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European Food Safety Authority

EFSA Journal 2012;10(1):2513

SCIENTIFIC OPINION

Guidance on Risk Assessment for Animal Welfare[1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The document provides methodological guidance to assess risks for animal welfare, considering the various husbandry systems, management procedures and the different animal welfare issues. The terminology for the risk assessment of animal welfare is described. Risk assessment should not be carried out unless the relevant welfare problem is clearly specified and formulated. The major components of the problem formulation are the description of the exposure scenario, the target population and the conceptual model linking the relevant factors of animal welfare concern. The formal risk assessment consists of exposure assessment, consequence characterisation, and risk characterisation. The systematic evaluation of the various aspects and components of the assessment procedure aims at ensuring its consistency. All assumptions used in problem formulation and risk assessment need to be clear. This also applies to uncertainty and variability in the various steps of the risk assessment. The choice between qualitative, semi-qualitative or quantitative approaches should be made based on the purpose or the type of questions to be answered, data, and resource availability for a specific risk assessment. Quantitative data should be used whenever possible. Positive effects on welfare (benefit) could be handled within the framework of risk assessment if the analysis considers factors as having both positive and negative effects on animal welfare. The last section details the main components of risk assessment documentation.

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KEY WORDS

Animal welfare, risk assessment, risk, benefit, problem formulation, hazard, factor identification, exposure assessment, consequence characterisation, positive and negative welfare consequences.

1 On request from EFSA, Question No EFSA-Q-2007-168, adopted on 13 December 2011.

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3 Acknowledgements: The Panel wishes to thank the members of the Working Group on the Guidance on risk assessment for animal welfare: Moez Sanaa (Chairman), Donald Broom, Linda Keeling, Endre Szucs, Pascal Oltenacu, John Webster, Simon More, David Morton, Bosse Algers, Mo Salman, Antonio Velarde, Michael Sharp, Joerg Hartung and Hans-Hermann Thulke, Philippe Vannier, and Martin Wierup and EFSA staff Elisa Aiassa and Oriol Ribó for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Animal Health and Welfare (AHAW); Guidance on risk assessment for animal welfare. EFSA Journal 2012;10(1):2513. 29 pp. doi:10.2903/j.efsa.2012.2513. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

The aim of this Guidance is to provide a harmonised methodology for the assessment of risks for farm animal welfare, together with suggestions about the assessment of benefits for animal welfare. The guidance is intended to be applicable to all types of factors that affect welfare (i.e. housing characteristics, transport conditions, stunning and killing conditions), all types of husbandry systems and all animal categories.

The risks for animal welfare in EFSA scientific opinions have been considered since 2004 and the terminology used is explained in the Glossary. Risk assessment provides a science-based, transparent, and reproducible framework to address specific welfare problems within a limited time frame and with available scientific data. Benefit assessment should be possible with the same methodology. The definition of the target population, the exposure scenario and the conceptual model are the major components of the problem formulation. A conceptual model should be built in order to describe the exposure pathways and the different combination of events showing the relevant factors and their effects on the target population. Relevant factors related to, for example, genetic selection, housing and management, transport, stunning or killing, that are likely to improve or impair the welfare of the animals should be identified.

Risk assessment has three elements: exposure assessment, consequence characterisation and risk characterisation. Exposure assessment should provide a qualitative or quantitative evaluation of the strength, duration, frequency and patterns of exposure for the factors relevant to the exposure scenario(s) developed during the problem formulation.

Consequence characterisation involves assessing the magnitude (intensity and duration) of the negative and positive consequences for welfare and the probability of their occurrence at the individual level. Risk characterisation is the final step of risk assessment and is the qualitative or quantitative estimation of the probability of occurrence and magnitude of negative and positive welfare effects (known or potential) in a given population.

Uncertainty and variability in risk assessment, as well as all assumptions used in problem formulation and risk assessment, need to be clearly expressed. Quality of risk assessment includes the quality of the data input, the relevance of the assumptions and the quality of the final assessment in relation to uncertainty and variability.

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BACKGROUND AS PROVIDED BY EFSA

The European Food Safety Authority (EFSA) provides independent information regarding risks associated with food and feed, plant health, environment, animal health, and animal welfare (AW) by using, whenever possible, a risk assessment (RA). In addition, one of the tasks of the Authority is to promote and coordinate the development of uniform RA methodologies in the above-mentioned fields.

The Animal Health and Welfare (AHAW) Panel of EFSA has adopted 36 Scientific Opinions on Animal Welfare between 2004 and 2010, dealing with welfare of calves, fattening pigs, sows and boars, tail biting, seals, fish and dairy cows (EFSA, 2006a; 2007a,b,c,d; 2008a; 2009). Different approaches have been followed for these scientific opinions.

An EFSA Scientific Colloquium on "Principles of Risk Assessment of Food Producing Animals" was held in Parma in 2005 (EFSA, 2006[4]) and, subsequently, an EFSA workshop on "Risk Assessment Methodology in Animal Welfare" was held in Vienna in 2007. One of the main conclusions was that no specific standardised methodology exists in the field of risk assessment for animal welfare. The beneficial effects of some factors for animal health and for animal welfare in general were also discussed; however, only the assessment of risks was considered in detail. While specific guidelines have been published on animal diseases or chemical substances by the World Organisation for Animal Health (OIE, 2004 a,b) and the Codex Alimentarius Commission (CAC, 2002) respectively, there are currently no specific international guidelines on risk assessment for animal welfare.

A report on basic information for the development of guidelines on risk assessment for animal welfare was produced by the "Italian Reference Centre for Animal Welfare" (EFSA, 2007). The report includes a definition of risk assessment, a description of existing models, reviews the definition of animal welfare and different approaches for its evaluation. The report lists the main issues to be considered in the guidelines. These issues have been divided in the following three categories: i) slaughter, ii) transport, and iii) housing and management.

A "Framework for EFSA AHAW Risk Assessment" was produced (EFSA, 2008[5]) but a requirement for specific guidelines and standardised working methodology for risk assessment, including the assessment of beneficial effects of some factors applied to animal welfare has been clearly identified. Against this background, EFSA launched a self-mandate in 2007 to develop guidance on risk assessment for animal welfare.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The original terms of references for the self-mandate were amended in 2009, and were to define a comprehensive and harmonised methodology to evaluate risks and benefits in animal welfare, taking into consideration the various procedures, management and housing systems and the different animal welfare issues, with reference to the methodologies followed in the previous EFSA Scientific Opinions on various species.

The defined methodology for assessing risks and benefits in animal welfare should take into account and adapt current risk assessment methodologies, for example those for animal disease and food safety, and also the complex range of measurable welfare outcomes.

The guidance document should define concisely the generic approach for working groups, while addressing specific areas of assessment of risks and benefits in animal welfare.

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[4] http://www.efsa.europa.eu/en/supporting/pub/111e.htm
[5] http://www.efsa.europa.eu/fr/supporting/pub/233r.htm

Clarification of the Terms of Reference

While the original mandate exclusively focused on risk assessment (i.e. consideration of harmful factors), the 2009 terms of reference of the mandate included explicit consideration of benefit assessment. However, at its 55th plenary meeting[6], the AHAW Panel recognised that risk and benefit analysis in the context of animal welfare may require further conceptual and methodological refinement. The Panel recommended considering detailed aspects of benefit analysis for further work and possible future inclusion in its methodological framework. The Panel consequently proposed to concentrate on risk assessment aspects for the purpose of the Guidance. This was formally accepted by EFSA in April 2011.

