the variability in the phenotype is due to the additive part, meaning that the environment has no effect on the trait. Values close to 1 describe a high heritability (e.g., human height; Macgregor et al., 2006).

1.3 MACHINE LEARNING

The term “machine learning” was coined in 1959 by Arthur L. Samuel (Dec 1901 – Jul 1990) an American pioneer in artificial intelligence, and describes the branch of computer science that gives "computers the ability to learn without being explicitly programmed". Machine learning is based on the construction of algorithms that can learn how to make data-driven prediction or decision. The first computer learning program was written by the abovementioned Samuel while he was working for the IBM, and was a checkers game (Samuel, 1959). This software was perfected through time by other ingeneers and, eventually, the IBM’s Deep Blue chess-playing computer defeated in May 1997 the reigning world champion Garry Kasparov.

Machine learning can be classified into two main categories, based on different tasks and input given: supervised learning and unsupervised learning. In the former category, the programmer gives the computer both the input and its related desired output (labelled training data). Analysing the training data, the algorithm maps the given examples and will then apply what was learned to new data. This type of analysis is commonly used when the classification of data is already known, and the aim of the analysis is to attribute new records to the abovementioned data. In the latter category, no labels are given to the training data. These algorithms analyse data to discover hidden patterns and are used when it is of interest to search the data for common features that can cluster them together.

While being largely used in various fields of human medicine (e.g., Prosperi et al., 2013; Erus et al., 2014), sociological economy (e.g., Lee et al., 2014) and even in insurance companies surveys (e.g., Zhu and Welsch, 2015), machine learning is still scarcely exploited in animal sciences.

In this PhD thesis, three machine learning methods were used and are illustrated in the following paragraphs.

1.3.1 RANDOM FOREST

The Random Forest method (RF) is an ensemble learning method based, mainly, on decision-trees. RF develops a collection of tree-structured classification models: in each model, a multitude of decision trees are constructed, and each contributes an opinion of how the data should be classified (Breiman, 2001). The first RF algorithm was created in the late ‘90s (Ho, 1998) and it was later extended and perfected by Leo Breiman and Adele Cutler, that trademarked the term “Random Forests” in 2006 (U.S. trademark registration number 3185828, registered 2006/12/19).

RF algotihms build a forest of uncorrelated trees using a Classification And Regression Tree (CART) analysis, in which trees could be, as the term suggests, classification trees or regression trees. Classification trees analyse the input and operate with a categorical approach. Their predicted outcome is a class attribution. Differently, regression trees perform a regression on the input. Their predicted outcome is, therefore, a number. Each tree is composed by nodes, which represent one piece of information that is going to be processed, interconnected in an ordered way. According to the botanic-like nomenclature, each tree starts from a root (the input of the algorithm), moves through branches (“internal nodes” that receive one input and, after some processing, pass an output to other nodes), and, eventually, end in leaves (“external nodes” that receive an input, but return an output outside of the algorithm). In order to reduce bias, the RF creates a large number of trees applying a bootstrap aggregatin (or bagging) general technique: over a repeated number of sample selection from the input data, different trees are fitted. This procedure increases the performances of the model because it decreases the variance, still without increasing the bias. While a single decision tree could be prone to be biased by background noise in the training set, the average of many uncorrelated trees should be more robust. Overfitting is a minor problem with RF, because of the use of a large number of trees and due to the Strong Law of Large Numbers (i.e., the average of the results obtained from a large number of trials should be close to the expected value, and will tend to become closer as more trials are performed).

In Figure 1 is shown a simple example of a CART tree, showing the survival probability of a passenger during the sinking of the RMS Titanic (Apr 1912). The root node performs a first classification regarding the sex of the passenger. This node has two possible outcomes, the first leading to an internal node (i.e., a branch) with a new classification to perform, and the second to an external node (i.e., a leaf) giving a survival probability output for each data that correspond to class attributed by the root node (i.e., gender: female). Thus, a female passenger had a survival probability of 36%. This classification procedure will go through the whole tree, repeated for each data input.

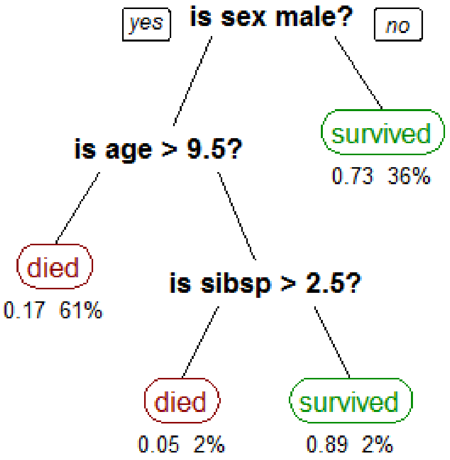

Figure 1. A tree showing survival of passengers on the Titanic ("sibsp" is the number of spouses or siblings aboard). The figures under the leaves show the probability of survival and the percentage of observations in the leaf. By Stephen Milborrow - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=14143467

1.3.2 NEURAL NETWORKS

The Neural Networks technique (NN) was developed in artificial intelligence field, with the aim to imitate the structure and function of the human brain, simulating human intelligence, learning independently and quickly, adapting continuously, and applying inductive reasoning to process knowledge (Zahedi, 1991). The theoretical bases of NN was defined in 1943 by Warren S. McCulloch (Nov 1898 – Sep 1969) and Walter Pitts (Apr 1923 – May 1969), which created the first computational NN model based on mathematics (McCulloch and Pitts, 1943). The first NN implementation dated back to 1957, when Frank Rosenblatt (Jul 1928 – Jul 1971) invented the perceptron algorithm. The perceptron is a particular type of classifier (supervised learning binary classifier), that can decide whether or not an input is to be assigned to a specific class or not.

NN is based on a collection of units called artificial neurons, in analogy with axons in a biological brain. Input neurons, similarly to root nodes in RF, are the starting point of the network and, thus, have no predecessors. They are the input interface between the network and the user. Output networks, differently, are the output interface of the network and, just like leaves in RF, do not pass the signal to other neurons. Each connection (or synapse) between artificial neurons can transmit a signal to another neuron, and the sum of these connections make the network.

NN neurons are organised in layers (Figure 2). Each layer usually gathers all the neurons that perform similar transformations on their inputs. Layers that contain neurons that are not input nor output neurons are called hidden layers, since they are not interfaced with the user outside the network.

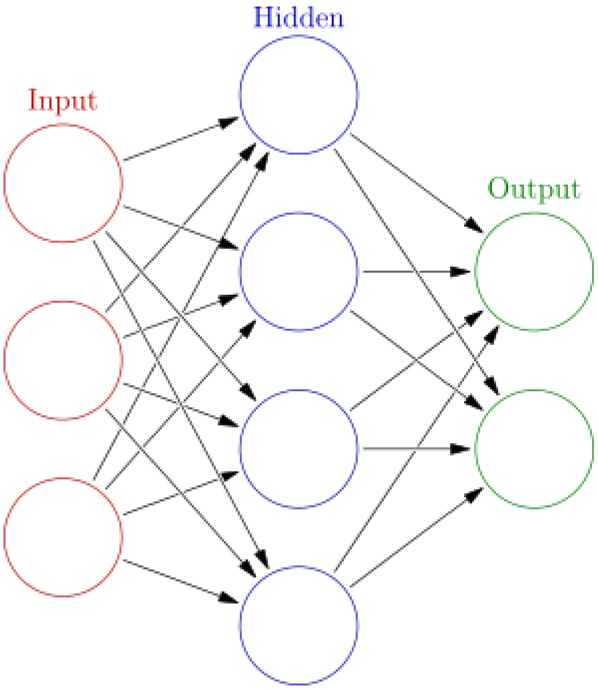

Figure 2. A Neural Network representation: each circular node indicates a neuron, and arrows are the connections between them. Three layers can be seen here, namely input (red), hidden (blue), and output (green). By Glosser.ca - Own work, Derivative of File: Artificial neural network.svg, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=24913461

Neurons and synapses may have a weight that can be defined by the programmer or, more commonly, varies as learning proceeds. Changes in weights can increase or decrease the strength of the signal that each neuron sends to the connected receiving neurons. Furthermore, each neuron may have an activation threshold. Only if the aggregated signal is below (or above) that threshold the downstream signal is sent.

1.3.3 LINEAR DISCRIMINANT ANALYSIS

Linear Discriminant Analysis (LDA) is a technique that works similarly to an ANOVA and a regression method, but uses categorical dependent variables instead of continuous ones (McLachlan, 2004). LDA is also related to principal component analysis and factor analysis, since they both search the data to find linear combinations of variables able to explain at best the data structure. LDA is a generalization of Fisher's linear discriminant (Fisher, 1936), which is a statistical method used to find a linear combination of features that separates two classes of objects. The obtained linear combination can be therefore used as a linear classifier. In addition, LDA is commonly used as a dimensionality reduction technique in association with other machine learning applications.

LDA searches for the best direction (or directions) in the variables space, which can highlight a distinct separation of the groups. LDA builds a new variable by linearly combining the original ones, making sure that the variability between groups remains greater than the one whithin groups. In Figure 3 is shown a made-up example of two groups of points (blue and red circlets) that are plotted on a 2D plot. With a linear combination of the original variables (i.e., by projecting the points on a new axis), and given the selection of the best discerning direction (i.e., following the dashed line), a new set of points will be obtained. Those new points can be used to divide the original data in the observed classes.

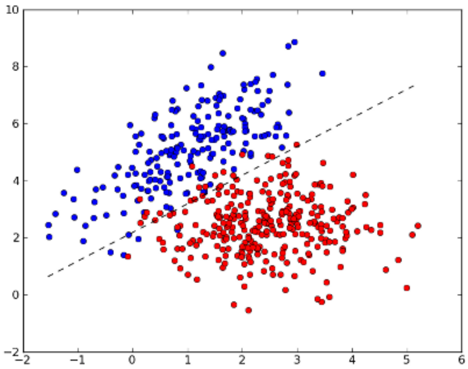

Figure 3. Linear discriminant analysis plot example: the two groups (blue and red circlets) can be identified when projecting the points following the dashed line.

2. AIM OF THE STUDY

Aim of this project was to apply statistical models to the prediction of the wellness status of dairy cows through the prediction of common diseases (namely mastitis, lameness, and dysentery) and other welfare threats (e.g., heat stress) reported in dairy farms. Different statistical approaches were applied, from simple regression to more advanced machine learning-based techniques. The relationships between rumination and other variables recorded from automatic recording systems were evaluated, since few information could be obtained from bibliographic searches. Furthermore, the genetic components of rumination and its correlation with well-studied variables (e.g., milk production) were investigated.

We selected phenotypes to be used as predictors among the ones commonly available in commercial farms. The rationale behind this choice is the will to build the basis for a future tool able to guide breeders in their work. Therefore, despite their potential importance, predictors that are usually obtained with experimental protocols or that needed expensive equipment to be measured (for example, the daily intake of each animal in the farm or blood metabolites analysis) were not considered.

The papers presented in Part II represent the main topics analysed:

1. Heat stress effects on rumination
2. Effects of diseases[1] on rumination and their prediction
3. Heritability of the rumination trait

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[1] namely mastitis, lameness, and dysentery

3. MATERIAL AND METHODS

In this chapter are described the generic materials and methods used through all the analyses performed during the PhD project. Specific details, parameters, and/or other peculiar materials and methods can be found in their respective papers in Part II.

3.1 FARMS AND ANIMALS

Five farms, one experimental and the others commercial, were monitored during this project (see Table 1 for summarised details).

Farm code	Type	Location	N° total animals	Monitored period
BUL	commercial	Pegognaga (Lombardy)	454	24/09/14 - 30/04/17
MIL	commercial	Spino d'Adda (Lombardy)	468	24/09/14 - 30/04/17
PAS	commercial	Spilamberto (Emilia-Romagna)	174	24/09/14 - 30/04/17
SAV	commercial	Pavullo nel Frignano (Emilia-Romagna)	228	08/02/16 - 23/05/17
TAD	experimental	Gariga di Podenzano (Emilia-Romagna)	269	24/09/14 - 31/03/17

Table 1. List of farms information: type of farm, location, number of total animal monitored during the project, and the range of monitored days.

Farms were selected according to the two following criteria:

- Breed (Holstein Friesian);
- Presence of an automatic recording system (see Paragraph 3.2.1).

The farms are all located in Northern Italy (Figure 4), two in Lombardy and three in Emilia-Romagna regions. The farms share a similar rearing system (which is the most common in that area) consisting in freestall barns, often open on two to four sides, with small open-space areas. The area in which the farms are located is the Po Valley, the major Italian plain, which originated by the sediments transported by the river Po. This area was chosen because of the large diffusion of Holstein dairy cattle farming and the high temperatures and humidity during summer.

Figure 4. Northern Italy, map detail. Red pointers mark the location of the farms.

Data from all the animals in the farms equipped with the automatic recording system sensor were gathered.

3.2 DATA COLLECTION

With few exceptions, described in the Material and Methods section of the relative papers, data was collected from three main sources. Animal-based information (e.g., RT and milk production) were gathered from the automatic recording system used in the farms, along with anagraphic data from the annexed herd management software. Environmental data was recorded with weather stations we installed in each farm. Lastly, sanitary information (available only for BUL farm) were gathered from the farmer's logbook.

3.2.1 AUTOMATIC RECORDING SYSTEM

The automatic recording system used in all the studies presented in this thesis is the Heatime® Pro system (SCR Engineers Ltd., Hadarim, Netanya, Israel). The system is composed of a neck collar with a tag containing a microphone with a microprocessor to monitor rumination and a 3-axis accelerometer to quantify activity. Each milking stall entrance in the farms was equipped with a single long-distance antenna, which read the specific signal coming from the tag on each cow. Schirmann et al. (2009) performed a third-party validation on this technology and confirmed the monitoring accuracy of the recorded variables. The herd management software, DataFlow™ II (SCR Engineers Ltd.), subsequently processes the raw data from the tag. The farmer can, furthermore, insert in the software additional information about each cow (e.g., ID number, age, parity …), along with the daily milk production registered by the parallel milking parlour. Customisable reports are then downloadable from the software in .xls format.

As per our farm selecting criteria, the automatic recording system was already in use in the selected farm when the PhD project started. Therefore, no habituation period for the cows to get used to the neck tag was needed. Furthermore, since this is a common management tool and not a veterinary nor an invasive device, no authorizations from an ethical committee were required.

A new feature was added to this system with a new tag released during 2015 that contains a termometer in addition to the michrophone and the accelerator. This new release allows the system to evaluate heat stress in animals. Since this update was not available at the beginning of this project, we provided weather stations and installed them on farm.

3.2.2 WEATHER STATIONS

Ambient temperature and relative humidity were recorded using a HOBO® Micro Station Data Logger (Onset®, Cape Cod, MA, USA) installed inside the barn (next to the pen, just high enough to be out of cows' reach) and equipped with a 12-bit temperature/RH sensor cable. Both ambient temperature and relative humidity were measured once a minute and recorded as a mean value every 5 min. The guaranteed working range, as indicated in the manufacturer manual, was from 0°C to 50°C with an accuracy of ± 0.2 °C for the ambient temperature and from 10% to 90% with an accuracy of ± 2.5% for the relative humidity.

The first weather station was installed in the experimental farm (TAD) on the 15th of April 2015. In the commercial farms, the remaining weather stations were installed on the 22nd and 25th of February (MIL and BUL, respectively), and on the 7th of June 2016 (PAS and SAV, on the same day).

3.3 STATISTICAL ANALYSES

Statistical analyses were performed using the free software R (version 3.2.5; R Foundation for Statistical Computing, Vienna, Austria). R is an open source programming language and software environment for statistical computing and graphics that stemmed from the S statistical language, developed in 1976 by researchers of the Bell Laboratories (Becker et al., 1988). Basic functions in R can be extended via packages, collections of new R functions, data, and compiled code in a well-defined format. Packages are available for download from the Comprensive R Archive Network (CRAN), a network of web servers around the world that store identical, up-to-date, versions of code and documentation for R.

Mixed models and machine learning algorithms were applied to the gathered data in this PhD project. Mixed models are statistical models that contain both fixed effects and random effects. While the fixed effects represent the observed quantities of the variables (treated as if they were non-random), random effects represent those variables whose effects arise from random causes. This type of statistical models, first introduced by the British statistician and biologist Ronald Fisher to study the correlation of different traits between relatives (Fisher, 1919), are particularly useful with datasets containing repeated measures on the same statistical unit. In all the papers, mixed models were fitted using the *lme4* package (Bates et al., 2015), and their statistical significance was then checked with the *lmerTest* package (Kuznetsova et al., 2015).

3.4 MACHINE LEARNING TECHNIQUES

All machine learning techniques were implemented using the *caret* package (Kuhn, 2016). For all the techniques, the original dataset was subsetted in a training and a validation datasets, consisting of the 80% and the 20% of observation, respectively. A 3 times repeated 10-folds cross-validation method was performed for the training phase of the models. Trained models were used to predict presence/absence of the disease in the validation phase. For each model, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated to evaluate the performance of the techniques.

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PART II

PAPER I

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Running title: Heat stress effects on Holstein cows' rumination.

