
DETECTION, CONSEQUENCES AND PREVENTION OF THERMAL DISCOMFORT FOR CATTLE KEPT OUTDOORS IN BELGIUM

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Submitted in fulfilment of the requirements for the degree of Doctor in
Veterinary Sciences, Ghent University, 2015

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ISBN: 9789058644220

2015

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LIST OF ABBREVIATIONS

AICC: Corrected Akaike Information Criterion
ALP: alkaline phosphatase
AS: artificial shelter
BB: Beninksberg
BPM: breaths per minute
CCI: Comprehensive Climate Index in °C
Cl⁻: chlorine
DIM: days in milk
EB: Ename Bos
H: Shannon Wiener Index
HB: Heidebos
HLI: Heat Load Index
HP: Hobokense Polder
KE: De Kevie
KH: Katershoeve
LCT: lower critical temperature
LSM: least square mean
MS: Molenstede
MYX: summed milk yield of the morning of same day and evening before
NEFA: non-esterified fatty acids
NS: natural shelter (in Chapters 2 and 3)
 OR treatment 'no shade' (in Chapters 4 and 5)
OA: open area
PS: Panting Score
Rad: solar radiation in W/m²
RH: % air humidity
RR: respiration rate
RT: rectal temperature
S: structural diversity index (in Chapters 2 and 3)
 OR treatment 'shade' (in Chapters 4 and 5)
SD: standard deviation
SE: standard error
SEM: standard error of mean
Ta: air temperature in °C
Tg: Black Globe Temperature in °C
Tbg: Black Globe Temperature in °C
THI: Temperature Humidity Index
THI_adj: adjusted version of the Temperature Humidity Index
UCT: upper critical temperature
VV: Velpvallei

WBGT: Wet Bulb Globe Temperature in °C
WS: wind speed in m/sec

CHAPTER 1

GENERAL INTRODUCTION

Adapted from: IMPORTANCE OF OUTDOOR SHELTER FOR CATTLE IN
TEMPERATE CLIMATES

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LIVESTOCK SCIENCE (2014) 159: 87–101



Abstract

It is well documented that health, welfare and productivity of cattle in (sub)tropical and cold regions can be improved by measures that mitigate the adverse effects of extreme climatic conditions. In temperate regions, however, the need for and effectiveness of such measures has received much less attention. The aim of this review is to give an overview of the most relevant climatic factors, animal characteristics and adaptation strategies that have to be taken into account when assessing the need for mitigating measures for cattle on pasture, more specifically in temperate areas. Belgian climatic data are used to show that conditions outside the thermo-neutral zone of certain cattle types, possibly leading to cold or heat stress and impairment of production if persistent, occasionally occur even in temperate climates. Such thermal stress is likely to become more common in the future, due to global warming and cattle's decreased capacity for thermoregulation caused by selection for high productivity. Recent research is reviewed to show that the traditional climatic indices and threshold values of the associated heat stress risk classes are outdated, too strongly focused on hot climates, and too general to evaluate heat stress in the different (mainly high-producing) cattle types bred in temperate areas nowadays. Nonetheless, the (currently limited) knowledge on the effect of adverse weather on pastured cattle in temperate climates suggests that providing shelter will benefit their welfare and productivity. Further research is needed, however, to estimate the effectiveness of different types of shelter for different types of cattle (for instance those differing in age, breed, experience and productivity).

KEYWORDS: Cattle, cold, heat, temperate, shelter, shade

1 INTRODUCTION

In most temperate regions, beef and dairy cattle are kept on pasture for at least some part of the year. Pasturing has some important benefits for animal health and welfare, like a decrease in claw and leg problems (Haskell et al., 2006; Hernandez-Mendo et al., 2007). Timing, duration and synchronisation of different behaviours are less restricted on pasture (Bracke and Hopster, 2006; O'Connell et al., 1989) and the greater space allowance also reduces aggression (Kondo et al., 1989; Wierenga and Hopster, 1990). Pasturing can also have benefits related to farm profitability (Dillon et al., 2005), environmental sustainability (Peyraud et al., 2010) and the public image of the beef and dairy sector (van den Pol-van Dasselaar, 2005) as well. On the other hand, it poses certain disadvantages and risks, such as additional labour to move animals (e.g. for milking), a less stable ration quantity and quality, a higher exposure to endoparasites like lungworms and liver fluke, and exposure to adverse weather conditions (van den Pol-van Dasselaar, 2005).

In comparison with temperate, mid-latitudinal areas, summers and winters are long and severe in (sub)tropical and high-latitudinal areas, respectively. In these regions, both livestock keepers and the public as well as scientists have since long been aware of the effects of exposure to cold and heat on livestock behaviour, physiology, welfare and productivity (Collier et al., 1982a; Kadzere et al., 2002a; Silanikove, 2000; Young, 1981) and the effectiveness of preventive measures thereupon (Armstrong, 1994; Blackshaw and Blackshaw, 1994; Gregory, 1995). The importance of prevention of cold and heat stress for cattle in temperate regions, however, is sometimes contested.

Cattle may adapt to chronic situations of relatively mild cold by accumulating energy reserves (body fat and muscle tissue) and by growing subcutaneous fat and thicker coats which provide increased insulation. The potential for such adaptation depends on environmental factors and animal phenotypic and genetic traits. Energy demand and efficiency are determined by body weight and growth rate, as well as by cattle type or breed. Robust and slow growing livestock breeds like the Scottish Highlander, Galloway, Hereford and Aberdeen Angus are characterised by low energy demands and a high potential to accumulate fat on a poor quality diet. As such, they are assumed to be relatively resistant to cold conditions, even under nutritional limitation. Therefore, these breeds are often kept outdoors year-round, for example for the purpose of grazing management in nature reserves (Wallis de Vries, 1994).

On the other hand, faster growing and highly productive commercial beef and dairy breeds such as the Holstein, Jersey, Charolais, Limousin, Blonde d'Aquitaine and Belgian Blue, have higher basal metabolic rates, growth rates and thus higher energy requirements (Wallis de Vries, 1994). These breeds are considered less suited to be kept in a wide range of climatic conditions and, in deep winter, they are generally kept indoors. Summer conditions are generally – but maybe unduly – considered less problematic for cattle in temperate areas, and the animals often stay on pasture for most of the time. However, on the hottest summer days, unsheltered outdoor conditions can be assumed to be difficult to cope with, especially for high producing dairy cattle, as will be elaborated further in this review. Next to seasonal challenges to thermal tolerance, livestock may also suffer thermal stress during intermittent extreme weather events such as hot spells, cold spells or storms. In these cases there is much less potential for adaptation. However, for livestock keepers, such extreme weather events seem to pose a greater challenge in terms of management, since they are unpredictable and they will thus require provisions for mitigation to be present at all times, requiring labour and economic investment that will not necessarily or immediately pay off. Also the public expresses concerns about the welfare of outdoor-housed cattle when climatic conditions are, or appear to be, severe. Although governmental services and animal protection organizations raise awareness and provide advice related to thermal comfort, legislation is often lacking, inconclusive or unclear about which measures (indoor or outdoor housing, with or without additional measures such as shade or shelter on pasture) ought to be taken when in order to prevent thermal stress.

2 CLIMATIC VARIABLES THAT CONTRIBUTE TO THERMAL STRESS

The physiological responses of animals to low and high temperatures are often presented on a bidirectional continuum divided into different zones (Fig. 1). Within the zone of thermal comfort an animal has an optimal experience of comfort in relation to environmental temperature. Within the thermo-neutral zone, i.e., when the ambient temperature is between the lower critical temperature (LCT) and the upper critical temperature (UCT), it has to invest only a minimum of energy in maintaining its body temperature (e.g. vasodilatation of peripheral blood vessels provides enough cooling) (Silanikove, 2000). Once the ambient temperature ventures outside of the thermo-neutral zone, the animal is required to increasingly invest metabolic energy in heat dissipation or heat production. The energy available for other

bodily functions will diminish. If this situation persists, the animal experiences stress, and health and production are impaired. Outside the zone of homeothermy the thermoregulatory mechanisms fail to keep body temperature within the normal range. Health declines even further, which may eventually lead to death.

Traditionally, the boundaries of the thermo-neutral zone are defined in terms of (ambient) temperature and are species-specific, but they do not take other climatic variables or animal factors such as age, productivity and linked metabolic rate into account. Whether or not an animal experiences thermal stress does not only depend on air temperature, but on other weather factors as well. For instance, the UCT of cattle is assumed to lie around 25-28°C (e.g. Collier et al. (1982)). But as humidity and solar radiation contribute to thermal comfort too (Hahn et al. (2003))(Rosselle et al., 2013), lower temperatures may already induce heat stress in case of high humidity or intense solar radiation. Although adult cattle in general are quite resistant to low ambient temperatures (Table 1), rain or snow wets their coats, thereby decreasing their insulation value and greatly increasing evaporative heat loss. Especially in combination with convective heat loss (wind chill), such exposure to precipitation may drastically reduce skin temperature (Schutz et al., 2010) and can thus cause cold stress at higher ambient temperatures.

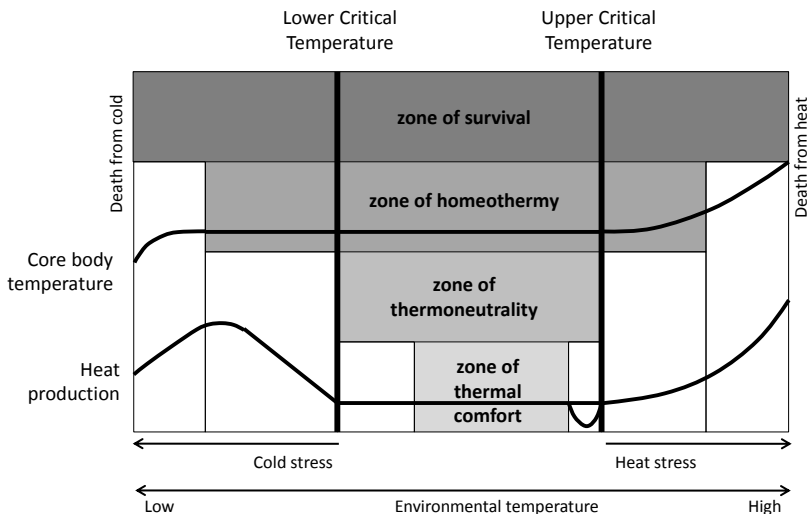


FIG. 1. Schematic representation of different temperature zones in relation to thermal stress. Adapted from Silanikove (2000).

TABLE 1. Lower Critical Temperatures (LCT) in dry and still air for different cattle types.

Cattle type		LCT (°C) ^a
Calf	New-born	9
	1 month old	0
Store	Maintenance	-16
	0.4kg LWG/d	-30
	0.8kg LWG/d	-32
Beef cow	1.5kg LWG/d	-32
	Maintenance	-21
Dairy cow	9l milk/d	-17
	23l milk/d	-26
	36l milk/d	-33

^a Source: Australian Agricultural Council. Ruminants Subcommittee (1990).

In order to make sound management decisions, different climatic parameters can be combined into a single measure to quantify the degree of discomfort and potential production loss. This has resulted in the continued development of climatic indices. These indices are usually associated with risk classes reflecting the effect of the transgression of threshold values on biological response functions such as body temperature, respiration rate or milk production (Hahn et al., 2003). At present, the standard for classifying moderate to hot conditions in livestock research and management is the Temperature-Humidity Index (THI) (Equation 1), developed by Thom (1959). It forms the basis for the Livestock Weather Safety Index, that defines four heat stress risk classes (Table 2), as a guide for heat stress mitigation (LCI, 1970). For example, in the 'alert' and higher risk zones provision of shade and cow sprinklers is recommended and livestock personnel is advised to regularly check cattle behaviour and breathing rate and to limit handling and moving of animals (Hahn et al., 2003) <http://www.coolcows.com.au/go-on-alert/take-action-in-the-heat.htm>; accessed on 21/08/2013).

TABLE 2. Heat stress risk classes according to the Livestock Weather Safety Index (LCI, 1970)

THI value ^a	heat stress class
THI < 74	normal
74 ≤ THI < 79	alert
79 ≤ THI < 84	danger
THI ≥ 84	emergency

^a THI= Temperature Humidity Index (equation 1)

Cold stress is most often quantified by means of the Wind Chill Index (WCI), originally developed to assess the risk of frostbite on human skin (Siple and Passel, 1945). An adapted formula is used in cold stress research in cattle (Equation 2)(Tucker et al., 2007). WCI values can be interpreted as an apparent temperature, and are usually expressed in degrees Celsius or Fahrenheit. For humans, Environment Canada’s WCI risk classes predict uncomfortable conditions with risk of hypothermia and greater risk of hypothermia and frostbite, at WCI’s below -10 and -28 respectively (<http://www.ec.gc.ca/meteo-weather/default.asp?lang=En&n=5FBF816A-1>; accessed on 21/08/2013). For cattle, to our knowledge, no scientifically validated cold stress risk classes in terms of WCI have been developed. We can only compare its values to established LCT’s for cattle to get a rough idea of the potential impact on comfort and physiology.

$$THI=0.8 \times T + [RH \times (T - 14.4)] + 46.4 \quad (1)$$

$$WCI = 13.12 + 0.62 \times T - 13.17 \times [WS]^{0.16} + 0.40 \times T \times [WS]^{0.16} \quad (2)$$

where T = air temperature in °C, RH = relative air humidity in decimal form (e.g. 0.60, not 60%), and WS is wind speed in km/h

Recently, both the THI and WCI have been criticised for not taking into account all climatic parameters that influence thermal comfort (Hahn et al., 2003). More recent climatic indices – such as the Comprehensive Climate Index (Mader et al., 2010) or the Heat Load Index (Gaughan et al., 2008) - incorporate the effects of temperature, humidity, wind speed as well as solar radiation in order to improve the assessment of cold or heat stress risk. Finally, also the duration of exposure to aversive conditions will greatly influence animals’ responses. This is frequently overlooked. For example, even though most heat stress research uses the original Livestock Weather Safety Index’s heat stress categories, it is unclear for how long the THI must have exceeded the threshold values in order to cause what effects, so after what time we can speak of alert, dangerous or emergency situations. In practical management situations it seems logical to use the instantaneous values of this heat stress index, regarding the focus on immediate measures when thresholds are transgressed. In more fundamental research, the duration of climatic stress has received somewhat more attention. For example, this has led to the development of an Accumulated Heat Load (AHL) model coupled to transgression of threshold values for the Heat Load Index (HLI) of Gaughan et al. (2008). As there seems to be a lack of consensus on the optimal index, a wide range of climatic indices have been developed and used (Eigenberg et al., 2005; Gaughan et al., 2008; Mader et al., 2006), which complicates comparisons between different studies.

3 ANIMAL FACTORS INFLUENCING THERMAL TOLERANCE

Thermal tolerance depends on several animal characteristics and their interactions with environmental factors (Berman, 2005). For example, it is widely accepted that young animals, due to the higher body surface/volume ratio, gain and lose heat from the environment more easily. In addition, neonatal calves cannot yet rely on heat production by ruminal fermentation in cold conditions (Collier et al., 1982a). Consequently they are much more susceptible to thermal stress compared to adults (Table 3).

TABLE 3. Animal factors influencing tolerance to thermal conditions.

Acclimatisation		
	Cattle acclimatised to cold conditions respond to a sudden elevation of environmental temperature with highly elevated rectal temperatures and respiration rates. No such reaction in cattle adapted to thermoneutral conditions.	(Robinson et al., 1986) (Webster et al., 1970)
Own (basal) metabolic heat production		
Breed	Better heat tolerance for <i>Bos indicus</i> than <i>B. taurus</i> (due to lower basal metabolic rate).	(Johnston et al., 1958)
	Thermoregulatory behaviour differs between breeds.	(Langbein and Nichelmann, 1993)
Productivity	Lower heat tolerance for high-producing dairy cattle due to high heat increment of milk production.	(Collier et al., 1982a; Fuquay, 1981; Kadzere et al., 2002b)
	Thermoregulatory behaviours differ between production stages.	(Tapkl and Sahin, 2006)
Efficiency of heat exchange		
Breed	Sweat glands lie closer to the skin surface and have a greater size and higher density in <i>Bos indicus</i> than in <i>B. Taurus</i> .	(Nay, 1956)
Ratio body surface/volume	Inversely related to the size of the animal → Lesser heat and cold tolerance for calves than adult cattle.	(Collier et al., 1982a)
Coat colour	Lower heat tolerance for dark animals (absorb more radiation).	(Becerril et al., 1993; Cena and Monteith, 1975; Hansen, 1990)
Coat insulation efficiency	Greater heat tolerance for calves with very short sleek hair than for calves with a deeper coat.	(Olson et al., 2003)
Body condition	Greater tolerance to cold, rainy and windy conditions for dairy cows with higher body condition.	(Tucker et al., 2007)
	Lower heat tolerance for beef cows with higher body condition scores.	(Brown-Brandl et al., 2006)
Other factors	e.g. temperament, health history, nutrition and hydration.	(Brown-Brandl et al., 2006)

As there are many such animal factors (Table 3), thermal tolerance may vary considerably between different cattle breeds, herds, and even individuals within the same herd.

Productivity is an animal-related factor of major interest, as the deliberate selection for high-producing animals is likely to render some typical temperate cattle breeds more susceptible to heat stress. In the most common dairy breed used in temperate regions, the Holstein, genetic selection for high milk yield has doubled the yield per cow in the last 40 years (Oltenucu and Broom, 2010). Such a high productivity requires a high metabolic rate, but in early lactation energy intake is typically insufficient to keep up with the energy demand and the cow has to mobilise body reserves. Early lactation is thus characterised by a 'negative energy balance' status (Oltenucu and Broom, 2010). In addition, the high metabolic rate results in the production of considerable metabolic heat (Collier et al., 1982a; Fuquay, 1981; Kadzere et al., 2002b). In case of additional heat load imposed on the animal by the environment, body heat is dissipated insufficiently. This is compensated by a lowered feed intake and associated decline in metabolic rate, both decreasing own heat production. By consequence, the cow will draw on body reserves so that a high milk yield can be retained, which aggravates the 'negative energy balance' of cows in early lactation. These factors increase high-producing dairy cows' susceptibility to heat stress. In addition, it must be mentioned that also other physiological cooling mechanisms, such as increased respiration and heart rate require some extra energy from the animal. A similar issue regarding productivity arises in beef cattle. In the Belgian beef cattle industry, for example, the selection for leaner meat and more beef per animal resulted in selection for double-muscled beef animals or their use in cross-breeding. But the double muscled condition, caused by an aberrant myostatin gene (Grobet et al., 1998), reduces oxygen transport efficiency (Lekeux et al., 2009) and pulmonary and cardiac function (Amory et al., 1992; Gustin et al., 1988). In addition, heat transfer from the animal to the environment is reduced due to the decreased surface/volume ratio and the increased muscle mass and double-muscled beef cattle are thus more susceptible to heat stress (Halipre, 1973).

Because several animal factors influence the tolerance of cattle to aversive weather conditions, the need for preventive measures may vary greatly between breeds and individuals, even within the same herd. Animals can also adapt to climatic conditions to some extent, and particularly so when changes are gradual. Nonetheless, the high productivity of beef and dairy cows increases the risk for heat stress, making it an increasingly important consideration in temperate regions.

4 POTENTIAL AVERSIVE EFFECTS OF THERMAL STRESS

When temperature increases, cattle will first react behaviourally. They will avoid orientating their long axis, i.e. their flanks, to the sun and prefer windy locations such as ridge tops or wind-exposed slopes (Senft et al., 1985). They will seek shade from trees, constructions or even companions in order to reduce heat absorption. Choice tests have shown the importance of such shade seeking behaviour. At high air temperatures, dairy cows prefer to stand in the shade rather than to lie outside shade, even after a lying deprivation of 12h (Schutz et al., 2008). Increased water intake will also have a direct cooling effect, as indicated by the decrease in respiration rate (Lanham et al., 1986). Cattle are also reported to submerge their body (fully or partially) in pools or drinking troughs to cool down (e.g. Clarke and Kelly (1996)). In addition, general activity is reduced to minimise the animal's own heat production and feed intake declines, which reduces heat production by ruminal fermentation.

The observed behavioural effects are intertwined with several physiological alterations. Heat is dissipated through increased vasodilatation, sweating and panting (West, 2003). Mild and severe heat stress increase energy requirements by 7% and 25%, respectively (NRC, 2001). However, heat production is lowered by a reduced metabolic rate (Bernabucci et al., 2010), which can be related to a decrease in production of thyroxin and triiodothyronine (Muller et al., 1994; Scott et al., 1983). Since feed intake is reduced as well when the ambient temperature rises, the animal is at risk of entering a state of negative energy balance and must rely on mobilisation of reserves from adipose tissue and skeletal muscle (Bernabucci et al., 2010). A negative energy balance reduces milk yield and composition (Collier et al., 1982b; Gwazdauskas, 1985; West, 2003) and the metabolic and respiratory adaptations may trigger changes in blood acid-base chemistry and blood minerals (Abeni et al., 2007; Calamari et al., 2007). Hyperventilation can cause respiratory alkalosis, which may be corrected by bicarbonate loss via the urine. Sometimes, however, overcompensation leads to acidosis. In combination with reduced fibre intake, slug feeding and decreased salivary buffering caused by excessive drooling may also reduce ruminal pH. On the longer term, subclinical ruminal acidosis can contribute to the development of laminitis or other lameness problems (Nocek, 1997; Shearer et al., 1999; Stone, 2004). Complex endocrine alterations will reduce reproductive performance (Gwazdauskas, 1985). Thus, it is clear that heat stress may affect behaviour and physiology, with detrimental effects on welfare and production in both dairy and beef cattle.

Negative effects on production are generally assumed to start at a THI value of 72 or even 74, based on mid-20th century studies performed on cows which produced much less milk than modern dairy cows do (Zimbelman et al., 2009), primarily in the USA and tropical regions. But also Eastern European studies used the same threshold to illustrate the relevance of heat stress for milk production in Mediterranean-temperate climate. In Croatia, for example, daily average THI values measured in the stable regularly exceeded 72, causing a significant decrease in milk yield (from 17.7 to 16.8 kg/day/cow), milk fat and protein content (Gantner et al., 2012). More recent research on dairy cows yielding more than 35 kg of milk per day showed that a daily average THI of 68 already results in a milk loss of 2.2 kg/day, suggesting that 68 is a better heat stress threshold for such cows (Zimbelman et al., 2009). It should be noted, however, that this threshold value – like the thresholds used in the Livestock Weather Safety Index - remains rather arbitrary and is likely too general to evaluate heat stress in different cattle types. Cows bred in, and adapted to, temperate conditions could be more sensitive and thus have an even lower heat stress threshold, as suggested by recent Western European studies. For example, Brügemann et al. (2011) identified a daily average THI value of 60 as the threshold for declining milk protein content in German Holstein cows. Hammami et al. (2013) evaluated six heat stress indices and for each of them they identified a new threshold beyond which milk production, fat and protein content start to decrease. Subsequently, they calculated the rate of decline per index unit, for each of these variables. In terms of the classical THI, a value of 62 was proposed as a new threshold for Western European Holstein cows, below which milk yield declines with 0.164 kg/day/cow. The above large-scale studies already provide evidence that the commonly used threshold values to define heat stress risk classes should be adapted or differentiated for different cattle types and productivity levels.

In addition, the existing heat stress thresholds have specifically been developed for dairy and beef cattle in farming settings. Cattle in grazing management of nature reserves, on the other hand, are often of different breeds. Especially year-round grazing management is mostly carried out by robust cattle breeds, originally intended as beef or work breeds (such as the Galloway, Scottish Highlander or Aberdeen), that are assumed to be relatively resistant to cold conditions, even under nutritional limitation (Wallis de Vries 1994). However, some characteristics - such as their thick hair coat, heavy posture and dark colour (Brown-Brandl et al., 2006) - might render them less tolerant to heat load than other breeds. For these cattle, specific heat and cold stress thresholds in terms of THI and/or other climatic indices, still have to be developed and validated.

Whereas the recent dairy cattle heat stress thresholds in terms of the THI were based on heat stress effects on productivity (milk yield and milk composition), heat stress thresholds for cattle in grazing management (especially year-round grazing management) ought to be based on other heat stress effects, because these cattle serve no production purpose. For robust cattle breeds in nature reserve management, heat stress thresholds could be based on externally detectable indications thermal discomfort (such as panting and drooling), like the developers of the Heat Load Index did for Angus beef cattle (Gaughan et al. 2008). Other physiological indicators of discomfort or stress, such as systemic glucocorticoid levels as a measure of HPA axis activity (Mormède et al., 2007), can also be used. However, the collection of samples might pose a challenge because cattle grazing free in nature reserves might be difficult to approach.

Another limitation of many heat stress studies –including the above-mentioned European studies on dairy cattle – is the lack of differentiation between different types of housing and heat alleviation strategies. For example, heat stress thresholds -in terms of THI or other indices - should vary with exposure to solar radiation and thus indoor vs. outdoor housing, with or without additional heat abatement. Thus, further research would be useful to establish more scientifically validated threshold values for different heat stress risk classes for different cattle types under different management conditions, based on effects on welfare, health and production.

It is reasonable to assume that cold stress, as opposed to heat stress, is a lesser problem in temperate regions because cattle are generally kept indoors during winter (e.g. Krohn et al., 1992) and because adult cattle can endure relatively low temperatures (Table 1). However, in late autumn and early spring, exposure to rain and wind may cause conditions below the LCT of adult cattle, even in temperate regions. The most important effect of sub-LCT conditions is an increase in maintenance energy requirement to maintain body temperature. Feed digestibility is reported to decrease, due to an increased passage rate of feed through the digestive tract (Kennedy et al., 1976). For young calves (especially under three weeks of age), energy requirements quickly increase with decreasing ambient temperature (Table 4). The energy requirement of adult beef cows is assumed to increase with $0.0007 \text{ Mcal/BW}^{0.75}$ for each degree that the ambient temperature differs from 20 °C (NRC, 2000). The change in energy requirement for lactating dairy cows in cold environments is probably minimal because of their high heat production, at least if they are kept dry and unexposed to wind (NRC, 2001).

TABLE 4. Effect of environmental temperature on calves' energy requirement.