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[6] http://www.efsa.europa.eu/en/events/event/ahaw110224-m.pdf

ASSESSMENT

1. INTRODUCTION

This Guidance provides a structured methodological framework based on existing EFSA practices as well as OIE and Codex Alimentarius risk assessment methodologies, for addressing risks to animal welfare related to any factors having the potential to affect the welfare of animals in any husbandry system.

The purpose of this Guidance is to provide a practical and generic procedure on how to conduct an assessment of the risks of poor animal welfare, and facilitate comparability of animal welfare risk assessments. The Guidance includes some suggestions concerning the assessment of benefits for animal welfare. The intention is to apply this Guidance in the working of the Animal Health and Welfare (AHAW) Panel of EFSA.

In this Guidance more explanatory text is given in some places to clarify the complex issues that are specifically related to animal welfare so that the reasons for taking certain actions are clear.

The main terms used in this guidance are defined in the Glossary.

2. PRINCIPLES OF ANIMAL WELFARE RISK ASSESSMENT

Risk assessment is one of the three components of risk analysis (Regulation (EC) 178/2002[7]). Risk assessment considers different types of factors within specific exposure scenarios (see the definitions in the Glossary) and it provides a scientific basis for appropriate risk analysis (i.e. the assessment, communication and management to reduce, eliminate or prevent the risks that can lead to poor welfare in animals).

Good communication between risk assessors, risk managers, and all interested parties, is essential to the risk analysis process.

At the inception of the assessment, risk assessors should consider the terms of reference and background information provided by those requesting the risk assessment. Risk assessors may request an initial planning stage to clarify the goals, scope, and focus of the risk assessment, and the major issues that will need to be addressed within the framework of a risk assessment (see Section 3.1, problem formulation).

Uncertainty and variability in risk assessment, as well as all assumptions used in problem formulation and risk assessment, need to be clearly expressed.

The choice between qualitative, semi-qualitative or quantitative approaches should be made according to the purpose or the type of questions to be answered, and the data and resources available for a specific risk assessment. Quantitative data should be used whenever possible without diminishing the utility of available qualitative information and expert knowledge.

2.1. ANIMAL HEALTH RISK ASSESSMENT

The World Animal Health Organisation (OIE) has developed standards for risk analysis related to the importation of animals and animal products. The recommended steps of risk assessment are first and foremost designed to consider the risk of infectious agent introduction into an importing country.

[7] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, 1-24.

The Terrestrial Animal Health Code (OIE, 2011), which governs animal import risk assessment describes four steps: (i) Release assessment, (ii) Exposure assessment, (iii) Consequence assessment, and (iv) Risk estimation.

The OIE approach assumes that the hazard has already been identified. If the hazard has already been identified then further discussion should focus on how the hazard is released from its source(s), the pathways by which the population at risk becomes exposed, the consequence of the contact between the hazard and the susceptible hosts, and integration of the previous steps to estimate the risk associated with the specified hazard.

Hazard identification is a pre-risk assessment activity, to determine whether exposure to an agent (biological or infectious agent) might cause an adverse health effect (disease) in animals or in humans. It is a qualitative step where evidence in the literature is collated and presented in a logical and rational manner to justify the concern regarding the perceived risk of a particular hazard. This step entails examination of the evidence in the literature for disease causation.

Since the OIE guidance is only related to import risk analysis to prevent introduction of infectious diseases, it needs to be modified for use in relation to animal welfare and animal diseases in general.

2.2. Animal welfare risk assessment

Problem formulation, including factor identification, is a prerequisite for any risk assessment (see Figure 1). The next stage is formal animal welfare risk assessment which comprises three steps: (1) exposure assessment; (2) consequence characterisation; and (3) risk characterisation.

Box 1. *Examples of single input/single consequence, multiple input/single consequence, and multiple input/multiple consequence risk assessment approaches (SISC, MISC, MIMC)*

Single input		**Single consequence**
A microbial agent	→	One disease
Mycobacterium bovis		Bovine tuberculosis
Listeria monocytogenes		Listeriosis

Multiple inputs		**Single consequence**
Various microbial agents		
Milking hygiene factors	→	Mastitis
Housing system factors		
Nutrition factors		
Etc.		

Multiple inputs		**Multiple consequences**
Various microbial agents		
Milking hygiene factors		Mastitis
Housing system factors	→	Lameness
Nutrition factors		Infertility
Chemical agents		Injuries
Animal handling		Abnormal behaviour

Factor identification in animal welfare risk assessment is equivalent to hazard identification, which considers whether particular factors have the potential to improve or impair directly or indirectly the animal welfare in the target population.

Animal welfare risk assessment usually considers simultaneously several factors within an exposure scenario, where each factor could affect one or several of the four welfare principles (see the Glossary).

Some risk assessments consider one single hazard and one single consequence (SISC: single-input-single-consequence, see Box 1). However, for animal welfare risk assessment, the questions often make it necessary to consider multiple factors *vs.* single consequences (MISC: multiple-inputs-single-consequences) and multiple factors *vs.* multiple consequences (MIMC: multiple-inputs-multiple-consequences).

Animals can be exposed simultaneously or successively to one or more factors. Factors may contribute to the same consequence or a variety of consequences. Risk assessment for multiple factors may evaluate the risks one at a time, or may take into account possible interactions among factors (antagonisms, synergisms and feedback).

3. Operational guidance

The workflow to conduct a risk assessment is presented in Figure 1.