Heat stress effects on Holstein dairy cows' rumination

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Abstract

The objective of this study was to investigate the relationship between Temperature-Humidity Index (**THI**) and Rumination Time (**RT**) in order to possibly exploit it as a useful tool for animal welfare improvement. During summer 2015 (1 June – 31 August), data from an Italian Holstein dairy farm located in the North of Italy were collected along with environmental data (i.e., ambient temperature and relative humidity) recorded with a weather station installed inside the barn. Rumination data were collected through the Heatime® HR system (SCR Engineers Ltd., Hadarim, Netanya, Israel), an automatic system composed of a neck collar with a Tag that records the RT and activity of each cow. A significant negative correlation was observed between RT and THI. Mixed linear models were fitted, including animal and test-day as random effects, and parity, milk production level and date of last calving as fixed effects. A statistically significant effect of THI on RT was identified, with RT decreasing as THI increased.

Keywords: dairy cow, heat stress, temperature-humidity index, rumination time, animal welfare

Implications

Heat stress in dairy cows is a topic of high economic importance, since it reduces both productive and reproductive performances and health and welfare. This is especially true in the Mediterranean basin, where a gradual increase in temperature and humidity is expected in the coming years. Cooling systems help in reducing heat stress in dairy cows, but could be expensive if used when unnecessary. This study aims to deepen the knowledge about the effects of heat stress on rumination, electronically monitored, and to establish the basis for future research investigating the possibility of using this relationship as a useful alert.

Introduction

One of the major external factors that can negatively affect the performance of dairy cows is the thermal environment in which they live (Nardone et al., 2010). This finding is especially true in high-yielding animals of high genetic merit, which are very sensitive to heat stress (Bernabucci et al., 2014). Several studies hypothesized the "thermo-neutral zone" (i.e., the thermal equilibrium between the animal and the environment where it lives) for lactating dairy cows to be between 5 and 25-26°C (Berman et al., 1985; Roenfeldt, 1998). Whenever the temperature exceeds this thermal zone, trespassing either the upper or lower limit, animal physiology changes to a disorder status in which, for example, milk production declines and its composition changes (Johnson, 1980).

Heat stress is an important threat to cattle breeding, especially in the Mediterranean basin, which is supposed to undergo a gradual increase in temperature and humidity in the coming years up to 2050 (Segnalini et al., 2013). Moreover, the current trend in the dairy industry is towards fewer but larger farms, rearing a great number of animals in the same structure (Winsten et al., 2010). Overcrowding and higher temperatures and humidity can indeed result in harsh conditions for dairy cows.

Responses of dairy cows to heat stress are copious: e.g., raised respiration rates (Omar et al., 1996), and panting and sweating (Blazquez et al., 1994). However, to date, few studies have analysed the effects of heat stress on health and rumination activity of cattle. Rumination, described by Erina et al. (2013) as the process of regurgitation, remastication, salivation, and swallowing of ingesta to reduce the particle size of feedstuffs and enhance fibre digestion, is a key physiological function in ruminants, and is associated with cow welfare (Bar and Solomon, 2010). Feeding variables (e.g., feed intake) have the largest effect on rumination, being intrinsically connected to this function. However, in commercial farms, recording of feeding information is not so common, and it's almost impossible to calculate feed intake for each animal. It is, instead, quite common among breeders to group animals on their productive level, optimizing the quantity and quality of feed (Spahr et al., 1993).

Despite its importance, the effects of length, severity, and timing with respect to stage of lactation of heat stress on health and rumination are still unknown. The overall effect of heat stress has been assessed by measuring body temperature of dairy cows, which shows a high susceptibility to hot environments (Araki et al., 1984). The Temperature-Humidity Index (**THI**) combines ambient temperature and relative humidity into a single value, and is a commonly used index (Hahn et al., 2003) to assess the effects of environmental temperature on dairy cows. However, THI does not include in its equation the effects of other environmental variables (e.g., solar radiation, wind). Several studies have already highlighted the negative relationship between THI and productive and reproductive performances in dairy cows (Bouraoui et al., 2002, García-Ispierto et al., 2007, Bernabucci et al., 2014, Biffani et al., 2016), but few studies investigated the relationship between THI and Rumination Time (**RT**). Soriani et al. (2013) found a significant negative correlation ($r = -0.32$; $p < 0.05$) between THI and RT, but the study was conducted monitoring only 21 cows.

Based on the aforementioned relationships, THI might be useful as a welfare index. THI recording could be implemented into automatic system programmed to send an alert directly to farmers, who can subsequently act to resolve or reduce the heat stress suffered by their animals. Several studies have already shown that the use of a cooling system helps in reducing the negative effects of heat stress in dairy cows (Frazzi et al., 2000, Calegari et al., 2016), thus an automatic activation based on a THI threshold could lead to further improvements. Indeed, some commercially available systems that can activate cooling apparatus based on THI levels already exist.

THI can be easily obtained at farm level by installing devices for temperature and humidity recording, but their distribution is still limited. On the other hand, automated recording devices to monitor cow behaviour (e.g., RT) are becoming increasingly common at farm level. Information could indeed be paired and used to disentangle their relationship. Eventually, evidences can be gained whether RT might be used as a potential and more comprehensive indicator of animal welfare than THI. The objective of the present paper is to investigate the relationship between THI and RT in dairy cows using data collected during a hot summer in a temperate area of the Mediterranean basin.

Material and Methods

Animal and environmental data collection

Data were collected from 122 Italian Holstein cows, reared in the Tadini Dairy Park experimental farm, located in Gariga di Podenzano, province of Piacenza, Northern Italy (geographical position: 44° 58’ 55.0” N, 9° 40’ 58.8” E; 68 m above the sea level). Cows were located in a freestall barn, North-South oriented, without any ventilation system, and open on all of the four sides. All of the animals inside the barn were used for this study. The mean age (± SD) of the animals was 46.67 ± 17.56 months and the average (± SD) daily milk production was 28.17 ± 9.60 kg. Animals were classified by parity as "1", "2" and "3plus" (all the cows after the third calving) and were 48, 34, and 44, respectively. Although recent studies confirmed a significant relationship between RT and both respiration rate and panting (Magrin et al., 2016), this information is not available in commercial farms and was not considered in the present study.

Ambient Temperature and Relative Humidity (**AT** and **RH**, respectively) were recorded from the 1 June to the 31 August 2015 using a HOBO® Micro Station Data Logger (Onset®, Cape Cod, Massachusetts USA) installed inside the barn (next to the pen, just high enough to be out of cows' reach) in April 2015 and equipped with a 12-bit Temperature/RH sensor cable. Schüller and Heuwieser (2016) suggested that climate conditions should be obtained at cow level because of microclimatic differences that occur in dairy barns. However, since the barn was open on all of the four sides, fresh air supply and air recirculation was similar for all the animals and areas with different microclimates were less likely to generate. Any variability due to this issue was absorbed by the random effects of the models (namely animal and date-test effects).

Both AT and RH were measured once a minute and recorded as a mean value every 5 minutes. The guaranteed working range, as indicated in the manufacturer manual, was from 0 to 50°C with an accuracy of ± 0.2°C for the AT and from 10 to 90% with an accuracy of ± 2.5% for the RH.

Descriptive statistics for the three months (June, July, and August) were calculated and the difference between the means was statistically evaluated with a Tukey's Honest Significant Difference (HSD) test, a multiple comparison followed by a statistical test with a distribution similar to a t distribution. The HSD test was performed using the HSD.test function from the R package *agricolae* (de Mendiburu, 2016).

The RT was measured and summarised in 2-h intervals using the Heatime® HR system (SCR Engineers Ltd., Hadarim, Netanya, Israel). The system is composed of a neck collar with a tag containing a microphone to monitor rumination and an accelerometer to quantify activity, as validated by Schirmann et al. (2009). The raw data are subsequently processed by the DataFlow™ II software (SCR Engineers Ltd.) provided by the farm with additional information about each cow, namely ID number, age, parity, dates of calving, days in milk (DIM), and the daily milk production registered by the linked parallel milking parlour. Each stall entrance was equipped with a single long-distance antenna, which read the specific signal coming from the rumination monitoring tag on each cow. A possible critical point of this study was the possible issues due to mis-identification of the animals at the milking parlour. Given the small dimensions of the herd and the milking parlour, the probability for cows to switch their positions once passed the identification antennae was low. We acknowledge that this could be an issue in larger farms. Furthermore, in order to check possible wrong association between cow ID and production/rumination data, consecutive recordings from the same cow were visually inspected in order to identify unexpected patterns and to remove potential outliers.

Temperature Humidity Index

THI was calculated according to the equation from Vitali et al. (2009):

$$THI= (1.8 \times AT +32) - (0.55 -0.55 \times RH) \times [(1.8 \times AT+32)-58],$$

where AT is expressed in degrees Celsius and RH as a fraction of the unit. The (1.8 x AT + 32) term is used for the conversion from degree Celsius to Fahrenheit. Following Hahn et al (2003), six THI thresholds for heat stress classification were used, namely “safe” ($THI < 68$), “mild discomfort” ($68 \le THI < 72$), “discomfort” ($72 \le THI < 75$), “alert” ($75 \le THI < 79$), “danger” ($79 \le THI < 84$), and “emergency” ($THI \ge 84$). After merging weather and rumination data, a mean value for each 2-h interval was estimated for THI, using AT and RH information.

Statistical analyses

The correlation between THI and RT was calculated using the *cor* function in R (R Core Team, 2014). The effect of THI on RT was analysed with a 2-step procedure: in the first step, RT was adjusted for a set of fixed and random effects (excluding the THI), fitting the following mixed linear model (model 1):

$$RT_{ijklm} = animal_i + par_j + prod\_lvl_k + calv_l + date_m + \varepsilon_{ijklm},$$

where RT_{ijklm} is the rumination time in 2-h intervals; $animal_i$ is the random effect of the i^{th} animal (in order to take account of the correlation between measurements recorded from the same animal); par_j is the fixed effect of the parity order j; $prod\_lvl_k$ is the fixed effect of the animal milk production level k, classified based on the calculated production tertiles (low, mid and high) of animals grouped by parity and lactation phase (DIM < 60, 60 <= DIM < 100, DIM >= 100); $calv_l$ is the fixed effect of the month and the year of the last calving event class l (format mmyyyy); $date_m$ is the random effect of the m^{th} test day; and ε_{ijklm} is the random residual effect.

In the second step, THI (factor with 6 classes, as previously defined) were added as a fixed effect to model 1:

$$RT_{ijklmn} = animal_i + par_j + prod\_lvl_k + calv_l + date_m + THI_n + \varepsilon_{ijklmn} \text{ (model 2)},$$

where *THI_n* is the fixed effect of the n^{th} THI class.

Both models were performed using the lmer function of R package *lme4* (Bates et al., 2015) which fits mixed model by Restricted Maximum Likelihood (REML) t-test using Satterthwaite approximations to degrees of freedom.

Subsequently, statistical significance of the model was checked with the *lmerTest* package (Kuznetsova et al., 2015). The package updates the existing function anova with the capability to evaluate statistical significance of fixed effects in a mixed model adding both Least Squares Means (LSM) and confidence intervals. Q-Q plots were used to check residuals distribution. Finally, in order to test the effect of THI on RT, model 1 and model 2 were compared by a simple ANOVA.

Results

Environmental data

In order to check the logger recording activity, AT and RH recorded during April 2015 were compared with data collected from an external professional weather station, located nearby (< 1 km). The two datasets showed a Pearson correlation rate of 98%, with the values of on-farm temperature and humidity always being slightly higher than the external ones, most likely due to the heat dissipation mechanisms (e.g., sweat evaporation and breathing) of the animals. Schüller et al. (2013) stated that to assess heat stress accurately is mandatory to measure the relevant climate data inside the barn to avoid underestimation of heat stress. However, we did not find such a strong underestimation in our study: this is most likely due to the different distance between the meteorological station and the barn in our and their study (< 1 km and 18 km, respectively).

A total of 288 AT and RH records per day were collected in June (n = 8640), July (n = 8928) and August (n = 8928). For each record, THI was calculated. Descriptive statistics for the three months are summarised in Supplementary Table S1. For each of the three months, the monthly THI mean was over the "safe" condition threshold: specifically, "mild discomfort" in June, "discomfort" in August and "alert" condition in July, which was the hottest summer month. Furthermore, the daily THI mean reached the "danger" condition threshold in three consecutive days, from the 5th to the 7th of July. The numbers of total days and maximum consecutive ones per THI category are reported in Table 1.

Rumination Time and fitted models

Descriptive statistics for the RT are summarized in Supplementary Table S2. Pearson correlation between RT and THI showed a significant unfavourable correlation (-0.22, $p < 0.001$), in agreement with Soriani et al. (2013).

The fixed effects from Model 1 (estimated values, SEM and p-values reported in Table 2) were statistically tested, and the results are summarised in Table 3. Random effect variances are in Table 4. Cow and test-day accounted for approximately 5.4% and 1.3% of the overall variance, respectively. The adjusted R2 of this model was 0.07 (calculated as suggested by Xu, 2003). The fitted values from the model were plotted versus the residuals of the same (calculated using the resid function of R), showing a symmetrical distribution without any clear pattern, suggesting that a linear model was suitable for our data.

Milk production level and the date of last calving had significant associations with RT ($p < 0.001$ for both the variables), while parity did not ($p = 0.106$). Using mid-production level as reference class, both high- and low-production levels showed a significant association with RT: the former increasing by 1.147 min ± 0.212 per unit ($p < 0.001$), and the latter decreasing by 1.492 min ± 0.207 per unit ($p < 0.001$). This result, together with a correlation of 20.2% ($p < 0.001$), confirmed the hypothesis validated by Moallem et al. (2010), in which RT and milk production are positively related. Differently, Byskov et al., 2015, conducted a similar study in experimental farms where feeding data was available. A negative correlation was found between milk production and RT in minutes per kilogram of DMI. Although accounting for DMI in RT could result in more precise results, it is important to keep in mind that usually, in commercial farms, it is not possible to quantify DMI for each animal. Regarding the month/year of last calving event, all of the different classes resulted in a statistically significant difference from the 092014 (i.e., September 2014) reference level.

In the second step of this work, Model 2 (which included the THI effect) was fitted, its fixed effects (estimated values, SEM and p-values reported in Table 3) were statistically tested, and the results are summarised in Table 4. THI effect was statistically significant and RT decreased by 9.36, 13.33, 19.44, 25.64 and 32.19 min/2h per unit by a mild, discomfort, alert and danger THI status, respectively. Changes in RT due to different milk production in Model 2 were smaller than in Model 1. The greater effect that THI has on RT could have partially masked milk production effect on the dependent variable of the study, resulting in the observed minor effect on RT. As expected, parity didn't have a significant effect on RT in Model 2 either. With the inclusion of the THI effect in model 2 (Table 5), the variance explained by both test-day and animal effect increased. Indeed, the residual variance of model 2 was lower than the one in model 1, confirming that the new added variable explained part of the remaining variability of the first model. Over the total variance, in the second model, the between-cow variation accounted for approximately the 5.7%, while between-date variation accounted for the 5.2%. LSM of RT by THI levels were calculated and presented in Figure 1.

The differences between the two models were tested by ANOVA, resulting statistically significant ($p < 0.001$). Both the AIC and the BIC were smaller for model 2, suggesting a better fit of the data. Adjusted R2 for model 2 (calculated as suggested by Xu, 2003) was 0.13, showing a two-fold increase with respect to model 1.

Discussion

The results presented in this study confirm a significant unfavourable association between THI and RT in Holstein dairy cows: when THI increases (i.e., increasing heat stress), a reduction in the RT occurs. An intuitive explanation could be that heat stress is known to activate physiological and metabolic responses to cope with the not optimal environmental conditions. These responses might involve, for example, a reduction in feed intake which eventually causes a reduced RT. This negative correlation between RT and THI is furthermore confirmed by similar findings from a previous study (Soriani et al., 2013).

The two fitted models (with and without THI as fixed effect, respectively) were statistically different. This result supports the hypothesis that a significant association does exist between THI and RT, the latter showing a decrease as the THI classes shifted from “safe” to “emergency”. The THI classes adopted here are effectively describing a different response to a growing non-optimal climate, as highlighted by the statistical difference between the effects on RT at different classes. Furthermore, adding the THI to the other factors statistically improved the goodness of fit of the model (two-fold increase in adjusted R2).

The effect of milk production was included in the model as a categorical effect using 3 classes (namely, low-, mid- and high-productive) and not as a linear or non-linear covariate. The rationale behind the use of such an approach was mainly due to an attempt to reduce model complexity, especially because of the limited number of available records. Nevertheless, both model 1 and 2 were also fitted including milk production as linear and/or quadratic effect but results did not change. Milk was included as an independent variable in the model to assess the association between variations in RT and changes in milk production. There is a positive correlation between the two variables, with RT increasing in high-productive animals and decreasing in low-productive ones. This association confirmed the results previously obtained by other authors (Moallem et al., 2010). However, a different result was reported by Byskov et al., 2015, were the correlation between RT (expressed in minutes per kilogram of DM intake) was negative. The association presented here could be therefore biased due to the absence of feed intake information. Nevertheless, Byskov et al., 2015 reported that variation in feed intake took into account for 32% of the variation in RT, whereas 48% of the total variation in RT was found between-cows. Considering that our model took into account the between-cows variation, which represents almost half of the total variation, introduced bias due to the absence of feed intake recording should not invalidate our results and, therefore, could be a reasonable compromise between scientific precision and commercial farm needs, where feed intake is rarely recorded.

The correlation between THI and RT presented in this study suggests that rumination could be a valuable tool for evaluating the heat stress effect on Holstein dairy cows. Further studies are needed to confirm the potential predictive use of the RT changes.

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Supplementary material

Supplementary material for this article can be found in Annex I.

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Table 1. Numbers of total days and consecutive ones per Temperature-Humidity Index (THI) category: “safe” condition (THI < 68), “mild discomfort” (68 ≤ THI < 72), “discomfort” (72 ≤ THI < 75), “alert” (75 ≤ THI < 79), “danger” (79 ≤ THI < 84), and “emergency” (THI ≥ 84).