Temperature (°C)	% increase in Maintenance Energy Requirement (MER) ^a	
	Birth to 3wk of age ^a	>3wk of age ^b
20	0	0
15	13	0
10	27	0
5	40	13
0	54	27
-5	68	40
-10	86	54
-15	94	68
-20	108	81
-25	121	94
-30	134	107

^a Source: NRC (2001).

^{a,b} Calculated on the basis of lower critical temperatures of 20° C and 10° C, respectively.

Adult cattle will usually adapt to the increased energy demand by increasing their feed intake and reducing energy expenditure by reducing activity (Olson and Wallander, 2002; Malechek and Smith, 1976) and seeking shelter. For example, in winter cattle avoid exposure to wind (Houseal and Olson, 1995; Senft et al., 1985) and maximise radiant heat absorption by orienting the major axis of their bodies at a right angle to the sun (Malechek and Smith, 1976). But they may have difficulties to adapt in severely or persistently cold and rainy, snowy or windy conditions, or when feed of sufficient quantity and quality is lacking. For example, Webster et al. (2008) reported an increased thermal stress response in unsheltered cows in cold, windy and rainy conditions (Table 5). Eventually, the cold stress effects may lead to an increased incidence of weak calf syndrome and reduced fertility and lactation (Young, 1981). As calves are more susceptible to cold stress they are usually kept indoors in winter. Especially in rainy conditions this is very important, as contact with cold water may induce severe clinical conditions such as subcutaneous oedema and haemorrhages in peripheral tissues (Olson et al., 1980).

It is clear that heat and cold stress are relevant problems for cattle health, welfare and production. Yet a thorough assessment of the prevalence and the severity of the resulting problems and associated costs in temperate climate is lacking. Such information would be highly useful when evaluating the need for substantial capital investment in the provision of shelter.

5 OCCURRENCE OF EXTREME CONDITIONS IN TEMPERATE CLIMATES

Heat stress conditions have been shown to occur regularly in the European Mediterranean regions (Abeni et al., 2007; Gantner et al., 2012). Research on the occurrence of extreme weather events in cooler temperate areas is sparse. Belgian weather data are presented here as a case study to assess the prevalence of cold and heat stress risk conditions in a typical temperate region, according to established climatic indices. Belgium has a typical temperate climate due to its position at average latitude at the western edge of the European continent. The prevailing westerly winds carry humidity landward, thus providing a rainy climate characterised by cool and humid summers and relatively mild and rainy winters (<http://www.meteo.be/meteo/view/nl/357714-Algemeen.html>; accessed on 21/08/2013). Daily weather data (maximum, minimum and average temperature, maximum and average wind speed over 24 hours, and one measurement of relative humidity per 24 hours) were obtained for the period between February 1994 and May 2005, from a weather station located in Melle (central Belgium). Per day, average THI and WCI and, subsequently, percentages of days exceeding different heat and cold stress thresholds were calculated in order to evaluate the occurrence, frequency and severity of heat and cold stress. These daily averages were compared to established thresholds. In case of cold conditions we used the boundaries of Environment Canada's WCI risk classes, since for cattle no risk classes in terms of WCI have been developed. In the case of warm conditions we use the THI values of 62 and 68, since an objectively and readily detectable physiological effect - milk yield reduction – has been shown to occur to start at a daily average THI of about 62 according to Hammami et al. (2013) and 68 according to Zimbelmann et al. (2009). This informs us about the potential risk for what most will agree to call thermal stress. The concept of stress is subject to personal interpretation, however. Most scientists distinguish between stress or distress and discomfort and will agree that a feeling of discomfort can arise well before readily, physiologically detectable signs of stress or distress arise (e.g. Silanikove, 2000). In the case of thermal stress or discomfort, for example, animals may experience discomfort and their homeostatic mechanisms may be challenged temporarily on the coldest or hottest moment of the day. This would go unnoticed when we would only evaluate daily averages of THI and WCI. Therefore, we also calculated daily maximum THI and minimum WCI, to assess the occurrence of thermal conditions that might be uncomfortable and during which animals could already benefit from shelter.

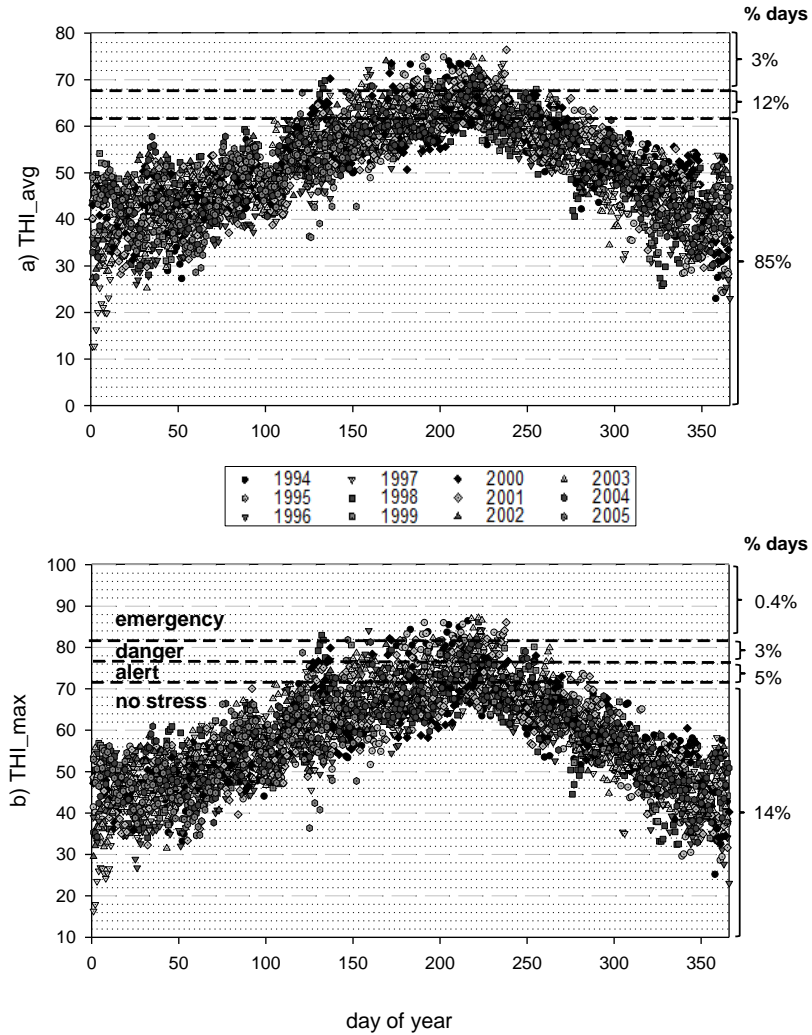


FIG.2. Yearly course of daily average (a) and daily maximum (b) Temperature Humidity Index (THI, according to Equation (1)) in Melle, Central Belgium (Biocentre Agri-Vet, Ghent University) for 1994 until 2005. The percentage of days above and below the heat stress thresholds of 62 and 68 (a) and the percentage of days in the different heat stress risk classes of the classical LWSI (b) are indicated on the left hand of the figure.

More detailed supplementary figures, with the course of the daily average THI (Fig. S1) and daily maximum THI (Fig. S2) per individual year, can be found at the end of Chapter 1.

Figure 2a shows that, over the course of the examined period, the daily average THI mostly fell below 62, as expected. However, high-producing dairy cows may sometimes have experienced heat stress (production losses), since daily average THI exceeded 62 and 68 on 15% and 3% of the days, respectively. For 28 of occasions (22%) where daily average THI exceeded 68, the heat stress situation lasted for more than one day, while it lasted for about a week (six, seven or eight days) in seven of these cases (5%). Daily maximum THI exceeded 74 and 79 (Livestock Weather Safety Index thresholds to define an alert situation and a dangerous situation) on respectively 8% and 3% of all days studied (Figure 2b).

Though from the LWSI it is unclear for how long the THI must have exceeded these threshold values in order to cause what effects, Brown-Brandl et al. (2005) demonstrated at least some physiological indications of thermal stress or discomfort (like elevated respiration rate and body temperature) during the hottest part of the day on days where maximum THI exceeded 74. We can thus assume that the observed Belgian outdoor summer conditions will quite regularly cause temporal discomfort and that provision of shelter to prevent this can be useful.

The daily average WCI fell below zero on 20% of the days studied (Figure 3a). It rarely reached below -10 (1% of the days; restricted to the period between late autumn and early spring). The daily average WCI very exceptionally dropped below -15 and this was restricted to the mid-winter period (end of December and beginning of January). Daily average WCI never dropped below -20. The daily minimum WCI fell surprisingly often below zero, 56% of the days studied (Figure 3b). It reached below -10 on 14% of the days (from early autumn till late spring). In winter (mid November till mid March) the daily minimum WCI dropped relatively often to -20 and occasionally below that. Daily minimum WCI never dropped below -28. If we compare these WCI values (to be interpreted as apparent temperatures) to the LCT for different cattle types (Table 1), we can conclude that daily average WCI never fell below the LCT of adult cattle. The lowest range of observed values for daily minimum WCI, however, did occasionally fall below the LCT of beef cattle and lower producing (or dry) dairy cattle.

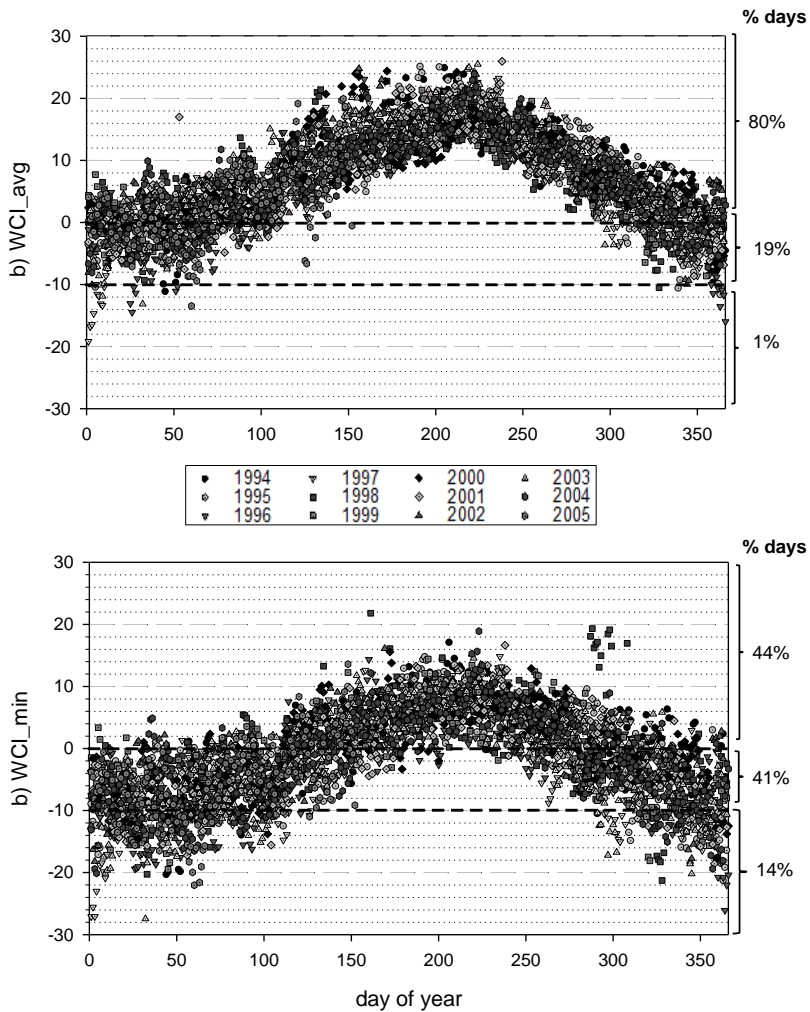


FIG.3. Yearly course of daily average (a) and minimum (b) Wind Chill Index (WCI, according to Equation (2)) in Melle, Central Belgium (Biocentre Agri-Vet, Ghent University) for 1994 until 2005. The percentage of days above and below Environment Canada’s WCI thresholds to predict slight discomfort and greater discomfort and risk of hypothermia, 0 and -10 respectively, are indicated in the table on the right of each figure.

More detailed supplementary figures, with the course of the daily average WCI (Fig. S3) and daily minimum WCI (Fig. S4) per individual year, can be found at the end of Chapter 1.

We can thus assume that the Belgian outdoor winter conditions observed in this case study would not cause real stress for healthy and well fed adult cattle, but might temporarily cause discomfort. In addition, previous research (Table 5) has indicated that a WCI between -10 and -20 falls below the LCT of calves and adult cattle that are not well adapted. At such WCI values, the use of shelter will increase or - if shelter is absent and these conditions persist (e.g. a week or longer) - physiological stress responses will be triggered (Table 5). In our case study, for seven days out of the 33 (21%) where daily average WCI reached below -10, this condition lasted for more than one day, while it lasted for nine days on one occasion. For cattle in less than optimal health or body condition or not adapted to outdoor life in cold conditions, extreme winter conditions in temperate areas may thus be stressing and require provision of shelter. In addition, the occurrence of extreme weather conditions is expected to increase with global warming (IPCC, 2007). In Europe and other temperate areas the occurrence of high temperatures and heat waves has clearly increased over the latest decades. These trends are expected to continue into the 21st century (Table 6).

TABLE 5. Examples of potential effects of persistence of low Wind Chill Indices (WCI's) on beef and dairy cattle's welfare.

Study	Webster et al., 2008	Morgan et al., 2009
Cattle type & location	Non-lactating Holstein-Friesian cows, North New Zealand	Aberdeen x Limousin suckler cows, South Scotland
Duration of exposure	1 week	4 * 3 weeks
Min WCI	-7.7	-14,1 ^a
Max WCI	6.8	-9.4 ^a
Mean WCI	-0,3	-11,3 ^a
Shelter?	No	Yes
Effect on cattle	Several physiological stress responses: reduced lying time, increased plasma and faecal cortisol and decreased white blood cell numbers	In the first two periods effective outside temperature fell below estimated LCT ^b , and the use of shelter (trees, ring feeder and straw bales) increased

^a Based on mean WS and minimum temperature, given by the author, ^b Calculated according to Blaxter (1977) and NRC (2000), based on estimates of the animals' total insulation value (based on tissue and coat insulation value and wind speed) an heat production (based on calculations of metabolizable energy intake, energy for maternal and foetal growth and body surface area) (Morgan et al., 2009)

TABLE 6. Gross lines of ongoing (last decades) and expected future changes in occurrence of extreme climatic conditions - as potential causes of cold and heat stress for livestock kept in pasture – for temperate areas in general and Europe in specific.

	Trends over last decades		Expected future trends	
High temperatures				
Temperate areas	↑ frequency and intensity of hot days + nights Very likely (>90%) (IPCC, 2007)	↑ frequency of heat waves/warm spells Likely (>66%) (IPCC, 2007)	↑ frequency and intensity of hot days + nights Virtually certain (>99%) (IPCC, 2007)	↑ frequency of heat waves/warm spells Very likely (>90%) (IPCC, 2007)
Europe	↑ mean annual temp. (+ 0,8 °C) (Maracchi et al., 2005)		↑ summer temperature (stronger in N than S) (Meehl and Tebaldi, 2004)	↑ frequency, duration and intensity of heat waves (Meehl and Tebaldi, 2004)
Low temperatures				
Temperate areas	↓ frequency and intensity of cold days + nights Very likely (>90%) IPCC (SRES-based) projections		↓ frequency and intensity of cold days + nights Virtually certain (>99%) IPCC (SRES-based) projections	
Europe	↑ mean annual temperature (+ 0,8 °C) (Maracchi et al., 2005)		↑ winter temperature (stronger in E than in W) (Maracchi et al., 2005)	
Heavy rainfall				
Temperate areas	↑ frequency of heavy rainfall Likely (>66%) IPCC (SRES-based) projections		↑ frequency of heavy rainfall (especially in high and mid-latitude areas) Very likely (>90%) IPCC (SRES-based) projections	
Europe	North: ↑ rainfall (up to +40%) South: ↓ rainfall (up to -20%) (Maracchi et al., 2005)		North: ↑ rainfall South: ↓ rainfall (Maracchi et al., 2005)	

The Belgian case-study and global warming predictions indicate that cold and heat stress are increasingly relevant risks for cattle in the western European climate if kept outdoors without shelter. Unsheltered high-producing dairy cows will (occasionally) experience heat stress and discomfort (more regularly) in the typical temperate summer. Thus, we confirm the need for heat stress mitigation strategies. There are no validated cold stress risk zones for cattle, based on WCI, and there are no clear predictions for future cold stress incidence. Based on the comparison of the observed WCI values in our case study to established LCT's for different cattle types, it seems that dry outdoor winter conditions in Belgium will rarely cause real cold stress for healthy adult cattle, but might still temporarily cause discomfort. It can be expected that this discomfort increases (and potentially develop into a condition of actual cold stress) when adding the effects of precipitation in an unsheltered pasture.

6 MITIGATION OF HEAT STRESS BY MAN-MADE SHELTER

Heat stress can be mitigated by actively cooling or by providing shade. Wetting animals with sprinklers, showers or fine mist is most effective in dry climates (Armstrong, 1994). It has a rather limited use in more humid temperate areas, although its effectiveness can be improved when combined with convective cooling (Armstrong, 1994). Providing shade, to the contrary, is an easier and more cost-efficient protection against heat stress on pasture. Many studies have illustrated the beneficial effects of providing shade to heat-stressed cattle in hot climates. Cows with access to shade eat more and rest, drink or linger around the drinking trough less (Shultz, 1984). Lower concentrations of corticosteroids in blood plasma (Ingraham, 1979; Muller et al., 1994) indicate that cows with access to shade have lower stress levels than cows without access. Shade can also reduce core body temperature, respiration rate and panting (Brown-Brandl et al., 2005; Hansen, 1990; Valtorta et al., 1997). Positive effects of shade on the performance of cattle kept in hot climates include increased grazing and dry matter intake, resulting in increased weight gain (Gaughan et al., 2010; McDaniel and Roark, 1956; Mitlohner et al., 2001). Conception rate, calf birth weight, milk yield, and milk fat and lactose yield are also increased by shade provision, whereas somatic cell counts are decreased (Collier et al., 1982b; Davison et al., 1988; Roman-Ponce et al., 1977). The effects of shade provision have been studied far less in temperate regions. Nonetheless, in such regions less elevated body temperature and higher milk yield have been reported for cows with access to shade (Kendall et al., 2006; Tucker et al., 2008).

Artificial shelter design is subject to a number of critical factors. First, the shelter should be spacious enough for the size of the herd. Generally, 3.5 up to 6.5 m² of shade per animal is recommended (Armstrong, 1994; Higgins, <http://www.ca.uky.edu/agc/pubs/aen/aen99/aen99.pdf>; accessed on 21/08/2013). These dimensions seem to be arbitrarily determined, although they do roughly correspond with the recommended minimum amount of space needed to allow the animals to lie, rest and pass each other comfortably. For example, the International Commission of Agricultural and Biosystems Engineering recommends 5.7m² of lying space per animal for Holstein-Friesian cows (CIGR, 1994b) and 3.28 m² of lying space per animal for beef cows of 700kg (CIGR, 1994a) in bedded freestalls. A cost-benefit analysis for determining the optimal (in terms of animal welfare and productivity) amount of shade depending on herd size would be a useful avenue for further research. Overcrowding and inappropriate shelter design

may also hinder air movement and thus lead to heat accumulation due to insufficient convective cooling (Mader et al., 1999). In addition sufficient ventilation is needed to allow the floor surface to dry out and for providing convective cooling, especially in humid conditions (Armstrong, 1994) Shelter material also affects the effectiveness of artificial shade constructions. Metal roofs are often used because of their durability and low cost and maintenance requirements. However, care must be taken that they emit as little solar radiation to the cows as possible, which is achieved by placing the roof sufficiently high above the animals, by applying a reflective coating on the upper surface, and/or by insulating the roof (Armstrong, 1994; Blackshaw and Blackshaw, 1994). Hay and straw are very effective roof materials due to their high insulating value, but these are not very durable and may attract and harbour pests (Blackshaw and Blackshaw, 1994). An alternative to solid shelter constructions for preventing heat stress is shade cloth. Eigenberg et al. (2010) evaluated different types of polyethylene shade cloth based on predicted cattle respiration rate (from climatologic measurements under and outside of shade), grouped into three heat stress categories: normal, alert and danger (Figure 4). All of the shade cloths (nearly) eliminated the 'danger' situation that was observed when animals were exposed to the sun. Cloths reducing solar radiation by 100% fitted with a reflective coating on top reduced the time in the 'alert' zone by 41%, whereas cloths that reduced solar radiation by 60 % with and without a reflective coating reduced the time in the 'alert' zone by 3% and 8% respectively. An important advantage of shade cloth is the low weight, which allows construction of simple portable or movable structures. These are ideal for rotational grazing systems and to prevent over-trampling and manure build up in one specific place (Armstrong, 1994).

7 MITIGATION OF COLD STRESS BY MAN-MADE SHELTER

Comparisons of weight gain (McCarrick and Drennan, 1972), carcass quality, estimations of energy demand, immune function and behaviour (Hickey et al., 2002) between steers housed indoors and on outdoor out-wintering pads, suggest that in Irish winters growing beef cattle can be housed outdoor, even without wind-shelter. Scandinavian research, on the other hand, stresses the importance of rain shelter, wind breaks and lying places with dry bedding for dairy heifers (Redbo et al., 2001), dairy bulls (Tuomisto et al., 2009), beef cows (Manninen et al., 2007) and suckler cows (Manninen et al., 2008). It was concluded that if such facilities are provided, cattle can be housed outside

year-round without severe negative effects on productivity, health or welfare. It should be noted, however, that these studies took place in conditions with low wind speed and relatively little precipitation, in forest paddocks where vegetation likely served as an additional windbreak. The need for shelter is likely greater in more open pastures, especially in windy and rainy conditions. In Canadian winter conditions, weaned beef bull calves provided with shelter have higher gain rates than calves without shelter (Kubisch et al., 1991). Under moderately cold New Zealand winter conditions (six weeks with minimum and maximum wind chill of -3.9 and -9.9°C respectively and minimum and maximum rainfall of 35 and 118 mm/24h, respectively) shelter significantly improved animal welfare (i.e. it increased the time spent lying down and decreased faecal glucocorticoid, thyroxin and NEFA concentrations). Dairy heifers also grew faster when provided shelter in their paddocks under New Zealand winter conditions (Holmes et al., 1978).

Whereas the roof of an artificial shelter provides protection against precipitation, walls can be added on one or more sides to break cold and wet winds. As such, these walls are best placed perpendicular to the direction of prevailing winds. Shelters are preferably located somewhat higher - never lower - than the surrounding terrain, to prevent water from flowing towards the shelter and accumulating inside. Sufficient individual space and ventilation is important to ensure a dry floor surface. In comparison to lightweight shade constructions for use in warm conditions, a movable winter shelter is more difficult to construct. Moving a construction with a roof and walls is relatively labour-intensive. Nonetheless, Swedish researchers estimated the cost per cow - infrastructure plus labour - for a rotational grazing system with movable shelter lower than for conventional indoor winter housing for beef suckler cows (Salomon et al., 2012).

8 MITIGATION OF THERMAL STRESS BY NATURAL SHELTER

Vegetation can also protect livestock against solar radiation in summer and against wind and precipitation in winter. Trees or shrubs create shade and have an additional cooling effect originating from moisture evaporating from their leaves. They reduce wind speed and may create a small 'rain shadow' – an area where the amount of precipitation reaching the sheltering animals is lower - on the leeward side. The magnitude of these effects depends on the height and the porosity or density of the vegetation, and thus on the species composition (Brandle et al., 2004; Gregory, 1995; McArthur, 1991).

However, during the past five decades, in many agricultural regions, the presence of trees and shrubs in pastures has decreased because these are considered as obstacles that interfere with large-scale production and mechanisation of agriculture (Björklund et al., 1999; Le Coeur et al., 2002). Furthermore, they potentially constitute a habitat for pest insects and parasites (Gregory, 1995). When planting new vegetation to provide shade and shelter, plant species that are poisonous for livestock or that may induce photosensitisation (e.g. Morton and Campbell (1997)) should obviously be avoided. So-called shelterbelts may also limit stock carrying capacity if there is excessive trampling, which renders the area unsuitable for grazing. Trampling under shelter could be prevented by provision of adequate space and/or - locally - bedding material (Gregory, 1995). Another problem, however, is excessive manure deposition on locations sheltered by vegetation (Gregory, 1995), which makes the area no longer suitable for grazing or resting and constitutes a risk factor for mastitis in lactating cows.

The effect of vegetation on the productivity of the surrounding pasture is not entirely clear, because it is influenced by many different factors. Trees or shrubs may reduce forage productivity and feed quality due to competition for space, soil nutrients and moisture. These effects become more important as vegetation density increases (Hawke, 1991; Lewis et al., 1983). Forage productivity may also be decreased by reduction of photosynthesis in plants growing in shaded areas. However, research indicates that by selecting shade tolerant forages and pruning to maintain light levels at 40% to 60% of that in the open, yields equal to or greater than in open pastures can be attained (Garrett et al., 2004). Potential positive effects on forage are reduced evaporation from grass tissue and soil - particularly important in dry periods -, reduced physical damage and growth limitation by wind, reduced soil erosion and leaching of soil nutrients (Gregory, 1995). Woody habitats on grassland provide greater biodiversity than regular pastures (McAdam et al., 2007) and enhance landscape connectivity (Jose, 2009). In the context of eco-agriculture, agroforestry systems like silvopasture - grazing livestock on forested pastures - are gaining popularity again, also in temperate climate (e.g. Rigueiro-Rodriguez and MacAdam (2009)).