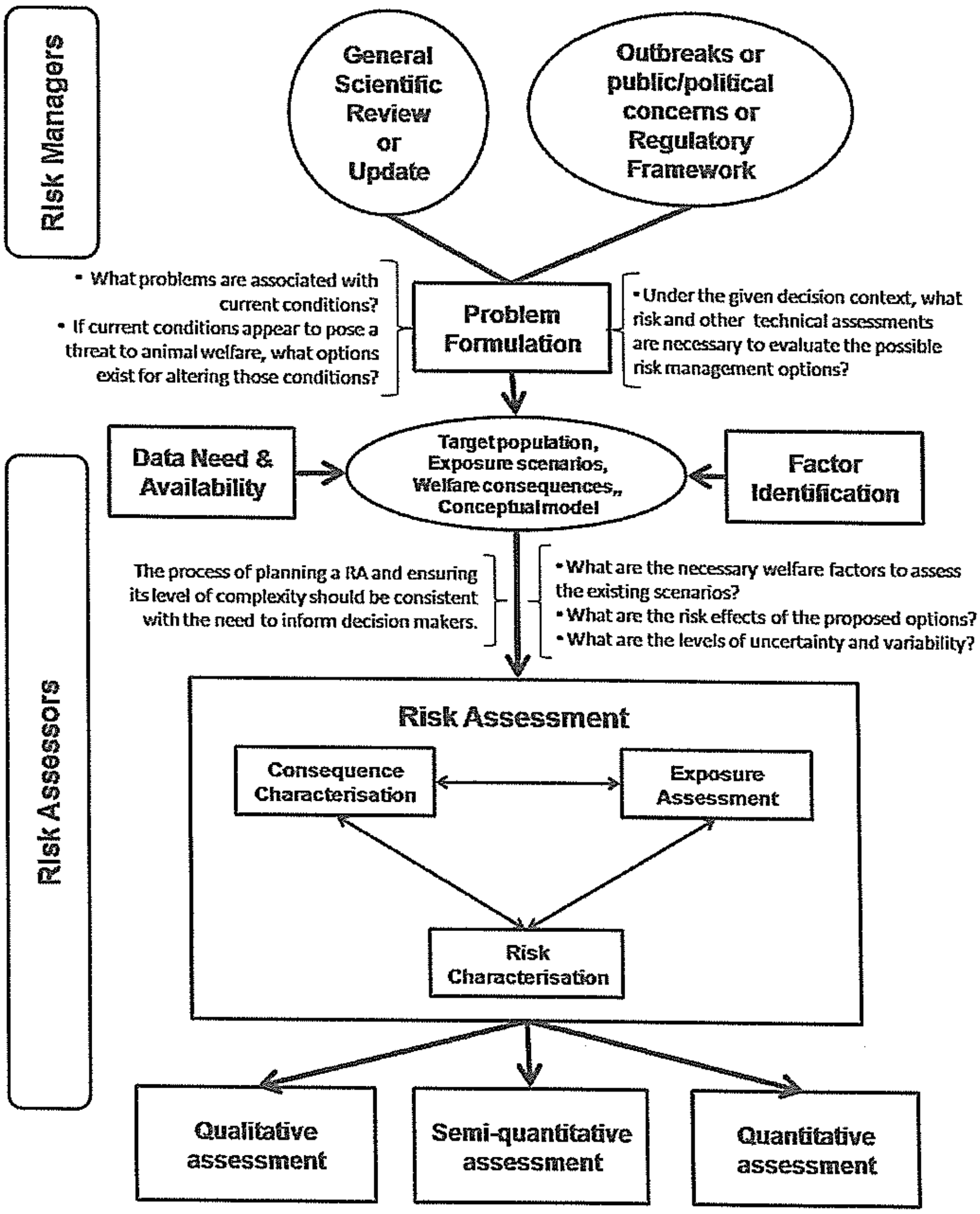

Figure 1. Workflow to conduct a risk assessment

3.1. Problem formulation

Problem formulation precedes the formal risk assessment and defines the original question that needs addressing. It establishes the purpose, breadth, and focus of the animal welfare risk assessment. Problem formulation comprises the following steps.

1. Clarify the risk question(s)
2. Identify the target population
3. Identify factors of animal welfare concern
4. Identify exposure scenarios
5. Identify the known animal welfare consequences and their measurement
6. Build a conceptual model, including identification of the relevant methodology and the data needs

Risk question(s): During problem formulation, the aim of the exchanges between the risk managers and the risk assessors is to achieve precise and clear formulation of the risk questions (see Box 2).

The questions may arise within the management context of enforcing a new policy or procedure or defining requirements for the application of alternative policies or procedures.

Target population: The population considered in the risk assessment is a subset of the animal population, and is defined by a set of common characteristics (e.g. geographical area, and intrinsic attributes such as age, breed, sex, etc.) in relation to the risk question(s).

As an example, depending on the risk question, the target population could be dairy cows in general or dairy cows farmed in a particular system (dairy cows kept in cubicle houses; dairy cows kept in tie stalls; dairy cows kept in straw yards; and dairy cows kept at pasture) in a particular region.

In the case of the transport of animals, the target population can be defined by: the species of animals being transported; animal categories within each species; and the mode of transport (e.g. truck, boat, aeroplane).

Box 2. A risk question can typically be:

- A factor-based question: for example, how does a potential management option compare with an existing option regarding the risk for the welfare of the animals?
  Examples: welfare consequences of changing transport duration; consequences for welfare of reducing ante-mortem inspection procedures; consequences for welfare when rearing laying hens in large cages.
- A consequence-based question: for example, what is the welfare consequence of changing an existing management system to an alternative system?
  Examples: how to transport animals in order to minimise heat stress; identifying the risks when animals are killed by Method A vs Method B; the best way to minimize the risk of injuries during pre-slaughter.

Factor identification: Factors are defined as any aspect of the environment of the animals in relation to housing and management, animal genetic selection, transport and slaughter, which may have the potential to impair or improve their welfare. A hazard is a factor with the potential to cause poor welfare.

Identification of factors should be based on the scientific literature. In this step, in accordance with the risk question(s), as well as the target population and exposure scenario, the aim is to list all the relevant factors that have the potential to influence the animals' welfare.

Factor selection commences with the preparation of a list of the needs of the animals under consideration which is compiled using the scientific literature on the biological functioning and strengths of preferences of the animals (EFSA, 2008; p.30). It is then necessary to draw up a clear description of the selected factors related to their known welfare consequences (see the EFSA report on the welfare of dairy cows (EFSA, 2009)).

Factors may have both negative and positive effects, and there may be more than one effect. A scientific literature review should then be undertaken to collate all the available studies identifying the associations between factors and animal welfare effects. Such an analysis highlights the factors likely to influence animal welfare. These are then discussed and prioritised within the target population and the risk questions.

Data describing the magnitude and estimating the probability of occurrence of welfare consequences are extracted from published studies.

Exposure scenarios: An exposure scenario is a sequence or combination of events in relation to the risk question that includes, in general, all information on housing, nutrition, genetic selection, transport, farming and management procedures, slaughter procedures and husbandry to which animals of the target population are subjected.

Relevant combination(s) of the identified factors and their exposure levels are defined at this stage. It may be necessary to describe a reference scenario for comparison with the scenario under investigation (e.g. barren *versus* enriched cages for laying hens).

The list of factors may be revised after consideration of the different exposure scenarios.

Animal welfare consequences and their measurement: At this stage, risk assessors propose what animal welfare consequences are important for the risk question and how they can be measured. Welfare consequences are changes in welfare that result from the effect of a factor or factors (see Figure 2). During this step it should be decided whether or not the assessment will simultaneously include negative (risk) and positive (benefits) consequences. The assessment of the eventual positive consequences is appropriate when: (i) a particular factor or a group of factors could have positive and negative consequences for the same scenario of exposure; or (ii) an exposure scenario can include groups of factors that have both positive and negative consequences.

Animal-based measures (indicators) are necessary to assess the welfare consequences, and their interpretation and assessment will depend on their magnitude (Figure 2). Those animal-based measures of welfare consequences that can be used by a farmer, veterinarian or other trained inspector (welfare measures) are of particular value and these are the subject of a series of EFSA Opinions, such as for dairy cows (EFSA, 2012a), and pigs (EFSA, 2012b).

Conceptual model, including identification of the relevant methodology and the data needs: A conceptual model in problem formulation is a written description and visualisation of a model of known or supposed relationships between factors and animal welfare. It considers logically how the changes made to the scenario under consideration will affect animal welfare. Subsequently, the model shows how the risk questions will be addressed, the relevant information needed, the method that will be used to analyse the data, and the assumptions inherent in the analysis.