THI category	Number of total days			Number of maximum consecutive days		
	Jun	Jul	Aug	Jun	Jul	Aug
safe	5	0	3	2	0	2
mild discomfort	16	1	12	12	0	8
discomfort	6	4	8	4	2	3
alert	3	23	8	3	18	6
danger	0	3	0	0	3	0
emergency	0	0	0	0	0	0

Table 2. Model 1. Fixed effects estimates, standard error of the means and significance by p-value

Fixed effects	Estimate	SEM	p-value	Significance[†]
Intercept	39.389	1.625	< 0.001	***
par: 1	0	-	-	-
par: 2	1.536	1.233	0.216	ns
par: 3plus	-1.271	1.211	0.297	ns
prod_lvl: mid	0	-	-	-
prod_lvl: high	1.147	0.212	< 0.001	***
prod_lvl: low	-1.492	0.207	< 0.001	***
calv: 092014	0	-	-	-
calv: 082014	4.669	2.175	0.032	*
calv: 102014	5.196	2.145	0.016	*
calv: 112014	9.334	1.841	< 0.001	***
calv: 122014	8.482	2.492	< 0.001	***
calv: 012015	10.523	2.303	< 0.001	***
calv: 022015	5.274	2.370	0.028	*
calv: 032015	11.462	2.076	< 0.001	***
calv: 042015	10.691	2.181	< 0.001	***
calv: 052015	9.004	3.103	0.005	**
calv: 062015	7.060	3.365	0.039	*
calv: 072015	11.281	1.832	< 0.001	***
calv: 082015	7.010	1.611	< 0.001	***

[†] The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 <

Table 3. Model 2. Fixed effects estimates, standard error of the means and significance by p-value

Fixed effects	Estimate	SEM	p-value	Significance[†]
Intercept	52.081	1.685	< 0.001	***
par: 1	0	-	-	-
par: 2	1.654	1.239	0.185	ns
par: 3plus	-1.198	1.218	0.328	ns
prod_lvl: mid	0	-	-	-
prod_lvl: high	1.078	0.205	< 0.001	***
prod_lvl: low	-1.466	0.200	< 0.001	***
calv: 092014	0	-	-	-
calv: 082014	4.793	2.141	0.025	*
calv: 102014	5.460	2.120	0.011	*
calv: 112014	9.599	1.839	< 0.001	***
calv: 122014	8.719	2.500	< 0.001	***
calv: 012015	10.777	2.308	< 0.001	***
calv: 022015	5.558	2.375	0.021	*
calv: 032015	11.726	2.077	< 0.001	***
calv: 042015	10.962	2.184	< 0.001	***
calv: 052015	9.210	3.119	0.004	**
calv: 062015	7.322	3.383	0.033	*
calv: 072015	11.560	1.813	< 0.001	***
calv: 082015	7.230	1.581	< 0.001	***

[†] The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns

Table 3 (continued). Model 2. Fixed effects estimates, standard error of the means and significance by p-value

Fixed effects	Estimate	SEM	p-value	Significance[†]
THI: safe	0	-	-	-
THI: mild discomfort	-9.360	0.221	< 0.001	***
THI: discomfort	-13.334	0.244	< 0.001	***
THI: alert	-19.441	0.265	< 0.001	***
THI: danger	-25.640	0.327	< 0.001	***
THI: emergency	-32.192	1.145	< 0.001	***

[†] The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns

Table 4. Statistical test for fixed effects from both Model 1 (including parity, par, milk productive level, prod_lvl, and the month and the year of the last calving event, calv, in the observed Italian Holstein herd) and Model 2, including the Temperature-Humidity Index (THI) in addition to the previous model parameters.

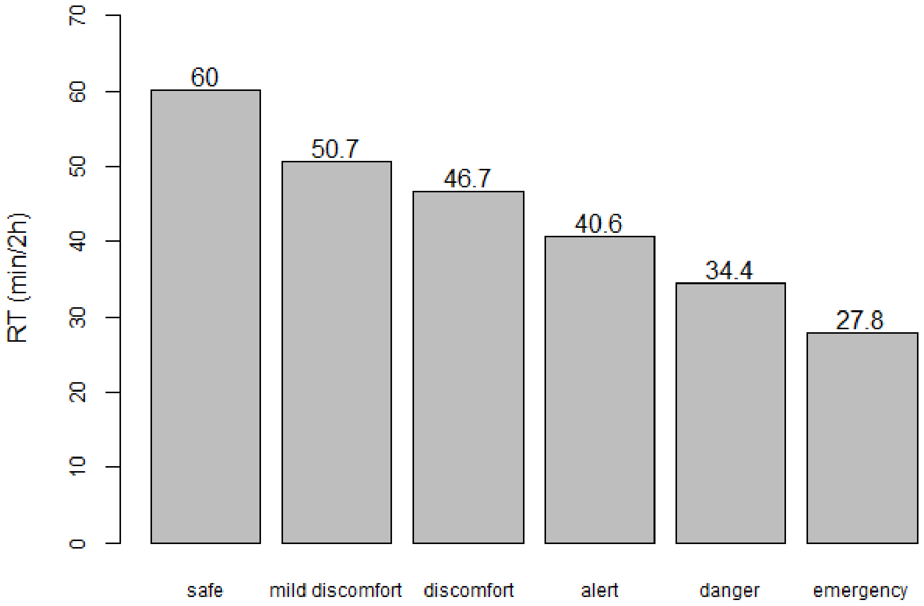
Fixed effects	Sum Sq	*P*-value	Significance[1]
Model 1			
par	2113	0.1063	ns
prod_lvl	52024	< 0.001	***
calv	30317	< 0.001	***
Model 2			
par	2016	0.1008	ns
prod_lvl	48215	< 0.001	***
calv	30047	< 0.001	***
THI	3098050	< 0.001	***

[1] The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns.

Table 5. Random effects, variances and standard deviations from both Model 1 (including parity, par, milk productive level, prod_lvl, and the month and the year of the last calving event, calv, in the observed Italian Holstein herd) and Model 2, including the Temperature-Humidity Index (THI) in addition to the previous model parameters.

Random effects	Variance	Std. Dev.
Model 1		
$animal_i$	26.729	5.170
$date_m$	6.313	2.513
residual	460.319	21.455
Model 2		
$animal_i$	27.22	5.22
$date_m$	24.94	4.99
residual	428.95	20.71

Figure 1. Least Squares Means of Rumination Time (**RT**) by Temperature-Humidity Index status in the observed Italian Holstein herd.



PAPER II

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Rumination time as a potential predictor of common diseases in high-productive Holstein dairy cows

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Summary

We examined the hypothesis that rumination time (RT) could serve as a useful predictor of various common diseases of high producing dairy cows and hence improve herd management and animal wellbeing. We measured the changes in rumination time (RT) in the days before the recording of diseases (specifically: mastitis, reproductive system diseases, locomotor system issues, and gastroenteric diseases). We built predictive models to assess the association between RT and these diseases, using the former as the outcome variable, and to study the effects of the latter on the former. The average Pseudo-R^2 of the fitted models was moderate to low, and this could be due to the fact that RT is influenced by other additional factors which have a greater effect than the predictors used here. Although remaining in a moderate-to-low range, the average Pseudo-R^2 of the models regarding locomotion issues and gastroenteric diseases was higher than the others, suggesting the greater effect of these diseases on RT. The results are encouraging, but further work is needed if these models are to become useful predictors.

Key words: rumination, animal welfare, dairy cow, predictive model

Over recent years animal welfare has become a major research area in animal husbandry. This increasing interest has been driven both by ethical concerns and by the increasing attention of consumers on product quality rather than quantity (Thornton, 2010). One of the most accepted definitions of the animal welfare concept is the ‘five freedoms’, formalised in July 1979 in a report by the UK’s Farm Animal Welfare Advisory Committee. A review by Webster (2001) is available, where these ‘freedoms’ are listed and explained. Nevertheless, being a multidimensional factor (Fraser, 1995), animal welfare assessment is not straightforward and it is dependent on different human cultures, traditions and religious faiths (Szücs et al. 2012).

Rumination is described as the process of regurgitation, re-mastication, salivation, and swallowing of feed to reduce the particle size and enhance fibre digestion (Erina et al. 2013). Rumination Time (RT, i.e., the number of minutes spent by a cow during a determined time interval) has been associated with rumen welfare, since it increases the production of saliva, which acts as a buffer for the ruminal pH (Beauchemin, 1991). The development in the early 2000s of automatic systems able to record and store a large amount of different parameters related to milk yield and cow activity, including RT, increased the possibility to investigate changes in RT and its relationships with other animal-related factors. Some studies have shown that a RT decrease might be an indicator of unfavourable psychological (acute stress: Herskin et al. 2004; anxiety: Bristow & Holmes, 2007) and pathological (hypocalcaemia: Hansen et al. 2003) conditions. More recently, RT has been further investigated to assess its relationship with the physiological changes linked with calving and oestrus events. Clark et al. (2015) correlated RT and activity time, concluding that there was a distinct decline in the duration of rumination pre-partum, which could be successfully used to predict the cows’ day of calving. Dolecheck et al. (2015) described the oestrus-related changes in parameters automatically recorded by different commercial systems and assessed the potential use of this data collecting technology for oestrus detection. The relationship between RT and diseases has not been fully investigated yet. Some recent studies showed that common dairy farm diseases significantly decrease the RT (Van Hertem et al. 2013; Liboreiro et al. 2015; Talukder et al. 2015). Stangaferro et al. (2016a, b, c) demonstrated that metabolic and digestive disorders, mastitis, and metritis have a negative effect on RT and could be predicted by analysing patterns in RT changes.

The hypothesis tested in this study is that, by predictive modelling, a trait recorded by automatic systems (e.g., RT) could be used as predictive tools for incoming diseases. Furthermore, the aim of this study was also to describe changes in RT in the days before the recording of different diseases.

Material & Methods

Data collection

The animals monitored in this study were 259 Italian Holstein cows reared in a commercial farm located in Mantua province, Lombardy (Northern Italy). All the animals were fed total mixed ration (TMR), milked twice a day and grouped in pens (lactating, pre-calving, and infirmary). RT data were recorded using the Heatime HR system (SCR Engineers Ltd., Netanya, Israel) from the 24th of September 2014 to the 6th of October 2015, for a total of 377 consecutive days. This system is composed of a neck collar with a tag containing a microphone to monitor rumination and an accelerometer to quantify activity (as validated by Schirmann et al. 2009). The raw data are then processed and summarised as 2-h intervals by the herd management software DataFlow II (SCR Engineers Ltd.), where all the information regarding each single animal (e.g., ID number, age, parity) is recorded, and then downloaded in a spreadsheet file.

The list of diseases was obtained from the farm management software, where they were recorded soon after the veterinary diagnosis, both during routine or requested visits to the farm. Their incidence is reported in Table 1. All of the recorded diseases were used in this analysis, regardless of their known effect or association with RT changes. Excluding mastitis, other diseases recorded in the software were grouped into three main classes, according to a veterinary classification: reproductive system diseases (i.e., metritis, retained foetal membranes, and ovarian cysts), locomotor system issues (i.e., lameness and generic leg infections), and gastroenteric diseases (i.e., abomasal displacement and dysentery). Other than the disease presence, no other information was available (e.g., no specific details on which type of mastitis or infection was diagnosed). In order to create a case-control dataset, for each disease, only the cows that manifested a disease at least once were kept in the dataset, hence removing all the animals that did not experience any disease during the study. Furthermore, all of the diseases were then summarised in a 'generic disease' variable, which described with 1/0 (i.e., presence/absence, respectively) the occurrence of at least one sanitary event.

Statistical analysis

This study was composed of two main parts: in the first one, mixed models were used to analyse the effects of diseases on 2-h rumination time. All the models were fitted using the lme4 package (Bates et al. 2015) in R (version 3.2.5; R Foundation for Statistical Computing, Vienna, Austria). Subsequently, the statistical significance Q3 of the model was checked with the lmerTest package (Kuznetsova et al. 2015). Model 1 was fitted with the general disease variable:

Model 1) $rum\_mean_{ijk} = generic\_disease_i + date_j + animal_k + \varepsilon_{ijk},$

where rum_mean_{ijk} is the mean rumination time for animal k affected by a generic disease in test-day j; $generic\_disease_i$ is the presence or absence of an unhealthy status; $animal_k$ is the random effect of the k^{th} animal; $date_j$ is the random effect of the j^{th} test day; and ε_{ijk} is the random residual effect.

Model 2 was fitted including as independent variables each disease category:

Model 2) $$rum\_mean_{ijklmn} = reprod_i + mast_j + locom_k + gastroent_l + animal_m + date_n + \varepsilon_{ijklmn},$$

where rum_mean_{ijklmn} is the mean rumination time for the animal m in the test-day n, affected or not by $reprod\ i$, $mast\ j$, $locom\ k$, and $gastroent\ l$; $animal_m$ and $date_n$ are the random effects; and ε_{ijklmn} is the random residual effect.

In the second part of this study, a sliding windows approach was applied to the data to investigate the change in rumination time in a total of six different windows before and after the disease event (i.e., generic disease, reproductive diseases, mastitis, locomotor system issues, and gastroenteric diseases): the windows dimensions were of 1, 3, and 5 d, symmetrically set around the disease event. This approach is widely used in genomic analyses (e.g., linkage disequilibrium and signatures of selection identification), but is seldom applied outside of this field. On each window, the 2-h rumination mean, standard deviation (SD), and slope (from a linear regression of the rumination on the days in the window) were calculated. Furthermore, for each of these new parameters, summary statistics (i.e., mean ± SD) were calculated. Four different generalised linear models (Logistic regression) were then fitted on the window before the sanitary record, each with the disease event as a binary response (i.e., presence/ absence: 1/0) and the afore-mentioned calculated parameters as predictors (Models 3.a to 3.d):

3.a) $disease = rum\_mean$

3.b) $disease = rum\_sd$

3.c) $disease = rum\_slope$

3.d) $disease = rum\_mean + rum\_sd + rum\_slope$

where *disease* is the presence or absence of one of the five cases analysed; *rum_mean* is the averaged rumination time in the window; *rum_sd* is the standard deviation of the rumination in the window; and *rum_slope* is the coefficient from the regression of the RT on the days in the window. AIC (Akaike information criterion) and McFadden's Pseudo R^2 (McFadden, 1974) were calculated to compare the models and assess which predictors and which window best fitted the data.

Results

Single Variable Comparison

The mean (±SD) RT of the animals in the herd, throughout the whole 377 d, was 46·99 ± 11·07 min/2 h. The effect of the disease presence on the 2-h RT was significant in every analysed case (P = 0·001 and P < 0·001, locomotor issues and all the other cases, respectively). Gastroenteric diseases had the largest effect, lowering RT by 9·91 min/2 h, while reproductive ones had the smallest, 1·08 min/2 h. Only three cows suffered from gastroenteric diseases, therefore, even if highly significant, the result must be interpreted with caution. The differences between the means (in min/2 h), the number of cows analysed, the ratio between positive and negative cases (case-control ratio), and the P-value from at-test, performed to assess if the differences between the two statuses were significant, are reported in Supplementary Materials, Table S1.

Multiple Variables Comparison.

The fixed effects of Model 1 (estimated values, SEM and P-values) are reported in Table 2, and their analysis of variance is reported in Table 3a. The random effects variances and standard deviations are reported in Table 3b. In this model, the diseased status had a significant effect (P <0·001) on RT, lowering it by 2·22 min/2 h. The inclusion of the effect of the parity as predictor in the models was considered. However, with the inclusion of this effect in a preliminary test, the resulting model had the worst fit on our data (probably because this effect was confounded with the animal and date random effects), and parity was therefore removed. The variance explained by the animal effect was 12·33 % of the total variance explained, and it was 6·30 times larger than the day effect variance. McFadden's Pseudo-R^2 of the model was 14·8 %. The fixed effects of Model 2 (estimated values, SEM and P-values are reported in Table 2) were statistically tested in the same way as Model 1, and the results are summarised in Table 3a, while the random effects variances and standard deviations are reported in Table 3b. The featured diseases in this model negatively affected RT, with a RT decrease which ranged from −1·73 to −5·76 min/2 h (reproductive and gastroenteric diseases, respectively). Similarly to the results of the general disease model, the variance explained by the animal effect (12·27 %of the total variance explained) was larger than the date effect (6·25 times larger). Pseudo-R^2 of the model was 14·8 %. Least Square Means (LSM) of 2-h RT by the different diseases are reported in Fig. 1. LSM were calculated for each single couple of diseased/non-diseased animals and were, therefore, different for different classifications. Variance inflation factor (VIF) was used as a diagnostic for multicollinearity within the models. In both of the models, multicollinearity was negligible. ANOVA was subsequently performed to assess which model (general disease variable vs. grouped diseases) better fitted the data: the two models were statistically different ($P < 0·001$).