9 CATTLE'S PREFERENCES FOR DIFFERENT SHELTER TYPES

A factor that is often undervalued when humans choose how to protect livestock against adverse weather conditions is the preference of the animals themselves. An animals' choice among different sheltering possibilities is generally assumed to be determined by the capacity to increase thermal comfort. For example Shearer et al. (1991) stated that cows in hot conditions would prefer natural shade from trees rather than man-made shade constructions, since the former combines protection from sunlight with cooling by moisture evaporating from the leaves. Studies that actually offer a choice between different types of shade or shelter are rather scarce. When simultaneously offered shade cloth blocking 99%, 50% or 25% of solar radiation dairy cows indeed preferred cloth with the two highest blocking percentages (Schutz et al., 2009). Yet more factors than the capacity to decrease temperature stress influence cattle's preference. For instance, dairy cows were unexpectedly found to prefer shade from an iron roof over shade from trees or vine leafs when given the choice (Gaughan et al., 1998), although the location of the different shelter types (proximity to water and feed troughs) may explain this preference. Although the domestication process (selection for tameness and protection against predators by humans) may have reduced the bovine sensitivity to predators in the strict sense, vigilance against predators in a wide sense remains relevant. For example Welp et al. (2004) showed that dairy cows increase vigilance in a novel feeding enclosure and in response to a dog or a person who had handled them aversively. Visual obstruction by vegetation in grazing allotments has also been shown to increase vigilance in Angus x Hereford cross-bred cows (Kluever et al., 2008). Thus vigilance may also influence cattle's sheltering behaviour, causing preference for more open types of shelter. Cattle are also sensitive to rapid movements (Grandin, 1999), thus constructions with cloths or plastic waving in the wind might scare them. Furthermore, cattle's strong gregarious tendency may influence their individual shelter seeking behaviour. For instance, in a mixed herd, heat tolerant *Bos taurus* x *Bos indicus* cows adapted their thermoregulatory behaviour on pasture (location and timing of grazing, activity and resting) to that of their less tolerant *Bos taurus* herd mates to realise group cohesion. When both breeds were kept on separate pastures, *Bos taurus* x *Bos indicus* cows spent less time in the shade, grazed more and were more active than the *Bos taurus* cows (Langbein and Nichelmann, 1993). This illustrates that motivational priorities are also highly dependent on social context. They also depend on age, reproductive stage, season, previous experience and by the number and type of resources that

are simultaneously available. Preferences can also depend on the length of time the animal has access to it. If a certain environment is preferred in the greater part of a limited time span, this does not mean that the animal would prefer to be in this environment all of the time (Bateson, 2004). Thus, when studying cattle's preferences for shade and shelter types, short term choice tests results must always be interpreted with caution and data are preferably gathered over a sufficient time and a wide range of environmental and social circumstances, from many different individuals (different breeds, ages, sexes, colours etc.)

10 CONCLUSION

Many climatic factors and animal characteristics have to be taken into account when assessing the need for preventive measures against cold and heat stress in cattle kept outdoors in a temperate climate. Thermo-tolerance may vary greatly according to factors such as breed, age, productivity, body condition, and coat condition even within the same herd. In addition, animals may – to a certain extent and particularly when the weather changes gradually - adapt to climatic conditions by physiological and behavioural mechanisms. Nonetheless, there is a substantial body of evidence of negative effects of hot (high temperatures, high humidity and intense solar radiation) and cold conditions (low temperature combined with precipitation and wind) on the well-being and performance of cattle. Belgian climatic data illustrated that such conditions outside the thermo-neutral zone of cattle are rather exceptional in typical temperate winters, but less so during typical summers. Global warming and the selection for high productivity will increase the risk of heat stress in cattle in the future. Recent research on high-producing cows in temperate climate has already shown that the traditional climatic indices and threshold values of the associated heat stress risk classes are outdated and too general to evaluate heat stress in cattle from different origins. Further research would be useful to establish widely validated threshold values for different heat and also cold stress risk classes based on effects on welfare, health and production in different cattle types. In order to be able to make a well-supported choice between different sheltering types, cattle owners and policy-makers would benefit from more scientific research evaluating different types and designs of shelter in terms of efficiency, cattle's preferences, cost and potential for integration into an economically and ecologically sustainable farming system.

11 ACKNOWLEDGMENTS

The authors would like to thank the experimental farm of Ghent University, Biocentre Agri-Vet, Melle, for the provision of the weather data from the RMI (Royal Meteorological Institute) weather station. We thank S. Buijs and M. Levenson for proof reading this paper. This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RT 10/13 PASSTRESS).

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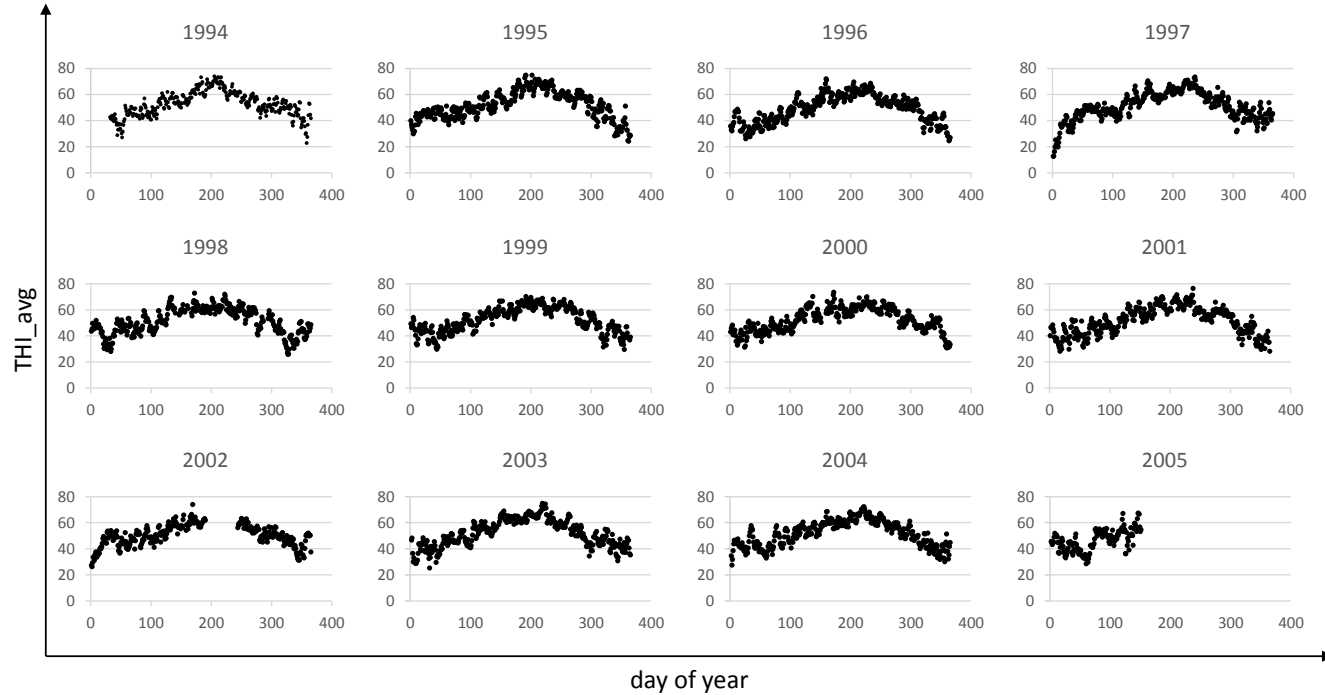
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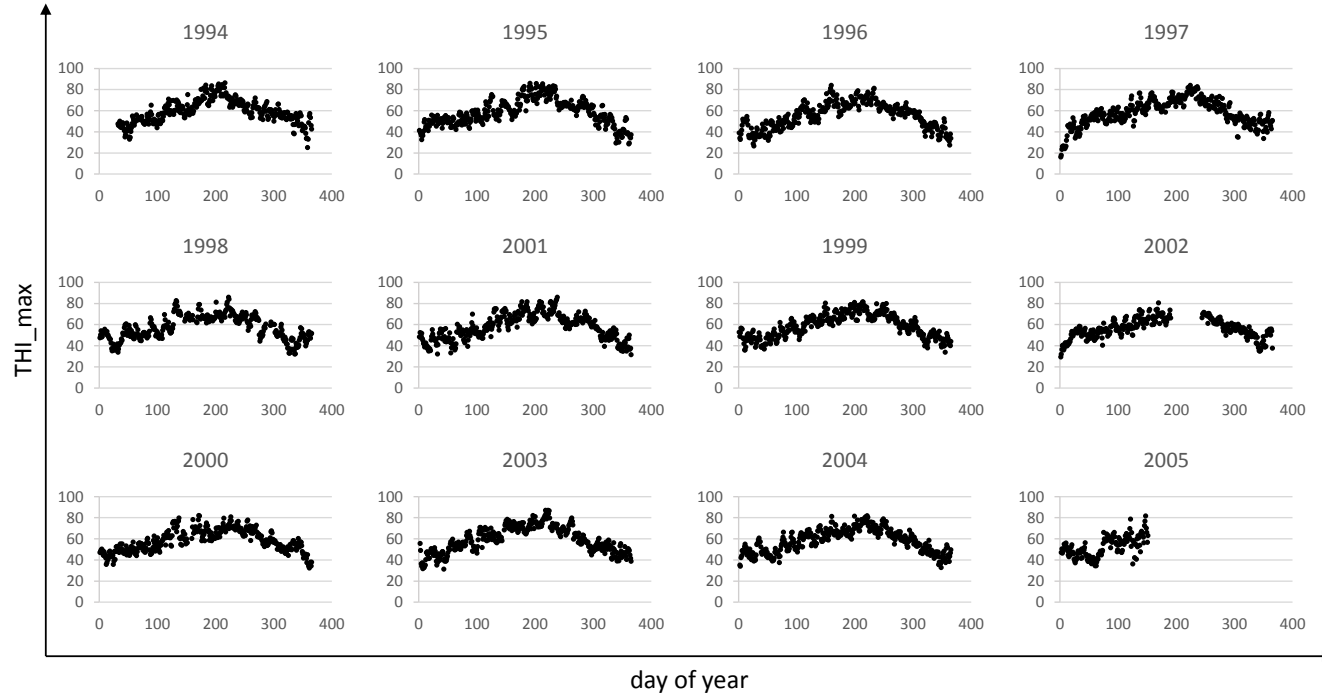
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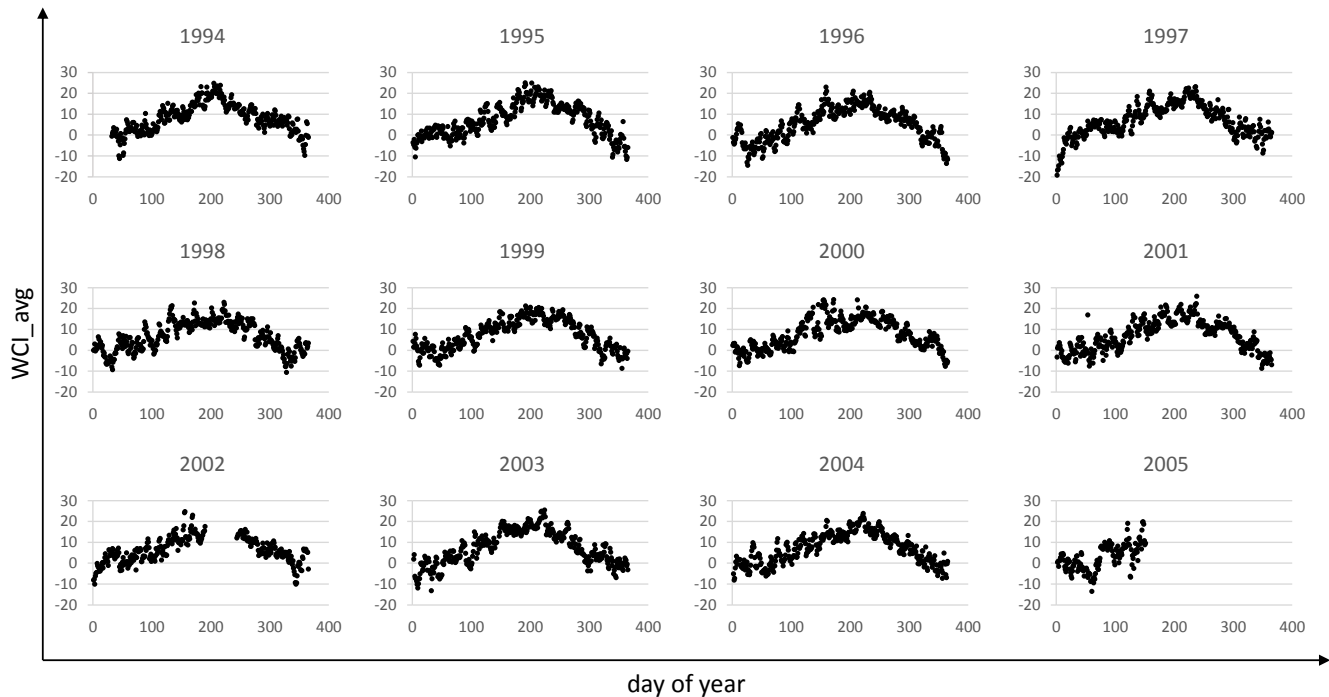
SUPPLEMENTARY FIGURE S1. Yearly course of daily average Temperature Humidity Index (THI, according to Equation (1)) in Melle, Central Belgium (Biocentre Agri-Vet, Ghent University) for 1994 until 2005.



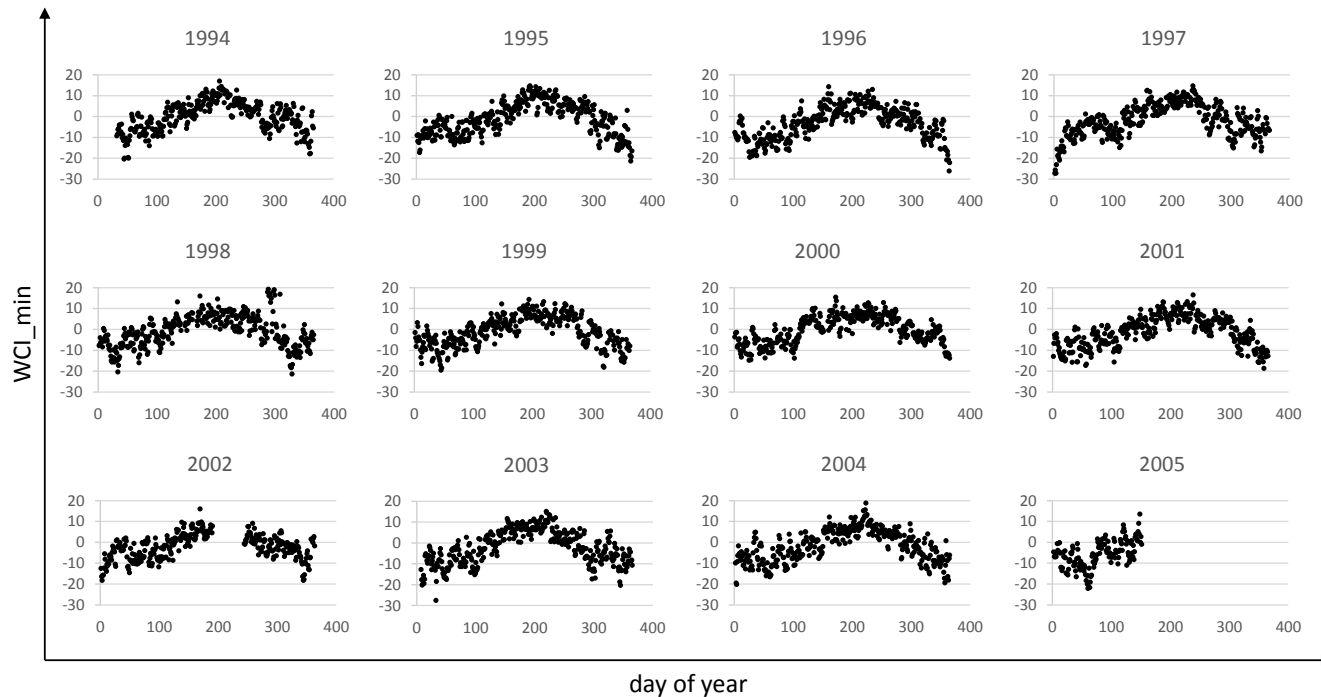
SUPPLEMENTARY FIGURE S2. Yearly course of daily maximum Temperature Humidity Index (THI, according to Equation (1)) in Melle, Central Belgium (Biocentre Agri-Vet, Ghent University) for 1994 until 2005.



SUPPLEMENTARY FIGURE S3. Yearly course of daily average Wind Chill Index (WCI, according to Equation (2)) in Melle, Central Belgium (Biocentre Agri-Vet, Ghent University) for 1994 until 2005.



SUPPLEMENTARY FIGURE S4. Yearly course of daily minimum Wind Chill Index (WCI, according to Equation (2)) in Melle, Central Belgium (Biocentre Agri-Vet, Ghent University) for 1994 until 2005.



CHAPTER 2

RESEARCH OBJECTIVES AND THESIS OUTLINE

The main aims of this doctoral research are twofold. Firstly, we wanted to examine the impact of cold and hot conditions in a temperate climate (specifically, Belgium) on thermal comfort of cattle kept outdoor. Secondly, we wanted to investigate several types of shelter (natural and artificial shelter) as protection against cold and heat stress.

Whether and when artificial shelter (man-made), in addition to natural shelter (vegetation), should be provided to cattle grazing in nature reserves in temperate regions, is an ongoing debate among reserve managers, animal protection organisations and the public. The current research aimed to contribute scientific observation to this debate, by studying the summertime and wintertime sheltering behaviour of cattle in eight Belgian year-round grazing projects. More specifically, in the first part of this doctoral research project, we investigated the effect of heat load in summer (→ **Chapter 3**) and cold in winter (→ **Chapter 4**) on the use of natural versus artificial shelter by cattle in these nature reserves.

Outdoor shelter against cold stress was not studied in dairy and beef cattle, given that in Belgium, these cattle types are generally kept indoors during winter (e.g. Krohn et al., 1992).

However, in the second part of the research project we did investigate the value of shade as protection against heat stress for dairy and beef cattle on pasture. More specifically, we studied the effect of heat load and shade on the welfare and productivity of Holstein dairy and Belgian Blue beef cattle. The main objectives of this study were to

- determine the extent of negative effects of heat load on
 - thermal comfort, for Holstein dairy cows and Belgian Blue cows and calves on pasture (→ **Chapter 5**)
 - the energy metabolism, milk yield and milk composition of Holstein dairy cows on pasture (→ **Chapter 6**)
- evaluate the effectiveness of shade as protection against heat stress
 - by evaluating the effect of shade on microclimate (→ **Chapter 5**)
 - by relating the voluntary use of shade on pasture to the degree of heat load (for the three types of cattle) (→ **Chapter 5**)
 - by determining the extent to which shade can reduce the negative effects of heat load (→ **Chapter 6**)

CHAPTER 3

SUMMERTIME USE OF NATURAL VERSUS ARTIFICIAL SHELTER BY CATTLE IN NATURE RESERVES

Adapted from: SUMMERTIME USE OF NATURAL VERSUS ARTIFICIAL SHELTER BY CATTLE IN NATURE RESERVES

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ACCEPTED FOR PUBLICATION IN ANIMAL WELFARE



ABSTRACT

Whether artificial shelter (man-made), in addition to natural shelter (trees and shrubs), ought to be provided to cattle grazing in nature reserves in temperate summers is a topic of debate. We have investigated the effect of heat load on the use of natural versus artificial shelter (with a roof and three walls) by cattle in eight nature reserves in Belgium. We used GPS collars to monitor use of open area, natural and artificial shelter during one or two summers (per 30 min). Cattle location data were coupled to same-time values of climatic 'heat stress indices' calculated from local weather stations' measurements of air temperature, air humidity, solar radiation and wind speed. Use of open area decreased as heat load increased. The strength of the effect, and whether the cattle sought natural or artificial shelter, were associated with the amount and spatial distribution of natural shelter in the reserve. When natural shelter was sparse, a more scattered distribution tempered the increased use of shelter with increasing heat load. If sufficiently available, cattle rather used natural than artificial shelter. When little natural shelter was available, cattle did use the artificial shelter and particularly so with increasing heat load. Microclimatic measurements indicated that vegetation blocked solar radiation at least as well as artificial shelter, and allowed more evaporative cooling. In conclusion, we found no evidence for added value of additional artificial shelter to protect cattle from heat load in temperate nature reserves, as long as adequate natural shelter is available.

KEYWORDS: Animal welfare, artificial shelter, cattle, nature conservation, temperate climate, vegetation

1 INTRODUCTION

Cattle (*Bos taurus*) kept outdoors are sometimes exposed to aversive weather. In (sub)tropical and cold regions, the health, welfare and productivity of cattle can be impaired considerably and at least some form of shelter is obviously needed (Silanikove 2002). In temperate regions, however, the need for shelter against aversive weather conditions has received less attention. In addition, most attention has been directed toward farming settings (see also Van laer et al. 2014). For example, Graunke et al. 2011 demonstrated that outdoor-wintered beef cattle on Scandinavian farms sought protection from cold and precipitation in forest on and around their pastures. Roselle et al. (2013) demonstrated that, in Belgian summers, beef cattle on pasture increased their use of shade (natural vegetation or artificial) with increasing ambient temperature, air humidity and solar radiation.

The thermal comfort and sheltering behaviour of cattle used for grazing management in nature reserves, however, has been studied far less. Year-round grazing management in nature reserves is seldom done with dairy cattle, but mostly with robust cattle breeds (originally intended as beef or work breeds) such as the Galloway, Scottish Highlander or Aberdeen. These breeds are characterised by low energy demands and a high potential to accumulate fat on a poor-quality diet. As such, they are assumed to be relatively resistant to cold conditions, even under nutritional limitation (Wallis de Vries 1994). However, some characteristics - such as their thick hair coat (Finch et al. 1984; Yeates 1955), heavy posture or fatness (Brown-Brandl et al., 2006) and dark colour (Brown-Brandl et al., 2006) (e.g. in case of the Aberdeen and Heck) - might render them less tolerant to heat load than other breeds. They may thus be more inclined to seek shelter on warm days, even under temperate summer conditions.

In forested reserves, animals can find shelter under trees and shrubs (from here on: natural shelter). But also reserves where typically less natural shelter is present and the landscape is more open, such as riverine areas (e.g. Wallis de Vries 1994) or marshes (e.g. Andresen et al. 1990), are sometimes grazed by cattle. Whether and when additional shelter (in addition to the existing vegetation) must be provided, and whether artificial shelter (man-made) or natural shelter (vegetation) is the best choice, is a topic of debate.

First, people have varying opinions on which degree of animal discomfort or suffering is ethically acceptable. Grazing management mostly involves at least minimal and temporal discomfort for the grazers. Cattle grazing as a tool for management of nature reserves, relies on cattle's effect on the landscape and

vegetation (mainly) by selective grazing, which creates structural diversity (a mosaic pattern) in the vegetation, and thereby a greater biological diversity (Plachter and Hampicke, 2010). In some cases, cattle grazing management even aims for the cattle to graze a specific, less attractive target species or habitat, which requires the depletion of plant species or habitats that are more attractive, according to the cattle's inherent dietary preferences (Olson, 1999). From the animal's point of view, this situation deviates from the optimum. This may be considered as discomfort, but this is minimal and probably acceptable by most people.

Advocates of the concepts of 'rewilding' (i.e. restoration of natural processes such as flooding and biological processes such as grazing; Vera 2000) and 'de-domestication' (i.e. introduction of domesticated animals into 'the wild' with the aim of making them become self-reliant; Gamborg et al. 2010), even accept greater infringements the comfort of grazers. They justify the suffering and even death of the weaker animals by the utilitarian view that natural selection is needed to increase the fitness and coping ability of the population in the long term. Animal rights advocates, on the other hand, object to suffering of animals which are still in human care during the first stages of de-domestication (Gamborg et al. 2010). For example, the death by starvation of a part of the population of 'rewilded' Heck cattle and Konik ponies in the Dutch polder reserve De Oostvaardersplassen during an unusually harsh winter was intensely criticised (Lorimer & Driessen 2013). Yet also in less dramatic situations, but when the public judges the weather conditions to be aversive, reserve managers are sometimes confronted with citizens' concern about grazers' thermal comfort and welfare.

Little scientific literature is available to deduce whether cattle prefer artificial or natural shelter and which provides the most effective protection against excessive heat load. Thus, in order to contribute to the debate about the need to provide artificial shelter to cattle in nature reserves in temperate areas, the current study aimed to investigate the effect of summer climatic conditions, mainly heat load, on the use of natural versus artificial shelter by cattle in several year-round grazing projects in Belgium. We hypothesised that the use of natural and/or artificial shelter, as an indication of thermal discomfort in open area, would increase in increasingly hot conditions. The relative degree of the increase in the use of natural versus artificial shelter, would inform us about the cattle's potential preference for either type of shelter. Alternatively, a lack of a consistent relationship between climatic conditions and the use of freely available (natural or artificial) shelter, would indicate that the Belgian summer conditions are not hot enough to initiate substantial thermal discomfort in the studied cattle in nature reserves.

2 MATERIALS AND METHODS

2.1 RESERVES AND ANIMALS

The study took place during the summers (April-October) of 2012 and 2013 in eight nature reserves in Flanders (northern region of Belgium). The nature reserves had varying amounts of natural shelter and all had one or two artificial shelters. All reserves were grazed by cattle year-round. Table 1 gives their location, an overview of the most important characteristics in terms of the vegetation or landscape type, the availability of natural shelter (trees and shrubs) and artificial shelter; the abbreviations we use throughout this publication for each individual reserve.

Four reserves already had an existing artificial shelter of which the reserve manager chose the size, design, position within the reserve and orientation of the shelter; in the remaining reserves we placed an identical artificial shelter five to eight months before the start of the first summer in which the corresponding reserve was studied. In one reserve (VV) two artificial shelters were used, one installed by the reserve manager and another installed by us. All shelters that were installed by us, had three closed walls made of wooden planks or boards and a slightly slatted roof out of galvanized steel plates, coated with 25 μ m white polyester and insulated with 2 cm of polyurethane foam. In addition, there was an 18cm gap between the roof and either of the three bearing walls, to allow a minimum of ventilation. Of the artificial shelters which were installed by the reserve manager, only one had three walls of stone and an insulated gable roof out of brick tiles. The three remaining artificial shelters installed by the reserve manager, were constructed with three closed walls made of wooden planks and a slightly slatted roof out of uninsulated galvanized steel plates (n=2) or an uninsulated gable roof out of brick tiles (n=1).