Problem formulation is not just a literature review and a description of all the available information about a risk issue. It should also determine the type of risk assessment to be used - qualitative, semi-quantitative or quantitative (see the Glossary). Both of these approaches can be valid: the criteria for

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Animal (2014), 8:4, pp 667–674 © The Animal Consortium 2014
doi:10.1017/S1751731114000032

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Seasonal variations in the composition of Holstein cow's milk and temperature–humidity index relationship

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(Received 25 February 2013; Accepted 17 December 2013; First published online 11 February 2014)

A retrospective study on seasonal variations in the characteristics of cow's milk and temperature–humidity index (THI) relationship was conducted on bulk milk data collected from 2003 to 2009. The THI relationship study was carried out on 508 613 bulk milk data items recorded in 3328 dairy farms form the Lombardy region, Italy. Temperature and relative humidity data from 40 weather stations were used to calculate THI. Milk characteristics data referred to somatic cell count (SCC), total bacterial count (TBC), fat percentage (FA%) and protein percentage (PR%). Annual, seasonal and monthly variations in milk composition were evaluated on 656 064 data items recorded in 3727 dairy farms. The model highlighted a significant association between the year, season and month, and the parameters analysed (SCC, TBC, FA%, PR%). The summer season emerged as the most critical season. Of the summer months, July presented the most critical conditions for TBC, FA% and PR%, (52 054 ± 183 655, 3.73% ± 0.35% and 3.30% ± 0.15%, respectively), and August presented higher values of SCC (369 503 ± 228 377). Each milk record was linked to THI data calculated at the nearest weather station. The analysis demonstrated a positive correlation between THI and SCC and TBC, and indicated a significant change in the slope at 57.3 and 72.8 maximum THI, respectively. The model demonstrated a negative correlation between THI and FA% and PR% and provided breakpoints in the pattern at 50.2 and 65.2 maximum THI, respectively. The results of this study indicate the presence of critical climatic thresholds for bulk tank milk composition in dairy cows. Such indications could facilitate the adoption of heat management strategies, which may ensure the health and production of dairy cows and limit related economic losses.

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Keywords: dairy cattle, bulk milk characteristics, season, temperature–humidity index

Implications

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An increase in somatic cell count (SCC) and total bacterial count (TBC) and a decrease in fat and protein content were observed during summer months. Critical climatic thresholds for milk composition have been established. Temperature–humidity index breakpoints for milk SCC, TBC, fat and proteins were established. The results of this study could be helpful to dairy cow producers, allowing them to adopt specific heat-abatement measures to counter the unfavourable consequences of heat stress at farm level.

Introduction

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Climate change, defined as the long-term disbalance of customary weather conditions such as temperature, wind and rainfall characteristics of a specific region, is likely to be one of the main challenges mankind faces during the current century. The increasing concern with the thermal comfort of agricultural animals is justifiable not only for countries occupying tropical zones, but also for nations in temperate zones where high-ambient temperatures are becoming an issue (Nardone *et al.*, 2010).

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Climatic conditions may affect the welfare and productive performance of livestock species. In dairy cows, high temperatures experienced during the hot season have an effect on physiology, metabolism, production and reproduction of the animal (Jordan, 2003; Bernabucci *et al.*, 2010). In some studies, seasonal variations in milk yield and composition have been investigated. Renna *et al.* (2010) compared the milk production of grazing cows recorded in 2003 and 2004. Those authors reported a decrease in milk, fat and protein yields during the summer months of the hottest year, 2003. Bouraoui *et al.* (2002) observed a significant decrease in milk, fat and protein yield and a significant increase in the somatic cell count (SCC) of lactating Holstein cows during the summer (temperature–humidity index (THI) = 78) compared with spring (THI = 68). In a 4-year retrospective study conducted on Holstein cows, Olde Riekerink *et al.* (2007) analysed the

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seasonal variations in SCC in individual and bulk milk samples. The authors reported a significant increase in the SCC during August and September. In a study carried out in Israel, cows that calved in December produced the highest milk and milk protein yields, and those that calved in June produced the lowest, 92.8% of the maximum (Barash *et al.*, 2001). Information on THI milk fat and protein percentage and SCC relationships are scarce or lacking.

Reduction in milk production related to the increase in THI has been reported (Ravagnolo *et al.*, 2000; West *et al.*, 2003). Ravagnolo *et al.* (2000) have observed a decrease of 0.009 kg and 0.012 kg in protein and fat yield, respectively, for each unit of THI above the threshold of 72. Our study carried out on Holstein cows housed in climatic chambers and exposed to THI = 84 during the day and THI = 78 during the night (Nardone *et al.*, 1992) highlighted a 25% decrease in milk yield and an 11.6% decrease in protein percentage, when compared with a control group exposed to thermo-neutral conditions (THI = 65). Furthermore, on comparing milk production during summer and spring in a dairy herd located in central Italy, we found a lower milk yield (−10%), and also lower protein and casein percentages in summer (3.01% *v.* 3.31% and 2.18% *v.* 2.58%, respectively; Bernabucci *et al.*, 2002).

Most of the previous studies focused their attention on milk, protein and fat yield changes under hot conditions. To date, very little information is available in the literature regarding the seasonal variation of total bacterial count (TBC) and THI relationship for SCC, TBC, and fat and protein percentage. The study was thus aimed at investigating annual, seasonal and monthly variations in milk characteristics (SCC and TBC, fat and protein percentage) and THI–milk characteristics relationships in Holstein dairy farms.

Materials and methods

The retrospective studies described below (seasonal pattern and THI milk composition relationships study) were conducted on bulk tank milk tests recorded over a 7-year period (2003 to 2009) in Holstein dairy farms located in the Po valley (Region of Lombardy, North Italy), one of the most important areas in Europe for milk and cheese production. Italian Holstein cattle are an interesting case study because they represent a large and highly selected dairy cow population reared in a warm area of the Mediterranean basin, that is becoming one of the hot spots in the world, owing to climate change (Segnalini *et al.*, 2010). Dairy farms located in the study area were highly homogeneous in terms of the production system adopted (intensive), the number of cows (110 cows in lactation on average), the genetic merit of cows, the average milk yield per cow (~9000 kg/lactation in 305 days on average), and the barn design and management (total confinement free barn housing with no time at pasture, TMR feeding practices and year-round calving patterns). Moreover, ~95% of the farms were provided with heat abatement measures. The following information was associated with each record reported: test date, farm code, SCC, TBC, fat and protein expressed as percentages (FA% and PR%, respectively).

Milk samples were analysed for SCC (5% and 8% intra- and inter-assay coefficients of variation, respectively) using a fluoro-opto-electronic counter (Fossomatic™ FC, Foss, Hillerød Denmark), for TBC (11.9% and 18.4% intra- and inter-assay coefficients of variation, respectively) using a fluoro-opto-electronic counter (BactoScan™ FC, Foss, Hillerød Denmark), and for FA% (0.014 g/100 ml and 0.045 g/100 ml intra- and inter-assay coefficients of variation, respectively) and PR% (0.014 g/100 ml and 0.035 g/100 ml intra- and inter-assay coefficients of variation, respectively) using FTIR spectrophotometry (MilkoScan™ FT 6000, Foss, Hillerød Denmark). Bulk tank milk was analysed at farms twice a month under a milk-quality payment system.

The values referring to SCC and TBC, expressed as cells/ml and colony-forming units (cfu)/ml, respectively, were transformed. The SCC was transformed into somatic cell score (SCS) by means of the following formula (Ali and Shook, 1980): SCS = [$\log_2$ (somatic cell count/100 000) + 3], and TBC values were natural log-transformed (LnTBC). The corresponding thresholds for SCS and LnTBC are indicated in REGULATION (EC) No 853/2004, as shown: LnTBC of 11.51 corresponds to 1.10^5 cfu/ml (rolling geometric average over a 2-month period, with at least two samples per month); SCS of 5.00 corresponds to 4.10^5 cells/ml (rolling geometric average over a 3-month period, with at least one sample per month). Italian Law 169/89 fixes a lower SCC limit in its application in Ministerial Decree 185/91 for the commercialization of high-quality drinking milk: SCS of 4.58 corresponds to 3.10^5 cells/ml. It is as rolling geometric average over a 3-month period with at least two samples per month.

Data were tested for non-normality by the Shapiro test by using SAS 9.2 software package (SAS/STAT, 2008). The analysis of data distribution indicated that all parameters analysed followed a normal distribution ($P < 0.05$). Complete descriptive statistics of the studies are reported in Table 1.