Sliding windows analysis

Regarding the sliding window analysis, summary statistics of the three disease predictors related to RT (i.e., mean, SD, and slope), for both the window before and after the disease event, are shown in Supplementary Materials, Table S2. In order to investigate if the pathological event changed the observed parameters ‘before’ and ‘after’ event occurrence, a t-test with a threshold of 0·05 for the P-value was used. For the generic disease analysis, only the slope was statistically different from before to after the event (different in all the three window’s sizes). In the reproductive system diseases analysis, significant differences were identified only in the slope for windows’ sizes of 3 and 5 d. In the mastitis analysis, the 1 d window mean and all the slopes were statistically different. In the locomotor system issues analysis, the 5 d window mean and all the slopes excluding the one in 1 d window were statistically different. Lastly, in the gastroenteric diseases, a similar pattern as in the locomotor system issues were observed, i.e., all the slopes excluding the one in 1 d window were statistically different. Logistic models were also fitted to the data: the estimate of the β, the odds ratio for the disease presence, the AIC of the model, and its Pseudo-R^2 are reported in Supplementary Materials, Table S3. In all of the five cases, the best model (i.e., lower AIC and higher Pseudo-R^2) was always Model 3.d, which fitted all the three considered predictors. For the generic disease analysis, mean, SD, and slope models showed significant effects in models from 3.a to 3.c (with a maximum Pseudo-R^2 of 2·99, 0·95, and 6·02 %, respectively), with the only exception of the 1 d window SD models, in which the effect is not significant. In Model 3. d, SD was never significant. Nevertheless, this model had the highest Pseudo-R^2 and the lowest AIC for all the three windows’ sizes. The reproductive system diseases model analysis showed a similar situation of the general disease analysis, although with lower Pseudo-R^2 values. Another important difference was the complete non-significance of all the models using SD as a predictor (Model 3.b). The mastitis model analysis had a similar pattern as the general disease one: the only non-significant window’s size in the single-predictor models (i.e., Model 3.a to 3.c) was the Model 3.b, window’s size of 1 d (SD). The maximum Pseudo-R^2 were 1·50, 1·14, and 4·10 %, respectively. Regarding Model 3.d, with window’s size of one, SD was not significantly effective on RT. The locomotor system issues analysis showed a different pattern from the previous ones: the mean RT model (Model 3.a) had Pseudo-R^2 tenfold higher than generic disease, reproductive diseases and mastitis ones. Similarly to the reproductive diseases analysis, however, Model 3.b was never statistically significant. Lastly, the gastroenteric diseases model analysis had, on average, the highest Pseudo-R^2 of all the analyses. The only non-significant window’s size was the 1 d slope window. In the models with the three predictors (i.e., Model 3.d), both SD and slope were never statistically significant. Nevertheless, the Pseudo-R^2 ranged from 43·89 to 58·81 %.

The best models for each case, selected using the AIC and the Pseudo-R^2 as criteria, were always Model 3.d, but with a window's size of 5 d for the generic disease (Pseudo-R^2 = 6·47 %), a window's size of 3 d for reproductive diseases (Pseudo-R^2 = 7·16 %), a window's size of 1 d for mastitis (Pseudo-R^2 = 5·61 %), a window's size of 5 d for locomotor issues (Pseudo-R^2 = 16·17 %), and a window's size of 1 d for gastroenteric diseases (Pseudo-R^2 = 58·81 %).

Discussion

Automated rumination and activity monitoring could be used to identify diseases earlier than through clinical diagnosis performed by trained personnel. This confirms the results obtained by Stangaferro et al. (2016a, b, c). All the comparisons between mean RT of diseased and non-diseased animals resulted statistically significant. Nonetheless, the differences were small and this type of comparison does not account for any interaction between variables and, therefore, it can identify large effects only. However, all of the different diseases' effects were confirmed as statistically significant in the multiple variable approach too. The difference between the effects in the single and in the multiple variable comparison comes from the effect of the cow and the test-day, taken into account as random effects using the mixed model in the latter. These random effects should reduce the bias due to the correlation between the repeated measures.

The variance explained by the animal effect was larger than the date effect variance in both cases (i.e., general and grouped disease), suggesting that the observed variability is mainly due to the animal effect rather than to the testday. This result is in accordance with the one from Byskov et al. (2015), where the authors observed that the 48 % of the total variation in RT was due to the animal effect, whereas feed intake accounted for the 32 %.

Compared through ANOVA, the two models were statistically different. Specifically, the model including specific variables for each disease fits the data better. Using mixed models, the effect of diseases on RT was confirmed in this study, though the model could be improved by adding further predictors (e.g., feed intake and diet of the animals, which was not available for this experiment). Results obtained with mixed models are in accordance with, and further expand, the results by Stangaferro et al. (2016a, b, c).

With the sliding windows approach, we wanted to test if different features of RT in the days before a disease diagnosis could be predictive of the disease itself. This predictability would be desirable, since the detection of a disease as early as possible allows for a more immediate sanitary intervention. The features selected were the mean, the SD, and the regression slope of RT on time to disease. A difference in the slope before and after the diagnosis means that the rumination changes its trend (negative or positive). In the significant cases, the ‘after’ windows showed a positive (or less negative) trend, while the ‘before’ window had always a negative one: these results could suggest that these diseases affect rumination time, lowering it, and, since in our data the recorded event corresponds to the veterinary visit and the treatment beginning, we saw the improving of the rumination in the ‘after’ window due to medical treatment. From a descriptive point of view, SD of the RT was never statistically different before and after the disease event, while the mean showed a difference in the day before mastitis only. Of the selected feature, then, only the slope should be considered as a predictor, though the best model was always the one using all of the three features. The benefits from including mean and SD in the model are larger than the disadvantages, and this is probably due to a better representation of the phenomenon.

Different diseases could be predicted using different window size. Specifically, mastitis and gastroenteric diseases are better described by the models using one single day before the clinical diagnosis, while reproductive diseases and locomotor issues by the ones using 3 and 5 d, respectively. The Pseudo-R^2 of the reproductive diseases and mastitis predictive models was low. This could be due to the moderate ability to identify mild cases of metritis (Stangaferro et al. 2016c) and mastitis caused by pathogens other than Escherichia coli through rumination changes. As stated by Stangaferro et al. (2016b), intramammary infections caused by E. coli are more easily identified because they are characterized by a severe inflammatory response, including sudden shock, sepsis, and often death. On the other hand, even with a small number of animals with gastroenteric disease, models predictive for these diseases had the highest Pseudo-R^2, in accordance with the high sensitivity detected by Stangaferro et al. (2016a). Locomotor issues, which were not analysed in the abovementioned studies, showed a Pseudo-R^2 in between the other cases.

The difference in the window size in each different disease could be due not only to the higher or lesser effect of each disease on RT, but also to the different reaction time of the farmer in response to the different symptoms detected on his animals. Different diseases are perceived differently by farmers (e.g., mastitis is, from a commercial point of view, a greater concern than other diseases) and, therefore, they could require veterinary intervention with different urgency. Moreover, in order to obtain accurate estimates at enough distance from the event it is necessary for the farmer to carefully consider the occurrence of the events.

Conclusion

In this study we observed that common farm diseases (i.e., reproductive diseases, mastitis, locomotor system issues, and gastroenteric diseases) significantly affect the 2-h interval RT, lowering it in comparison to the one of healthy animals. Further studies are needed to fully assess the suitability of RT for predicting the onset of these diseases in individual animals. The growing presence of automatic recording systems, even in medium-small farms, will allow researchers to have larger datasets for modelling studies.

Supplementary material

Supplementary material for this article can be found in Annex II.

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Table 1. Recorded diseases and relative incidence in the data, in descending order.

Disease	Incidence
Generic diseases	57.14 %
Reproductive diseases	34.36 %
Mastitis	32.43 %
Locomotor issues	8.11 %
Gastroenteric diseases	1.16 %

Table 2. Models 1 and 2, 2-h interval. Fixed effects estimates, standard error of the means and significance by p-value. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < . < 0.1 < ns.

Fixed effects	Estimate	SEM	p-value	Significance
Model 1				
Intercept	47.46	1.63	< 0.001	***
generic_disease	-2.22	0.15	< 0.001	***
Model 2				
Intercept	47.41	0.26	< 0.001	***
reprod	-1.73	0.17	< 0.001	***
mast	-4.07	0.33	< 0.001	***
locom	-1.76	0.65	0.007	**
gastroent	-5.76	0.95	< 0.001	***

Table 3. Models 1 and 2, 2-h interval. a) Statistical test for fixed effects. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < . < 0.1 < ns. b) Table of random effects, with their variances and standard deviations.

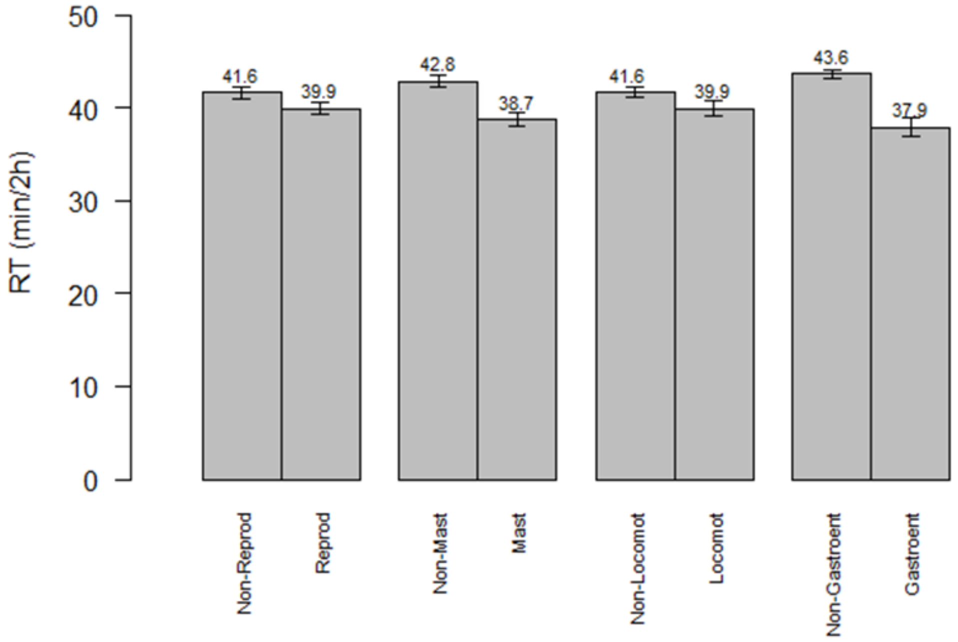
a)

Fixed effects	Sum Sq	Mean Sq	df	F-value	p-value (> F)	Significance
Model 1						
generic_disease	23387	23387	1	223.22	< 0.001	***
Model 2						
reprod	11305.8	11305.8	1	107.969	< 0.001	***
mast	15909.8	15909.8	1	151.938	< 0.001	***
locom	761.5	761.5	1	7.273	0.007	**
gastroent	3862.2	3862.2	1	36.883	< 0.001	***

b)

Random effects	Variance	Std. Dev.
Model 1		
$animal_i$	15.065	3.881
$date_j$	2.392	1.547
residual	104.772	10.236
Model 2		
$animal_i$	14.983	3.871
$date_j$	2.396	1.584
residual	104.713	10.233

Figure 1. LSM of Rumination Time (RT) by specific disease presence (i.e., "Diseased") or absence (i.e., "Non-diseased"), calculated for each single couple of diseased/non-diseased animals. The number of diseased animals for each disease is 126 (generic diseases), 66 (reproductive diseases), 68 (mastitis), 16 (locomotor issues), and 3 (gastroenteric diseases).



DRAFT I

DRAFT (to be submitted)

Heritability and genetic correlations between rumination and production traits in Holstein dairy cows during different lactation phases

Running title: Heritability of rumination in Holstein dairy cows

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SUMMARY

So far, researchers and farmers used rumination only as a key monitor of dairy cow health at farm level. However, investigating its genetics parameters and its correlation with other important productive features may turn this management tool in a new informative selection criterion for the dairy cattle breeding strategies. Recently, rumination heritability was estimated in few papers, along with its genetic correlation with feed efficiency. However, no evaluation in scientific literature are available on its genetic correlation with milk production and composition parameters. Therefore, the objective of this study was to estimate the heritability of rumination and its genetic correlation with milk production, milk composition (i.e., protein yield, fat yield, and the fat/protein ratio), and the somatic cells count. The estimated rumination heritability was 0.34. Regarding milk production and composition traits, the heritabilities were 0.35 (milk production), 0.53 (protein yield), 0.44 (fat yield), and 0.32 (fat/protein ratio). The somatic cells count heritability was 0.23.

KEYWORDS

Bayesian analysis, heritability, genetic correlation, Holstein dairy cow, rumination

1. INTRODUCTION

Rumination is the process of regurgitation, remastication, salivation, and swallowing of ingesta (Erina et al., 2013). Rumination is a key physiological function that provides for effective mechanical breakdown of roughage, and thereby increases substrate surface area to fermentative microbes. The microbial community composed by bacteria, protozoa, and fungi break down cellulose and lignin, aiding their digestion and producing volatile fatty acids, essential amino acids, and proteins, which are then absorbed into the rumen. This production supplies about 60-80% of the cow's energy. Rumination is nowadays often included in automatic recording systems that monitor the welfare status of the dairy cows, and these systems are commonly available in commercial farms (Borchers & Bewley, 2015). Since the abovementioned automatic recording systems record information on a high frequency (often recording every minute and then summarising data on an hourly basis), there is a large amount of data to be used as a new informative selection criterion in dairy cattle breeding strategies.

Although rumination process has been studied thoroughly from both the physiological and the pathological point of view, its heritability is still almost completely uninvestigated. To be a valuable selection criterion, rumination should have a moderate-to-high heritability and should be genetically correlated to other meaningful factors. Only recently, a first estimate of rumination heritability was calculated in Danish Holstein dairy cows and it was 0.32, a value usually considered as moderate heritability (Byskov, Fogh, & Løvendahl, 2017; average value between two heritabilities estimated in two different herds). In the abovementioned study, they calculated the genetic correlation between rumination and feed-related variables. It was showed that rumination is negatively correlated with dry matter intake and residual feed intake. However, the genetic correlation with other variables (namely energy-corrected milk) were not statistically significant.

Other meaningful parameters in dairy cow's selection system are the ones related to milk production and composition. Variables like milk yield (MY), protein (PY) and fat (FY) content, and somatic cells count (SCC) have been studied thoroughly, and their heritabilities were evaluated in various herds, breeds, and environments. As a few examples, MY heritability value was 0.30 in Japanese Holstein cows (Suzuki & van Vleck, 1994), 0.39 in Holstein reared in the UK (Kadarmideen, Thompson, & Simm, 2000), and 0.48 in a Holstein Fresian and Dutch Fresian crossbred population (Hoekstra, van der Lugt, van der Werf, & Ouweltjes, 1994). In the abovementioned works, PY heritability was 0.26 and 0.33 (Japanese and Dutch Holsteins, respectively), while FY heritability was 0.30 and 0.36 (again, Japanese and Dutch Holsteins, respectively). SCC heritability values from scientific literature are lower than the values of milk production and composition heritabilities. As examples, SCC heritability was 0.08 in Swedish Holstein and Canadian dairy cows (Emanuelson, Danell, & Philipsson, 1988, and Kennedy, Sethar, Moxley, & Downey, 1982, respectively) and 0.18 in Danish dairy cows (Lund, Miglior, Dekkers, & Burnside, 1994).

As of today, the genetic correlation between these milk production parameters and rumination has not been evaluated yet. Thus, the aim of the study was to investigate the genetic variation and to estimate the heritability of rumination time and its genetic correlation with MY, PY, FY, and SCC.

2. MATERIALS AND METHODS

2.1 Data

The Heatime HR system (SCR Engineers Ltd., Netanya, Israel) automatic recording system was used in this study. This system is composed by a neck collar with a tag (placed on the left side of the neck) containing a microphone to monitor rumination and a 3-axis accelerometer to quantify neck activity. Third-party validation on this technology was performed Schirmann et al. (2009), which confirmed the monitoring accuracy of the recorded variables. Other information regarding the animal (e.g., ID number, age, parity) were collected from the annexed herd management software.

Records for 710 Holstein dairy cows were available in the dataset, coming from four different farms, all located in Po Valley, Northern Italy. For each record, sire, dam, parity and age at last calving were gathered from the herd management software, while rumination time, MY, and days in milk (DIM) were gathered from the automatic recording system. PY, FY, and Log SCC data were gathered from the functional controls performed by the Italian Breeder’s Association. Contemporary groups were defined by merging herd, year, and month of the test-day, and groups containing less than five animals were not studied further.

Records were split in three subsets, according to the lactation phase in which the test-day was. The three lactation phases were defined as early phase (0-60 days from calving), mid phase (61-150 days from calving), and late phase (151-300 days from calving). Data editing and descriptive statistics were performed using R (version 3.2.5; R Foundation for Statistical Computing, Vienna, Austria).

2.2 Statistical models

The (co)variance and breeding values were estimated by bi- and tri-characteristic Bayesian models using the Gibbs sampler of the GIBBS3F90 software (Misztal, 2012). The model used in this study included random direct genetic effects, the random animal effect, the age at last calving and DIM (as covariate variables), the parity and the effect of contemporary groups (as factor variables). Rumination, MY, PY, FY, FY/PY, and LogSCC were the traits to be evaluated. The matrix model used for rumination was:

$$y = X\beta + Za + e$$

where y = vector of observations of the dependent variable (rumination); β = vector of contemporary group, associated with y through the incidence matrix of X; a = vector of random effects of additive genetic value of the animal associating a with y through the incidence matrix Z; and e = vector of residual effects.

For the Bayesian analysis, the effects included in the model were considered to be random variables. For the value of b, an informative *a priori* distribution based on bibliographic reference values for the breed in the study was assumed.