All artificial shelters had one open side. Four reserves were grazed by Galloway cattle, two by Aberdeen-Angus cattle, one by a local Flemish breed (Oost-Vlaams Wit-Rood), and one by Heck cattle. The maximum number of animals in each reserve, can be found in Table 1. In five out of the eight herds, fertile males were present, and thus also calves or young cattle were in the herd during at least a part of the study.

TABLE 1. Location and characteristics of the eight nature reserves under study in Belgium. Four reserves already had an existing artificial shelter installed by the reserve manager; in the remaining reserves we placed artificial shelter five to eight months before the start of the experiment. In one reserve, one artificial shelter (A) was installed by the reserve manager and another (B) was installed by us

	total area (ha)	vegetation or landscape type	% natural shelter	artificial shelter							max n ^o cattle	cattle breed	H	S
				recently installed?	orientation open side	roof material	roof insulated?	roof height	area (m ²)					
KH: 51° 5'N, 5°21'O	7.4	lowland pasture	17	yes	SE	galvanised steel	yes	2.8 m	24	5 ²	Aberdeen-Angus	0.93	0.22	
VV: 50°52'N, 4°59'O	63.0	alluvial meadows and some deciduous forest	31	A: no, B: yes	A: S-SE, B: SE	A and B: galvanised steel	A: no B: yes	A: ± 2.8 m B: 2.8 m	79	10 ²	Aberdeen-Angus	2.88	0.68	
MS: 51° 0'N, 5° 1'O	15.8	meadows and deciduous forest	37	no	S-SE	galvanised steel	no	± 2.5 m	46	5 ²	Galloway	1.88	0.45	
EB: 50°51'N, 3°38'O	63.2	deciduous forest surrounded by scrubs and grassland	54	yes	SE	galvanised steel	yes	2.8 m	24	15	Oost-Vlaams Wit-Rood	0.99	0.24	
KE: 50°46'N, 5°29'O	29.0	mosaic of marshes, grassland and scrubland	55	no	W	brick tiles	no	± 3.0 m ¹	24	10	Heck	2.29	0.55	
HB: 51°11'N, 3°53'O	143.4	wooded heath landscape	73	yes	SE	galvanised steel	yes	2.8 m	24	16	Galloway	1.30	0.31	
HP: 51°11'N, 4°20'O	34.7	mosaic of reeds, scrubs, grassland and deciduous forest	74	yes	SE	galvanised steel	yes	2.8 m	24	10	Galloway	2.39	0.57	
BB: 50°56'N, 4°48'O	47.5	mosaic of scrubs, grassland and deciduous forest	83	no	E-SE	brick tiles	yes	± 4.0 m ¹	60	10	Galloway	1.10	0.27	

KH: Katershoeve, VV: Velpvallei, MS: Molenstede, EB: Ename Bos, KE: De Kevie, HB: Heidebos, HP: Hobokense Polder, BB: Beninksberg. H= Shannon Wiener Index, S= structural diversity index. ¹ Height of the ridge of the gable roof. ²In these herds, no fertile males and thus no calves were present.

2.2 COLLECTION OF ANIMAL LOCATION DATA

In each reserve, a Lotek Wildcell M5 GPS collar with GSM communication function (Lotek Wireless Inc., Newmarket, Canada) was fitted onto one animal to monitor terrain use during one summer (2013; in two reserves: KE and VV) or two summers (2012 and 2013; in six reserves: BB, EB, HB, HP, KH and MS). The manufacturer guarantees an accuracy between of 0 and 10m, with an average of 5 m in open area. We verified this claim by determining ten times the deviation from a reference point in open area, a reference point under natural shelter (vegetation) and under the artificial shelter in each of the eight study reserves. We found an average deviation of 3.4 m (SE = 0.4 m) in open area, 10.1 m (SE = 0.4 m) under natural shelter and 6.8 m (SE = 0.4m) under artificial shelter.

We followed only one animal per reserve, because the relatively small herds (consisting of about 15 animals maximum) were known to travel through the reserves as a group. This was based on the observations of the conservators of the reserves, who were in charge of the regular inspection of the herd. The GPS collars were attached when cattle were caught for annual veterinary care. At the end of the study, the collars were removed upon the first annual veterinary care after the end date (Table 1). We strived to collar animals without obvious ailments or health problems, that were assumed (by the local reserve conservators who carry out regular health and welfare check-ups) to have a dominant (or at least not subordinate) position in the herd hierarchy, so that it can be assumed that they would have access to shelter if they felt the need to. These were usually female animals of intermediate age.

Animal positions were registered every 30 minutes (around the clock) and were plotted onto digital maps of the reserves, using ArcMap 2010. For each animal position, we determined if it took place in (1) open area (= no shelter), (2) natural shelter, including the surrounding 5m because we assumed cattle would still find protection (e.g. from wind or solar radiation) within 5m of trees or shrubs, or (3) artificial shelter (including the surrounding 5 m). These data were coupled to the climatic variables and indices registered by the closest or the most representative weather station, in the 15 minutes before the animal position was registered. The digital maps of the reserves were based on the most recent detailed orthographic aerial images available at the Flemish Agency for Geographical Information. The patches vegetated by trees and/or shrubs were mapped as natural shelter and the location of the artificial shelters was added manually. To correct for potential changes in

vegetation after the date of capture of the aerial images, these maps were checked in the field and adapted accordingly.

The area of each separate patch of natural and artificial shelter was determined (measured on site with a tape measure for artificial shelter, and determined with ArcMap 2010 for natural shelter) and the sum of the patches of natural shelter was divided by the total reserve area to obtain the percentage of natural shelter per reserve (Table 1).

2.3 QUANTIFICATION OF THE SPATIAL DISTRIBUTION OF SHELTER

The amount and spatial distribution of natural and artificial shelter across the reserve likely influences the cattle's use of the shelter. Therefore, we had to quantify the spatial distribution in order to include it into the analysis of the effect of climatic variables and indices on the use of shelter. We used a 'structural diversity index' based on the Shannon Wiener index (H), which is widely used in ecology to assess the diversity of species or habitats in a given area (Magurran, 1988). Here we used it to quantify the spatial distribution of 'areal units' of the three different location types— separate patches open area, natural and artificial shelter, in each reserve - according to Equation (1) below.

The Shannon Wiener index's value ranges from 1 to 4 and increases with an increasing number of and greater scatter of 'shelter units'. However, this value provides little information about the relative differences regarding the spatial distribution of shelter between the different reserves. This is why we used a method equivalent to the calculation of the Shannon evenness measure, traditionally used to quantify the difference in abundance between different species in a given area (Magurran, 1988). More specifically, we divided each reserve's Shannon Wiener index (H) by the maximum Shannon Wiener index (for the reserve with the maximum number of shelter units) (Equation 2), to obtain a relative measure per reserve, which we named the 'structural diversity index' (Table 1).

$$(1) H = - \sum_{i=1}^s [(n_i/N) * \ln (n_i/N)]$$

$$(2) S = H/H_{\max}$$

where i = i th unit of the location type (open area, natural shelter and artificial shelter)

s = number of location type units

n_i = area of the i th unit of the location type

N = total area of location type

$H_{\max} = \ln S_{\max}$ and S_{\max} = the maximum number of location type units in one reserve

2.4 COLLECTION OF CLIMATIC DATA

Custom-built Campbell Scientific BWS200 weather stations (Campbell Scientific Inc., Logan, Utah, US) in six out of eight reserves recorded the average air temperature, air humidity, solar radiation, wind speed and total precipitation every 15 minutes. For the two reserves without a weather station (MS and BB), climatic variables were used from the closest (max. 43 km distance) weather station (KH in 2012, VV in 2013).

In livestock heat stress research and livestock management, the effect of different climatic variables is often combined into a single measure to quantify the degree of discomfort and potential production loss. This has resulted in the development of climatic indices, which are usually associated with risk classes or threshold values reflecting the effect on biological response functions such as body temperature, respiration rate or milk production (Hahn et al. 2003). In our research we used six such heat stress indices (Table 2), for which we calculated 15-minute values based on the measurements of the weather stations.

The Temperature Humidity Index (THI) is at present still the most commonly used index for classifying moderate to hot conditions in livestock research and management. In addition, we used more recent climatic indices – such as an adjusted version of the THI, the Heat Load Index (HLI) and the Comprehensive Climate Index (CCI) – which incorporate the effects of temperature, humidity, wind speed as well as solar radiation to improve the assessment of heat stress risk (Table 2). The black globe temperature (T_{bg}) incorporates the effect of air temperature and solar radiation and the Wet Bulb Globe Temperature (WBGT) incorporates the effect of air temperature and solar radiation via the T_{bg} and the effect of solar radiation and wind speed via the wet bulb temperature (Table 2).

TABLE 2

Overview of climatic heat stress indices used in cattle research to quantify the effects of heat load.

Heat stress index + formula	Associated 'heat stress' threshold
$THI = 0.8 * Ta + [(RH/100) * (Ta - 14.4)] + 46.4$ (Thom 1959)	68, based on milk production losses (Zimbelman <i>et al.</i> 2009)
$THI_{adj} = 4.51 + THI - 1.992 * WS + 0.0068 * Rad$ (2.5) (Mader <i>et al.</i> 2006)	68, cfr. conventional THI
$CCI = Ta + Eq.1 + Eq.2 + Eq.3$	25 °C, based on elevated respiration rates (Mader <i>et al.</i> 2010)
$Eq.1 = e^{((0.00182 * RH + 1.8 * 10^{(-5)} * Ta * RH)) * (0.000054 * Ta^2 + 0.00192 * Ta - 0.0246)} * (RH - 30)$ $Eq.2 = (-6.56) / e^{(1 / (2.26 * WS + 0.23))^{0.45}} * (2.9 + 1.14 * 10^{(-6)} * WS^{2.5} - \log_{0.3} (2.26 * WS + 0.33)^{(-2)}) - 0.00566 * WS^2 + 3.33$ $Eq.3 = 0.0076 * Rad - 0.00002 * Rad * Ta + 0.00005 * Ta^2 * \sqrt{Rad} + 0.1 * Ta - 2$	
(Mader <i>et al.</i> 2010)	
$Tbg = 1.33 * Ta - 2.65 * Ta^{0.5} + 3.21 * \log(Rad + 1) + 3.5$ (Hahn <i>et al.</i> 2003)	25 °C, cfr. upper critical temperature for cows (Van laer <i>et al.</i> 2014)
$HLI = 8.62 + 0.38 * RH + 1.55 * Tbg - 0.5 * WS + e^{(2.4 - WS)}$ if Tg > 25 $HLI = 10.66 + 0.28 * RH + 1.3 * Tbg - WS$ if Tg < 25 (Gaughan <i>et al.</i> 2008)	70 (Gaughan <i>et al.</i> 2008)
$WBGT = 0.7 * Twb + 0.2 * Tbg + 0.1 * Ta$ (Schröter & Marlin 1996)	25 °C, cfr. upper critical temperature for cows (Van laer <i>et al.</i> 2014)

THI= Temperature Humidity Index, THI_adj= adjusted version of the Temperature Humidity Index, CCI= Comprehensive Climate Index in °C, Tg= Black Globe Temperature in °C, HLI= Heat Load Index, WBGT= Wet Bulb Globe Temperature in °C, Ta= air temperature in °C, Rad = solar radiation in W/m², RH= % air humidity, WS = wind speed in m/sec.

2.5 EFFECT OF NATURAL VS. ARTIFICIAL SHELTER ON MICROCLIMATE

In order to evaluate the effectiveness of natural vs. artificial shelter as protection against heat load we conducted microclimatic measurements on three days of high heat load in open area (n= 3 per day and per reserve), and under natural shelter area (n= 3 per day and per reserve) and under artificial shelter (n= 3 in the same shelter, per day and per reserve). Within each measurement session (per reserve), we aimed to minimize the time interval in which we took the microclimatic measurements by means of convenience sampling. The order of the nine measurements within a session was thus determined by the order of which we came across suitable patches along our path through the reserve. Patches of open area were selected to lie at least 25m from the nearest patch of (natural or artificial) shelter. For natural shelter, we selected places that were clearly regularly used by the cattle, as evident from trampling, fouling with excreta and/or the absence of a herb layer and low branches under dense foliage, as also described by Hauck & Popp (2010) and illustrated in Figure 1.

In each artificial shelter, we took three measurements per session, one in the centre and two in the inner corners of the shelter. Air temperature, wet bulb temperature, T_{bg} and WBGT were measured with Testo 400's WBGT probe (Testo AG Inc., Lenzkirch, Germany). Wind speed and relative air humidity were measured with a Testo 410-1 Pocket Vane Anemometer (Testo AG Inc., Lenzkirch, Germany). These manual measurements were also used to calculate the HLI.

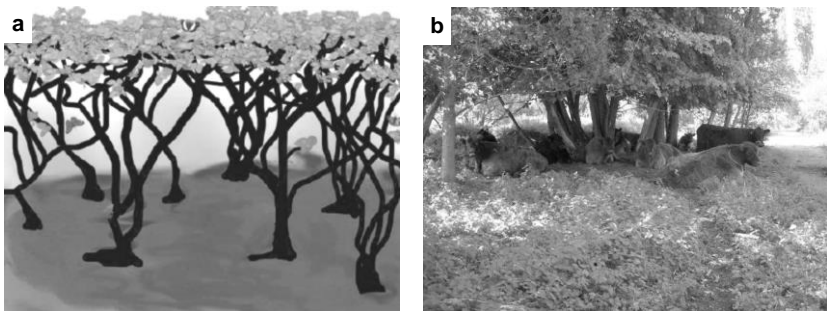


Figure 1. Image of a typical patch of natural shelter used by cattle as described by Hauck & Popp (2010) (a) and as observed in our study (b).

2.6 DATA ANALYSIS

The difference in air temperature, wind speed, Tbg, and HLI measured in open area, under natural shelter and under artificial shelter was modelled by means of a mixed model ANOVA (proc MIXED, in SAS 9.4) to correct for the effect of repeated measurements within each reserve. In post-hoc tests, Tukey-Kramer adjustments were used to account for multiple comparisons. In addition, we checked the correspondence between the manual measurements of climatic variables in open area (used in the evaluation of the effectiveness of natural vs. artificial shelter) with the measurements of the closest weather station at the same time. Therefore, we conducted a mixed model ANOVA (in SAS 9.4, proc GLIMMIX) that compared the average air temperature, Tbg, HLI and wind speed measured (a) manually and (b) by the closest weather station during the three measurement sessions per reserve. A random factor was used to correct for the effect of repeated measurements within each reserve.

To determine if a certain location type is generally preferred (across all climatic conditions) we compared the expected use of the three location types – i.e. the expected distribution of GPS registrations over the three location types - with their observed use (the observed distribution of GPS registrations over the three location types), per reserve (n=8). Per reserve, the expected use is defined as the proportion of reserve area covered by the location type multiplied by the total number of GPS registrations in the respective reserve. The ratio of observed/expected use can be either between 0 and 1 (indicating avoidance of the location type) or between 1 and infinity (indicating preference for the location type). The closer the ratio is to 0, the stronger the avoidance; the closer the ratio to infinity, the stronger the preference.

To investigate the effect of climatic conditions on the use of open area (as opposed to shelter, natural or artificial), eight mixed model logistic regressions were fitted (in SAS 9.4, proc GLIMMIX), which modelled the use of open area (binomially distributed) in function of rain intensity (in mm per 15 minutes) and each of the six climatic heat stress indices in Table 2. Each logistic regression modelled the probability of use of open area as a function of (1) the effect of rain intensity or the heat stress index under focus, (2) the effect of the amount of natural shelter, (3) the effect of the structural diversity index (Table 1), the two-way interaction between (1) and (2), the two-way interaction between (1) and (3), and the three-way interaction between (1), (2) and (3). The amount of natural shelter expressed as deviation (+ or -) from the situation where half of the area is covered by natural shelter,

thus theoretically ranging between -50 (if there would be no natural shelter at all) and 50 (if the whole area would be covered by natural shelter). All models included time of day as random factor to correct for repeated measurements per day, nested within the experimental unit, i.e. the reserve. All analyses were conducted in SAS 9.4 (SAS Institute Inc., Cary, NC).

Out of all models, the model with the HLI yielded the lowest pseudo-AICC (Corrected Akaike Information Criterion), and thus the best fit (Table 3). Consequently, we assumed that, out of the six climatic heat stress indices tested, the HLI was best suited to explain the observed trends in function of climatic conditions, and further only report results of the HLI model.

TABLE 3

Pseudo-AICC value (lower = better fit) for the analyses of the use of open area in function of the different heat stress indices

Climatic variable or heat stress index¹	Pseudo- AICC
THI	266 582
THI_adj	264 510
Tg	266 450
CCI	264 348
HLI	264 295
WBGT	264 374
rain intensity	266 298

THI= Temperature Humidity Index, THI_adj= adjusted version of the Temperature Humidity Index, CCI= Comprehensive Climate Index in °C, Tg= Black Globe Temperature in °C, HLI= Heat Load Index, WBGT= Wet Bulb Globe Temperature in °C, rain intensity in mm per 15 min.

Because the use of open area is modelled in function of three variables (HLI, amount of natural shelter and structural diversity) it is impossible to plot the relations in one graph. This is why the relationship between the HLI and the modelled use of open area is plotted for specific and existing cases of certain combinations of availability of natural shelter and structural diversity, i.e. for the eight studied reserves. The modelled relation was plotted on top of the plot of the raw data – i.e. the mean (uncorrected for repeated measurements) use – of the three different location types. As such, we illustrate that an overall model may not always predict reserve specific patterns precisely and roughly sketch the relationships between the relevant climatic index and the use of natural and artificial shelter in the reserves where they were not modelled. Only for the reserves where artificial shelter was used relatively frequently (>1% of observations), the probability of use of natural shelter and probability of use of artificial shelter were modelled (in SAS 9.4, proc GLIMMIX) as a function of the HLI, per reserve.

3 RESULTS

An overview of the climatic conditions measured by the weather stations in the study reserves, is presented in Table 4. The air temperature, T_{bg} and HLI registered manually were generally higher in comparison with the registrations of the closest weather station at the same time (respectively 3.8 °C (P < 0.0001), 5.6 °C (P < 0.0001) and 4.9 units (P = 0.0204). The wind speed registered by the manual measurements vs. weather stations did not differ significantly (P = 0.7236), though the absolute value of the difference between both averaged 0.6 ± 0.1 m/sec. This difference is probably due to the weather stations' measurements being an average over the entire hour whereas the manual measurement being an average of three instantaneous measurements spread over moments within this hour.

TABLE 4

Overview of climatic conditions measured by the weather stations, across the whole dataset, per reserve.

		KH	VV	MS	EB	KE	HB	HP	BB
min.	air temperature (°C)	-3.0	-1.6	-1.6	-2.8	0.0	-4.3	-2.6	-1.6
	wind speed (m/sec)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	mm of rain per 15 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	THI	26.6	29.1	29.1	26.9	32.0	24.3	27.3	29.1
	HLI	13.6	34.6	34.6	10.7	35.8	34.6	10.7	34.6
mean	air temperature (°C)	15.2	15.6	15.5	15.6	15.1	15.5	15.0	15.6
	wind speed (m/sec)	0.9	0.9	0.8	1.3	0.4	0.9	0.5	0.9
	mm of rain per 15 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	THI	58.5	59.2	58.9	59.4	58.4	59.2	58.4	59.1
	HLI	47.4	56.7	56.7	46.2	59.4	56.3	45.2	56.7
max.	air temperature (°C)	35.3	35.2	35.2	34.1	35.6	33.9	35.8	35.2
	wind speed (m/sec)	6.0	6.3	6.3	6.4	2.9	5.1	3.3	6.3
	mm of rain per 15 min	5.6	4.0	4.2	4.6	2.8	4.6	5.8	4.8
	THI	83.9	85.1	85.1	84.2	85.7	84.0	85.5	85.1
	HLI	96.7	97.2	97.2	97.3	102.3	95.5	97.5	97.2
% of observations where									
	mm of rain per 15 min > 0	5.70	4.50	4.13	4.76	4.20	5.12	5.15	4.47

KH: Katershoeve, VV: Velpvallei, MS: Molenstede, EB: Ename Bos, KE: De Kevie, HB: Heidebos, HP: Hobokense Polder, BB: Beninksberg. THI= Temperature Humidity Index, HLI= Heat Load Index.

3.1 EFFECT OF NATURAL VS. ARTIFICIAL SHELTER ON MICROCLIMATE

The micro-climatic measurements indicate that during high heat load several climatic parameters and indices differed between open area, natural and artificial shelter (Table 5). The Tbg, which combines the effect of air temperature and solar radiation, was not significantly different between artificial and natural shelter. In open area, however, it was about 8.5 °C higher than under natural or artificial shelter. Wind speed was generally highest in open area, lower under natural and lowest under artificial shelter and these differences were all highly significant. The HLI was highest in open area. The difference between open area and natural shelter and the difference between open area and artificial shelter were highly significant and were 12.7 HLI units and 9.0 HLI units, respectively.

TABLE 5

Effect of location type on three climatic variables: black globe temperature (Tbg), air temperature, wind speed and the heat load index (HLI).

shelter type	Tbg (°C)	Air temperature (°C)	Wind speed (m/sec)	HLI
	P<0.0001 LSM ± SEM	P<0.0001 LSM ± SEM	P <0.0001 LSM ± SEM	P <0.0001 LSM ± SEM
OA	35.1 ± 0.9 a	28.8 ± 0.7 a	1.1 ± 0.1 a	84.0 ± 2.2 a
NS	26.5 ± 0.9 b	24.6 ± 0.7 b	0.6 ± 0.1 b	71.3 ± 2.2 b
AS	26.6 ± 0.9 b	25.0 ± 0.7 b	0.1 ± 0.1 c	75.0 ± 2.2 c

OA = open area, NS = natural shelter, AS= artificial shelter. Least square means (LSM) ± standard errors (SEM) without a common letter differ significantly according to Tukey-Kramer corrected post-hoc comparisons, $P < 0.05$.

3.2 GENERAL PREFERENCES

In all reserves the artificial shelter covered < 1 % of the total reserve area. In six reserves (EB, HB, HP, KE, MS and VV), the artificial shelter was also used ≤ 1 % of the time (mean ± SE: 0.10 ± 0.05 %), and the ratio of observed use/expected use ranged between 0 and 4.7 (Figure 2). In one of these three reserves, KE, the artificial shelter (including the 5 m around it) was never used at all (Figure 2).

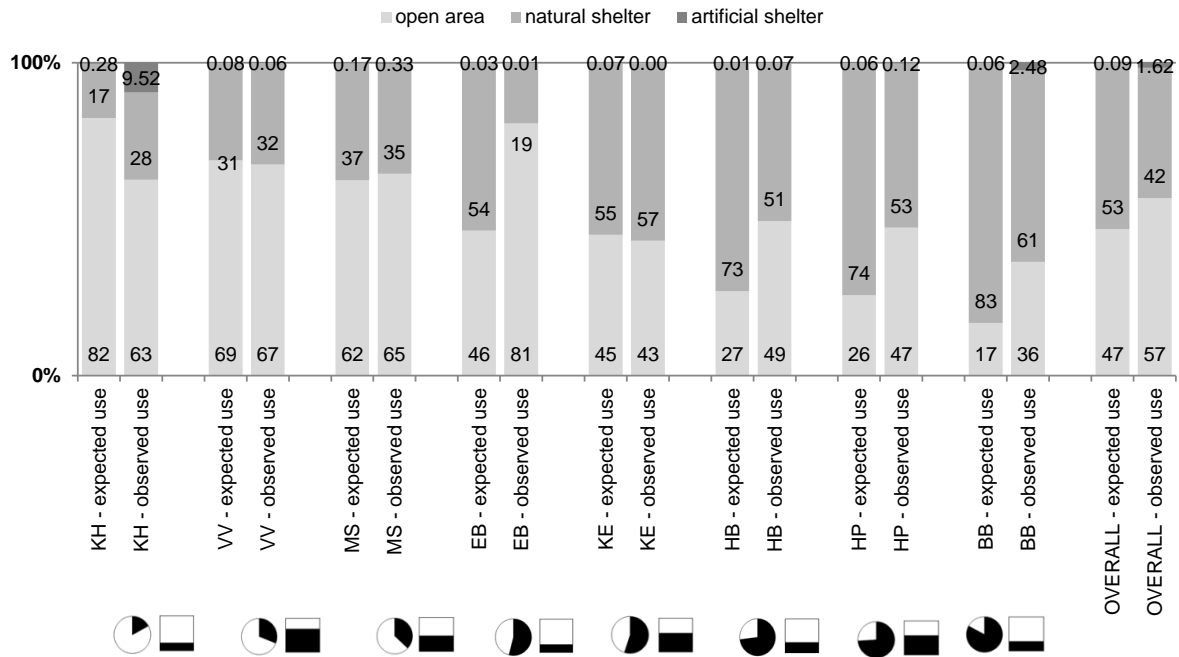


Figure 2. Comparison of the expected use (percentage of the reserves covered by the different location types) with the observed use (percentage of GPS registrations that took place within them), per reserve. Abbreviations for study reserves are explained in Table 1. For each reserve, circular symbols represent the percentage of natural shelter and square symbols the structural diversity, with more shading indicating higher values. OVERALL gives the average over all reserves.

Only in two reserves (KH and BB), artificial shelter was used more than 1 % of the time. In the most open and least structurally diverse reserve (KH), artificial shelter was used 9.5 % of the time (Figure 2) and 34 times more than expected. In one other reserve (BB; % NS >80) artificial shelter was used 2.5 % of the time (Figure 2) and 45 times more than expected. Natural shelter was slightly avoided (observed use/ expected use ca. 0.7) and open area was preferred, in the three reserves with the most natural shelter (% NS > 60: HB, HP and BB). In the most open and least structurally diverse reserve (KH) open area was avoided and natural shelter was generally (averaged over all climatic conditions) preferred.

3.3 EFFECT OF CLIMATIC CONDITIONS ON THE USE OF SHELTER

Rain intensity did not significantly influence the cattle's use of open area, nor did the interaction of rain intensity with either the amount of natural shelter or the structural diversity or their three way interaction (Table 6). The effect of HLI on the use of open area was influenced by the interactions with the amount of natural shelter and the structural diversity (Table 6). The use of open area decreased with increasing HLI (estimated effect of HLI is negative, Table 6) but a positive effect of the interaction between the HLI and structural diversity was found as well (Table 6).