Table 1 *Descriptive statistics of the studies*

Study period (7 years)	2003 to 2009
Geographical area	North Italy
Area	Po valley
Region	Lombardy
Annual, seasonal and monthly pattern study	
Number of dairy farms	3727
Milk characteristic records	656 064
Number of lactating cows	365 246
THI–milk quality relationship study	
Number of dairy farms	3328
Milk characteristic records	508 613
Number of lactating cows	316 160
Number of weather stations consulted	40
Weather station-farm distance (mean ± s.d.), km	10.92 ± 6.01

THI = temperature–humidity index.

Seasonal pattern study
The seasonal pattern was studied by utilizing 656 064 milk composition data items recorded in 3727 dairy farms (365 246 lactating cows). The months of December, January and February were defined as winter; March, April and May as spring; June, July and August as summer; and September, October and November as fall. The annual, seasonal and monthly variation in bulk tank milk composition (SCS, LnTBC, FA% and PR%) were evaluated by means of a GLM where THI, SCS, LnTBC, FA% and PR% were set as dependent variables, and year, season and month as independent variables. Farm was included into the model and considered as random effect. The differences were analysed by the Tukey test and the significances were set at a value of $P<0.05$. The analysis was carried out using the SAS 9.2 software package (SAS/STAT, 2008).

THI relationship study
The study was carried out on 508 613 milk composition data items recorded in 3328 dairy farms (316 160 lactating cows). The National Reference Centre for Animal Welfare (Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna, Brescia, Italy) provided the latitude and longitude for all farms. The useful weather stations belonged to the following institutions: Regional Environmental Protection Agency of the Lombardy Region and the Research Unit for Agricultural Climatology and Meteorology (CRA-CMA). The distances between farms and 40 weather stations were calculated and each farm was associated with the nearest weather station, located within a minimum radius of 0.3 km and maximum radius of 30 km. The average distance between any given farm and weather station was 10.92 ± 6.01 km. Supplementary Figure S1 displays a map of the study area where the weather stations and dairy farms are located.

Daily maximum temperature and minimum relative humidity and daily minimum temperature and maximum relative humidity data from the 40 weather stations were used to calculate maximum (maxTHI) and minimum (minTHI) daily THI, respectively. The maximum daily THI was chosen because it represents the worst environmental conditions experienced by the cows. Moreover, the maximum daily THI was also chosen in agreement with indication of Ravagnolo and Misztal (2000) who found that maximum THI fits best to data from public weather stations, and with results by Brügemann *et al.* (2012) who indicated higher sensitivity of test-day milk yield to extreme values of maximum THI compared with daily average THI. The minTHI represents the night-time conditions. The THI was used for characterizing the climate conditions of the farms. The max and minTHI recorded 1, 2, 3, 4 and 5 days before the sampling date were attributed to each milk record. The best relationship between THI and milk parameters was found for the THI recorded 2 days before the sampling date with r-value of 0.92, 0.72, 0.93 and 0.97 for SCS, TBC, PR% and GR%, respectively. The maximum and minimum daily THI recorded 2 days before the sampling date were chosen for the THI relationship study.

The THI was calculated using the formula suggested by Kelly and Bond (1971):

$$\mathrm{THI} = (1.8 \times \mathrm{AT} + 32) - (0.55 - 0.55 \times \mathrm{RH}) \times [(1.8 \times \mathrm{AT} + 32) - 58];$$

where AT is the ambient temperature (°C), and RH is the relative humidity as a fraction of the unit.

The THI relationship analysis was based on the calculation of a two-phase linear regression procedure (Nickerson *et al.*, 1989), which detected an inflection point (where one exists) in the relationship between max and minTHI, as the independent variable, and milk composition parameters, as the dependent variable. The following model was used:

$$V_2 = \text{costant} + \text{slope1} \times V_1 + \text{slope2} \times (V_1 - \text{break point}) \times (V_1 > \text{break point});$$

where V_1 is the independent variable and V_2 is the dependent variable.

The analysis was carried out using the Statistica 7.0 software package (StatSoft, 2004).

Results

THI pattern
Changes in monthly maxTHI are reported in Figure 1a. In general, over the 7-year period (2003 to 2009), July was the hottest month with an average maxTHI of 77.8 ± 3.6 ($P<0.01$). Within the period considered, 2003 was the hottest year ($P<0.001$) with an average monthly maxTHI of 80.4 ± 3.5, 79.6 ± 4.1 and 81.9 ± 4.3 for June, July and August, respectively. Year 2005 was the coolest one with an average monthly maxTHI of 74.9 ± 4.9, 76.8 ± 4.6, 73.4 ± 4.7 for June, July and August, respectively. The high-positive association between maximum and minimum daily THI was noted (Figure 1a and b).

Annual, seasonal and monthly variations in milk composition
The model demonstrated a significant association ($P<0.001$) between year, season and month and the parameters analysed (SCS, LnTBC, FA%, PR%). Figure 2 reports the annual patterns of SCS, LnTBC, FA% and PR%. Year 2003 was the year with the highest ($P<0.001$) levels of SCS (4.50 ± 0.79, corresponding to $331\,570 \pm 232\,649$ cells/ml) and LnTBC (10.35 ± 0.9, corresponding to $59\,534 \pm 165\,825$ cfu/ml), as well as the lowest ($P<0.001$) levels of FA% (3.81 ± 0.43) and PR% (3.35 ± 0.17). Milk protein content increased ($P<0.01$) from 2003 to 2009.

Of the seasons (Table 2), summer emerged as the most critical for all the parameters analysed, demonstrating the highest values ($P<0.001$) of SCS (4.61 ± 0.74, corresponding to $349\,489 \pm 23\,581$ cells/ml) and LnTBC (9.99 ± 1.05, corresponding to $52\,972 \pm 192\,896$ cfu/ml), and the lowest values ($P<0.001$) of FA% (3.75 ± 0.35) and PR% (3.32 ± 0.15) content.

Monthly and annual × month patterns are reported in Supplementary Figure S2 and S3, respectively. The monthly

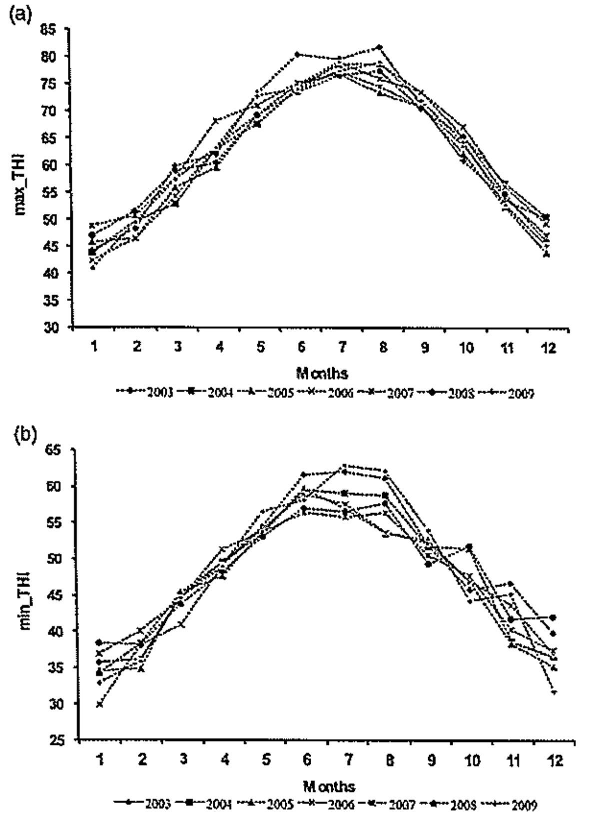

Figure 1 Monthly least squares means (±s.e.m.) of the maximum (A) and minimum (B) temperature humidity index (THI) recorded during the 7-year period (from 2003 to 2009).