Initially, for all traits, a single chain of 400,000 iterations was used, with a period of disposal (i.e., "burn-in") of 40,000 iterations, and a sampling interval (i.e., "thinning") of 10. However, these values in some cases have been altered according to the need to adjust for convergence (Raftery & Lewis, 1992). The convergence of the Gibbs chain was therefore tested by the criterion of Geweke. The convergence of the algorithm was verified with a significance level of 0.05 for the test, under the null hypothesis. In this case, the test considers the null hypothesis to be the convergence of the chain; therefore the larger the p-value (> 0.05) the greater is the convergence of the chain (Geweke, 1992). The Bayesian Output Analysis (BOA version 1.1.5) package (Smith, 2005) was used to calculate the mean and the SD for all parameters from the individual marginal posteriors.

The coefficient of direct heritability for all variables was estimated by:

$$h^2 = \sigma^2 a / \sigma^2 p$$

3. RESULTS

Descriptive statistics of the variables and analysed traits are presented in Table 1. ANOVA confirmed that rumination is statistically different ($P < 0.05$) between the 3 lactation phases. Specifically, rumination is higher during an early lactation phase, and drops shifting to the late phase. Regarding the analysed traits, MY is different between lactation phases ($P < 0.05$) and is higher during the mid phase. PY is statistically different as well ($P < 0.05$), increasing through the phases. FY in early and late phases is not statistically different ($P = 0.1$). However, it is statistically lower ($P < 0.05$) during the mid phase. The FY/PY ratio statistically decreases through the lactation phases ($P < 0.05$).

Lastly, the LogSCC is statistically different ($P < 0.05$) through all the phases, with the highest value during the late phase.

The heritability of rumination was evaluated using both REML and Bayesian approaches. The two approaches gave similar results, thus only the GIBBS sampling method results (Bayesian approach) are reported here (Table 2). The mean heritability of rumination trait, on average, was 0.34. Specifically, it was 0.32 in early lactation phase, 0.34 in mid lactation phase, and 0.35 in late lactation phase. This results confirms what obtained by Byskov et al. (2017).

The genetic correlation between rumination and milk production parameters were generally moderate-to-low, and ranged from -0.40 (rumination vs FY/PY ratio, early lactation phase) to 0.13 (rumination vs MY, mid lactation phase). Specifically, the genetic correlation between rumination and MY during the early phase was slightly negative (-0.04), while it was positive in both the mid and late phases (0.13 and 0.12, respectively). The genetic correlation with PY and FY was negative for all the lactation phases (0.00, -0.05, and -0.17 in PY, and -0.39, -0.22, and -0.33 in FY for early, mid, and late phases respectively), and, straightforwardly, the genetic correlation between rumination and FY/PY ratio was similarly negative (-0.40, -0.23, and -0.32 for early, mid, and late phases respectively). The genetic correlation with LogSCC ranged from -0.10 (late lactation phase) to 0.30 (early lactation phase), with a value of -0.05 for the mid phase.

The heritabilities of milk production parameters had much more variability through the lactation phases than the rumination one. MY heritability, on average, was 0.35, but increased through the phases (0.14, 0.39, and 0.53 for early, mid, and late lactation, respectively). The same increasing trend was shown by PY (mean value of 0.53, with 0.25, 0.50, and 0.83 for early, mid, and late lactation, respectively), FY (mean value of 0.44, with 0.18, 0.46, and 0.68 for early, mid, and late lactation, respectively), and FY/PY ratio (mean value of 0.32, with 0.14, 0.33, and 0.50 for early, mid, and late lactation, respectively). Lastly, LogSCC heritability, on average, was 0.23, with a similar heritability in early and late phases (0.25 and 0.26, respectively) and a lower one (0.19) in mid phase.

4. DISCUSSION

The descriptive statistics did not show any abnormal behaviour regarding variables and traits. The higher value of rumination in the early phase compared to the other two phases' ones is easily explained by the fact that cows need a high amount of energy during this phase. Thus, they will eat more and, subsequently, ruminate more. MY reached its highest value during the mid lactation phase, which covered the productivity peak of the lactation. Furthermore, PY and FY were lower in the phases with a higher MY, which is a known phenomenon. Laevens et al. (1997), showed that in bacteriologically negative cows the SCC is not affected by the lactation stage. However, they found a significant effect of lactation phase when bacteriologically positive cows were involved. This second result, which was obtained in similar conditions, is in agreement with our results.

The heritability estimated in the analysed population was similar to the only other heritability value that was present in scientific literature, which was estimated by Byskov, Fogh, & Løvendahl (2017) in a population of Danish Holstein cows. The average value found in this study (0.34), is considered a moderate value for heritability, meaning that it could be possible to use this trait in animal selection. Furthermore, we showed that the heritability of rumination trait is nearly constant during the whole lactation, with a difference of 0.03 points between the early and late phases of lactation. This result further confirm the possibility to use this trait in a selection strategy.

The average genetic correlation between rumination and MY during the entire lactation was close to zero (0.07), meaning that the two traits have an almost null genetic association. Therefore, rumination could not be a proxy for MY selection. The MY heritability value estimated in this study changed heavily during the lactation phases, with the highest value during the late phase. This means that persistency of milk production is a highly heritable trait. The average value through the lactation was 0.35, similar to the values obtained in other Holstein herds in Europe.

The average genetic correlation between rumination and PY was -0.11, indicating a weak negative genetic association. PY heritability in our population was 0.53, much higher than what is reported in literature (0.26 and 0.33, Japanese and Dutch Holsteins, respectively). FY genetic correlation with rumination was negative as well, but it was higher in absolute value (-0.31). This correlation is unfavourable, since an increase in daily rumination time would lead to a decrease in FY in milk. FY heritability was 0.44, higher than what reported for other Holstein populations (e.g., 0.30 and 0.36, Japanese and Dutch Holsteins, respectively). Regarding the FY/PY ratio, which is used as indicator for subclinical ketosis diagnosis (Jenkins et al., 2015), its correlation with rumination was -0.32, and its heritability was 0.32. Given this negative association, selection for higher daily rumination time would have negative effects on this ratio.

Lastly, the heritability of LogSCC was 0.23, similar to what reported in Danish Holstein. LogSCC average genetic correlation with rumination was weak (0.05), although it was higher during the early phase of lactation (0.30).

5. CONCLUSIONS

In conclusion, rumination was found to have a moderate heritability in Holstein dairy cows reared in Northern Italy. However, its negative (although weak) genetic correlations with milk production traits, along with the positive association with somatic cells count, showed that daily rumination time is not a suitable proxy for production performances selection.

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Table 1. Descriptive statistics (mean ± SD) of the variable available in the datasets, divided in early (0-60 days), mid (61-150 days), and late (151-300 days) lactation phases.

Phase	Parity	Age at calving (m)	DIM	Rum (min/ d)	MY	PY	FY	FY/PY	LogSCC
Early	2.1 ± 1.2	40.2 ± 17.5	32.7 ± 9.9	512.9 ± 109.5	37.4 ± 10.2	3.1 ± 0.3	4.0 ± 1.0	1.3 ± 0.3	237.9 ± 647.1
Mid	2.0 ± 1.2	44.4 ± 21.5	103.9 ± 13.5	508.9 ± 101.4	38.5 ± 8.4	3.2 ± 0.3	3.8 ± 0.6	1.2 ± 0.2	226.0 ± 465.1
Late	1.9 ± 1.1	48.5 ± 24.2	250.4 ± 59.7	487.3 ± 108.9	30.8 ± 6.9	3.5 ± 0.3	4.0 ± 0.6	1.1 ± 0.1	268.6 ± 458.9

Abbreviations: DIM, days in milk; Rum, rumination time; MY, milk yield; PY, protein yield; FY, fat yield; FY/PY, fat and protein yields ratio; LogSCC, somatic cell count on a logarithmic base.

Table 2. Heritability (h^2, mean ± SD) and correlation (*r*) for rumination (Rumin) versus milk yield (MY), protein yield (PY), fat yield (FY), fat/protein (FY/PY) ratio, and Log somatic cell count (LogSCC) in the three lactation phases (namely, early: 0-60 days; mid: 61-150 days; and late: 151-300 days). For each couple of traits, additive (σ_a^2) and residual (σ_e^2) variances are reported.

		Rumination			Second trait			
Parameter	Phase	σ^2_a	σ^2_e	h^2	σ^2_a	σ^2_e	h^2	*r*
Rumin vs MY	Early	2399.4 ± 425.9	5164.8 ± 358.3	0.32 ± 0.05	8.35 ± 2.79	51.57 ± 3.20	0.14 ± 0.04	-0.04
	Mid	2543.9 ± 414.2	4967.6 ± 330.8	0.34 ± 0.05	15.70 ± 2.39	24.84 ± 1.73	0.39 ± 0.05	0.13
	Late	2830.7 ± 487.7	5442.0 ± 386.7	0.34 ± 0.05	14.91 ± 1.70	13.52 ± 0.99	0.53 ± 0.04	0.12
Rumin vs PY	Early	2437.4 ± 412.9	5154.7 ± 352.1	0.32 ± 0.05	0.02 ± 0.00	0.06 ± 0.00	0.25 ± 0.04	0.00
	Mid	2504.6 ± 419.6	4998.9 ± 338.9	0.33 ± 0.05	0.02 ± 0.00	0.02 ± 0.00	0.50 ± 0.04	-0.05
	Late	2961.8 ± 493.4	5379.9 ± 377.4	0.36 ± 0.05	0.05 ± 0.00	0.01 ± 0.00	0.83 ± 0.02	-0.17
Rumin vs FY	Early	2437.4 ± 412.9	5154.7 ± 352.1	0.32 ± 0.05	0.10 ± 0.03	0.46 ± 0.03	0.18 ± 0.04	-0.39
	Mid	2504.6 ± 419.6	4998.9 ± 338.9	0.33 ± 0.05	0.16 ± 0.02	0.19 ± 0.01	0.46 ± 0.04	-0.22
	Late	2961.8 ± 493.4	5379.9 ± 377.4	0.36 ± 0.05	0.21 ± 0.02	0.10 ± 0.01	0.68 ± 0.03	-0.33

Table 2 (continued). Heritability (h^2, mean ± SD) and correlation (*r*) for rumination (Rumin) versus milk yield (MY), protein yield (PY), fat yield (FY), fat/protein (FY/PY) ratio, and Log somatic cell count (LogSCC) in the three lactation phases (namely, early: 0-60 days; mid: 61-150 days; and late: 151-300 days). For each couple of traits, additive (σ_a^2) and residual (σ_e^2) variances are reported.

		Rumination			Second trait			
Parameter	Phase	σ^2_a	σ^2_e	h^2	σ^2_a	σ^2_e	h^2	*r*
Rumin vs FY/PY ratio	Early	2465.1 ± 420.5	5127.9 ± 350.4	0.33 ± 0.04	0.01 ± 0.00	0.06 ± 0.00	0.14 ± 0.04	-0.40
	Mid	2556.4 ± 417.0	4958.9 ± 329.4	0.34 ± 0.05	0.01 ± 0.00	0.02 ± 0.00	0.33 ± 0.04	-0.23
	Late	2949.4 ± 509.0	5378.8 ± 385.9	0.35 ± 0.0	0.01 ± 0.00	0.01 ± 0.00	0.50 ± 0.04	-0.32
Rumin vs LogSCC	Early	2372.6 ± 425.9	5183.7 ± 358.5	0.31 ± 0.05	0.85 ± 0.18	2.62 ± 0.17	0.25 ± 0.05	0.30
	Mid	2525.9 ± 417.8	4973.4 ± 336.4	0.34 ± 0.05	0.58 ± 0.14	2.51 ± 0.15	0.19 ± 0.05	-0.05
	Late	2863.3 ± 486.0	5423.2 ± 379.7	0.35 ± 0.05	0.58 ± 0.11	1.67 ± 0.11	0.26 ± 0.04	-0.10

DRAFT II

DRAFT (to be submitted)

Prediction of mastitis, lameness, and dysentery in Holstein dairy cattle using machine-learning techniques

Running title: Machine-learning-based disease prediction

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ABSTRACT

The aim of this study was to determine prediction efficacy of 3 common dairy cows' diseases (i.e., mastitis, lameness, and dysentery) using different machine-learning-based techniques (namely Random Forest, Neural Networks, and Linear Discriminant Analysis). Data recorded from automatic systems available for farmers and data from herd management software (e.g., age, parity, and DIM) were used as predictors. The best predictive results were obtained for dysentery, while mastitis prediction showed lower performances. We showed in this work that, even with sanitary information recorded by farmers, it is possible to have a first attempt of disease prediction. Using experimental data in future studies may improve results, towards the realization of a prediction tool that would be valuable to farmers, giving them the possibility to start with the treatments on an early stage of the disease.

INTRODUCTION

Animal health is one of the key factors affecting the efficiency of farms (Heikkilä et al., 2012). Mastitis, lameness, and dysentery are three common diseases that afflict dairy farms worldwide. Mastitis is considered the most costly disease in dairy farming (Seegers et al., 2003), but the economic impact of the other two diseases is not ignorable (Bennett et al., 1999). Although disease prevention is a fundamental element in livestock production system (Schwabenbauer, 2012), it is a goal often difficult to pursue. The early detection of the abovementioned diseases is, therefore, important (de Mol et al., 1999; de Mol et al., 2013), and finding some indicators able to predict their onset before the clinical symptoms appear would be greatly beneficial for the farm management.

Precision dairy monitoring technologies are a valuable tool to gather a large amount of data regarding a variety of variables, and are a valid alternative to the time-consuming subjective observation. Many of these technologies, developed in early 2000s, are nowadays commonly used on dairy farm (Borchers and Bewley, 2015). Recent studies have shown that variability in traits recorded with these systems (e.g., rumination time) is related to pathological statuses. For example, Van Hertem et al. (2013) associated changes in rumination, milk yield, and neck activity to lameness and validated a logistic regression model to detect clinical symptoms. Through machine-learning approaches, data coming from those automatic monitoring systems have been already used for the detection of mastitis (Cavero et al., 2008), oestrus (Firk et al., 2003) and calving (Borchers et al., 2017). However, to our knowledge, there are few or even no studies in scientific literature regarding the use of these techniques to predict diseases instead of detecting them.

Aim of this study was to determine the efficacy of different machine-learning-based techniques in predicting mastitis, lameness, and dysentery using data coming from automatic recording systems and other herd management software.

MATERIALS AND METHODS

Data Collection and Editing

The automatic recording system used in this study was the Heatime HR system (SCR Engineers Ltd., Netanya, Israel). This system is composed by a neck collar with a tag (placed on the left side of the neck) containing a microphone to monitor rumination and a 3-axis accelerometer to quantify neck activity. Schirmann et al. (2009) performed a third-party validation on this technology and confirmed the monitoring accuracy of the recorded variables. The herd management software DataFlow II (SCR Engineers Ltd.) then processes the raw data, considering all the information regarding each single animal (e.g., ID number, age, parity). Data from the automatic recording system were collected for 950 consecutive days, from the 24th of September 2014 to the 30th of April 2017. The herd consisted of 454 Italian Holstein cows reared in a commercial farm, located in the Po Valley, Northern Italy. All the animals were fed total mixed ration (TMR), milked twice a day and grouped in pens (lactating, pre-calving, and infirmary). The system was already in use in the selected farm when we started collecting data, therefore no habituation period was needed.

Ambient temperature and relative humidity were recorded throughout the whole period using a weather station installed inside the barn. The weather station was equipped with a 12-bit temperature/RH sensor cable. Temperature–Humidity Index (THI), which combines ambient temperature and relative humidity into a single value, is a commonly used index (Hahn et al., 2003) to assess the effects of environmental temperature on dairy cows. THI was calculated according to the equation from Vitali et al. (2009):

$$THI = (1.8 \times T + 32) - (0.55 - 0.55 \times RH) \times [(1.8 \times T + 32) - 58]$$

where the ambient temperature (T) is expressed in degrees Celsius and the relative humidity (RH) as a fraction of the unit. The $(1.8 \times T + 32)$ term is used for the conversion from degree Celsius to Fahrenheit.

The final variables considered in this study were age (months) and parity of the animal, DIM, Rumination Time (RT, min/day), Activity Time (AT, units/day), daily milk production (kg/day), and the THI. Sanitary records were obtained from the farm management software, where they were recorded soon after the diagnosis of the veterinary, both during routine or requested visits to the farm. Other than the disease presence or absence (binary variable 1/0), no additional information was available (e.g., no specific details on which type of mastitis or how severe the lameness was). Among the available diseases, mastitis, lameness and dysentery were selected. These 3 diseases are widely common in dairy farms. Furthermore, they were selected to have diseases with a different number of cases in our dataset (namely, high number of cases for mastitis, medium number of cases for lameness, and low numerosity for dysentery). The complete dataset was divided in 2 subsets, grouping primiparous animals and pluriparous ones, respectively. The total number of the animals is not equal to the sum of primiparous and pluriparous animals since some animals calved during the monitoring period and thus shifted between parity classes. Since in our datasets the numerosity of disease and non-disease records was highly unbalanced, we decided to remove records regarding days when all the cows were non-diseased, thus keeping only days with at least one disease record. We are aware that reducing the number of observations could lead to a less precision of the predictive modelling results. However, the models could not manage the original high-unbalanced data. Furthermore, in order to create a case-control dataset, for each disease, only the cows that manifested it at least once were kept in the dataset, hence removing all the animals that did not experience any disease during the study. Finally, 3 different datasets were selected after data pruning, in which RT, AT, milk and THI were summarized as means of the day before (1-day window), from 3 to 1 days before (3-days window), and from 5 to 1 days before (5-days window) each recording day.