TABLE 6

Effect of rain intensity and the heat load index (hli) and their interactions with the quantitative availability and spatial distribution (structural diversity) of natural shelter on the use of open area.

Effect	Estimate	SE	P
Intercept	0.823	0.064	<0.0001
rain intensity (mm per 15 min)	-0.067	0.208	0.746
availability NS	-0.020	0.001	<0.0001
structural diversity	-0.892	0.150	<0.0001
rain intensity *availability NS	0.013	0.008	0.098
rain intensity *structural diversity	-0.608	0.533	0.254
rain intensity *availability NS *structural diversity	-0.045	0.023	0.055
Intercept	3.855	0.204	<0.0001
HLI	-0.056	0.004	<0.0001
availability NS	0.024	0.003	<0.0001
structural diversity	-4.157	0.491	<0.0001
HLI*availability NS	<- 0.001	< 0.001	<0.0001
HLI*structural diversity	0.056	0.009	<0.0001
HLI*availability NS *structural diversity	<- 0.001	< 0.001	<0.0001

OA= open area, NS = natural shelter, AS= artificial shelter.

Thus if natural shelter was more clustered, the use of open area remained higher than when natural shelter was more scattered. Compare, for example, EB (lower structural diversity) and HP (higher structural diversity) in Figure 3. The availability of natural shelter had a positive effect on the use of open area (Table 6) but also the three way interaction between HLI, the availability of natural shelter and structural diversity had a negative effect on the use of open area (Table 6). Thus, if natural shelter was more sparse, the decrease of use of open area with increasing heat load was less pronounced or even absent when the structural diversity was high (Table 6). Compare for example EB (lower structural diversity) with KE (higher structural diversity) and MS (lower structural diversity) with VV (higher structural diversity) in Figure 3. However, the effects were associated with rather large standard errors (Table 6), which is not surprising when comparing the predicted probability of use of open area with the raw data (Figure 3).

In six reserves, the probability of use of artificial shelter could not (reliably) be modelled, as there were $\leq 1\%$ of observations in the artificial shelter. For these reserves probability of use of natural shelter can be assumed to be as good as complementary to the probability of use of open area, so it would be redundant to model. For the other two reserves (BB and KH) - where artificial shelter was used $> 1\%$ of observations - the probability of both artificial and natural shelter use was modelled in function of the HLI. In both reserves, the use of artificial shelter increased with increasing HLI (Figure 4 and Table 7). In the reserve with the most natural shelter (BB), however, an increasing HLI was associated with a greater increase in the use of natural shelter than artificial shelter. In KH the use of natural shelter decreased with increasing HLI (Figure 4 and Table 7).

TABLE 7

Effect of the heat load index (HLI) on the use of natural and artificial shelter in Katershoeve (KH) and Beninksberg (BB).

Reserve	Effect	Estimate \pm SE	P
Use of natural shelter			
KH	Intercept	-0.027 \pm 0.124	<0.0001
	HLI	-0.022 \pm 0.004	<0.0001
BB	Intercept	-5.808 \pm 0.242	<0.0001
	HLI	0.112 \pm 0.004	<0.0001
Use of artificial shelter			
KH	Intercept	5.447 \pm 0.245	<0.0001
	HLI	0.055 \pm 0.004	<0.0001
BB	Intercept	7.378 \pm 0.681	<0.0001
	HLI	0.043 \pm 0.011	<0.0001

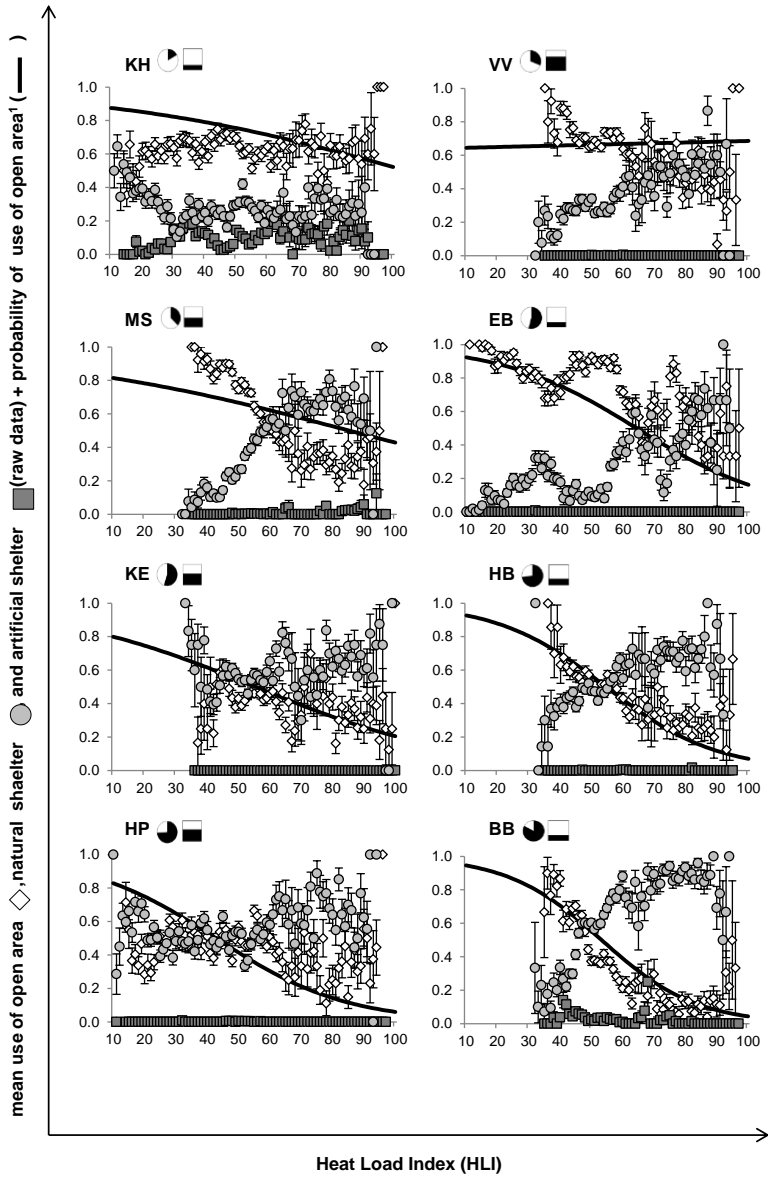


Figure 3. Plots of uncorrected (for repeated measures) means (\pm SE) of the use of open area (OA; \diamond), natural shelter (NS; \circ) and artificial shelter (AS; \square) and the probability of use of open area predicted by the logistic model (taking the amount and spatial distribution of natural shelter into account), at rounded values of the Heat load Index (HLI), per reserve. Abbreviations for study reserves are explained in Table 1. For each reserve, circular symbols represent the percentage of natural shelter and square symbols the structural diversity, with more shading indicating higher values. ¹According to the logistic model.

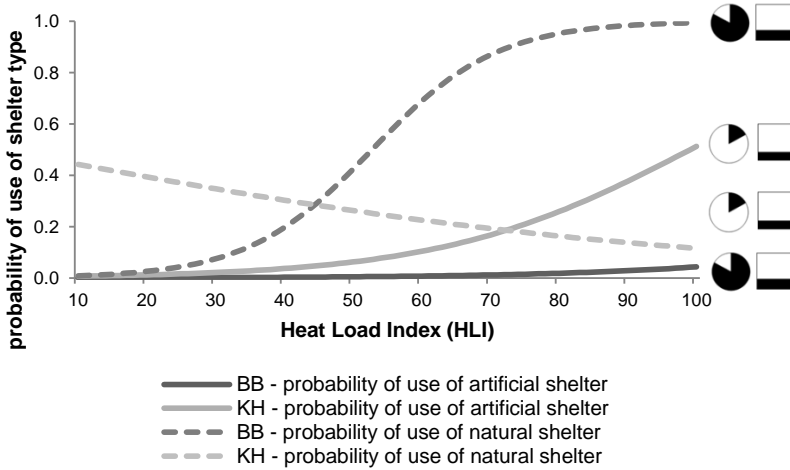


Figure 4. The relation between the Heat Load Index and the use of artificial and natural shelter predicted by the logistic regressions for the two reserves where artificial shelter was used for more than 2% of the time. Abbreviations for study reserves are explained in Table 1. For each reserve, circular symbols represent the percentage of natural shelter and square symbols the structural diversity, with more shading indicating higher values.

4 DISCUSSION

In order to contribute to the debate about the need to provide artificial shelter to cattle in nature reserves in temperate areas we investigated (1) the effectiveness of natural and artificial shelter against heat load, and (2) the changes in the use of open area vs. (natural or artificial) shelter according to climatic conditions in eight nature reserves in Belgium. The results do not provide conclusive evidence that additional shelter is needed to protect (adult, healthy) cattle from heat in temperate nature reserves as long as adequate natural shelter is available.

Our measurements of microclimatic conditions in open area and under natural and artificial shelter during high heat load point out that sufficiently dense natural shelter blocks solar radiation quite well, as the black globe temperature was usually not higher under natural than under artificial shelter. At the same time, natural shelter allows more evaporative cooling as compared to artificial shelter, owing to increased air circulation (higher wind speed). In open area, wind speed was generally higher than under natural and artificial shelter. Therefore, when solar radiation is a less important

contributor to heat load, e.g. in cloudy but warm weather conditions or during warm nights, this higher wind speed may provide for a better cooling environment in open area. This might be one of the reasons why we observed large variations in the use of open area at elevated HLI values.

Most cattle in our study did not seem to show a clear preference for artificial shelter either. Artificial shelter was in most reserves (EB, HB, HP, KE, MS and VV) used very rarely ($\leq 1\%$ of all observations). Consequently, we had an insufficient number of observations to model it in function of heat load in these reserves. Only in the smallest reserve with the least natural shelter (KH), the use of artificial shelter increased markedly and the use of natural shelter decreased with increasing HLI. In conclusion, only the cow in the reserve reserve with the least natural shelter preferred artificial shelter for protection against heat load.

Although cattle's tendency to use or avoid the artificial shelter might also depend on previous experience (Bateson, 2004), we did not observe that the time the cattle had known the shelters before the start of the study was related to the general tendency to use the artificial shelter. Of the two artificial shelters that were used more than 1% of the time, one (in BB) was installed more than ten years before versus the other (in KH) only seven months before the start of the study. In the one reserve where we had one recently-installed and one older (more than ten years old) artificial shelter (VV), both were hardly used. Furthermore, animals' preferences are not only influenced by experience in a quantitative but also in a qualitative sense, thus by the association between a given choice and a pleasant or unpleasant consequence. For example, Grandin et al. (1994) found that cattle resisted to change a choice once they had learned to associate one given option with restraint in a squeeze chute or cattle crush. But the opposite is also possible. In our study, in the reserve with the most natural shelter (BB), cattle were occasionally fed hay inside the shelter, at times when the reserve manager judged natural feed availability to be too low (e.g. during prolonged snow cover). Although hay was never provided during the study period, the association with feed might still have contributed heavily to the observed general preference for the artificial shelter. Nevertheless, at high heat load, the cow in this reserve did not seek the artificial shelter but rather used natural shelter for protection against heat.

As also discussed in Van laer et al. (2014), cattle's preference for a certain type of shelter may also be influenced by anti-predatory or vigilance behaviour. Although the long domestication process may have reduced the bovine sensitivity to predators in the strict sense, vigilance against predators in a wide sense remains relevant. For example, visual obstruction by vegetation in grazing allotments has also been shown to increase vigilance in Angus x Hereford cross-bred cows (Kluever et al., 2008). Welp et al. (2004) showed that dairy cows increase vigilance in a novel feeding enclosure and in response to a dog or a person who had handled them aversively. Thus vigilance may also be a factor influencing cattle's apparent preference for more open natural shelter, versus an artificial shelter with three closed sides. Furthermore, closed walls hinder air movement, and thus allow less convective cooling and more heat accumulation than an open type of shelter (Mader et al., 1999). This was also reflected in our microclimatic measurements under artificial versus natural shelter, during high heat load. Consequently, if our study would have used artificial shelters with a more 'open design' (e.g. without walls), we might have obtained different results, with regard to the micro-climatic measurements as well as the cattle's use of the artificial shelter.

In the current summertime study, the heat load threshold at which cattle start to seek (natural or artificial) shelter and thus decrease their use of open area, as well as the strength of the effect, was associated with the amount of natural shelter and its spatial distribution across the grazed area. When natural shelter was abundant, the use of open area decreased notably and gradually with increasing heat load, but if natural shelter was less abundant, a greater scatter of it seemed to buffer the decrease of use of open area. At first glance this may seem counterintuitive. If shelter is highly scattered, cattle in open area are usually closer to shelter than they would be if shelter were more clustered, and thus they could be expected to make use of it more easily. But if open area and shelter regularly alternate when an animal is moving through the terrain (at random or in function of motivations other than shelter seeking), the animal may have less opportunity to accumulate heat load and thus its motivation to seek shelter may remain lower.

Moreover, the structural diversity in the terrain may influence its thermal dynamics. Air temperature is higher in open area than in patches of natural shelter (as confirmed by our microclimatic measurements). But this might be more extreme in large patches than in small patches of open area sharing more edge surface with patches of natural shelter. In urban environments, this is known as the "heat island" effect (Santamouris 2001). For example, a vegetated patch of 60 by 60 meters has a cooling effect of 2.9 °C on the immediate, non-shaded surroundings, and even cools 1.1 °C at a distance of

40 m (Shashua-Bar & Hoffman 2000). This can be another possible explanation why grazers would be less motivated to seek shelter in areas with highly scattered vegetation as heat load increases.

It must be mentioned that the above effects and trends are associated with rather large standard errors and variation. This is inherent to observational studies like the current one, as many hard-to-avoid differences between reserves or individual cows may influence the relation between climatic conditions and use of open area, natural and artificial shelter. For example, different breeds were used in different reserves, but not enough replicates were available to allow a proper between-breeds comparison. On the other hand, there is little indication for differing susceptibilities to heat load between the breeds used in the present study. Another factor that potentially influences cattle's sheltering behaviour is the use of specific locations for activities with greater priority than shelter seeking, such as drinking or grazing. For instance, Gaughan et al. (1998) unexpectedly found cattle preferred shade from an iron roof over shade from trees or vine leaves due to its proximity to water and feed troughs. Water is known to be one of the most important factors determining grazers' terrain use (e.g. Senft et al. 1987, Stuth 1991). However, motivational priorities are not fixed (Bateson, 2004). The degree to which terrain use is determined by the location of water or feed sources, can increase when heat load and thus the motivation to seek shelter declines. Furthermore, physical barriers to animal movement, such as steep slopes, impenetrable vegetation, or water courses potentially influence use of shelter (Stuth, 1991). The presence of water courses and pools (which also varied among the study reserves) can also lessen the motivation to seek shelter from heat load because cattle are known to partially submerge themselves in water to cool off (e.g. Clarke and Kelly, 1996).

In spite of the multiple other factors which may have influenced terrain use of the cattle we studied, our research does confirm that, even in temperate summers, across all studied reserves, heat load made cattle avoid open area and increase their use of the available (natural or artificial) shelter. As these relations were gradual, a general threshold value at which the cattle start to seek shelter could not be identified.

5 ANIMAL WELFARE IMPLICATIONS

A decrease in the use of open area cannot readily be translated in terms of 'need for shelter' or 'reduced animal welfare in absence of shelter', even in these specific reserves. On the other hand, application of the precautionary principle does argue in favour of providing additional shelter to avoid any potential welfare derogation in reserves with little natural shelter. Our study indicates that cattle would prefer natural shelter (additional vegetation), but new plantations need time to grow and may not always be appropriate for a number of reasons (expense, practical feasibility, management of the reserve, etc.). In these cases, one or several well-designed artificial shelters placed in strategic locations can also provide heat load relief.

6 CONCLUSIONS

Our findings indicate that, even in a temperate climate such as Belgium, cattle in nature reserves increasingly avoid open area and seek shelter at high heat load in summer. The strength of this response differed between nature reserves and was associated with the amount and spatial distribution of natural shelter across the reserve. Furthermore, this study documents that sufficiently dense natural shelter (vegetation) blocks solar radiation quite well, and at the same time allows more evaporative cooling as compared to an artificial shelter with three closed walls and one open side. In the current study, the (healthy and adult) cattle rarely used this type of artificial shelter as protection against high heat load, except in one nature reserve that contained little natural shelter. If sufficiently available, cattle preferred natural shelter to artificial shelter. Therefore, this study provides no evidence for added value of such an artificial shelter to protect (healthy and adult) cattle from heat load in nature reserves in temperate climate zones, as long as adequate natural shelter is available.

7 ACKNOWLEDGEMENTS

The authors thank the many people who helped with the field work for this study, including employees and local volunteers of Natuurpunt and the Government of Flanders' Agency for Nature and Forest (ANB), the ILVO staff who built the artificial shelters, and especially Thijs Decroos (ILVO Animal Sciences Unit). Furthermore, we thank the Research Institute for Nature and Forest for helping to recover a lost GPS collar and prof. Maurice Hoffmann for sharing his useful ideas concerning data analysis. Thanks to Miriam Levenson (ILVO) for English-language editing. This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RT 10/13 Pastress). The experiment was approved by ILVO's Ethical Committee (EC 2011/148 and EC 2011/148bis).

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CHAPTER 4

WINTERTIME USE OF NATURAL VERSUS ARTIFICIAL SHELTER BY CATTLE IN NATURE RESERVES IN TEMPERATE AREAS

Adapted from: WINTERTIME USE OF NATURAL VERSUS ARTIFICIAL SHELTER
BY CATTLE IN NATURE RESERVES IN TEMPERATE AREAS.

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APPLIED ANIMAL BEHAVIOUR SCIENCE (2015) 163: 39-49



ABSTRACT

The current study contributes scientific observation to the ongoing debate about whether and when artificial shelter (man-made), in addition to natural shelter (vegetation), should be provided to cattle grazing in nature reserves during temperate winters. In several year-round grazing projects in Belgium, we have investigated the effect of winter climatic conditions on cattle's use of natural versus artificial shelter. In eight nature reserves with varying amount and spatial distribution of natural shelter and an artificial shelter, GPS collars were used to monitor terrain use during one, two or three winters (per 30 min, 24h per day). Cattle location data were related to instantaneous open-field measurements of the Comprehensive Climatic Index (CCI). In addition, the effects of the time of day (day-time versus night-time) and the amount of natural shelter and its spatial distribution on these relationships were investigated. In most nature reserves, cattle increasingly avoided open area and sought shelter at CCI values $<0^{\circ}\text{C}$. The strength of the effect, and whether cattle used natural or artificial shelter differed between day-time and night-time and between reserves, and was partially explained by the amount of natural shelter and its spatial distribution across the reserve. Although artificial shelters with three closed walls provided better protection against cold (especially wind chill), cattle were more likely to use natural shelter as long as it was sufficiently available. Providing an artificial shelter in nature reserves appears to have added value for cattle's thermal comfort only when natural shelter is scarce.

KEYWORDS

Artificial shelter, natural shelter, vegetation, thermal comfort, temperate climate, cold stress

1. INTRODUCTION

In winter, cattle used in year-round grazing management of nature reserves are sometimes exposed to cold, rainy and windy conditions, even in temperate climate zones. This may cause discomfort in absence of adequate shelter, and if such conditions are extreme and/or persistent thermal stress responses (including reduced lying times, increased plasma and faecal cortisol and decreased white blood cell numbers) are triggered, indicating impaired welfare (Webster et al., 2008). Nevertheless, adult cattle in general are quite resistant to low ambient temperatures (Australian Agricultural Council, Ruminants Subcommittee, 1990). Moreover, in European nature reserves winter or year-round grazing management is mostly realised by cattle breeds (like Galloway, Aberdeen-Angus and Scottish Highland cattle) that are assumed to be relatively resistant to cold due to their thick hair coat and low energy demand, allowing them to accumulate fat easily (even on a poor quality diet) (Wallis de Vries, 1994). However, when rain or snow wets an animal's coat, the latter loses insulation value and consequently evaporative heat loss greatly increases. Especially in combination with convective heat loss (wind chill), this may drastically reduce skin temperature (Schutz et al., 2010) and cause discomfort, even at air temperatures higher than an animal's estimated Lower Critical Temperature (LCT)(the air temperature below which heat production and conservation mechanisms are switched on to retain a normal body temperature). Negative climatic effects are further exacerbated in young animals (Australian Agricultural Council, Ruminants Subcommittee, 1990) and when food availability is scarce. In the latter case ruminal fermentation - an important source of metabolic heat- is decreased. In the long term a poor body condition increases cold stress susceptibility (Tucker et al., 2007). For example, malnourishment in combination with harsh climatic conditions (for example at the end of the winter of 2004-2005) repeatedly caused the death of a significant part of the population of 'rewilded' Heck cattle and Konik ponies in the Dutch polder reserve De Oostvaardersplassen. These deaths were intensely criticised (Lorimer & Driessen 2013). However, even in less extreme winter conditions concerns have been raised about grazers' thermal comfort and welfare.

Under summer conditions, trees and shrubs can protect animals in forested nature reserves from heat load in summer conditions. Under winter conditions, the same vegetation can serve as wind break and can create a 'rain shadow' – an area where less precipitation reaches the sheltering animals (Van laer et al., 2014). In most reserves, trees and shrubs (from here on: natural shelter) are presumed to provide adequate protection against cold, rainy and windy conditions. On the other hand, also reserves where typically less natural shelter is present – such as polders and marshes

(Andresen et al. 1990) or riverine areas (Wallis de Vries 1994) - are sometimes grazed. Especially in such cases, the reserve managers, local governments and animal protection instances may be questioned about the need for additional shelter to assure the thermal comfort and welfare of the grazing animals.

In order to assess the degree of discomfort or stress caused by the combination of low temperatures and high wind speed, the Wind Chill Index (WCI), was developed. Currently the WCI is still widely used as the most relevant cold stress measure in both human and livestock research. A specific formula is available for cattle (formula 1)(Tucker et al., 2007). WCI values can be interpreted as an apparent temperature, and are usually expressed in degrees Celsius or Fahrenheit. To our knowledge no scientifically validated cold stress risk classes for cattle have been developed to be able to appraise WCI values. Thus, in order to assess whether a certain WCI value might impact thermal comfort and physiology, we just have to compare WCI values to established LCT's for cattle (as in Van laer et al. 2014). The Comprehensive Climate Index (CCI), developed by Mader et al. (2010), (formula 2) incorporates the effects of temperature, air humidity, wind speed and solar radiation in one measure to improve the assessment of cold (and heat) stress risk. This index is to be interpreted as an apparent temperature as well. According to Mader et al. (2010) cattle with a low susceptibility (i.e. healthy and well-adapted to cold) may experience mild, moderate and severe cold stress from values below 0°C, -10°C and -20°C onwards, respectively. For highly susceptible cattle these threshold values shift to 5°C, 0°C and -5°C, respectively.

The aim of the present study was to investigate the relationship between wintertime climatic conditions and the use of natural (existing vegetation) versus artificial (man-made) shelter by cattle in several year-round grazing projects in Belgium. In addition we investigated (1) the effect of the availability (amount) of natural shelter and its spatial distribution and (2) the effect of the time of day (day-time versus night-time) and on these relationships. Finally, we evaluated the effect of natural and artificial shelter on microclimate by taking microclimatic measurements inside and outside of each type of shelter, on several cold days.

2. MATERIALS AND METHODS

2.1. RESERVES AND ANIMALS

The study took place in eight nature reserves in Flanders (the northern part of Belgium). The same reserves and animals were used in our study into summertime sheltering behaviour, described in Chapter 3. For more information on the reserves and the cattle, we refer to **paragraph 2.1 and Table 1 of Chapter 3**.

2.2. DATA COLLECTION

In each study reserve, a Lotek Wildcell M5 GPS collar with GSM communication function (Lotek Wireless Inc., Newmarket, Canada) was fitted onto one cow to remotely monitor terrain use during one, two or three winters (Table 1). For more information on the GPS collars and the cattle wearing them, we refer to **paragraph 2.2 of Chapter 3**.

TABLE 1

Overview of the (winter) periods in which each reserve was studied.

reserve	study periods		
	winter 1	winter 2	winter 3
KH	1/10/2011 - 15/4/2012	1/10/2012 - 15/4/2013	1/10/2013 - 13/01/2014
VV	n.a.	1/10/2012 - 15/4/2013	1/10/2013 - 13/01/2014
MS	n.a.	1/10/2012 - 15/4/2013	1/10/2013 - 13/01/2014
EB	1/10/2011 - 15/4/2012	1/10/2012 - 23/12/12 ^a	1/10/2013 - 13/01/2014
KE	n.a.	n.a.	1/10/2013 - 10/12/2013 ^b
HB	1/10/2011 - 15/4/2012	1/10/2012 - 01/02/2013 ^a	1/10/2013 - 13/01/2014
HP	1/10/2011 - 15/4/2012	1/10/2012 - 15/4/2013	1/10/2013 - 13/01/2014
BB	n.a.	1/10/2012 - 15/4/2013	1/10/2013 - 13/01/2014

KH: Katershoeve, VV: Velpvallei, MS: Molenstede, EB: Ename Bos, KE: De Kevie, HB: Heidebos, HP: Hobokense Polder, BB: Beninksberg. n.a.: reserve not studied in the relevant winter. ^aOn these dates the GPS-tagged animals lost their GPS collars, which could not be re-attached before the end of the relevant winter period. ^bOn this date management by grazing was stopped and all Heck cattle were removed from this reserve.

Between 1/10/2011 and 13/02/2012 we had registered the animals' positions every 15 minutes between 8:00 a.m. (GMT) and 8:00 p.m. (GMT). However, because also night-time sheltering behaviour was deemed relevant, as of 14/2/2012, we started tracking the cattle's terrain use over 24h, but with only one registration per 30 minutes. The 30-min intervals were chosen as a measure to guarantee GPS battery life until the batteries could be replaced at the annual veterinary check-up.