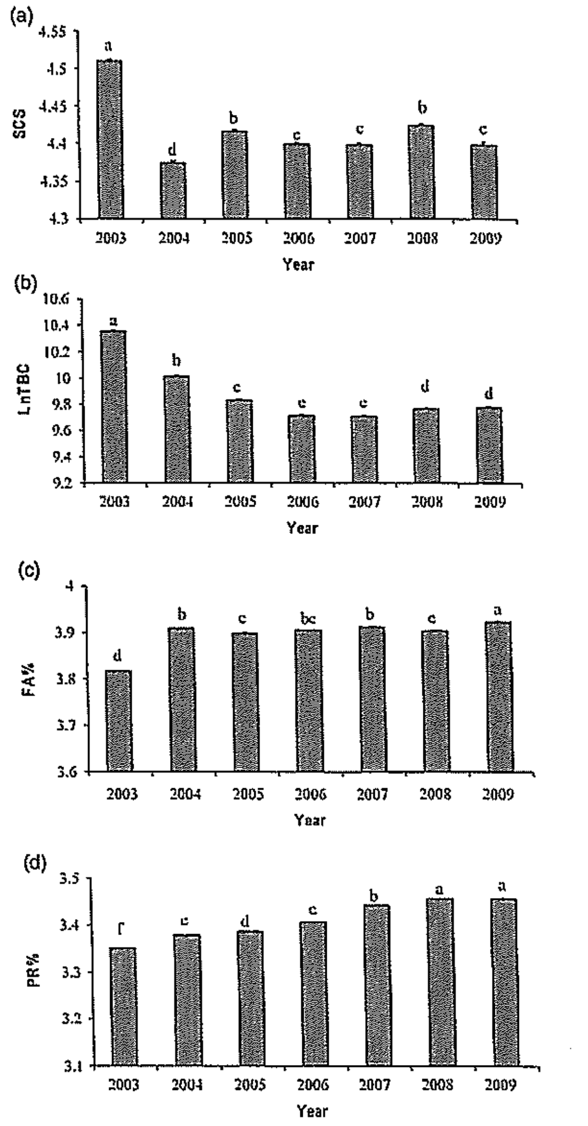

Figure 2 Annual least squares means (±s.e.m.) in reference to the entire period of the study (from 2003 to 2009) for milk (a) somatic cell score (SCS), (b) natural log-total bacterial count (LnTBC), (c) fat percentage (FA%), and (d) protein percentage (PR%). a, b, c, d, e, f = $P<0.01$ between years.

pattern identified July as the month with the most severe climatic conditions ($P<0.001$) for LnTBC, FA% and PR%, with average values of 10.00 ± 1.04 (corresponding to 52 054 ± 183 655 cfu/ml), 3.73 ± 0.35 and 3.30 ± 0.15, respectively. August emerged as the most critical month for SCS with an average value of 4.69 ± 0.73 (corresponding to 369 503 ± 228 377 cells/ml).

THI milk composition relationship

Results of the two-phase regression analysis computed for milk characteristics (SCS, LnTBC, FA% and PR%), with average daily maxTHI as the independent variable, are shown in Figure 3. The model indicated a break point at 57.3 ($r=0.98$) for SCS with a final loss of 0.034 (the lowest total sum of square errors). The model provided the slope and intercept of two distinct regression lines: the first line was $Y_1 = 4.29 + 0.0003 \times \text{THI}$ ($R^2 = 0.0058$; $P = 0.7$) before the breakpoint (35 to 57.3 THI range); and the second line, after the breakpoint (57.3 and 88 THI range), was $Y_2 = 3.55 + 0.0133 \times \text{THI}$ ($R^2 = 0.95$; $P<0.001$). A breakpoint was detected at 72.8 THI for LnTBC ($r = 0.96$) with a final loss of 0.079 (the lowest total sum of square errors). The slope and intercept of two distinct regression lines were: $Y_1 = 9.68 + 0.002 \times \text{THI}$ ($R^2 = 0.41$; $P<0.01$) before the breakpoint (35 to 72.8 THI range) and

Table 2 *Seasonal least squares means (± s.e.m.) of milk characteristics refer to the entire period of the study (2003 to 2009).*

	Season				
	Winter	Spring	Summer	Fall	s.e.m.
SCS	4.287[c]	4.304[c]	4.613[a]	4.473[b]	0.002
LnTBC	9.823[d]	9.869[b]	9.986[a]	9.837[c]	0.003
FA%	4.014[a]	3.852[c]	3.749[d]	3.971[b]	0.001
PR%	3.4638[b]	3.3874[c]	3.3221[d]	3.4783[a]	0.0003

SCS = somatic cell score; LnTBC = natural log-total bacterial count; FA% = fat percentage; PR% = protein percentage.
[a,b c,d]$P<0.01$ between seasons.

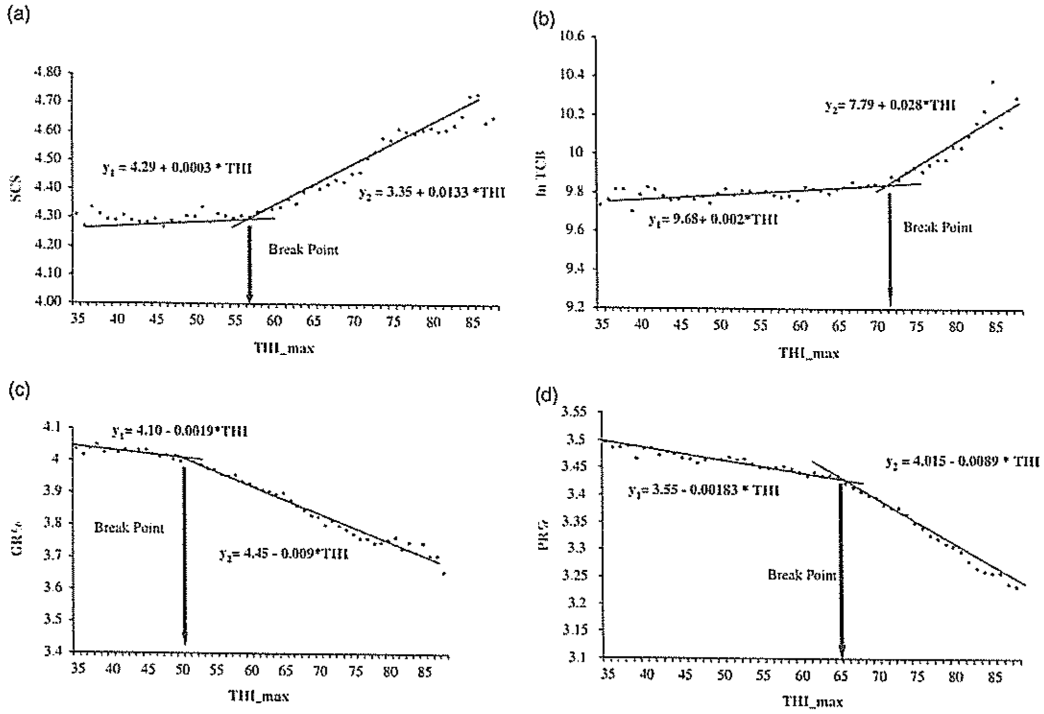

Figure 3 Cow's milk characteristics in relation to maximum temperature–humidity index (THI) recorded 2 days before sampling day. A breakpoint was detected at: 57.3 THI for (a) somatic cell score (SCS); 72.8 THI for (b) natural log-total bacterial count (LnTBC); 50.2 THI for (c) fat percentage (FA%); 65.2 THI for (d) protein percentage (PR%).