For each of the 3 diseases, we calculated the number of recorded cases. Regarding mastitis, we recorded 146 cases in 84 primiparous animals (of which 50 animals with single cases and 34 animals with, on average, 2.8 cases each), and 249 cases in 115 pluriparous ones (of which 51 animals with single cases and 64 animals with, on average, 3.1 cases each). Regarding lameness, we recorded 27 cases in 26 primiparous animals (of which 25 animals with single cases and 1 animal with 2 cases), and 48 cases in 35 pluriparous ones (of which 26 animals with single cases and 9 animals with, on average, 2.4 cases each). At last, regarding dysentery, we recorded 7 cases in 7 primiparous animals, and 10 cases in 9 pluriparous ones (of which 8 animals with single cases and 1 animal with 2 cases).

Statistical Analysis and Prediction Model

Statistical analyses were performed using R (version 3.2.5; R Foundation for Statistical Computing, Vienna, Austria). A relative importance analysis was performed on our predictors using the Partial Least Squares (PLS) method in the pls package (Mevik and

Wehrens, 2007). PLS is a dimension reduction technique that maps the predictor variables to a smaller set, which maximally explain the outcome variable.

Then, machine-learning techniques were applied to the datasets to predict mastitis, lameness, and dysentery. All machine-learning techniques were implemented using the caret package (Kuhn, 2016). Specifically, Random Forest (RF), Neural Network (NN), and Linear Discriminant Analysis (LDA) techniques were selected. The RF method is based on decision-tree classification and develops a group of tree-structured classification models. Each tree contributes an opinion of how the data should be classified (Breiman, 2001). NN technique was developed in artificial intelligence field, with the aim to imitate the structure and function of the human brain, simulating human intelligence, learning independently and quickly, adapting continuously, and applying inductive reasoning to process knowledge (Zahedi, 1991). LDA is a technique that works similarly to an ANOVA and a regression method, but uses categorical dependent variables instead of continuous ones (McLachlan, 2004). For all the techniques, the original dataset was splitted in a training and a validation datasets, consisting of the 80% and the 20% of observation, respectively. A 3 times repeated 10-folds cross-validation method was performed for the training phase of the models. Trained models were used to predict presence/absence of the disease in the validation phase. For each model, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated to evaluate the performance of the techniques.

RESULTS AND DISCUSSION

Data descriptive analysis

For each disease, only the animals that experienced it at least once were kept in the dataset, in order to obtain a case/control scenario. We decided to keep rows with missing data to minimize the loss of records in the first part of the analysis (i.e., descriptive and PLS). We calculated summary statistics of the predictors for primiparous and pluriparous animals (Table 1). Month of recording was initially used as predictor, but its effect introduced confounding into the model. This is due to the correspondence, at least in the Italian scenario, between high THI and summer months (namely, June, July, and August). Month of recording was therefore removed from the predictors used. We tested the correlation between predictors. Age and parity, as expected, showed a strong correlation (Mastitis dataset: 87.7%, $P < 0.001$. Lameness dataset: 89.7%, $P < 0.001$. Dysentery dataset: 92.9%, $P < 0.001$). However, they both were kept in the analysis, because in the predictive modeling part of this study we observed that models missing one of these two predictors showed lesser accuracy in comparison with models including both.

To assess which predictor was the most important relatively to each dataset, we performed a relative importance analysis using the PLS method (Supplemental Figures S1, S2, and S3). Regarding mastitis prediction, the most important predictor in the 1 and 3-days windows (for both primiparous and pluriparous cows) and in 5-days window (pluriparous only) was the THI. It was a positive predictor (risk factor), meaning that high levels of THI (i.e., heat stress) increased the probability for a cow to develop mastitis. In the same windows, for both primiparous and pluriparous cows, milk production and DIM were important negative predictors (protective factors). Daily milk was lower in mastitic animals (t-test, $P < 0.001$), and this result is quite straightforward. Animals that had a high milk production and an advanced phase of lactation in the days before the sanitary event have a lower probability to have mastitis. Differently, in the 5-days window of the primiparous animals, the most important predictor was milk production (negative predictor; protective factor). THI was the second most important variable and was a positive predictor (risk factor). This confirms the results reported in the review by Pragna et al. (2017): heat stress can cause immunosuppression in dairy cattle, and the higher udder temperature can lead to mastitis.

Regarding lameness prediction, the scenario was more various than in mastitis prediction. In pluriparous animals the most important positive predictor (risk factor) was always parity, probably due to the older age of those animals compared to the primiparous ones. THI as well was an important positive predictor (risk factor). From this analysis, age resulted being a negative predictor (protective factor). This result is counterintuitive, and could be due to the correlation between age and parity, where the latter absorbed the majority of the importance. In the primiparous cows, the most important predictor was different in each of the 3 windows datasets. In 1-day window, the most important was DIM (negative predictor; protective factor). During an early phase of lactation, primiparous animals are physiologically more stressed than pluriparous, and this could cause the higher number of lameness cases in association with low DIM. In both 3 and 5-days windows, the main predictors were THI (positive predictor; risk factor), milk production (positive predictor; risk factor), and DIM (negative predictor; protective factor), but their relative importance was different among the 2 datasets. THI was the most important predictor in the 3-days window, while milk production was the most important one in the 5-days window.

Lastly, regarding dysentery prediction, the most important predictors were age and parity (primiparous and pluriparous animals, respectively), and they both were negative predictors (protective factors). This result is straightforward, being dysentery mainly a juvenile disorder (except for viral forms, e.g., Winter Disentery caused by bovine coronavirus - Toftaker et al., 2017). THI was an important positive predictor (risk factor) in primiparous cows only. In our interpretation, heat stress, especially regarding gastroenteric issues, affects primiparous animals more strongly than pluriparous ones.

Predictive modelling

We then used RF, NN, and LDA machine-learning techniques on the 3 datasets (1, 3, and 5-days windows) in both primiparous and pluriparous per each disease. Even after the pruning described in the Materials and Methods paragraph, the mastitis data was unbalanced, with a number of non-diseased records 100-fold higher than the diseased ones. Therefore, we decided to reduce the number of non-diseased records by keeping only the 10-days range of non-diseased around diseased records. In the reduced dataset, non-diseased records were 6-fold more numerous than diseased ones. In Tables 2 and 3 (primiparous and pluriparous, respectively), we summarized the models' performance indicators, namely sensitivity (calculated as True Positives / (True Positives + False Negatives)), specificity (True Negatives / (True Negatives + False Positives)), positive predictive value (True Positives / (True Positives + False Positives)), and negative predictive value (True Negatives / (True Negatives + False Negatives)).

In primiparous animals, the machine-learning techniques we used performed poorly for mastitis prediction, with RF applied to the 5-days window as the best model (although with a sensitivity of 9.1%). While the model almost always correctly assigned non-diseased cases, this technique could not assign the diseased to their true class. NN and LDA could not identify any of the diseased cases, and therefore scored a sensitivity of 0% for all the datasets. This negative result could be due to different reasons: for example, the dataset, even if previously reduced and pruned of the excessive number of non-diseased cases, was still unbalanced. Furthermore, it is possible that the selected predictors were not enough or could not fully describe the mastitis event. Regarding lameness, NN and LDA had the same negative results as in the previous disease. All of the records in validation sets were assigned to the non-diseased class, even if they were diseased. RF, on the other hand, performed better than the same technique applied to mastitis. For all the 3 window datasets, the sensibility of the models was 12.5%. The Positive predicted value (i.e., the number of true positives divided by the number of positive calls) was 100%, meaning that all of the records assigned as diseased were truly diseased. The prediction efficiency of dysentery was positive. All the techniques correctly assigned each new diseased record in the validation set to the diseased class, while misassigning only a few non-diseased records. RF scored a 100% precision for both sensitivity and specificity, and was, therefore, the best technique to predict dysentery. This high precision in primiparous cows could be because dysentery is typically a juvenile disease, and therefore the predictors were enough to describe it in these datasets. We must say that it could be also possible that the better results in dysentery compared to mastitis and lameness could be due to overfitting, even though this possibility should be low because of the repeated cross-validation used on training datasets and the subsequent use of an external validation dataset.

In the pluriparous dataset, none of the techniques could predict mastitis correctly. The only dataset with a sensitivity different from 0 was the 5-days window in RF (sensitivity 2.4%). Surely, since we collected sanitary data recorded by the farmer, we were aware of their possible low accuracy. For example, the etiology of mastitis affects its harshness, and mastitis caused by E. coli, characterized by severe inflammatory responses, are more easily identified through changes in physiological activities, like RT (Stangaferro et al., 2016). Regarding lameness, similarly to primiparous cows, only the RF technique could predict the disease. However, in this second case, the RF models performed better, with a maximum sensibility of 72.9% in the 5-days window. This better result in older cows compared to the primiparous datasets could be because lameness is more common in old animals, and therefore more easily detected and predicted by the model. Dysentery prediction performed similarly to the primiparous scenario regarding RF, while NN and LDA had lower sensibility. We could apply the same reasoning used previously with the primiparous animals: being dysentery a typically juvenile disease, prediction performed better in young animals (i.e., primiparous) than in old ones (i.e., pluriparous).

CONCLUSIONS

In this work, we showed that it is possible to predict diseases using data automatically gathered by recording systems already largely available on dairy farms. The critical point of this study was the usage of non-experimental sanitary information, recorded by the farmer, which were not always accurate. Using machine-learning techniques, disease prediction is a possible goal to achieve, although this is not an easy task. Future works, in which experimental data regarding sanitary information (e.g., the etiology and the severity of diseases) will be used, would further improve the results obtained here.

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Table 1. Summary statistics of the variables used to predict the diseases in primiparous and pluriparous Holstein dairy cows. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns

	Primiparous	Pluriparous	Statistical significance of difference (t-test)
Variable[1]	Mean ± SD	Mean ± SD	
Age (months)	34.3 ± 12.6	57.6 ± 16.4	***
Parity	1	2.9 ± 1	***
DIM	185.8 ± 121.9	174.8 ± 115.8	***
RT (min/day)	564.7 ± 88	565.9 ± 87.1	**
AT (units/day)	612.7 ± 137.1	608.9 ± 134.1	***
Milk production (kg/day)	29.9 ± 8.5	30 ± 8.6	ns

[1] Variables abbreviations: RT = daily Rumination Time; AT = daily Activity Time.

Table 2. Summary of different machine-learning techniques (namely Random Forest, Neural Networks, and Linear Discriminant Analysis) predictive ability for mastitis, lameness, and dysentery in primiparous Holstein dairy cows.

Disease	Technique[1]	Subset	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	p-value[2]
Mastitis	RF	1-day	25.8	95.4	57.1	84.4	ns
		3-days	22.6	99.2	87.5	84.3	ns
		5-days	29.0	99.2	90.0	85.4	ns
	NN	1-day	0.0	100.0	-	80.8	ns
		3-days	0.0	100.0	-	80.8	ns
		5-days	3.2	100.0	100.0	81.3	ns
	LDA	1-day	6.5	96.9	33.3	81.3	ns
		3-days	9.7	96.9	42.9	81.8	ns
		5-days	12.9	96.9	50.0	82.4	ns
Lameness	RF	1-day	26.7	100.0	100.0	93.5	ns
		3-days	26.7	99.4	80.0	93.4	ns
		5-days	26.7	100.0	100.0	93.5	ns
	NN	1-day	0.0	100.0	-	91.3	ns
		3-days	0.0	100.0	-	91.3	ns
		5-days	0.0	100.0	-	91.3	ns
	LDA	1-day	0.0	100.0	-	91.3	ns
		3-days	0.0	100.0	-	91.3	ns
		5-days	0.0	100.0	-	91.3	ns

[1] Techniques abbreviations: RF = Random Forest; NN = Neural Networks; LDA = Linear Discriminant Analysis.

[2] p-value referred to the significance of the difference between the model accuracy and the no-information-rate. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns

Table 2 (continued). Summary of different machine-learning techniques (namely Random Forest, Neural Networks, and Linear Discriminant Analysis) predictive ability for mastitis, lameness, and dysentery in primiparous Holstein dairy cows.

Disease	Technique[1]	Subset	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	p-value [2]
Dysentery	RF	1-day	100.0	100.0	100.0	100.0	***
		3-days	100.0	100.0	100.0	100.0	***
		5-days	100.0	100.0	100.0	100.0	***
	NN	1-day	97.2	100.0	100.0	99.1	***
		3-days	100.0	100.0	100.0	100.0	***
		5-days	100.0	100.0	100.0	100.0	***
	LDA	1-day	100.0	100.0	100.0	100.0	***
		3-days	100.0	100.0	100.0	100.0	***
		5-days	100.0	100.0	100.0	100.0	***

[1] Techniques abbreviations: RF = Random Forest; NN = Neural Networks; LDA = Linear Discriminant Analysis.

[2] p-value referred to the significance of the difference between the model accuracy and the no-information-rate. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns

Table 3. Summary of different machine-learning techniques (namely Random Forest, Neural Networks, and Linear Discriminant Analysis) predictive ability for mastitis, lameness, and dysentery in pluriparous Holstein dairy cows.

Disease	Technique[1]	Subset	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	p-value[2]
Mastitis	RF	1-day	11.9	95.1	41.7	78.7	ns
		3-days	2.4	97.2	20.0	77.3	ns
		5-days	9.5	95.1	36.4	78.3	ns
	NN	1-day	0.0	100.0	-	77.4	ns
		3-days	0.0	100.0	-	77.4	ns
		5-days	0.0	100.0	-	77.4	ns
	LDA	1-day	0.0	100.0	-	77.4	ns
		3-days	0.0	100.0	-	77.4	ns
		5-days	0.0	100.0	-	77.4	ns
Lameness	RF	1-day	69.1	99.9	96.7	98.6	***
		3-days	69.1	99.9	96.7	98.6	***
		5-days	71.4	99.9	96.8	98.8	***
	NN	1-day	0.0	100.0	-	95.8	ns
		3-days	0.0	100.0	-	95.8	ns
		5-days	0.0	100.0	-	95.8	ns
	LDA	1-day	0.0	100.0	-	95.8	ns
		3-days	0.0	100.0	-	95.8	ns
		5-days	0.0	100.0	-	95.8	ns

[1] Techniques abbreviations: RF = Random Forest; NN = Neural Networks; LDA = Linear Discriminant Analysis.

[2] p-value referred to the significance of the difference between the model accuracy and the no-information-rate. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns .

Table 3 (continued). Summary of different machine-learning techniques (namely Random Forest, Neural Networks, and Linear Discriminant Analysis) predictive ability for mastitis, lameness, and dysentery in pluriparous Holstein dairy cows.

Disease	Technique[1]	Subset	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	p-value [2]
Dysentery	RF	1-day	100.0	99.2	97.0	100.0	***
		3-days	100.0	99.2	97.0	100.0	***
		5-days	100.0	99.2	97.0	100.0	***
	NN	1-day	76.9	91.5	70.4	93.8	***
		3-days	69.2	93.1	72.6	92.0	***
		5-days	81.5	91.1	70.7	94.9	***
	LDA	1-day	44.6	93.9	65.9	86.6	*
		3-days	46.2	93.9	66.7	86.9	*
		5-days	47.7	93.5	66.0	87.2	*

[1] Techniques abbreviations: RF = Random Forest; NN = Neural Networks; LDA = Linear Discriminant Analysis.

[2] p-value referred to the significance of the difference between the model accuracy and the no-information-rate. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns .

ABSTRACT I

ASAS-ADSA®-CSAS-WSASAS
2016 JAM
JOINT ANNUAL MEETING
JULY 19 - 23, 2016 • SALT LAKE CITY, UTAH

2016 ADSA Joint Annual Meeting (JAM)

19 – 23 July 2016, Salt Lake City (UT, USA)

Published in: *Journal of Animal Science 2016, 99 (s5): 187-188*

Oral communication

Daily rumination time in Italian Holstein cows: Heritability and correlation with milk production.

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* Presenting Author. "Breeding and genetics: Selection for health and fertility" Session, July 21, 2016.

Abstract text

The aim of the study was to investigate the genetic variation of daily rumination time (min) and its correlation with test-day milk production (kg). Data for the analysis consisted of 91,589 records for rumination time and milk yield from 398 cows (age: 43.21 ± 16.11 months), collected from September 2014 to October 2015 in two Italian Holstein herds (TAD and MIL). There were 493 calvings and data distribution across parities was 46.4%, 26.7% and 26.7% for first, second and later parities, respectively. DIM classes were defined as one class for every 30d resulting in 11 classes and there were a total of 378 herd-test day contemporary groups. The average rumination time was 513.51 ± 115.84 min and the average milk yield was 33.59 ± 9.18 kg.

Pedigree information was available for 11,634 animals. A Repeatability Animal Model was fitted using the AIREMLF90 software. Herd, year/month of calving, and DIM classes within parity were treated as fixed effects, while herd-test-day, permanent environmental, and the additive genetic cow effects were treated as random. Rumination time was longer in pluriparous than in primiparous cows and showed a decreasing trend across DIM. On average, at the beginning of the lactation, pluriparous cows ruminated 75 min longer than primiparous. As expected, pluriparous cows had a higher production levels across DIM than primiparous, with a peak around DIM class 2 and 3 (i.e., 60-90 d). The herd with the highest daily rumination time had the lowest milk production yield: the fixed effects solutions were 569.5 min and 25.8 kg (Herd TAD; rumination time and milk yield, respectively) and 446.4 min and 31.9 kg (Herd MIL; rumination time and milk yield, respectively). The heritabilities for test-day milk yield and daily rumination time were 0.13 (SE = 0.06) and 0.32 (SE = 0.09), respectively. Although the negative phenotypic correlation observed, genetic correlation between the two traits was 0.38 (SE = 0.47); this high standard error is possibly the consequence of the dataset dimension. So far, rumination time has been used as a key monitor of dairy cow health at farm level. Investigating its genetics aspect and the relationship with other important yields and health traits may turn this management tool in a new informative selection criterion for the dairy cattle breeding strategies.