Similar as in Chapter 3, animal locations were plotted onto digital maps of the reserves, to determine when different shelter types were used (in ArcMap2010). For more information on the digital maps, we refer to **paragraph 2.2 of Chapter 3**.

Custom-built Campbell Scientific BWS200 weather stations (Campbell Scientific Inc., Logan, Utah, US), placed in open area in six out of eight reserves, registered the average air temperature, air humidity, solar radiation and wind speed per 15 minutes. For the two reserves without a weather station (MS and BB), climatic variables were used from the closest weather station (VV; 15 km and 16 km from MS and BB, respectively). The weather stations' measurements also allowed calculation of 15-minute values of the WCI (formula 1) and the CCI (formula 2).

$$WCI = 13.12 + 0.62 * Ta - 13.17 * (WS * 3.6)^{0.16} + 0.40 * Ta * (WS * 3.6)^{0.16} \quad (1)$$

$$CCI = Ta + Eq. 1 + Eq. 2 + Eq. 3 \quad (2)$$

$$Eq.1 = e^{((0.00182 * RH + 1.8 * 10^{-5}) * Ta * RH)} * (0.000054 * Ta^2 + 0.00192 * Ta - 0.0246) * (RH - 30)$$

$$Eq.2 = (-6.56) / e^{[(1 / (2.26 * WS + 0.23))^{0.45}] * (2.9 + 1.14 * 10^{-6}) * WS^2.5 - \log 0.3 (2.26 * WS + 0.33)^{-2}} - 0.00566 * WS^2 + 3.33$$

$$Eq.3 = 0.0076 * Rad - 0.00002 * Rad * Ta + 0.00005 * Ta^2 * \sqrt{Rad} + 0.1 * Ta - 2$$

where Ta = air temperature in °C, RH = relative air humidity in decimal form (e.g. 0.60, not 60%), and WS is wind speed in m/sec.

Similar as in Chapter 3, evaluation of the effect of natural and artificial shelter on microclimate was done via additional microclimatic measurements. These were carried out during seven cold days throughout the first two winters, in open areas (in 3 open places per reserve), under natural shelter (in 3 patches with trees and shrubs, per reserve) and under artificial shelter (one per day per reserve). Air temperature, wet bulb temperature and black globe temperature were measured with the Testo 400 Wet Bulb Globe Temperature-probe (Testo AG Inc., Lenzkirch, Germany). Wind speed and relative air humidity were measured with a Testo 410-1 Pocket Vane Anemometer (Testo AG Inc., Lenzkirch, Germany). These variables were used to calculate and compare the WCI between the natural shelter, artificial shelter and open area.

2.3. DATA ANALYSIS

2.3.1. EFFECT OF NATURAL AND ARTIFICIAL SHELTER ON MICROCLIMATE

The differences in air temperature, black globe temperature, wind speed and the WCI measured in the open areas, under natural and under artificial shelter were modelled by means of a mixed model ANOVA (in SAS 9.4) which included a repeated statement to correct for the effect of repeated measurements within each reserve. The interaction between reserve and shelter type was included to reveal any existing differences in effectiveness of the shelter types between reserves. In the post-hoc tests, Tukey-Kramer adjustments were used to account for multiple comparisons.

2.3.2. QUANTIFICATION OF THE SPATIAL DISTRIBUTION OF SHELTER

The amount and spatial distribution of natural and artificial shelter across each reserve potentially influence the cattle's use of these shelters. The spatial distribution of shelter was quantified by means of a 'structural diversity index' per reserve. For more information on its calculation and its value per reserve, we refer to **paragraph 2.3 and Table 1 of Chapter 3**, respectively.

2.3.3. FACTORS INFLUENCING CATTLE SHELTERING BEHAVIOUR

For each animal position we determined, in ArcMap 2010, if it was located in (1) open area (= no shelter), (2) natural shelter, including the surrounding 5m because we assumed cattle would still find protection (e.g. from wind or shade) within 5m of trees or shrubs, or (3) artificial shelter including the surrounding 5m. These data were coupled to the local climatic variables and indices of the 15 minutes preceding the registration of the animal position. All records for which either the WCI or the CCI had a value $\geq 20^{\circ}\text{C}$, were removed from the dataset, to exclude possible periods where sheltering behaviour could have been determined by excessive heat load rather than cold. The threshold of 20°C was chosen to be low enough under the estimated Upper Critical Temperature of cattle in general (25°C ; Van laer et al., 2014) while retaining sufficient data certainly above the Lower Critical Temperature (only 6% of all data were removed, see Results section).

To determine if a certain type of shelter is generally preferred (across all climatic conditions) we compared the expected use of the three shelter types – i.e. the expected distribution of GPS registrations over the three types - with their observed use (the observed distribution of GPS registrations over the three types), per reserve (n=8). For more information on the definition of observed and expected use, we refer to **paragraph 2.6 of Chapter 3**.

The ratio of observed/expected use can be either between 0 and 1 (indicating avoidance of the shelter) or between 1 and infinity (indicating preference for the shelter). The closer the ratio is to 0, the stronger the shelter type is avoided; the closer the ratio to infinity, the stronger the shelter is preferred.

Because cattle are known to have pronounced circadian activity patterns (e.g. Arnold 1984) and because their use of open area vs. shelter may coincide with particular behaviours (e.g. grazing occurs mainly in grassland and thus open area), we examined if a distinct circadian pattern existed in the use of different types of shelter, before examining whether and how sheltering behaviour is affected by climatic factors. We plotted the percentage of animal locations (averaged over the entire study period), that fell within each type of shelter, per time of day (half hourly), per reserve. We considered the time between 9:00 a.m. and 6:00 p.m. LMT (GMT+2) as 'day' and the time between 9:00 p.m. and 6:00 a.m. LMT as 'night'. The two three-hour periods in between, i.e. those around sunrise and sunset, were considered 'dawn' and 'dusk', respectively. In HB, HP, KH and VV open area was used somewhat less at night. In these cases, mostly natural shelter was used at night. In the two reserves where artificial shelter was used frequently (BB and KH), usage occurred at different times of day. In the reserve with the most natural shelter (BB), the artificial shelter was used mainly during the day, whereas in the reserve with the least natural shelter (KH), the artificial shelter was used mainly in the later part of the night, at dawn and during the earlier part of the day. Because of these (limited) diurnal patterns in the use of open area and shelter, the final analyses were all carried out (1) for all registrations, during the day, night, dusk and dawn and (2) separately for registrations during day-time and during night-time.

First, we also carried out preliminary graphical analyses of the effect of climatic conditions on the use of open area. More specifically, we plotted the mean (\pm standard error) use of open area, natural and artificial shelter at rounded values (no decimals) for the two cold stress indices (uncorrected for repeated measurements). As such we determined that the CCI predicted the change in the use of the three shelter types better than the WCI. Based on this finding and the fact that the CCI includes the four climatic variables contributing to thermal discomfort – whereas the WCI includes only two of these - the final, statistical analyses used the CCI as predicting cold stress index.

In addition, the graphical analysis of the raw data showed that the mean use of open area generally (but not in KE, EB and VV) decreased and the use of natural shelter increased from CCI <0°C on. In the final analyses, a CCI of 0°C was thus used as threshold value below which the use of open area started to change.

For the final, statistical analysis of the effect of cold on the use of open area (as opposed to shelter, either natural or artificial), a mixed model logistic regression was fitted, which modelled the use of open area, each taking the effect of quantitative availability and spatial distribution (structural diversity) of natural shelter on this relationship into account. The use of open area (binomially distributed), was thus modelled as:

$$\text{probability of use of open area} = e^y / (1 + e^y)$$

where

$$\text{if CCI} \geq 0 \text{ value: } Y = a + \varepsilon$$

$$\text{if CCI} < 0 \text{ value: } Y = a + b * x_1 + c * x_2 + d * x_3 + e * x_1 * x_2 + f * x_1 * x_3 + g * x_2 * x_3 + \varepsilon$$

where

x1= the effect of the CCI

x2= the effect of the amount of natural shelter, expressed as deviation (+ or -) from the situation where half of the area is covered by natural shelter, thus theoretically ranging between -50 (if there would be no natural shelter at all) and 50 (if the whole area would be covered by natural shelter)

x3= the effect of the structural diversity index (Table 1)

We fitted the above model (1) for all registrations, during the day, night, dusk and dawn and (2) separately for registrations during day-time (9:00 a.m. – 6:00 p.m. LMT), during night-time (9:00 p.m. – 6:00 a.m. LMT). All mixed models included time of day as random factor to correct for repeated measurements per day, nested within the experimental unit, i.e. the reserve. All analyses were conducted in SAS 9.4.

3. RESULTS

3.1. RANGE OF CLIMATIC CONDITIONS

When considering all climatic data coupled to animal locations, over the eight study reserves, the observed air temperature ranged between -18.0 °C and 27.0°C, the mean was 5.6°C. The wind speed ranged between 0 and 27.4 m/sec, with a mean of 1.8 m/sec. The observed WCI and CCI ranged between -17.4°C and 28.5°C (mean = 5.6 °C) and between -21.6°C and 32.2°C (mean = 3.4 °C), respectively.

As the threshold for WCI and CCI has been established at 20°C, 6% of the data were removed to exclude possible periods where sheltering behaviour could have been motivated by heat load. In the remaining dataset, the WCI was <0°C in 21.7% of the observations, the CCI was <0°C in 33.8% of the observations. In KE, only a relatively short observation period could be covered (Table 1), during which WCI and CCI values ≤0°C were quite rare. This might explain why results from KE often differ from those from other reserves.

3.2. EFFECT OF NATURAL AND ARTIFICIAL SHELTER ON MICROCLIMATE

We found no significant effect of the interaction between reserve and shelter type on any of the climatic variables (Table 2). No difference was thus observed in the effectiveness of the shelter types against aversive winter weather among the reserves. Between open area, natural and artificial shelter we found no significant differences in air temperature, Black Globe Temperature or relative air humidity (Table 2), but wind speed and Wind Chill Index did differ between shelter types. Averaged over all reserves, wind speed was significantly higher in open area than in artificial shelter ($\Delta=1.25$ m/sec). In natural shelter, the wind speed was intermediate (1.04 m/sec higher than in open area) but not significantly different from that in artificial shelter. The Wind Chill Index was about 4.2 units higher in natural shelter than in open area and about 3.7 units higher in artificial shelter than in natural shelter.

TABLE 2.

Effect of shelter type, reserve, and the interaction between them on four climatic variables (black globe and air temperature, wind speed and air humidity) and the Wind Chill Index. In addition, least squares means for each climatic variable are compared between shelter types.

		black globe temperature (°C)	air temperature (°C)	wind speed (m/sec)	air humidity (%)	Wind Chill Index (°C)
shelter type		P =0.7847	P =0.996	P <0.0001*	P=0.3374	P <0.0001*
reserve		P =0.8229	P =0.9574	P =0.8202	P =0.1376	P =0.9199
shelter type *reserve		P =0.7901	P =0.9432	P =0.1958	P =0.9948	P =0.3093
		LSM ± SEM	LSM ± SEM	LSM ± SEM	LSM ± SEM	LSM ± SEM
mean	OA	3.15 ± 1.19 a	1.79 ± 1.15 a	1.56 ± 0.13 a	81.57 ± 3.72 a	-1.49 ± 1.42 a
(over all reserves)	NS	2.74 ± 1.19 a	1.79 ± 1.15 a	0.53 ± 0.13 b	83.16 ± 3.72 a	2.67 ± 1.42 b
	AS	3.06 ± 1.19 a	1.83 ± 1.15 a	0.31 ± 0.13 b	79.48 ± 3.72 a	6.33 ± 1.43 c

OA = open area, NS = natural shelter, AS= artificial shelter. Least square means (LSM) ± standard errors (SEM) without a common letter differ significantly according to Tukey-Kramer corrected post-hoc comparisons, P<0.05.

3.3. FACTORS INFLUENCING CATTLE SHELTERING BEHAVIOUR

3.3.1. GENERAL PREFERENCES

In five reserves, the artificial shelter was used slightly more than expected. In KE, the artificial shelter (including the 5m around it) was never used at all. Although artificial shelter was used slightly more than expected in VV, MS, EB, HB and HP, in these reserves, it was still used infrequently. Only in two reserves (KH and BB) was the artificial shelter used much more than expected by chance and for more than 2% of the time (Fig. 1).

There was no obvious association between % use of artificial shelter and reserve characteristics such as the amount and spread of natural vegetation. In six out of eight reserves (VV, MS, EB, HB, HP and BB) open area was used slightly more and natural shelter was used slightly less than expected based on their coverage. In two other reserves (KH and KE) natural shelter was used slightly more and open area was used slightly less than expected based on their coverage (Fig. 1).

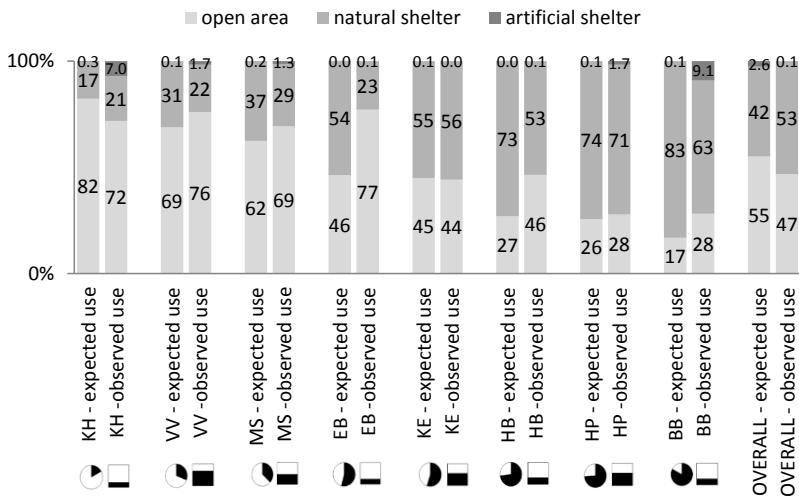


FIGURE 1. Comparison of the expected use (percentage of the reserves covered by the different types of shelter) with the observed use (percentage of GPS registrations that took place within them), per reserve. Abbreviations for study reserves are explained in Table 2. For each reserve, circular symbols represent the percentage of natural shelter and square symbols the structural diversity, with more shading indicating higher values. OVERALL gives the average over all reserves.

3.3.2. COLD STRESS INDICES

Considering the data from all reserves and all time periods (day, night, dusk and dawn), the effect of CCI on the use of open area was influenced by the interaction with the amount of natural shelter and the three way interaction with structural diversity (Table 3). In reserves with abundant natural shelter (such as BB, HP and HB), the use of open area indeed did decrease when CCI values fell below 0°C (Fig. 2). Also, if an intermediate amount of natural shelter was present and was more clustered (as in EB) the use of open area decreased when CCI fell below 0°C. If an intermediate amount of natural shelter was present and if it was more scattered (as in MS and KE), CCI decreasing below 0°C had a lesser influence on use of open area. If natural shelter was sparse, CCI decreasing below 0°C decreased the use of open area if shelter was more clustered (as in KH) but increased the use of open area if shelter was more scattered (as in VV).

TABLE 3

Effects of the Comprehensive Climatic Index (CCI), the amount of natural shelter (NS) and the structural diversity index (S) on the use of open area.

Effect	Estimate	SE	DF	t Value	Pr > t
overall					
Intercept	0.987	0.073	2877	13.48	<0.0001
INDEX ^a	0.125	0.021	6128	5.83	<0.0001
NS	-0.038	0.001	2858	-31.87	<0.0001
S	-1.437	0.178	2830	-8.06	<0.0001
INDEX ^a *NS	-0.004	0.001	5823	-4.54	<0.0001
INDEX ^a *S	-0.079	0.054	6197	-1.47	0.1423
INDEX ^a *NS *S	0.014	0.002	5455	6.61	<0.0001
day-time					
Intercept	1.019	0.136	920.9	7.49	<0.0001
INDEX ^a	0.022	0.042	1328	0.54	0.5901
NS	-0.033	0.002	926.9	-15.1	<0.0001
S	-1.348	0.33	906.3	-4.09	<0.0001
INDEX ^a *NS	-0.002	0.002	1458	-1.24	0.2159
INDEX ^a *S	-0.025	0.108	1406	-0.23	0.8158
INDEX ^a *NS *S	0.011	0.004	1375	2.44	0.0146
night					
Intercept	1.492	0.248	711.2	6.01	<0.0001
INDEX ^a	0.078	0.061	972.1	1.29	0.1982
NS	-0.053	0.004	697.9	-13.09	<0.0001
S	-2.723	0.591	691.2	-4.61	<0.0001
INDEX ^a *NS	-0.005	0.002	1030	-1.94	0.0525
INDEX ^a *S	-0.065	0.162	1039	-0.4	0.6896
INDEX ^a *NS *S	0.018	0.007	979.9	2.67	0.0077

^a=0 if ≥0, =original value <0

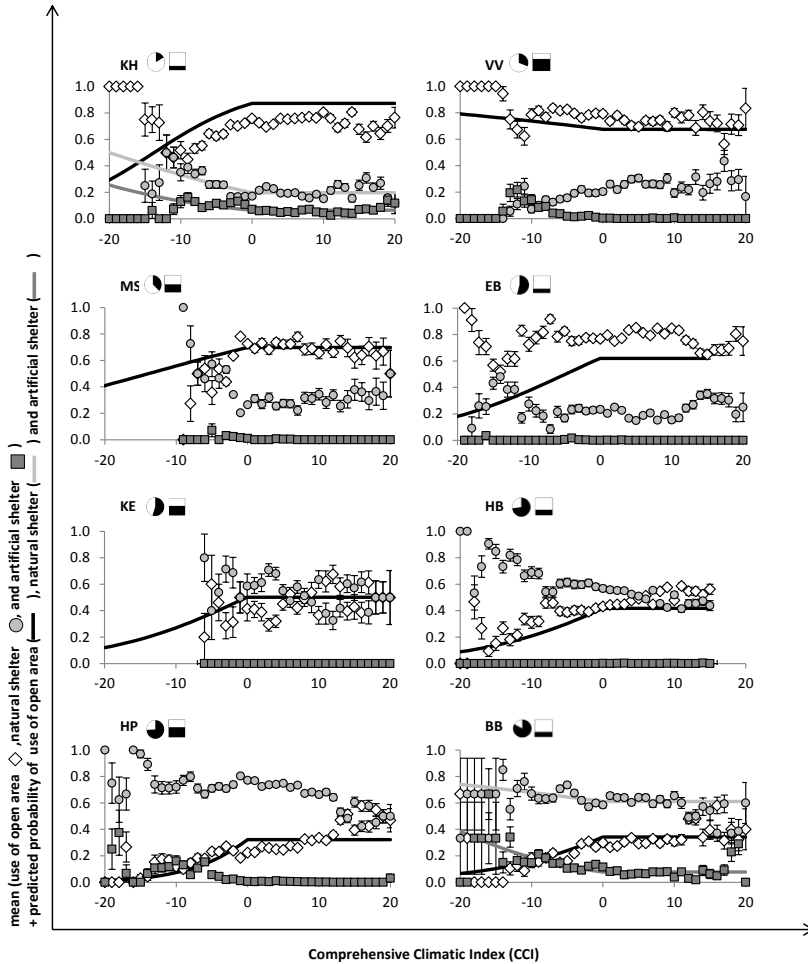


FIGURE 2. Plots of uncorrected (for repeated measures) means (\pm SE) of the use of open area (OA; \diamond), natural shelter (NS; \circ) and artificial shelter (AS; \square) and the probability of use of open area predicted by the logistic model (taking the amount and spatial distribution of natural shelter into account), at rounded values of the CCI, per reserve. Only for the two reserves where artificial shelter was used a lot ($>2\%$ of the time; KH and BB), the graphs also depict the predicted (by additional logistic regressions per reserve) use of artificial shelter and natural shelter. Abbreviations for study reserves are explained in Table 1. For each reserve, circular symbols represent the percentage of natural shelter and square symbols the structural diversity, with more shading indicating higher values.

When day and night data were analysed separately, the effect of CCI on the day-time use as well as night-time use of open area was influenced by the three-way interaction with the availability of natural shelter and structural diversity (Table 3). In the reserves with abundant natural shelter (BB, HP, HB and KE), the night-time use of open area was basically (in thermoneutral conditions) lower, and decreased more steeply with CCI decreasing below 0°C than during day-time (Fig. 3). When natural shelter was abundant, a greater scatter of shelter (a higher structural diversity) amplified the decrease of use of open area (during day-time as well as night-time). When natural shelter was scarce, the day-time use of open area was less influenced by CCI, and a greater scatter of shelter buffered the decrease of use of open area, during day-time as well as night-time (Fig. 3). During night-time, the use of open area was more influenced by CCI than during day time, also in the reserves with less natural shelter.

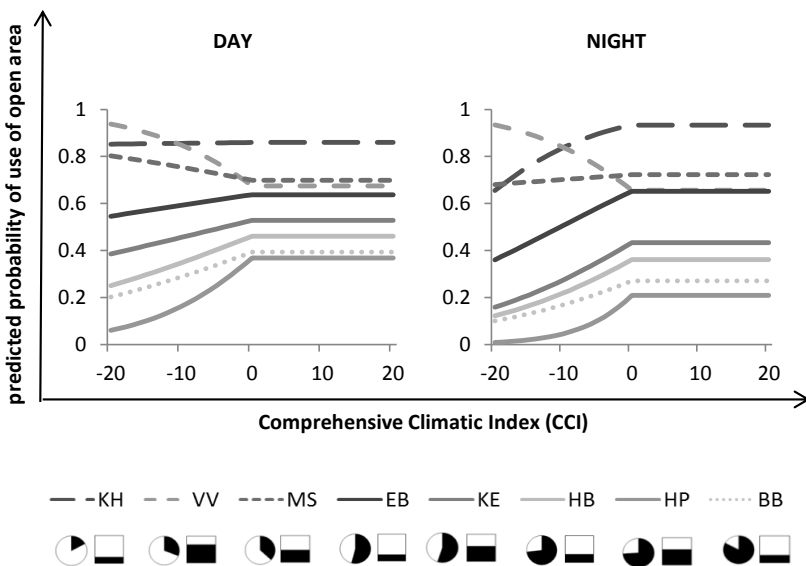


FIGURE 3. The probability of use of open area predicted by the logistic model (taking the amount and spatial distribution of natural shelter into account), in function of the Comprehensive Climatic Index (CCI), during day-time and during night-time, per reserve. Abbreviations for study reserves are explained in Table 1. For each reserve, circular symbols represent the percentage of natural shelter and square symbols the structural diversity, with more shading indicating higher values.

Only for the two reserves where artificial shelter was used in > 2% of observations (BB, and KH), day-time use of natural and artificial shelter in these two reserves was modelled in function of CCI (Fig. 4), for day-time and night-time, separately. In both reserves, the use of artificial shelter increased but the use of natural shelter decreased with CCI decreasing below 0°C during day-time. During night-time, the use of natural shelter as well as artificial shelter increased with CCI decreasing below 0°C in BB, although the use of artificial shelter always remained much lower than the use of natural shelter. In KH the use of natural shelter did not increase significantly but the use of artificial shelter did increase significantly with CCI decreasing below 0°C during night-time.

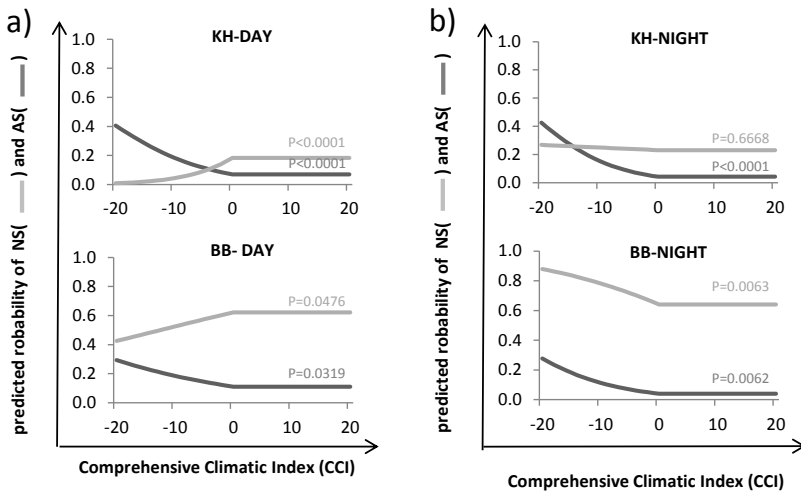


FIGURE 4. The probability of use of natural and artificial shelter predicted by the additional logistic models in function of the Comprehensive Climatic Index (CCI) during day-time (a) and night-time (b), for the two reserves where artificial shelter was used a lot (>2% of the time (>250 observations); KH and BB).

4. DISCUSSION

In order to contribute to the debate about the need to provide artificial shelter as protection against winter weather for cattle in nature reserves in temperate areas we investigated (1) the effectiveness of natural and artificial shelter against cold, and (2) the changes in the use of open area vs. natural or artificial shelter as a function of cold in eight nature reserves in Belgium.

From an apparent temperature (CCI) below 0°C on, the use of the three different shelter types generally started to change in comparison to assumed thermoneutral conditions ($CCI \geq 0^\circ C$). The CCI value of 0°C can thus be considered as a threshold value at which the presumed well adapted cattle in most reserves started to seek shelter. This is similar to the CCI threshold Mader et al. (2010) used to assess mild cold stress in low susceptible cattle. However, it is well above the usual estimates for the LCT of adult cattle, which lie between -16 °C and -30 °C (Australian Agricultural Council, Ruminants Subcommittee, 1990). Similarly, Rubio et al. (2008) found that rangeland cattle in New Mexico decreased their use of open pasture and increased their use of woodland with decreasing temperature (below the average of the past 12 days), even at WCI's between -2.5°C and 13.6°C. Furthermore, studies specifically investigating the effect of winter climatic conditions on rangeland cattle sheltering behaviour are rather rare. In addition they are mostly conducted in considerably colder conditions (e.g. Beaver and Olson, 1997), and thus the results are hardly comparable with ours. Even studies into cattle foraging behaviour - more generally – in relation to winter climatic are mostly limited to colder climates, e.g. the cold desert climate of northern Utah (US) in Malechek and Smith (1976) or the cold mountain winter climate of Montana in Olson and Wallander (2002). In our study, the lowest CCI values registered in open area, did fall below the estimated LCT of low producing (dairy and beef) cattle, which is at least an indication that the cattle in our study would have - occasionally and temporarily – experienced thermal discomfort when residing in open area.