$Y_2 = 7.79 + 0.028 \times \text{THI}$ ($R^2 = 0.85$; $P < 0.001$) after the breakpoint (72.8 to 88 THI range).

The two-phase regression computed for FA% indicated a breakpoint at 50.2 THI ($r = 0.99$) with a final loss of 0.0089 (the lowest total sum of square errors). The regression line before the breakpoint (35 to 50.2 THI range) was $Y_1 = 4.10 – 0.002 \times \text{THI}$ ($R^2 = 0.51$; $P = 0.05$); the regression line after the breakpoint (50.2 to 88 THI range) was $Y_2 = 4.45 – 0.009 \times \text{THI}$ ($R^2 = 0.98$; $P < 0.001$). For PR%, the analysis indicated a breakpoint at 65.2 THI ($r = 0.99$) with a final loss of 0.0014 (the lowest total sum of square errors). The two regression lines provided by the model were: $Y_1 = 3.55 – 0.0019 \times \text{THI}$ ($R^2 = 0.9$; $P < 0.01$) and $Y_2 = 4.01 – 0.0089 \times \text{THI}$ ($R^2 = 0.99$; $P < 0.001$) before (35 to 65.2 THI range) and after the break point (65.2 to 88 THI range), respectively.

Two-phase regression computed for milk characteristics (SCS, LnTBC, FA% and PR%), with average daily minTHI as the independent variable was also calculated. The minTHI break points were: 43.1 for SCS ($r = 0.99$) with a final loss of 0.014; 49.4 for lnTBC ($r = 0.95$) with final loss of 0.091; 38.0 for FA% ($r = 0.99$) with final loss of 0.0094; and 49.4 for PR % ($r = 0.99$) with final loss of 0.003.

Discussion

On average, the milk composition values recorded over the entire period of this study (2003 to 2009) were compliant with the Italian criteria for raw milk utilized for high-quality pasteurized milk production. The values for SCC recorded in July, August and September indicate that there is a risk of exceeding the criteria (3.10^5 cells/ml) for raw milk production utilized for high-quality pasteurized milk production. The TBC levels were always below the limit of 1.10^5 cfu/ml, as fixed by European legislation, whereas FA% and PR% were always above the limits fixed for high-quality pasteurized milk commercialization (3.5% and 3.2%, for FA% and PR%, respectively).

The retrospective study on the annual, seasonal and monthly variations in bulk milk composition demonstrates a significant association with climatic conditions. High SCC and TBC values and lower FA% and PR% values were recorded during the summer months, in correspondence with an increase in THI. This evidence was more pronounced for the year 2003, when the highest values of THI were recorded.

The increase in SCC during the summer has been reported (Schukken *et al.*, 1993; Bouraoui *et al.*, 2002; Green *et al.*, 2006), although the mechanism involved is not yet clear. Milk somatic cells are mainly leukocytes, including macrophages, lymphocytes and polymorphonuclear neutrophils. The major factor affecting SCC levels is the infection of the mammary gland. The main pathogens causing an increase in SCC include primary contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*, and environmental pathogens such as coliforms and *Streptococcus spp.* (Harmon, 1994). It is well known that high values of SCC in bulk milk are closely related to high individual SCC, indicating a high prevalence of subclinical mastitis (Waage *et al.*, 1998).

Some authors (Olde Riekerink *et al.*, 2007; Hogan and Smith, 2012) reported a high incidence of mastitis in dairy cows during the hot season. The increased incidence of mastitis during the summer might be related to the effect that high temperatures and humidity have on cows' susceptibility to infections, as well as to the increased number of pathogens or vectors to which the cows are exposed. The impact of heat stress on immune function in dairy cows has been reported. Lacetera *et al.* (2005) observed that summer conditions characterized by the occurrence of extreme events (heat waves) were associated with depressed cellular immunity. Moreover, peripheral blood mononuclear cells, isolated from dairy cows and exposed to incubation temperatures simulating conditions of hyperthermia, exhibited a decrease in DNA synthesis and an increase in heat-shock proteins (Lacetera *et al.*, 2006), indicating a depressed response of cellular-mediated immunity in heat-shock cells.

It is also known that during the summer, the growth and number of environmental bacteria in bedding material increase owing to favourable temperature and humidity (Harmon, 1994). Under these conditions, it is reasonable that bacterial contamination of the udder by environmental pathogens may increase between milking operations, when the udder comes in contact with bedding, soil, water and dung (Jayarao *et al.*, 2004). Therefore, it is rational to hypothesize that the high values of SCC recorded during the summer are because of the increase in temperature, simultaneously operating in two ways. On one hand, high ambient temperatures may be responsible for the animals' higher susceptibility to infection, by impairing the capacity of the immune system. On the other hand, high temperatures may encourage the growth and proliferation of environmental pathogens and their vectors, such as flies. It is acknowledged that a greater presence of environmental bacteria increases the risk of udder infection. The higher levels of bulk milk TBC observed during the summer testify the higher levels of udder contamination and could support this hypothesis.

A drop in fat and protein percentage and yield as temperature rises has been widely reported (Nardone *et al.*, 1992; Berabucci *et al.*, 2002; Renna *et al.*, 2010). Interestingly, it is the negative association between fat and proteins and spring found in the present study. Other authors (Barash *et al.*, 2001; Aharoni *et al.*, 2002) who analysed test-day records of different cow populations (Georgian and Israeli primiparous and Israeli multiparous) observed peaks in fat and protein concentration from October to January, an initial drop in spring and a significant decrease during summer in these parameters. The mechanism responsible for the decrease in fat and protein percentage in spring is not well understood. The farms located in the study area are highly homogeneous in terms of the genetic merit of animals and, with the exception of summer (high-energy diet and heat-abatement devices), dietary and management practices are consistent throughout the year. The two breakpoints found in this study for fat and protein, in correspondence with maxTHI values of 50 and 65, respectively, and the negative association of these parameters with spring, when temperatures start to increase but are not very high, indicate an initial reduction in fat and protein content during a period that is not considered hot for dairy cows. Moreover, in this study, the climatic conditions recorded over 7 years demonstrated the same range of THI for spring and fall; unlike in spring, however, fat and protein increase to reach their peak during the fall. This evidence suggests that during the spring, heat load is not the main factor accounting for the decrease in milk components.

One possible factor affecting FA% and PR% in milk is the lactation phase. Basically, it is widely known that during lactation, fat and protein are lower in correspondence with the peak in milk yield, and increase until the end of lactation. One possible hypothesis for the drop in fat and protein content in spring is the higher incidence of calving and, subsequently, the higher number of fresh cows compared with other seasons. Under the conditions of this study, this factor must be excluded because the incidence of calving was distributed throughout the year with lower values during the spring months and higher values during the fall months and early winter (Italian breeding association, official statistics available at http://bollettino.aia.it/bollettino/bollettino.htm). Another possible factor influencing the percentage of fat and protein in the milk is the level of milk yield. As is well known, milk yield is affected by photoperiod and increases in correlation with increasing day length (Stanisiewski *et al.*, 1985; Dalh and Petitclerc, 2003); it is therefore plausible to believe that for the latitudes considered in this study, the reduction in milk components recorded in spring might be related to the increase in milk yield as a response to the increasing photoperiod, with a consequent dilution effect on protein and fat concentrations. The higher milk yield in spring in the study area is testified by official statistic data (www.CLAL.it). Considering the period of 7 years (2003 to 2009) the average spring milk yield was 15% higher than that recorded during the fall season. Barash *et al.* (2001) reported that the elongation of daylight increased the average milk production by 1.2 kg/h. Therefore, the decrease in fat and protein in spring and their increase in fall, in correspondence with the same climatic conditions, are more the result of the increasing photoperiod or the stage of lactation than of heat stress, whereas the broad drop in fat and protein content recorded during the summer is related to the negative impact of hot conditions on the synthesis of these milk components.