Keywords: Rumination time, Milk production, Genetic variation

ABSTRACT II

#ASPA2017
22nd Congress of the Animal Science and Production Association
June 13-16, 2017 - Perugia

XXII Congress of the Animal Science and Production Association

13 – 16 June 2017, Perugia (Italy)

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Oral communication

Predictive models for locomotion issues in Italian Holstein dairy cows.

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* Presenting Author. "Husbandry, welfare and behaviour" Session, June 14, 2017.

Abstract text

Locomotion system issues are major issues in dairy herds, affecting both animal welfare and farm productivity. Early detection could improve the effectiveness of treatments and increase the chances to cure lame cows. Currently, locomotion issues detection requires direct observation of cows walking (locomotion score). However, this is a time-consuming task and is not always an available option in large dairy farms. Aim of this preliminary study was to build a predictive model for locomotion system issues in Italian Holstein dairy cows using some novel phenotypes from automatic recording systems (milking parallel parlour and SCR Heatime and DataFlow2 system) as predictors. Data was recorded from a commercial farm located in the province of Mantua (Lombardy, Northern Italy) for a total of 413 animals, daily monitored for two years (Sep. 2014 – Dec 2016). The response variable was binary (0/1: healthy and diseased, respectively). The selected variables were daily rumination time, parity, DIM, daily milk production, daily

activity and month of recording. Summary statistics (mean and SD) were calculated: rumination time, 563.28 ± 88.48 min/day; parity, 1.93 ± 1.28; DIM, 171.34 ± 124.14 days; milk production, 24.14 ± 13.74 kg/day; activity, 614.00 ± 134.64 min/day. DIM were classified in four classes to assess the potential effect of the lactation stage: dry, early, mid, and late lactation (no lactation, <120 days, 120-240 days, and >240 days, respectively). Three different datasets were prepared, where rumination, milk, and activity were averaged as means of 1, 3, and 5 days before the response variable record. On each dataset, two models were fitted: logistic regression and random forest. All the analyses were performed in R using the caret package. Data were divided into a training and a testing dataset (proportion 80/20). Training data was used to train two different algorithms which were used to predict the class variable. The two selected algorithms were: 1. a logistic regression 2. a random forest.

For all the datasets, logistic regression was not able to predict diseased individuals, assigning all to the 'healthy' class. Random forest performed better, although with a high-class error. The 5-days window had the lowest OOB error rate (0.24%) and the lowest class error (0.71). Further tuning of the selected models will be necessary to build a valuable tool to predict locomotion system issues.

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The authors thank SCR Engineers Ltd. (Hadarim, Netanya, Israel) and Milkline Srl (Gariga di Podenzano, Piacenza, Italy) for supplying the technologies used in this study, and "Bulgarelli Giacomo e Astore" dairy farm for the herd rumination raw data and health status recordings.

PART III

GENERAL DISCUSSION AND CONCLUSIONS

Different machine learning techniques were applied to the data gathered during this PhD project, and the relationships between rumination time and other variables were evaluated. In the first paper presented in this thesis, the effects of heat stress on rumination were assessed. A statistically significant unfavourable association was found: an increase in THI led to a reduction in rumination time. This association is likely to be due to the activation on responses to cope with the environment, which might involve a reduction of feed intake, leading to rumination decrease. Mixed models, including THI amongst the predictors for rumination changes, confirmed the abovementioned association. THI could be therefore suitable as predictor in a predictive model involving rumination changes prediction. This result highlights the need to consider heat stress when dealing with dairy cow's welfare. This is especially true in high-yielding cows, which are more sensitive to heat stress, since their termic balance is already stressed by the high metabolic requirements to produce large amounts of milk.

In the second paper, a step forward was made, and diseases were added in a mixed model to study if there was a change in rumination time before their onset. From the obtained results, it is plausible to assume that rumination time could be used as a predictor for diseases' onset. This could have important implications in farmed animal management, since the detection of a disease as early as possible allows for a more immediate sanitary intervention, usually leading to a better and faster recovering from the disease. Different diseases have different prediction efficacy, due to their different features. For example, diseases like mastitis and gastroenteric diseases predictive models performed better when using short-term data (i.e., one day before the veterinary diagnosis), while others (e.g., lameness) could be predicted with larger advance.

In Draft I and Abstract I, the heritability of rumination and its genetic correlations with productive parameters was studied. Rumination in the analysed population was moderate (0.34), but the genetic correlations were unfavourable. Specifically, rumination was negatively correlated with milk production traits and positively correlated with somatic cells count. Although weakly correlated, selection for a higher rumination could lead to negative results on milk productivity. Therefore, attention is needed regarding the introduction of rumination in a breeding selection plan.

In Draft II, machine learning techniques were used to build models to predict the onset of three different diseases (namely mastitis, lameness, and dysentery). A preliminary analysis was conducted on lameness alone, and it was presented as oral communication during the XXII Congress of the Animal Science and Production Association (Abstract II). In these works, the prediction efficacy of different machine learning techniques was tested. As expected, different techniques had different accuracy, due to the different types of algorithms and statistical approaches. Further tuning of the different algorithms will be performed before proceeding to the submission of the paper to an international journal for peer reviewing. One of the critical points of all the works in this project including diseases' prediction was the usage of non-experimental sanitary information, recorded by the farmer, which were not always accurate.

In conclusion, rumination is a valuable trait in cows, covering a key role in animal welfare. Furthermore, rumination is deeply interconnected to various other features, both animal-related (e.g., diseases and milk production) and environment-related (e.g., THI). In addition, automatic recording systems proved themselves a valuable source of low-cost data, which is usually available in commercial farms but is often underestimated and scarsely used. The large amount of available recorded data reduces the bias due to the usage of non-experimental data, and this proves valuable when dealing with commercial systems.

PART IV

1. LIST OF PUBLICATIONS

1.1 REFEREED SCIENTIFIC PAPERS

Moretti, R., S. Biffani, F. Tiezzi, C. Maltecca, S. Chessa, and R. Bozzi. Rumination time as a potential predictor of common diseases in high-productive Holstein dairy cows. J. Dairy Res. 84: 385-390.

Moretti, R., S. Biffani, S. Chessa, and R. Bozzi. 2017. Heat stress effects on Holstein dairy cows' rumination. Animal. 11 (12): 2320-2325.

Talenti, A., E.L. Nicolazzi, S. Chessa, S. Frattini, **R. Moretti**, B. Coizet, L. Nicoloso, L. Colli, G. Pagnacco, A. Stella, P. Ajmone-Marsan, G. Ptak, and P. Crepaldi. 2016. A method for single nucleotide polymorphism selection for parentage assessment in goats. J. Dairy Sci. 99:3646–3653.

1.2 CONFERENCE PAPERS

Moretti, R., S. Biffani, S. Chessa, and R. Bozzi. 2017. Predictive models for locomotion issues in Italian Holstein dairy cows. 22nd Congress of the Animal Science and Production Association (ASPA), June 13 – 16, 2017, Perugia (PG, Italy). Pages 50-51 in Ital. J. Anim. Sci. 16 (s1).

Moretti, R., R. Bozzi, C. Maltecca, F. Tiezzi, S. Chessa, D. Bar, and S. Biffani. 2016. Daily rumination time in Italian Holstein cows: Heritability and correlation with milk production. 2016 ADSA Joint Annual Meeting (JAM), July 19 – 23, 2016, Salt Lake City (UT, USA). Pages 187-188 in J. Anim. Sci. 99 (s5).

Chessa, S., O. Bulgari, F. Turri, P. Cremonesi, **R. Moretti**, S. Costa, S. Colombini, A. Caroli, L. Rapetti, and B. Castiglioni. 2015. Goat milk technological properties: milk coagulation properties and cheese yield. 21st Congress of the Animal Science and Production Association (ASPA), June 9 – 12, 2015, Milan (MI, Italy). Page 131 in Ital. J. Anim. Sci. 14 (s1).

Moretti, R., A. Criscione, S. Bordonaro, D. Marletta, B. Castiglioni, and S. Chessa. 2014. Genome-wide SNP assay in the Nero Siciliano pig to discover potential wild boar introgression. 21st International Colloquium on Animal Cytogenetics and Gene Mapping (ICACGM), June 7 – 10, 2014, Ischia (NA, Italy). Page 415 in Chromosome Res. 22 (3).

Chessa, S., A. Criscione, **R. Moretti**, S. Bordonaro, D. Marletta, and B. Castiglioni. 2013. Estimation of linkage disequilibrium in the Nero Siciliano italian autochthonous breed using the Illumina 60K SNP array. 8th International Symposium on the Mediterranean Pig, October 10 – 12, 2013, Ljubljana (Slovenija). Pages 37-40 in Acta Agric. Slov. 4 (s4).

Chessa, S., S. Bordonaro, **R. Moretti**, A. Criscione, D. Marletta, and B. Castiglioni. 2013. Genomic analysis for the valorization of Nero Siciliano swine breed. 20th Congress of the Animal Science and Production Association (ASPA), June 11 – 13, 2013, Bologna (BO, Italy). Page 14 in Ital. J. Anim. Sci. 12 (s1).

1.3 CONFERENCE POSTERS

Chessa, S., P. Cremonesi, **R. Moretti**, F. Turri, and B. Castiglioni. 2014. Using SNP to enhance fatty acids composition in goat milk. 10th World Congress of Genetics Applied to Livestock Production (WCGALP), August 17 – 22, 2014, Vancouver (BC, Canada).

Chessa, S., S. Bordonaro, **R. Moretti**, A. Criscione, D. Marletta, and B. Castiglioni. 2012. Il Suino Nero Siciliano allevato nel Parco dei Nebrodi: caratterizzazione genomica finalizzata alla valorizzazione dei suo prodotti tipici e alla conservazione della biodiversità. 10th Congress of the Italian Association of the Agricultural Science Societies (AISSA), November 28 – 29, 2012, Palermo (PA, Italy)

2. CURRICULUM VITAE

Riccardo Moretti was born on December 4th 1988 in Crema, Italy. After completing secondary education, he obtained his Bachelor (2010) and Master (2012) Degree in Veterinary Biotechnology at the University of Milan, Faculty of Veterinary Medicine, focusing on animal genetic improvement. After his Degree, he was employed by the Institute of Agricultural Biology and Biotechnology of the National Research Council of Italy (IBBA-CNR) to work as a research assistant (Feb. 2013 – Sep. 2013) on high-throughput genetic characterization of Italian autochthonous pig breeds for traceability purposes. His employment continued at IBBA-CNR as a research fellow (Dec. 2013 – Oct. 2014), working on genomic and bioinformatics analyses on swine and goat breeds.

In Nov. 2014 he started his PhD project in Agricultural and Environmental Sciences at University of Florence. The PhD project focused on animal welfare (specifically dairy cows welfare) and on bioinformatics topics. He worked with the statistical programming language R to develop predictive models to assess the welfare status of Holstein dairy cows. During his PhD, he spent a period of 7 months as a visiting research scholar at North Carolina State University (Raleigh, NC, USA), under the supervision of Prof. Christian Maltecca. During his visit, while working on his PhD project, he attended Courses in Animal Genetic Improvement and Quantitative Genetics and Breeding. Furthermore, he participated as a speaker in a series of Animal Breeding and Genetics Seminars (NC State University) and as a presenting author at the international meeting of the American Dairy Science Association in Salt Lake City (UT, USA).

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In Italy

Thank you to dr. Stefania Chessa and dr. Stefano Biffani, co-supervisors of the project. They were always there when I needed help.

In Raleigh

Thank you to Professor Christian Maltecca, co-supervisor of the project and supervisor of my stage at North Carolina State University (USA). Thank you to all the people (Italian, American, and international) I met during my American adventure.

At home

Thank you to my family, Love, and friends, who have encouraged me throughout these years.

"Surround yourself with people who make you happy.
People who make you laugh, who help you when you're in need.
People who genuinely care. They are the ones worth keeping in your life.

Everyone else is just passing through"

ANNEX I

Supplementary Material

Heat stress effects on Holstein dairy cows' rumination

Table S1. Descriptive statistics for Ambient Temperature (AT), Relative Humidity (RH), recorded inside the barn and the calculated Temperature-Humidity Index (THI) by month. All the means are in a "mean ± standard deviation (SD)" format and refer to the total of the monthly records

SUMMER 2015		June	July	August
AT (°C)	Mean ± SD	23.28^{a} ± 3.84	28.04^{b} ± 3.68	24.61^{c} ± 4.28
	Min	14.94	19.81	16.67
	Max	31.99	35.34	35.03
RH (%)	Mean ± SD	64.41^{a} ± 14.57	57.64^{b} ± 11.47	64.49^{a} ± 15.76
	Min	35.14	34.15	32.99
	Max	94.20	83.42	95.45
THI	Mean ± SD	70.32^{a} ± 4.43	76.43^{b} ± 3.96	72.15^{c} ± 4.66
	Min	58.77	66.17	61.48
	Max	80.00	84.81	82.17

[a,b] Values within a row with different superscripts differ significantly at $P<0.05$.

Table S2. Descriptive statistics for the observed 2-h and daily Rumination Time (RT) by month in the Italian Holstein herd. All the means are in a “mean ± standard deviation (SD)” format and refer to the total of the monthly records

SUMMER 2015		June	July	August
2-h RT (min/2h)	Mean ± SD	$48.79^{a} \pm 22.30$	$46.22^{b} \pm 21.78$	$48.93^{a} \pm 22.82$
	Min	0	0	0
	Max	120	120	120
Daily RT (min/day)	Mean ± SD	$585.41^{a} \pm 92.01$	$554.65^{b} \pm 103.52$	$586.78^{a} \pm 91.79$
	Min	37	61	97
	Max	853	860	1028

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

ANNEX II

Supplementary Material

Rumination time as a potential predictor of common diseases in high-productive Holstein dairy cows

Table S1. Differences between the mean Rumination Time (RT, expressed in min/2h) in absence or presence of the disease and their t-test p-value.

Disease	Interval	Num cows	case-control ratio	Mean RT (absence)	Mean RT (presence)	Mean difference	p-value
Generic diseases	2-h	126	1:2.35	47.00	45.73	1.27	< 0.001
Reproductive diseases	2-h	66	1:0.85	46.92	45.84	1.08	< 0.001
Mastitis	2-h	68	1:39.51	47.43	42.59	4.84	< 0.001
Locomotor issues	2-h	16	1:34.51	45.59	43.19	2.40	0.001
Gastroenteric diseases[1]	2-h	3	1:14.49	47.67	37.76	9.91	< 0.001

[1] Only three animals suffered from gastroenteric diseases, therefore, even if highly significant, caution should be used with this result.

Table S2. Mean ± SD of rumination mean, rumination SD, and slope (from the regression line) before and after different sanitary events (a – e). The differences between before and after the events were tested using t-test (row by row and couple-wise): the superscripts (i.e., a and b) identify the couples which elements were significantly different ($P < 0.05$).

a) Generic disease

Window size	Mean		SD		Slope	
	Before	After	Before	After	Before	After
1	42.01 ± 11.47	41.28 ± 10.21	9.61 ± 5.36	9.56 ± 5.75	-4.84^{a} ± 7.00	-2.39^{b} ± 7.01
3	43.83 ± 7.96	43.51 ± 7.82	10.54 ± 3.87	10.36 ± 3.43	-1.04^{a} ± 1.72	0.29^{b} ± 1.54
5	44.62 ± 6.90	44.61 ± 6.89	10.35 ± 3.34	10.17 ± 2.80	-0.54^{a} ± 0.78	0.28^{b} ± 0.81

b) Reproductive diseases

Window size	Mean		SD		Slope	
	Before	After	Before	After	Before	After
1	42.53 ± 11.13	43.29 ± 8.82	9.11 ± 4.73	7.91 ± 4.12	-3.56 ± 6.75	-3.60 ± 5.63
3	44.71 ± 7.21	43.39 ± 6.58	10.31 ± 4.45	9.16 ± 3.22	-1.23^{a} ± 1.82	-0.38^{b} ± 1.46
5	45.29 ± 6.05	43.86 ± 6.63	10.01 ± 3.61	9.26 ± 2.46	-0.52^{a} ± 0.81	-0.04^{b} ± 0.62

c) Mastitis

Window size	Mean		SD		Slope	
	Before	After	Before	After	Before	After
1	43.95^{a} ± 9.36	41.30^{b} ± 9.76	10.05 ± 5.98	11.19 ± 6.65	-5.64^{a} ± 6.50	-2.54^{b} ± 8.29
3	44.65 ± 6.72	43.73 ± 7.67	10.57 ± 3.68	11.11 ± 3.63	-0.77^{a} ± 1.53	0.33^{b} ± 1.40
5	45.33 ± 6.17	45.08 ± 6.35	10.51 ± 3.17	10.82 ± 2.89	-0.41^{a} ± 0.67	0.34^{b} ± 0.85

Table S2. (continued) Mean ± SD of rumination mean, rumination SD, and slope (from the regression line) before and after different sanitary events (a – e). The differences between before and after the events were tested using t-test (row by row and couple-wise): the superscripts (i.e., a and b) identify the couples which elements were significantly different ($P < 0.05$).