Whether an animal prefers artificial or natural shelter is often assumed to depend primarily on their relative effectiveness. Our measurements of microclimate in open area and under natural and artificial shelter during cold days indicated that the studied artificial shelters (with one open side and three closed walls out of wooden planks or boards) generally provided better protection against wind chill than natural shelter (vegetation). Thermal comfort should thus be superior in the artificial shelter than when using natural shelter. Despite its better protection against cold, artificial shelter was used for more than 2% of the time in two reserves only. Only in the reserve with the least natural shelter (KH), did the use of the artificial shelter, but not

the use of natural shelter, increase significantly as the apparent temperature (the cold stress indices) decreased below 0°C, especially during the night. In BB, the night-time use of natural shelter remained higher and increased more steeply than the use of artificial shelter as the apparent temperature decreased below 0°C. In the other reserves the use of artificial shelter could not (reliably) be modelled due to the low number (n=0-236) of observations. In BB, the use of artificial shelter during the day may very likely have been caused by the provision of additional feed on cold days, rather than by the effect of the climatic conditions on thermal comfort (cf. Harris et al., 2002). This was the only reserve where hay was provided inside the artificial shelter at times when the reserve manager judged natural feed availability to be too low (e.g. during prolonged snow cover). Hay was always placed in the shelter during the day. The reserve with the least (17%) natural shelter (KH) was therefore the only reserve for which we can reliably state that the observed animal was more likely to use the artificial than natural shelter as protection against cold, both during day-time and night-time. In the other six reserves the cattle were always more likely to use natural than artificial shelter, irrespective of the apparent temperature.

The degree to which cattle sought shelter as the apparent temperature decreased, differed between night and day. Day-time use of open area clearly decreased as the apparent temperature decreased below 0°C when natural shelter was relatively abundant. In reserves with less natural shelter, this effect was less clear, or even opposite. Night-time use of open area, on the other hand, decreased as the apparent temperature decreased below 0°C in all reserves, except when natural shelter was scarce and highly scattered (e.g. MS and VV). This suggests that, during day-time the motivation to seek shelter from cold is subordinate to motivation for other behaviours (such as grazing), or 'not worth the travelling effort', but becomes more important during night-time. Indeed, optimum foraging theories for ruminants assume that these animals' habitat use is basically a consequence of their foraging behaviour, which is geared towards a maximum ratio of energy intake (feed intake, quantitatively and qualitatively) and energy expenditure (Stuth, 1991). However, due to decision making at landscape-level (Senft et al. 1987), foraging decisions may be overshadowed by the time and energy costs of travel (e.g. WallisDeVries 1996) or other, non-foraging motivations. In such cases, foraging may occur in suboptimal habitats while the animal primarily moves among, for example, optimal feeding habitat, drinking places and sheltering sites (Senft et al. 1987). Indeed, the location of water is known to be one of the most important factors determining grazers' terrain use (e.g. Senft et al. 1987, Stuth 1991).

However, motivational priorities are not fixed (Bateson, 2004). The degree to which an animal's terrain use is determined by the location of water or feed sources is known to change seasonally (e.g. Harris et al., 2002), and decrease when climatic conditions transgress the animal's thermoneutral zone, and thus the motivation to seek shelter increases (Stuth, 1991). The other way around, it is also possible that in our study, the motivation of the cattle to seek shelter only overcame the required 'cost' - travelling effort and leaving preferred grazing sites (usually open area) - when their motivation to graze was low, i.e. at night.

The degree to which cold influenced the use of shelter, differed substantially between day and night and between reserves. However, this was partially explained by the amount of natural shelter and its spatial distribution across the grazed area. When natural shelter was scarce but more scattered this buffered the decrease of use of open area. This may be explained by the effort - time and energy costs of travel (e.g. WallisDeVries 1996) - required to reach such scarce natural shelter. Such an effort may simply not be worth the payoff provided by shelter.

Other factors might influence cattle's use of natural and artificial shelter, but are hard to avoid in observational field studies like the current one. Such factors include the breed of cattle (although there is little indication for differences in cold-stress susceptibility between the breeds used in the present study), or the proximity to preferred feeding or drinking sites. This possibility is illustrated by Gaughan et al. (1998), who unexpectedly found that cattle preferred shade from an iron roof over shade from trees or vine leaves, due to its proximity to water and feed troughs. Furthermore, physical barriers to animal movement, such as steep slopes, impenetrable vegetation, or water courses potentially influence the use of shelter (Stuth, 1991). In spite of these limitations, this study reveals that, even in temperate winters, cattle in most of our eight nature reserves increasingly avoided open area and sought shelter from an apparent temperature around 0°C on, and especially so during night-time.

Only when natural shelter was sparse, the artificial shelter rather than natural shelter was used as protection against cold. Yet, this does not necessarily mean that the animal's welfare is impaired in absence of shelter. On the other hand, animal welfare risk management very often draws from the 'precautionary principle', which is based on the view that the lack of full scientific certainty cannot be used as a reason for postponing measures to prevent potential negative effects on animal welfare (Croney and Millman, 2007). Viewing the results of the current study in this framework, they can be used to argue in favour of providing at least some form of additional shelter

in open reserves containing little natural shelter. Our study indicates that, in this case, cattle prefer natural shelter (extra vegetation), despite the better protection against wind chill offered by artificial shelter with at (at least) three closed walls. On the other hand, when providing additional natural shelter is incompatible with vegetation and landscape management objectives, one or more well-designed shelter(s) in (a) strategic location(s) can also provide shelter from the cold.

5. CONCLUSIONS

Our findings indicate that in winter, even in a temperate climate such as Belgium, cattle in nature reserves increasingly avoid open area and seek shelter when the apparent temperature (quantified by means of the Comprehensive Climatic Index) drops below 0°C, and especially so during the night. Yet our results do not provide conclusive evidence that additional shelter is needed to protect cattle from cold as long as adequate natural shelter- sufficiently dense and in a sufficient amount to allow the animals to shelter together - is available. Artificial shelters with one open side and three closed walls generally provided a better protection against wind chill and thus a higher apparent temperature. Despite this, with the exception of one reserve where natural shelter was sparse, the monitored cattle chose natural shelter as protection against cold instead of an artificial shelter.

6. ACKNOWLEDGEMENTS

The authors thank the many people who helped with the field work for this study, including employees and local volunteers of Natuurpunt and the Government of Flanders' Agency for Nature and Forest (ANB), the ILVO staff who built the artificial shelters, and especially Thijs Decroos (ILVO Animal Sciences Unit) and Arne Van Landschoot (an MSc student who helped with data collection and preliminary data analysis). Thanks to Miriam Levenson (ILVO) for English-language editing. This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RT 10/13 Pastress). The experiment was approved by ILVO's Ethical Committee (EC 2011/148 and EC 2011/148bis).

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CHAPTER 5

EFFECT OF HEAT LOAD AND SHADE ON BEHAVIOURAL INDICATORS OF THERMAL DISCOMFORT IN HOLSTEIN DAIRY AND BELGIAN BLUE BEEF CATTLE ON PASTURE

Adapted from: EFFECT OF HEAT LOAD AND SHADE ON BEHAVIOURAL INDICATORS OF THERMAL DISCOMFORT IN HOLSTEIN DAIRY AND BELGIAN BLUE BEEF CATTLE ON PASTURE

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ABSTRACT

Using behavioural indicators of thermal discomfort, i.e. shade seeking, panting scores (PS) and respiration rate (RR), we evaluated the effect of hot summer conditions and shade, for a herd of adult Holstein dairy cows and a herd of adult and juvenile Belgian Blue beef cattle kept on pasture in a temperate area (Belgium). During the summer of 2012, both herds were kept on pasture without access to shade (NS). During the summers of 2011 (for all three cattle types) and 2013 (for adult dairy and beef cows only; no calves in the 2013 trial) each herd was divided into one group with (S) and one without (NS) access to shade. Shade was provided by young trees with shade cloth (80% reduction in solar radiation) hung between them. For S animals, we investigated how shade use was related to hot conditions as quantified by six climatic indices. The Heat Load Index (HLI), which incorporates air temperature and humidity, solar radiation and wind speed, was the best predictor of the six indices tested. In 2011, there was a relatively high threshold for use of shade. When HLI = 90, shade use probability reached 17% for dairy cows, 27% for adult beef cows and 25% for beef calves. In 2013, however, at HLI = 90, shade use probability reached 48% for dairy cows and 41% for adult beef cows. For animals from the NS treatment we determined the effect of hot summer conditions on RR and PS (with 0 = no panting and 4.5 = extreme panting). In all three types of cattle, an increase in black globe temperature was the best predictor for increasing RR and PS. Furthermore, we determined how the effect of hot summer conditions on RR and PS was affected by the use of shade. Under hot conditions (black globe temperature $\geq 30^{\circ}\text{C}$), more than 50% of the animals under shade retained normal PS and RR (PS < 1 and RR < 90 BMP for adult cows and < 120 BPM for calves), whereas normal RR and PS were significantly less prevalent for animals outside shade. Our findings suggest that, even in temperate summers, heat can induce thermal discomfort in cattle, as evidenced by increases in shade use, RR and PS, and that shade increases thermal comfort.

KEYWORDS: Heat stress, shade, temperate climate, thermoregulatory behaviour, cattle

1 INTRODUCTION

In most temperate regions, beef and dairy cattle are kept on pasture for at least part of the year and especially during the summer. Pasturing has some important benefits for cattle health and welfare, but it also poses disadvantages and risks, including exposure to adverse weather conditions (van den Pol-van Dasselaar, 2005). In subtropical regions, heat stress (behavioural and physiological effects of hot ambient conditions) has been thoroughly documented to negatively impact the health, welfare and productivity of unsheltered cattle. Shade provision is known to alleviate many signs of heat-stress, as reviewed by e.g. Armstrong (1994).

In temperate regions, however, fewer studies have been done on the need for and effectiveness of shade (reviewed by Van laer et al., 2014). But, recent research (e.g. Hammami et al., 2013) has shown that traditional climatic indices and associated threshold values to define heat stress are outdated and too general to evaluate heat stress in cows currently kept in temperate areas. Observations based on new heat stress thresholds for traditional heat stress indices show that summer climatic conditions occasionally do fall outside highly productive cattle's thermoneutral zone, even in temperate areas, such as Belgium (Van laer et al., 2014).

In Holstein dairy cows, the most common dairy breed used in temperate regions, genetic selection has doubled the milk yield per cow in the last 40 years (Oltenuacu and Broom, 2010). Such a high production level requires a high metabolic rate, which results in considerable metabolic heat production (Fuquay, 1981; Kadzere et al., 2002), which makes it difficult for the cow to dissipate its body heat under hot ambient conditions. The double-muscled Belgian Blue breed (the dominant breed in the Belgian beef industry) is assumed to be more susceptible to heat stress than most other beef breeds (Halipre, 1973), owing to reduced oxygen transport efficiency (Lekeux et al., 2009) and reduced pulmonary and cardiac function (Amory et al., 1992; Gustin et al., 1988). This is caused by the relatively small volume of heart and lungs (in comparison to the body volume) and the aberrant myostatin gene (Grobet et al., 1998). Research on heat stress in Belgian Blue beef cattle is limited, however, to field studies on the sheltering behaviour of Belgian pastoral beef cattle (Roselle et al., 2012).

Two main strategies are used to assess the need for protection against heat stress: weather-based or animal-based measures. The panting score (PS) is an example of the latter and is based on visual evaluation of the presence and degree of two important heat stress symptoms in cattle, panting and drooling (Mader et al., 2006; Mader et al., 2010; Schütz et al. 2014). The score varies between 0 (no panting or drooling) and 4.5 (extreme panting and drooling). Meat & Livestock Australia advises cattle keepers to cease all handling and movement of cattle as soon as 10% of cattle have a PS of 2 or above (<http://www.mla.com.au/files/02daccf7-a8ef-4c2e-9288-9d5900e40fa9/heatload-in-feedlot-cattle.pdf>). Proactive planning of cattle handling and management based on weather-predictions, requires antecedent validation of climatic heat stress indices and associated heat stress thresholds (Table 1). Not all heat stress thresholds have been validated based on animal-based measures, but the more recent climatic indices, such as the Heat Load Index (HLI; Gaughan et al., 2008), an adjusted version of the Temperature Humidity Index (THI_{adj}; Mader et al., 2006) and the Comprehensive Climatic Index (CCI; Mader et al., 2010) do have validated heat stress thresholds (Table 1). In addition, Gaughan et al. (2010) have compared the tolerance to increasing HLI values, based on increasing PS, for several (n=17 total) *Bos indicus*, *Bos taurus* and *Bos indicus* x *Bos taurus* feedlot steers, during summertime in Australia, which is characterised by a warm climate. However, heat tolerance of cattle (even within the same breed) may also vary according to their degree of adaptation, which is different when the cattle are kept in warm versus temperate climate. Furthermore, the tolerance to increasing HLI values has not yet been evaluated, for Holstein dairy cows (very common in temperate climate) and Belgian Blue beef cows (very common in Belgium), based on increasing PS.

To address the above-mentioned lack of knowledge, an experiment was carried out over the course of three summers, to evaluate the need for and the effectiveness of shade as protection against hot summer conditions, as quantified by the HLI, specifically for Holstein dairy cattle and Belgian Blue beef cattle on pasture in a temperate region (Flanders, Belgium). Effects of hot summer conditions and shade on the body temperature, energy metabolism and productivity of the Holstein dairy cows in this experiment, are described in a separate publication. The current paper focusses on:

- (1) the assessment of the degree of thermal discomfort caused by the summer conditions for the Holstein dairy cows and Belgian Blue beef cows and calves on pasture, as indicated by elevated respiration rates (RR) and PS
- (2) the evaluation of the effectiveness of shade, by relating voluntary use of shade (by the three cattle types) to climatic conditions and by studying the effect of shade on RR and PS.

TABLE 1

Overview of climatic indices used in cattle research to quantify the effects of hot summer conditions

Climatic Index + formula	Associated 'heat stress' threshold according to literature
$THI=0.8*Ta+[(RH/100)*(Ta-14.4)]+46.4$	68, based on milk production losses (Zimbelman et al. 2009)
$THIadj = 4.51 + THI - 1.992 * WS + 0.0068 * Rad (2.5)$	68, cfr. conventional THI
$Tbg= 1.33 * Ta - 2.65 * Ta^{0.5} + 3.21 * \log (Rad+1) + 3.5$	25 °C, cfr. upper critical temperature for cows (Van laer et al. 2014)
$WBGT = 0.7*Twb + 0.2 * Tbg + 0.1 * Ta$	25 °C, cfr. upper critical temperature for cows (Van laer et al. 2014)
$HLI = 8.62 + 0.38 * RH + 1.55 * Tbg - 0.5 * WS + e^{(2.4 \cdot WS)}$ if $Tbg > 25$ $HLI = 10.66 + 0.28 * RH + 1.3 * Tbg - WS$ if $Tbg < 25$	70, 77 and 86 are used to define warm, hot and very hot conditions, respectively, based on Panting Score and body temperature data of unshaded Angus steers (Gaughan et al., 2008)
$CCI=Ta+Eq.1+Eq.2+Eq.3$	25 °C, based on elevated respiration rates (Mader et al. 2010)

$$Eq.1 = e^{((0.00182 * RH + 1.8 * 10^{(-5)} * Ta * RH)) * (0.000054 * Ta^2 + 0.00192 * Ta - 0.0246)} * (RH - 30)$$

$$Eq.2 = (-6.56) / e^{(1 / (2.26 * WS + 0.23))^{0.45}} * (2.9 + 1.14 * 10^{(-6)} * WS^{2.5} - \log_{0.3} (2.26 * WS + 0.33)^{(-2)}) - 0.00566 * WS^2 + 3.33$$

$$Eq.3 = 0.0076 * Rad - 0.00002 * Rad * Ta + 0.00005 * Ta^2 * \sqrt{Rad} + 0.1 * Ta - 2$$

THI= Temperature Humidity Index, THIadj= adjusted version of the Temperature Humidity Index, CCI= Comprehensive Climate Index in °C, Tbg= Black Globe Temperature in °C, HLI= Heat Load Index, WBGT= Wet Bulb Globe Temperature in °C, Ta= air temperature in °C, Rad = solar radiation in W/m², RH= % air humidity, WS = wind speed in m/s, Twb=Wet Bulb Temperature in °C.

2 MATERIALS AND METHODS

2.1. TIMING AND LOCATION OF THE STUDY

The study took place during three subsequent summers (2011, 2012, 2013; Table 2) and was approved by the Animal Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (application nr. 2011/151 and 2011/151bis). The experiment took place at ILVO's experimental farm (latitude 50°59'1"N, longitude 3°46'49"E). Holstein dairy cows were rotationally kept on four (in 2011) or two (in 2012 and 2013) different pastures. The Belgian Blue Beef cattle were kept on two adjacent pastures (in 2011, 2012 and 2013). Each pasture was neighboured by a shaded area surrounded by an electric fence. This shaded area could be accessed from either of the two adjacent pastures through a 3-5 m wide passage. The shade was provided by young trees and shade cloth (shading percentage = 80%) spanned between them (more details are given in Supplementary Figure S1). The two shaded areas for dairy cattle (625 m² each) were used by maximum 60 dairy cows on the adjacent pastures, thus they offered at least 10.5 m² of shade per cow. The shaded area for the Belgian Blue beef cows and calves was 900 m², and was used by a maximum of 15 cows and 9 calves. Therefore, it offered at least 37.5 m² of shade per cow or calf.

2.2. ANIMALS AND MANAGEMENT

The number of lactating Holstein-Friesian dairy cows used in this experiment varied between 60 and 125 due to dry cows leaving the herd and recently calved cows and heifers and cows nearing parturition being added. Cows and heifers were 199.3 ± 100.6 (mean ± SD) days in milk, parity ranged between 0 and 7 (mean ± SD: 2.2 ± 1.3) and the mean daily milk production was 27.7 ± 7.1 (mean ± SD) l/day.

All cows were milked twice daily (starting around 5.30 h and 15.30 h) and received half of the daily portion of concentrate during each milking. After milking they were fed the daily mixed ration of mainly corn silage (49% to 76%, 60% on average) and prewilted grass silage (9% to 29%, 21% on average), supplemented with a protein source (soybean meal or protected soybean meal) and wheat or corn cobb mix. Additionally, during some periods the ration was completed with pressed beet pulp (0% to 25%, 9% on average) and/or by-products from bio-ethanol or starch industry. This mixed ration was

provided in feed troughs located in a loose housing stable (in 2011 and 2012) or in an open-air passage to pasture (in 2013) located behind the milking parlour. During the entire study period, the dairy cows were kept on pasture where they could graze ad libitum, except for during milking.

A herd of 30 Belgian Blue beef cows was used in this experiment. These cows were between 0 and 209 days in milk (mean \pm SD: 60.1 ± 61.2), parity ranged between 1 and 4 (mean \pm SD: 1.6 ± 0.8) and age varied between 2 and 7.2 years (mean \pm SD: 3.4 ± 1.1). Their suckling calves were also included in the trial from two weeks of age until weaning (16 weeks) in 2011 (n=18) and 2012 (n=15). In 2013 no calves were included in the trial. Only at the end of each summer (starting at the end of August, in the three years), the beef cattle received some additional grass silage and/or maize silage, because the grass availability on their pastures was deemed to be low. The feed was provided in a mobile feed bunk in a non-shaded part of the pastures and at a time that did not coincide with the monitoring of cattle's use of shade. During the entire study period, cows and calves stayed on pasture permanently, except during the monthly veterinary check-ups (pregnancy detection and weighing), during artificial insemination (no bull was kept on pasture for safety reasons), and during the week of weaning.

In 2011 and 2012, water was provided at several (minimum two per allotment) watering points (large open troughs and additional individual drinkers) spread across the non-shaded parts of the pasture. In 2013, an additional large open water trough was placed inside each shaded area.

2. 3. EXPERIMENTAL TREATMENTS

During the summers of 2011 and 2013, the dairy herd was divided into two treatment groups. By randomly assigning the members of 'matched' pairs of cows to either treatment, the dairy treatment groups were as comparable as possible with regard to traits known to affect susceptibility to heat stress (i.e. productivity, parity, age and percentage of black coat). Similarly, the herd of 30 Belgian Blue beef cows (and their suckling calves in 2011) was divided into two treatment groups. Again, random assignment of 'matched' pairs of cows to either treatment, made the treatment groups as comparable as possible in terms of the distribution of parity, age, weight, percentage of black coat and, in 2011 and 2012, in terms of the number of suckling calves. During the summers of 2011 and 2013, in each herd one group (the S treatment) could always access the shaded area, whereas the other group (the NS treatment) never had access to shade when kept on pasture. In order to exclude potential confounding effects of allotment to either of the two or four (in case of dairy cattle in 2011) pastures available per cattle breed (e.g. pasture productivity

or composition, location of drinking troughs, etc.) on the cows' behaviour or productivity, NS and S groups were regularly (for dairy cows daily, for beef cows and calves weekly) switched between allotments (Fig. S1). During the summer of 2012, both herds (dairy and beef) were kept on the same pastures as in 2011 and 2013, but none of the animals had access to shade (the NS treatment). The same animal observations were made as in 2011 and 2013 and these data were pooled with those from the NS treatment in 2011 and 2013 to investigate the effect of climatic conditions as such on the RR and PS.

2.4. CLIMATIC DATA

A custom-built Campbell Scientific BWS200 weather station (Campbell Scientific Inc., Logan, UT, USA) located in open pasture, within 500m of all pastures used in the trial, registered the average air temperature, air humidity, solar radiation, and wind speed every 15 minutes. Based on these measurements, 15-minute values of six climatic indices were calculated (Table 1).

In order to evaluate the effect of shade on microclimate, additional measurements of T_{bg} were conducted, using Testo 400's Wet Bulb Globe Temperature probe (Testo AG Inc., Lenzkirch, Germany), under shade and outside of shade. During eight measurement sessions, on seven days for which the weather forecast predicted daily maximum temperatures $\geq 25^{\circ}\text{C}$, T_{bg} was measured at 1.5 m height, under shade and in open area nearby, for each of the shaded areas. Three measurement sessions took place between 1000 h and 1230 h, two sessions between 1200 h and 1430 h and three sessions between 1330 h and 1600 h. During each measurement session, three instantaneous measurements were taken inside and outside of each shaded area. The measurements outside of shade were taken on three locations 20-50m away from each shaded area.

2.5. ANIMAL OBSERVATIONS

2.5.1. USE OF SHADE

The use of shade by the individual animals from the S treatment was monitored between 10 h and the time of evening milking (approx. 15 h) for dairy cows, and between 10 h and late afternoon (ranging between 15 h and 18 h) for beef cows, on several days (Table 2) during the summers of 2011 and 2013, to include a range of climatic conditions between thermoneutral and hot. An unmanned camera (Sony HDR-CX220E) filmed the cow's passage to and from the shaded area. Based on the time recordings of each individual cow's 'entering' and 'leaving' events, individual shade use was determined per cow by one/zero recording at 15-minute intervals. This means that for

each 15-minute interval, each cow was classified as having used shade or not. Individual cows were identified from the video footage by numbers painted on their flanks using oil-based heat detection tail paint (Tell Tail, FIL, Mount Maunganui, New Zealand) in 2011 and based on the individual coat pattern in 2013.

2.5.2. RESPIRATION RATE (RR) AND PANTING SCORE (PS)

The RR and PS were monitored, in each experimental group, during the same time periods as for the monitoring of shade use (see above) and for almost all days on which shade use (of S cows) was recorded (2011 and 2013) and on 12 other thermoneutral and hot days in 2012 (Table 2). In the beef herd (max. 30 adult cows and 18 calves), each animal (S and NS) was sampled once per hour. In 2011 and 2013, the observer switched between the S and NS group every half hour. In the larger dairy herd (min. 60 cows, max. 110 cows), it was not possible to sample each animal every hour. Instead, in 2012, the observer aimed to sample 60 cows (all NS) during each hourly scan. In reality, 56 cows were sampled on average (SD = 7, min. = 33, max. = 76). Which cows were sampled, was determined semi-randomly, and based on their proximity and visibility to the observer. In 2011 and 2013, the observer aimed to sample 30 cows during every hourly scan in each treatment group, switching between the S and NS group every half hour. In the S group, the observer sampled as many animals in shade as possible. In the NS group, sampled cows were selected semi-randomly, based on their proximity and visibility to the observer.

The RR was determined by timing five respirations (flank movements) and converting this to the number of breaths per minute (BPM). PS was scored on a tagged visual analogue scale, labelled with the descriptors of Gaughan et al. (2008) (Fig.1), as in Tuytens et al. (2014). Over the course of the three summers, one permanent observer and five different additional observers scored RR and PS. All additional observers were trained by the permanent observer, based on repeated scoring of at least 20 different movies (in randomized order) of cattle with varying RR and PS, until there was sufficient agreement between the permanent and additional observer (less than 10% deviance in RR and PS).

TABLE 2. Overview of (1) the number of days on which shade use, panting score (PS), and respiration rate (RR) were observed, and (2) the climatic conditions on these ‘observation days’¹.

(1) Number of ‘observation days’		2011		2012		2013	
		shade use:	PS and RR:	shade use:	PS and RR:	shade use:	PS and RR:
dairy cows / beef cows / beef calves		15/ 21/ 19	13/ 15/ 13	n.a./ n.a./ n.a.	9/ 11/ 7	13/ 15/ n.a.	13/ 15/ n.a.
(2) Climatic conditions during these ‘observation days’ ¹		THI	THIadj	CCI	Tbg	WBGT	HLI
dairy cows	range	59.8 - 83.2	56.4 - 83.1	10.8 - 36.3	17.3 - 38.5	14.2 - 28.8	51.2 - 88.1
	mean ± SE	70.21 ± 0.03	71.14 ± 0.03	23.81 ± 0.03	26.78 ± 0.02	20.75 ± 0.02	71.72 ± 0.06
beef cows	range	59.4 - 83.2	55.5 - 83.1	10.4 - 36.3	17.3 - 38.5	12.7 - 28.8	45.4 - 88.1
	mean ± SE	71.11 ± 0.05	72.22 ± 0.05	24.9 ± 0.05	27.67 ± 0.04	21.19 ± 0.03	72.81 ± 0.09
beef calves	range	59.4 - 80.5	55.5 - 82.6	10.4 - 35.0	17.3 - 38.6	12.7 - 26.9	45.4 - 87.8
	mean ± SE	69.19 ± 0.11	69.98 ± 0.12	23.00 ± 0.11	26.46 ± 0.1	19.79 ± 0.07	68.04 ± 0.22

n.a.=not applicable, because shade or the animal type was not available. THI= Temperature Humidity Index, THIadj= adjusted version of the Temperature Humidity Index, CCI= Comprehensive Climate Index in °C, Tbg= Black Globe Temperature in °C, HLI= Heat Load Index, WBGT= Wet Bulb Globe Temperature in °C.