Interesting was the change of PR% from 2003 to 2009. The increase in PR% throughout the years is likely attributable to the genetic improvement. In fact, milk protein is the most important trait considered into the Holstein breed selection scheme in Italy (Canavesi *et al.*, 2009).

The retrospective analysis on the relationship between THI and milk characteristics demonstrated a significant association between maximum and minimum daily THI, recorded 2 days before the sampling day, and SCC, TBC, FA% and PR%. Other than by the analysis of data of the present study, the use of THI recorded 2 days before sampling was also suggested by an analysis of the literature and by other research carried out recently by the authors of this study (U. Bernabucci, unpublished data), which reported a lag time between exposition to climatic conditions and the animal's biological response. As an example, West *et al.* (2003) reported that under hot conditions, milk yield was more affected by THI recorded 2 days before the sampling day. Other authors (Spiers *et al.*, 2004) reported an increase in respiration rate and rectal temperature 1 day after exposure to heat stress conditions and a reduction in dry matter intake and milk yield 2 to 4 days after heat exposure.

The THI–SCC relationship indicated a positive association with SCC, in relation to increased values of THI, and indicated a breakpoint at 57.3 maxTHI and 43.1 minTHI. It should be noticed that the breakpoint identified for SCC could be affected by the SCC flat pattern recorded within the 74 and 83 maxTHI range. Constant values of SCC, recorded between 74 and 83 maxTHI, affect (reducing) the slope of the second regression line and lead to the identification of a breakpoint for the lower value of THI. This reduction could be estimated at 2 to 3 THI points, and thus it is more reasonable to hypothesize a breakpoint for SCC at around 60 maxTHI and 46 minTHI. The flat pattern is probably a consequence of the measures put in place for maintaining the levels of SCC below the limit of 3.10^5 cells/ml.

The analysis of the THI–TBC relationship showed a positive association and indicated a breakpoint at 72.8 maxTHI and 49.4 minTHI. Milk TBC starts to increase in correspondence with values of THI characteristics of the hot season, confirming what it has been said previously, namely, the increase of the content of TBC in the milk during the summer is strictly related to the greater bacterial growth and higher contamination of the udder compared with the other seasons. To date, there are no studies concerning the relationship between THI or other climatic variables and somatic cell and bacterial count, and this makes it difficult to compare the results of this study. The results provided by this study regarding the relationship between THI and SCC and TBC represent a first indication for understanding the relationship between climatic conditions and changes in these indices.

The two-phase regression analysis indicated a negative association between fat and protein and increasing values of THI and indicated a breakpoint, representing a significant change in the slope, in correspondence with 50.2 and 65.2 maxTHI and 38.0 and 49.4 minTHI for FA% and PR%, respectively. Studies aimed at assessing the threshold of maxTHI, above which milk yield starts to decrease, have been carried out (Igono *et al.*, 1992; Ravagnolo *et al.*, 2000; West *et al.*, 2003). Conversely, still less information is available regarding the relationship between THI and milk fat and protein percentage. In a New Zealand study, Bryant *et al.* (2007) reported patterns for fat and protein with a drop that occurred around the values of 50 and 60 THI, respectively. These results support the findings of this study insofar as they indicate the high sensibility of fat and protein to hot conditions. One should recall that the Bryant *et al.* (2007) study referred to different field conditions than the ones of this study, mainly because of the farming systems adopted. In particular, in the New Zealand study, the dairy cows were raised under a grazing system, whereas in this study the dairy cows were confined within free stall housing with no time at pasture.

Ravagnolo *et al.* (2000) analysed the association between THI and 249 430 first-parity test-day records of milk, fat and protein yields collected from 1990 to 1997. They identified 72 THI as critical threshold because above this point milk, fat and protein yield start to decrease. The discrepancy with our results could be explained by the different experimental conditions. First of all, they analysed first-parity test-day record, whereas we used bulk milk data collected from all parity cows. As is known, heifers generate far less metabolic heat than cows, have greater surface area compared with internal body mass and would be expected to suffer less from heat stress (West, 2003) than mature cows. In addition, one should recall the different genetic merit of the two cow populations analysed. The American study refers to a dairy cow population from the 1990s, whereas this study refers to a dairy cow population from the 2000s. The genetic improvement in milk yield experienced over the last few decades is often in conflict with maintaining homeothermy (Bernabucci *et al.*, 2010). This supports the evidence reported by Bryant *et al.* (2007): cows with high genetic merit were more susceptible to the effects of hot environments than their low genetic merit counterparts. Finally, in the American study, each test-day record was assigned to daily weather records, whereas the THI values used in this study were recorded 2 days before the sampling day.

Second, results of the two-phase regression analysis represent a contribution that pointed out the importance of the minimum THI during hot periods. Our results indicated that minTHI thresholds were similar for protein, SCC and TBC. When minimum daily THI was >46 to 49, an increase in SCC and TBC and a decrease in PR% occurred. In contrast, very low minTHI threshold was found for FA%. This last result confirms that milk fat percentage more than the other milk characteristics is strongly affected by the increasing photoperiod other than heat stress.

The findings reported in this and other studies suggest the existence of a climatic condition threshold, above which fat and protein start to decrease. However, the THI breakpoint values reported in these studies do not agree in indicating one exclusive onset critical condition. Further studies are necessary to understand the phenomena better, defining more accurately the climatic references at farm level that characterize the risk of heat stress, for better assisting farmers in the planning of useful heat management strategies.

Conclusions

Summer was the most critical season for milk characteristics, with negative consequences on the commercialization of high-quality milk. The maxTHI values of 57 and 73 and minTHI of 43.1 and 49.4 for SCC and TBC, respectively, represent the critical threshold above which SCC and TBC start to increase significantly in bulk tank milk. At the same time, the maxTHI values of 50 and 65 and the minTHI of 38.0 and 39.4 for FA% and PR%, respectively, represent the thresholds above which FA% and PR% start to decrease significantly. The THI breakpoints found for FA% and PR% suggests that during the year, heat load is not the main factor accounting for the decrease in bulk milk components.

Although the milk composition values identified in this study were always within the limits of the Italian law for the commercialization of pasteurized milk, there is a risk of exceeding the threshold for the commercialization of high-quality milk during the summer. Low quality leads to a decrease in the milk price paid at cowshed with subsequent economic losses for the farmers. The results of this study could be helpful to dairy cow producers, allowing them to adopt specific heat-abatement measures to counter the unfavourable consequences of heat stress at farm level.

Acknowledgements

This study was supported financially by MiPAAF-FILIGRANA Project (D.M. 25741/7303/11–01/12/2011) and co-supported by FACCE-MACSUR project (D.M. 2660–20.06.2012). The authors gratefully acknowledge the Lombardia Environmental Protection Agency and the Research Unit for Agricultural Climatology and Meteorology (MiPAAF-CRA-CMA) for providing temperature and relative humidity data.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1751731114000032.

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