d) Locomotor system issues

Window size	Mean		SD		Slope	
	Before	After	Before	After	Before	After
1	35.15 ± 12.42	38.35 ± 10.47	7.06 ± 3.75	8.39 ± 3.97	-3.41 ± 4.61	-0.17 ± 5.76
3	36.94 ± 8.61	42.15 ± 7.38	9.78 ± 3.37	9.61 ± 2.79	-0.79^{a} ± 1.49	0.95^{b} ± 1.62
5	37.76^{a} ± 6.95	42.51^{b} ± 5.86	10.17 ± 2.67	9.59 ± 2.55	-0.64^{a} ± 0.70	0.46^{b} ± 0.72

e) Gastroenteric diseases

Window size	Mean		SD		Slope	
	Before	After	Before	After	Before	After
1	19.81 ± 8.76	28.00 ± 12.72	14.15 ± 7.91	10.83 ± 8.05	-7.13 ± 16.26	2.83 ± 4.58
3	35.78 ± 6.23	36.47 ± 16.96	16.32 ± 3.66	11.28 ± 5.84	-4.55^{a} ± 0.83	1.55^{b} ± 1.75
5	39.51 ± 5.50	38.21 ± 16.31	15.56 ± 4.57	11.14 ± 4.28	-2.22^{a} ± 0.43	0.75^{b} ± 0.13

Table S3. Summary of the logistic regression models' output: from a) to e) the 5 cases (i.e., generic diseases, reproductive diseases, mastitis, locomotor system issues, and gastroenteric diseases) with their estimates of the β (value, SE in brackets, and the p-value), odds ratio, AIC (Akaike information criterion) of the model, and McFadden's Pseudo-R2.

a) Generic disease

Window size	Disease = RT mean				Disease = RT sd				Disease = RT slope				Disease = RT mean + RT sd + RT slope							
	Estimate	Odds ratio	AIC	Pseudo R²	Estimate	Odds ratio	AIC	Pseudo R²	Estimate	Odds ratio	AIC	Pseudo R²	mean Estimate	Odds ratio	sd Estimate	Odds ratio	slope Estimate	Odds ratio	AIC	Pseudo R²
1	-0.079 (0.009) p < 0.001	0.92	2096.9	2.99%	0.009 (0.017) ns	1.01	2161.1	0.01%	-0.087 (0.011) p < 0.001	0.92	2096.6	3.01%	-0.076 (0.009) p < 0.001	0.93	-0.038 (0.019) ns	0.96	-0.099 (0.014) p < 0.001	0.91	2034.4	6.07%
3	-0.073 (0.012) p < 0.001	0.93	2096.4	1.69%	0.115 (0.025) p < 0.001	1.12	2112.2	0.95%	-0.654 (0.052) p < 0.001	0.52	2016.7	5.43%	-0.054 (0.012) p < 0.001	0.95	0.021 (0.028) ns	1.02	-0.555 (0.066) p < 0.001	0.57	1999.2	6.45%
5	-0.061 (0.013) p < 0.001	0.94	2069.6	1.04%	0.119 (0.029) p < 0.001	1.13	2075.5	0.76%	-1.379 (0.104) p < 0.001	0.25	1965.6	6.02%	-0.039 (0.013) p = 0.002	0.96	-0.000 (0.033) ns	1.00	-1.280 (0.138) p < 0.001	0.28	1960.1	6.47%

b) Reproductive diseases

Window size	Disease = RT mean				Disease = RT sd				Disease = RT slope				Disease = RT mean + RT sd + RT slope							
	Estimate	Odds ratio	AIC	Pseudo R²	Estimate	Odds ratio	AIC	Pseudo R²	Estimate	Odds ratio	AIC	Pseudo R²	mean Estimate	Odds ratio	sd Estimate	Odds ratio	slope Estimate	Odds ratio	AIC	Pseudo R²
1	-0.065 (0.016) p < 0.001	0.94	708.6	2.11%	-0.024 (0.030) ns	0.98	723.2	0.09%	-0.062 (0.019) p < 0.001	0.94	712.6	1.56%	-0.064 (0.016) p < 0.001	0.94	-0.046 (0.033) ns	0.96	-0.076 (0.023) p < 0.001	0.93	699.3	3.96%
3	-0.047 (0.021) p = 0.022	0.95	693.0	0.73%	0.072 (0.046) ns	1.08	695.7	0.34%	-0.662 (0.082) p < 0.001	0.52	652.3	6.59%	-0.028 (0.020) ns	0.97	-0.071 (0.053) ns	0.93	-0.752 (0.128) p < 0.001	0.47	652.4	7.16%
5	-0.039 (0.022) ns	0.96	693.5	0.44%	0.045 (0.055) ns	1.05	695.9	0.10%	-1.314 (0.190) p < 0.001	0.27	660.1	5.27%	-0.023 (0.022) ns	0.98	-0.071 (0.060) ns	0.93	-1.453 (0.256) p < 0.001	0.23	661.2	5.68%

Table S3. (continued) Summary of the logistic regression models' output: from a) to e) the 5 cases (i.e., generic diseases, reproductive diseases, mastitis, locomotor system issues, and gastroenteric diseases) with their estimates of the β (value, SE in brackets, and the p-value), odds ratio, AIC (Akaike information criterion) of the model, and McFadden's Pseudo-R2.

c) Mastitis

Window size	Disease = RT mean				Disease = RT sd				Disease = RT slope				Disease = RT mean + RT sd + RT slope							
	Estimate	Odds ratio	AIC	Pseudo R^2	Estimate	Odds ratio	AIC	Pseudo R^2	Estimate	Odds ratio	AIC	Pseudo R^2	mean Estimate	Odds ratio	sd Estimate	Odds ratio	slope Estimate	Odds ratio	AIC	Pseudo R^2
1	-0.059 (0.011) $p < 0.001$	0.94	1565.6	1.50%	0.029 (0.019) ns	1.03	1586.9	0.15%	-0.101 (0.013) $p < 0.001$	0.90	1524.4	4.10%	-0.056 (0.011) $p < 0.001$	0.95	-0.033 (0.023) ns	0.97	-0.114 (0.017) $p < 0.001$	0.89	1504.4	5.61%
3	-0.064 (0.014) $p < 0.001$	0.94	1565.2	1.27%	0.120 (0.028) $p < 0.001$	1.13	1568.3	1.08%	-0.549 (0.069) $p < 0.001$	0.58	1533.8	3.26%	-0.050 (0.013) $p < 0.001$	0.95	0.067 (0.030) $p = 0.026$	1.07	-0.411 (0.072) $p < 0.001$	0.66	1518.8	4.46%
5	-0.053 (0.015) $p = 0.001$	0.95	1545.4	0.77%	0.141 (0.032) $p < 0.001$	1.15	1539.6	1.14%	-1.171 (0.129) $p < 0.001$	0.31	1497.2	3.87%	-0.037 (0.015) $p = 0.011$	0.96	0.068 (0.035) $p = 0.049$	1.07	-0.921 (0.147) $p < 0.001$	0.40	1490.6	4.56%

d) Locomotor system issues

Window size	Disease = RT mean				Disease = RT sd				Disease = RT slope				Disease = RT mean + RT sd + RT slope							
	Estimate	Odds ratio	AIC	Pseudo R^2	Estimate	Odds ratio	AIC	Pseudo R^2	Estimate	Odds ratio	AIC	Pseudo R^2	mean Estimate	Odds ratio	sd Estimate	Odds ratio	slope Estimate	Odds ratio	AIC	Pseudo R^2
1	-0.133 (0.021) $p < 0.001$	0.88	240.5	11.07%	-0.108 (0.060) ns	0.90	266.3	1.37%	-0.067 (0.033) $p = 0.043$	0.94	265.8	1.53%	-0.130 (0.021) $p < 0.001$	0.88	-0.165 (0.071) $p = 0.020$	0.85	-0.119 (0.054) $p = 0.028$	0.89	234.3	14.88%
3	-0.182 (0.031) $p < 0.001$	0.83	238.0	11.80%	0.076 (0.076) ns	1.08	268.4	0.34%	-0.532 (0.162) $p = 0.001$	0.59	261.1	3.08%	-0.177 (0.032) $p < 0.001$	0.84	-0.051 (0.081) ns	0.95	-0.374 (0.182) $p = 0.040$	0.69	237.2	13.62%
5	-0.196 (0.036) $p < 0.001$	0.82	227.6	11.22%	0.144 (0.084) ns	1.16	253.3	1.02%	-1.443 (0.265) $p < 0.001$	0.24	237.2	7.42%	-0.179 (0.038) $p < 0.001$	0.84	-0.075 (0.106) ns	0.93	-1.267 (0.411) $p = 0.002$	0.28	219.2	16.17%

e) Gastroenteric diseases

Window size	Disease = RT mean				Disease = RT sd				Disease = RT slope				Disease = RT mean + RT sd + RT slope							
	Estimate	Odds ratio	AIC	Pseudo R^2	Estimate	Odds ratio	AIC	Pseudo R^2	Estimate	Odds ratio	AIC	Pseudo R^2	mean Estimate	Odds ratio	sd Estimate	Odds ratio	slope Estimate	Odds ratio	AIC	Pseudo R^2
1	-0.235 (0.060) $p < 0.001$	0.79	26.9	49.02%	0.182 (0.091) $p = 0.046$	1.20	45.6	7.21%	-0.135 (0.077) ns	0.87	46.0	6.51%	-0.264 (0.080) $p = 0.001$	0.77	0.171 (0.122) ns	1.19	-0.056 (0.065) ns	0.95	26.5	58.81%
3	-0.188 (0.061) $p = 0.002$	0.83	41.2	17.00%	0.450 (0.125) $p < 0.001$	1.57	37.3	25.76%	-1.139 (0.269) $p < 0.001$	0.32	29.6	42.78%	-0.221 (0.102) $p = 0.030$	0.80	-0.554 (0.562) ns	0.58	-2.497 (1.474) ns	0.08	26.5	58.64%
5	-0.173 (0.074) $p = 0.020$	0.84	44.1	10.18%	0.464 (0.135) $p < 0.001$	1.59	38.2	23.39%	-2.347 (0.585) $p < 0.001$	0.10	30.1	41.54%	-0.063 (0.092) ns	0.94	-0.125 (0.328) ns	0.88	-2.697 (1.537) ns	0.07	33.1	43.89%

ANNEX III

Prediction of mastitis, lameness, and dysentery in Holstein dairy cattle using machine-learning techniques

Figure S1. Relative importance analysis (by Partial Least Squares method) of mastitis predictors in primiparous and pluriparous Holstein dairy cows, repeated for the 3 datasets (1, 3, and 5-days windows). Dark grey indicates positive predictors (risk factors), while light grey indicates negative predictors (protective factors).

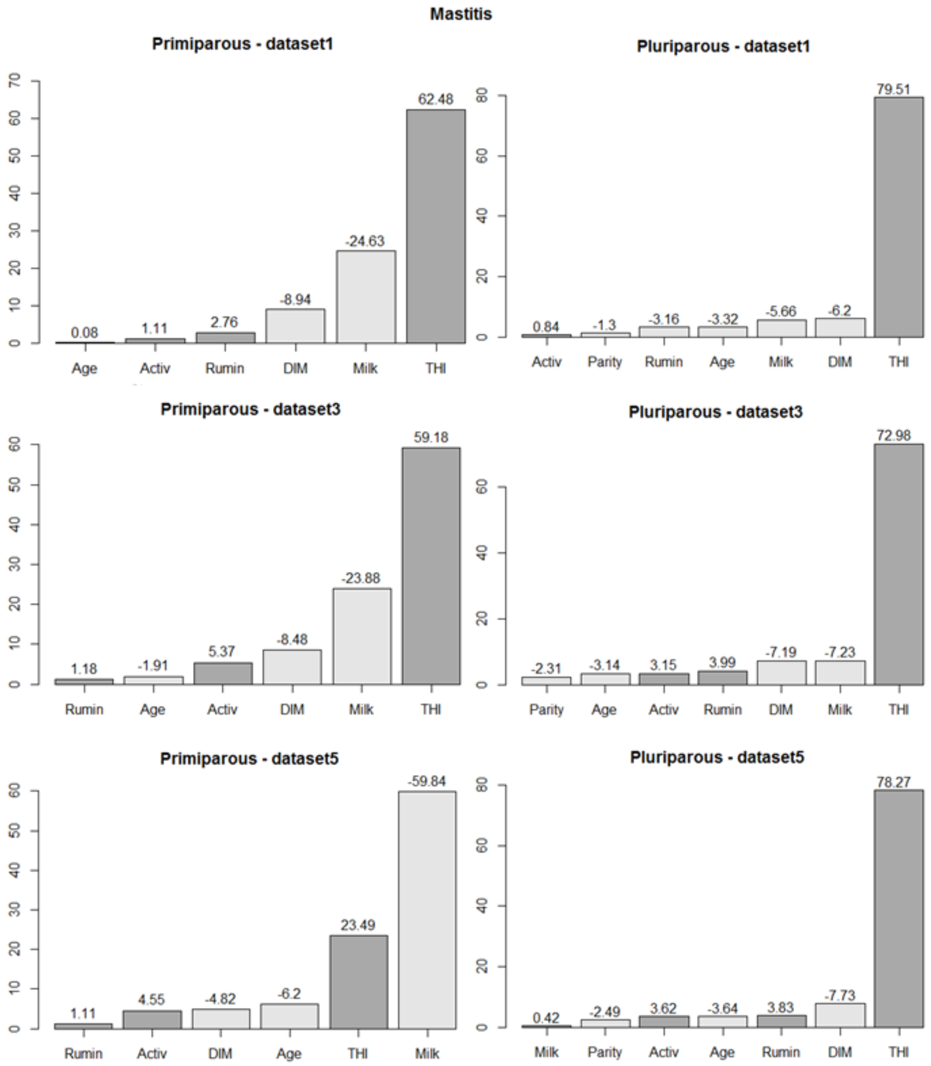

Figure S2. Relative importance analysis (by Partial Least Squares method) of lameness predictors in primiparous and pluriparous Holstein dairy cows, repeated for the 3 datasets (1, 3, and 5-days windows). Dark grey indicates positive predictors (risk factors), while light grey indicates negative predictors (protective factors).

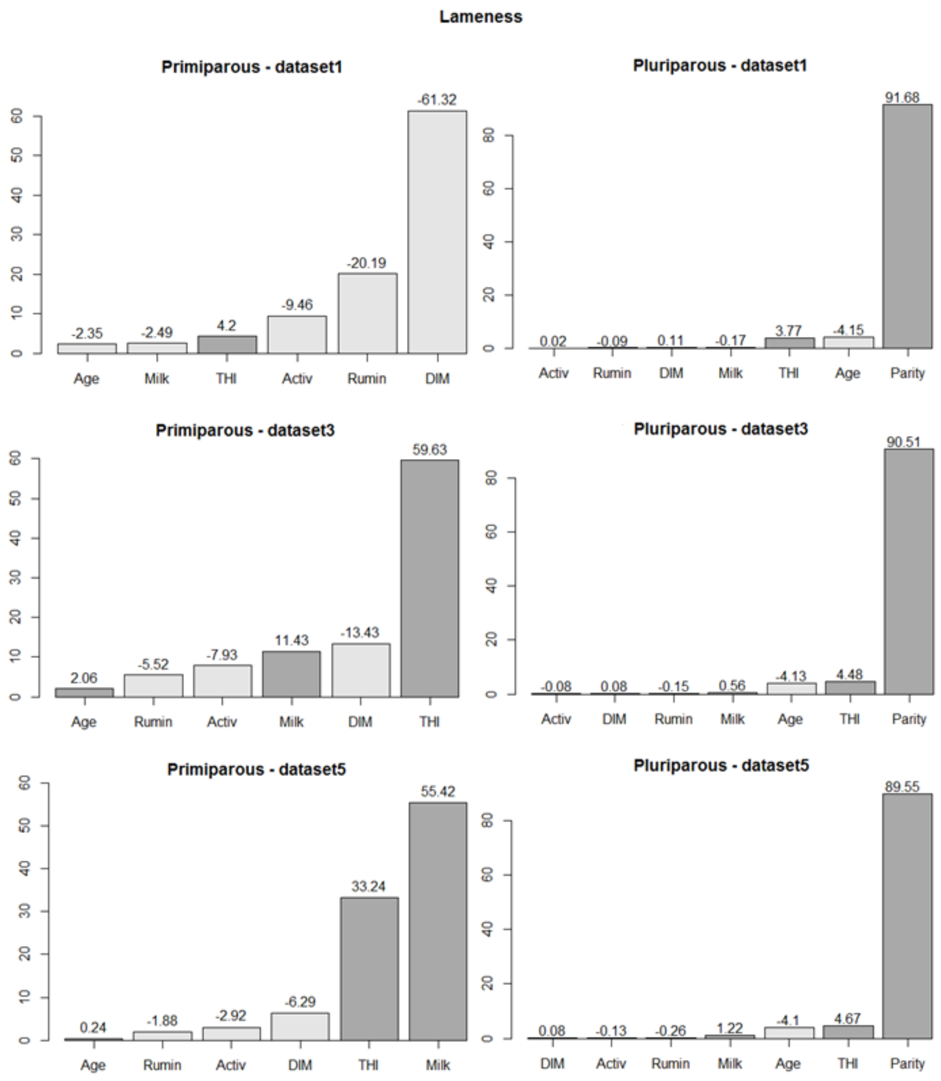

Figure S3. Relative importance analysis (by Partial Least Squares method) of dysentery predictors in primiparous and pluriparous Holstein dairy cows, repeated for the 3 datasets (1, 3, and 5-days windows). Dark grey indicates positive predictors (risk factors), while light grey indicates negative predictors (protective factors).