¹Range and mean (± SE) during observation hours are given for the pooled data from 2011, 2012 and 2013.

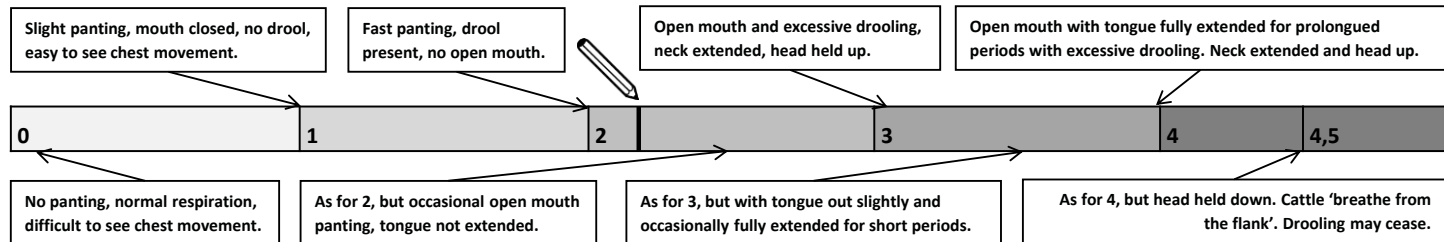


FIGURE 1. The tagged visual analogue scale labeled with descriptors to determine cattle PS. Adapted from Gaughan et al. (2008).

2.5. DATA ANALYSIS

2.5.1. EFFECT OF SHADE ON MICROCLIMATE

The difference in black globe temperature (T_{bg}) measured in open area and under shade was modelled using a mixed model ANOVA (proc mixed in SAS 9.3). Measurement session and shade area were included as random intercept effects.

2.5.2. USE OF SHADE

Per animal type (separately for dairy cows, and adult and juvenile beef cattle), for the animals from the S treatment we examined the effect of hot conditions, as quantified by the six abovementioned climatic indices, on the use of shade (per 15 minutes, binomially distributed) by means of a mixed model logistic regression (proc glimmix in SAS 9.4), which modelled the probability of use of shade as a function of the climatic index under focus and its interaction with the effect of year (2011 or 2013; not applicable for beef calves; their shade use was only studied in 2011). The potential year effect was thus treated as a fixed factor. In addition, these models all included a random factor to correct for repeated measurements per cow. For each animal type, all climatic indices had a highly significant ($P < 0.0001$) positive effect on the probability of shade use, but the Heat Load Index (HLI) yielded the best fit, i.e. the lowest corrected Pseudo-AICC (corrected Akaike Information Criterion) value (Table 3). Consequently, we only report on shade use as a function of HLI. The logistic regression models yield the probability of shade use as outcome variable. This probability can be interpreted as the probability that an individual cow will use shade at a given HLI value, which is essentially the same as the proportion of the group that can be expected to use shade at a given HLI value. We interpret a shade use probability $\geq 10\%$ as an indication of thermal discomfort outside shade.

2.5.3. RESPIRATION RATE (RR) AND PANTING SCORE (PS)

Per animal type, the effect of hot summer conditions (as quantified by the six climatic indices) on RR and PS of animals from the NS treatment (including pooled data from 2012, 2011 and 2013) was investigated by means of six mixed linear regressions (proc mixed in SAS 9.4), each of which modelled the RR and PS as a function of the climatic index under focus. For the animals from the S treatment, the effect of hot summer conditions and use of shade on RR and PS was investigated by means of a mixed linear regression, which modelled the RR and PS as a function of (1) the climatic index under focus, (2) the effect of using shade (1 if the observed animal was in shade, 0 if the observed animal was not in shade at the moment of observation) and the interaction between (1) and (2).

These mixed models all included a random factor to correct for repeated measurements per cow per day. Because of computational limitations of the analysis software, observations of the same individual in different years were regarded as independent. The models with Tbg consistently yielded the lowest pseudo-AICC value and thus the best fit (Table 3). Thus, we only report on RR and PS as functions of Tbg.

TABLE 3. Pseudo-AICC value for the different models (with different climatic indices) tested for the use of shade, the Panting Score (PS) and the respiration rate (RR).

		Dairy cows		Beef cows		Beef calves	
X= use of shade							
animals with access to shade only	Tbg	167075		77449		20038	
	THI	175426		78519		21903	
	THIadj	169659		74011		21889	
	HLI	154238		72320		17850	
	CCI	DNC ¹		72250		19991	
	WBGT	177639		79534		21550	
X=							
		PS	RR	PS	RR	PS	RR
animals with access to shade	Tbg	2229	25182	1960	16899	289	2714
	THI	2231	25198	1958	16899	295	2726
	THIadj	2242	25203	1969	16909	303	2731
	HLI	2244	25246	1987	16930	313	2743
	CCI	2259	25206	1973	16913	306	2733
	WBGT	2263	25259	1962	16908	297	2728
animals without access to shade	Tbg	4294	45110	2723	25963	765	7003
	THI	4299	45135	2728	25966	770	7005
	THIadj	4313	45160	2748	25985	777	7015
	HLI	4318	45192	2780	26023	801	7032
	CCI	4339	45162	2742	25978	776	7013
	WBGT	4335	45183	2724	25966	770	7010

¹DNC: Model did not converge. THI= Temperature Humidity Index, THIadj= adjusted version of the Temperature Humidity Index, CCI= Comprehensive Climate Index in °C, Tbg= Black Globe Temperature in °C, HLI= Heat Load Index, WBGT= Wet Bulb Globe Temperature in °C. The climatic index that yielded the lowest Pseudo-AICC (Corrected Akaike Information Criterion) value was considered the best explaining index and is shaded.

The subsequent analyses used data pooled over the three summers, S and NS treatment, but only from hours during which the average value of Tbg $\geq 30^{\circ}\text{C}$ (because the shade use model as a function of Tbg predicted a shade use probability of $\geq 10\%$ when Tbg $\geq 30^{\circ}\text{C}$). Per hourly scan with Tbg $\geq 30^{\circ}\text{C}$, and per animal type, we determined the percentage of observations where the PS <1 (normal), 1-2 (elevated) and ≥ 2 (strongly elevated), for animals under and outside of shade. Per animal type, we used three separate linear mixed models (proc mixed in SAS 9.4) to compare these prevalence percentages between animals in the shaded area and outside of it.

The same approach was used to compare the prevalence of normal, elevated and strongly elevated RR values, between animals in the shade and outside of it. The threshold values for the RR categories (per animal type) were based on the correlation between PS and RR scored in the same observation (Table 4). The mixed models all included a random factor to correct for repeated measurements per day. The data were sufficiently normally distributed, based on histograms and qq-plots of the residuals.

TABLE 4. Definition of Panting Score (PS) and respiration rate (RR) categories (per animal type) used in the comparison between animals in the shade and outside of it.

PS	Corresponding values of the RR (in BPM) ^a		Classification
	Dairy & beef cows -	Beef calves	
0-0.5	<60	<90	Normal
0.5-1	60-90	90-120	
1-1.5	90-120	120-150	Elevated
1.5-2	90-120	150-180	
2-2.5	120-150	180-210	Strongly elevated
2.5-3	150-180	180-210	
3-3.5	180-210	210-240	Very strongly elevated
3.5-4	210-240	240-270	

^aBased on the relationship (per animal type) between PS and RR, which was always relatively strong and quite alike: when no shade was used, $RR=35+50*PS$ ($R^2=0.61$) for dairy cows, $RR=40+49*PS$ ($R^2=0.71$) for adult beef cows and $RR=63+54*PS$ ($R^2=0.72$) for beef calves; when shade was used, $RR=32+57*PS$ ($R^2=0.53$) for dairy cows, $RR=31+50*PS$ ($R^2=0.52$) for adult beef cows and $RR=52+67*PS$ ($R^2=0.60$) for beef calves.

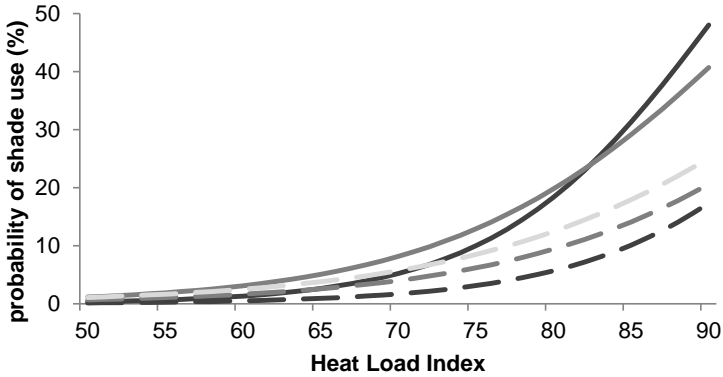
3 RESULTS

3.1. EFFECT OF SHADE ON MICROCLIMATE

Shade lowered T_{bg} by 3.8°C ($P = 0.004$). The mean (\pm SE) T_{bg} was 25.7 \pm 2.3°C under shade and 29.6 \pm 2.3°C outside of shade.

3.2. USE OF SHADE

The responses of dairy cows and beef cows to HLI differed between 2011 and 2013. In 2013 the probability of shade use increased more steeply with increasing HLI than in 2011 (Fig. 2). In 2011, shade use probability reached $\geq 10\%$ at an HLI of 85 for dairy cows, 81 for adult beef cows and 77 for beef calves (Fig. 2). In 2013, the shade use probability reached $\geq 10\%$ at an HLI of 75 for dairy cows and 72 for adult beef cows (Fig. 2).



Probability of shade use = $e^y / (1 + e^y)$, where
 $Y = a + b \cdot \text{HLI}$ (a and b are given $\pm \text{SE}$)

- dairy cows 2013: $a = -12.80 \pm 0.49, b = 0.12 \pm 0.01$
- dairy cows 2011: $a = -12.80 \pm 0.49, b = 0.14 \pm 0.01$
- beef cows: 2013: $a = -9.58 \pm 0.56, b = 0.09 \pm 0.01$
- beef cows: 2011: $a = -9.58 \pm 0.56, b = 0.10 \pm 0.01$
- beef calves 2011: $a = -8.80 \pm 1.07, b = 0.09 \pm 0.01$

FIGURE 2. Predicted use of shade by dairy cows, beef cows and beef calves according to the Logistic Mixed Models as a function of the Heat Load Index (HLI).

These models provide realistic estimates of the percentage of shade use during the hottest part of the day. The raw data (Fig.3) show that the mean percentage of shade use increased along with the mean value for HLI per time of day, for dairy cows (Fig. 3a), beef cows (Fig. 3b) and beef calves (Fig. 3c). In all cases, the HLI increased gradually from 10 h to 15 h. For dairy cows in 2011, the use of shade also increased gradually from 10 h onwards, to reach a maximum around 20% at about 1330 h (Fig. 3a1). In 2013, the increase in both HLI and shade use over the course of the day was less steep. In 2011, between 10 h and 15 h, shade use increased along with increasing HLI, to reach about 30% at 15 h for adult beef cows and about 40% at 1530 h for beef calves. After 15 h (for adult cows) or 16 h (for calves), average shade use decreased along with the decreasing HLI. In 2013, the increase in adult beef cows' use of shade increased along with increasing HLI as well, to reach about 45% at 15 h.

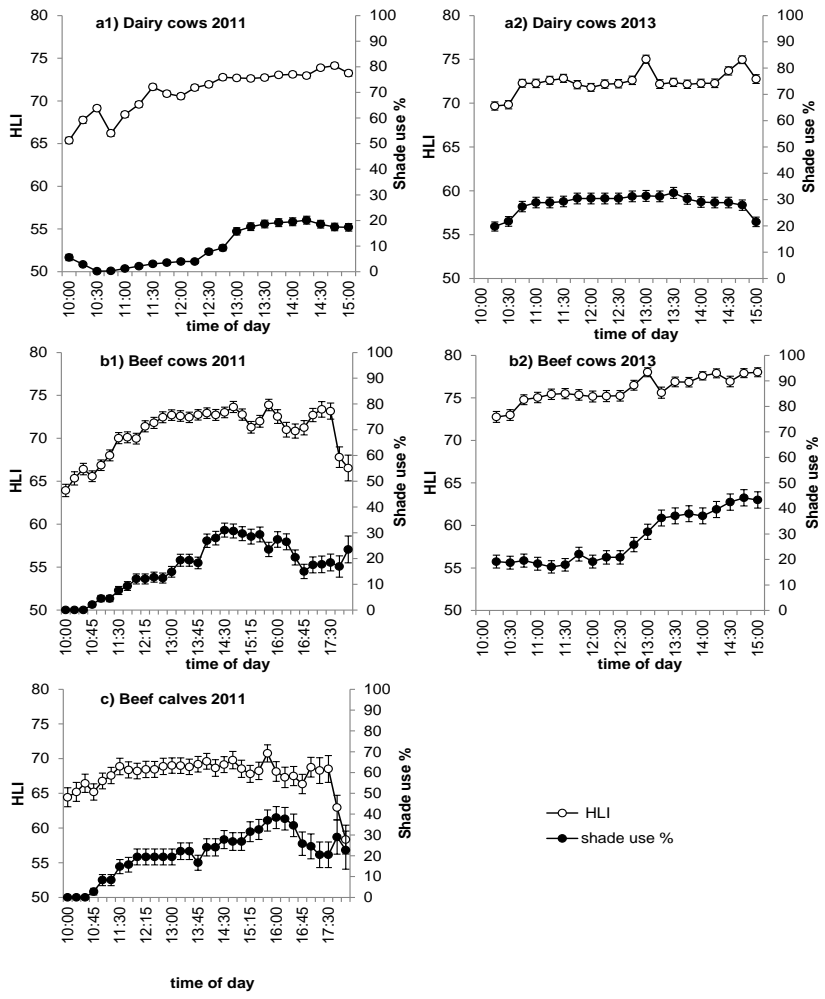


FIGURE 3. Mean percentage of shade use, i.e., the mean of all observed cows (individual values = 0 or 1), including all days, and the mean value for the Heat Load Index (HLI) (averaged over all observation days) plotted against the time of day, per 15 minutes, for dairy cows in 2011 (a1) and 2013 (a2), beef cows in 2011 (b1) and 2013 (b2) and for beef calves in 2011 (c).

3.3. RESPIRATION RATE (RR) AND PANTING SCORE (PS)

For NS animals, RR and PS increased with increasing Tbg, for the three cattle types (all $P < 0.0001$; Table 5). The RR of dairy cows and adult beef cows increased similarly with increasing Tbg (Table 5). Beef calves' RR increased more steeply with increasing Tbg. Beef calves' PS increased somewhat more steeply than that of adult beef cows (Table 5). With increasing Tbg, PS increased less steeply for dairy cows than for adult beef cows (Table 5). For S animals, both RR and PS of dairy cows, beef cows and beef calves increased with increasing Tbg (all $P < 0.01$; Table 5).

The use of shade, however, did not influence the relation between Tbg and PS, for the three cattle types (Table 5). For adult beef cows, the use of shade did not significantly influence the relationship between Tbg and RR (Table 5). For beef calves, the RR tended to increase more steeply for animals outside the shade than for animals in the shade ($P = 0.051$, Table 5). For dairy cows, the RR increased more steeply for animals outside shade than for animals in the shade ($P = 0.016$, Table 5). At the highest observed values of Tbg (40°C), shade reduced the average RR by 23 BPM (from 123 ± 5 BPM to 100 ± 5 BPM; $P < 0.0001$) for dairy cows and by 50 BPM (from 197 ± 17 BPM to 149 ± 20 BPM; $P = 0.0095$) for beef calves.

When all data (2011, 2012 and 2013, from NS and S) were pooled, we determined that for adult dairy and beef cows observations of $\text{BPM} \geq 150$ and $\text{PS} \geq 2.5$ were only made for (NS and S) animals outside the shaded area, not for S animals under shade at the moment of RR and PS determination. In addition, at $\text{Tbg} \geq 30^{\circ}\text{C}$, shade use significantly increased the prevalence of normal RR (< 90 BPM) and PS (< 1), so that both remained $> 50\%$ for both cattle types (Fig. 4). Use of shade reduced the prevalence of very high RR (≥ 120 BPM) for adult beef cows as well as dairy cows, and for adult beef cows shade use also reduced the prevalence of high RR (≥ 90 BPM) (Fig. 4). For beef calves, at $\text{Tbg} \geq 30^{\circ}\text{C}$, shade use also increased the prevalence of normal PS (< 1) and tended ($P < 0.01$) to increase the prevalence of normal RR (< 120 BPM), so that both remained $> 50\%$ (Fig. 4). However, for the calves, shade use had no significant effect on the prevalence of elevated and strongly elevated RR and PS (Fig. 4).

TABLE 5. Estimations of the effect of the black globe temperature (Tbg), shade use and their interaction on the Panting Score (PS) and respiration rate (RR).

NS treatment		PS as a function of Tbg					RR as a function of Tbg				
	Effect	Est. ²	SE	DF	t	P	Est. ²	SE	DF	t	P
dairy	Intercept	-0.86	0.19	115	-4.44	<0.0001	-10.86	8.00	137	-1.36	0.1771
cows	Tbg	0.06	0.01	122	9.17	<0.0001	3.44	0.29	149	11.78	<0.0001
adult	Intercept	-1.3	0.19	136	-6.78	<0.0001	-18.51	8.75	138	-2.12	0.0362
beef cows	Tbg	0.07	0.01	142	10.14	<0.0001	3.31	0.31	144	10.72	<0.0001
beef	Intercept	-1.52	0.28	60.2	-5.42	<0.0001	-71.08	18.99	47.5	-3.74	0.0005
calves	Tbg	0.08	0.01	60.4	7.93	<0.0001	6.28	0.69	45.5	9.05	<0.0001
S treatment		PS as a function of Tbg and use of shade					RR as a function of Tbg and use of shade				
	Effect	Est. ²	SE	DF	t	P	Est. ²	SE	DF	t	P
	Intercept	-0.80	0.31	242	-2.55	0.0115	-4.62	12.03	277	-0.38	0.7009
dairy	SU=0 ¹	0.01	0.22	2485	0.05	0.9626	-8.31	9.70	1919	-0.86	0.3917
cows	Tbg	0.05	0.01	220	4.47	<0.0001	2.63	0.41	234	6.36	<0.0001
	Tbg*SU=0 ¹	0.01	0.01	2414	1.45	0.1461	0.79	0.33	1863	2.4	0.0163
	Intercept	-1.01	0.28	227	-3.58	0.0004	-7.89	12.73	245	-0.62	0.5359
adult	SU=0 ¹	-0.26	0.24	747	-1.11	0.2666	-6.54	11.25	656	-0.58	0.5610
beef cows	Tbg	0.05	0.01	200	5.22	<0.0001	2.40	0.43	212	5.61	<0.0001
	Tbg*SU=0 ¹	0.01	0.01	737	1.49	0.1366	0.47	0.37	657	1.26	0.2082
	Intercept	-2.28	0.67	82.4	-3.42	0.001	-42.54	41.92	63.8	-1.01	0.3141
beef	SU=0 ¹	0.12	0.58	250	0.2	0.8415	-59.73	38.53	213	-1.55	0.1226
calves	Tbg	0.10	0.02	71.1	4.37	<0.0001	4.80	1.48	52.8	3.24	0.0021
	Tbg*SU=0 ¹	0.01	0.02	241	0.31	0.7589	2.69	1.37	202	1.96	0.0510

¹SU=0: effect of not using shade

²Est.: estimate of the effect

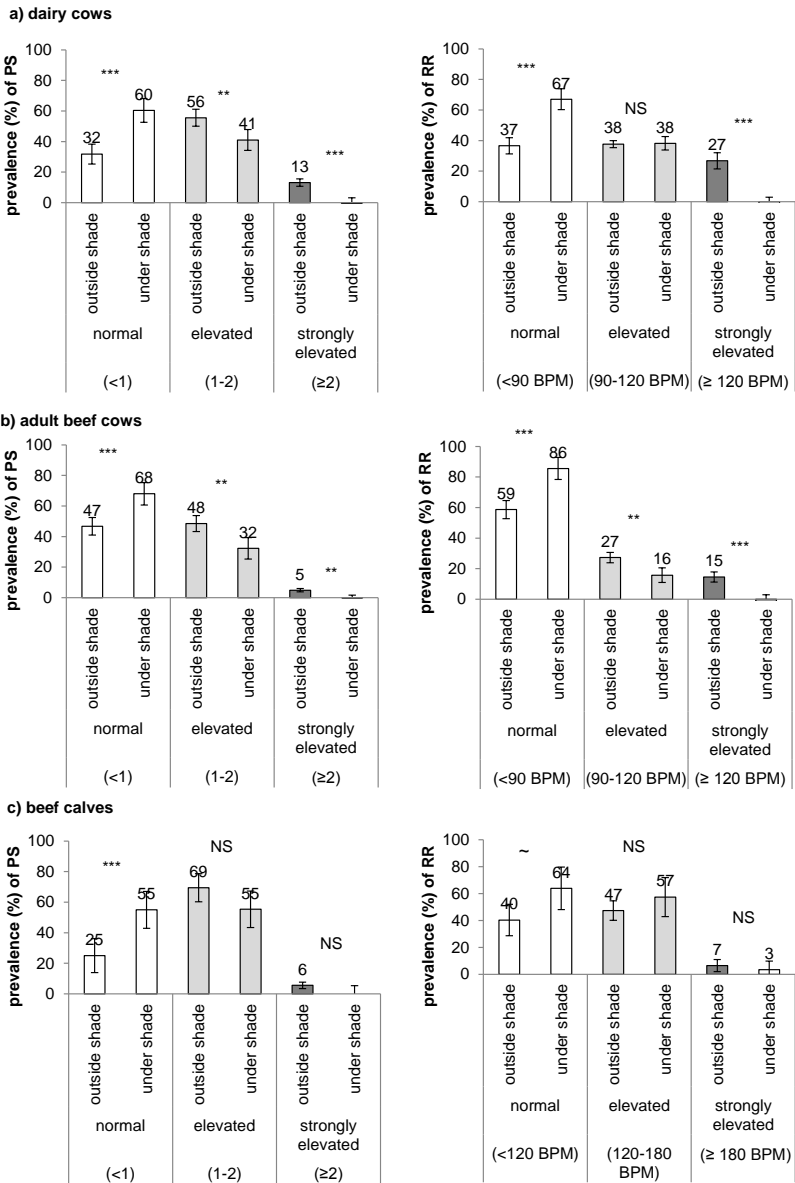


FIGURE 4. Prevalence (in %) of normal, elevated, and strongly elevated and very strongly elevated PS and BPM among animals outside shade and under shade, at black globe temperatures (T_{bg}) > 30°C. ***P < 0.001, **P < 0.01, *P < 0.05, ~P < 0.01, NS = P > 0.01 for the comparison of the prevalence under and outside of shade.

4. DISCUSSION

4.1. EFFECT OF HOT SUMMER CONDITIONS ON BEHAVIORAL INDICATORS OF THERMAL DISCOMFORT

Cattle increased their use of shade when the degree of heat increased. For all three cattle types, HLI predicted shade use best. This is in line with expectations because shade protects against heat stress mainly by reducing solar radiation and the HLI is greatly determined by the intensity of solar radiation. The traditional THI (Thom, 1959), which is not (directly) affected by the solar radiation intensity, was not a good predictor of shade use. To our knowledge, no studies have yet related shade use probability to HLI, based on 15 minute data, before. Therefore, our HLI threshold values for shade use can only be compared to HLI threshold values based on 'heat stress symptoms' other than shade use, e.g. to the threshold values in Table 1, based on PS and body temperatures of unshaded Angus steers. In 2011, the shade use probability reached $\geq 10\%$ at HLI values beyond or equal to the threshold of 77, which Gaughan et al. (2008) used to define moderate heat stress conditions. In 2013, the shade use probability already reached $\geq 10\%$ during mild heat stress conditions according to Gaughan et al. (2008).

This apparently high threshold for use of shade in 2011 could be due to several factors. In 2011, no drinking trough was provided inside the shade area, whereas in 2013 there was. Secondly, in 2011 the cattle were less habituated to the shaded area; the trees had been on their pastures for two years, but the shade cloth was hung only one month before the start of the study. Furthermore, animals using shade were physically separated from animals that did not use shade (by an electric fence with a relatively narrow (3-5 m) opening as entrance and exit). The motivation for shade use might thus be opposed to the cattle's strong gregarious tendency, which has already been shown to influence shade-seeking behaviour (Langbein and Nichelmann, 1993). In the present study, we did observe that individual cows quickly followed each other into and out of the shade, presumably to maintain group cohesion. In practice, a non-fenced shade area that allows easy access to all individuals at the same time would be better and likely encourage cattle to seek shade more than was observed in our study in 2011.

On the other hand, the experimental setup strengthens our hypothesis that the animals that did seek shade, probably did so primarily to seek shelter from the heat load imposed by intense solar radiation. In addition, thermal discomfort in unshaded cattle of the three types was also evident from the increasing RR and PS.

4.2. EFFECT OF SHADE ON RESPIRATION RATE (RR) AND PANTING SCORE (PS)

The increase in RR with increasing degree of heat was not as pronounced when dairy cows and beef calves were in the shade. No such effect was found, however, for adult beef cows. Neither did shade use buffer the increase of PS with increasing degree of heat. Yet, when all data (2011, 2012 and 2013, NS and S treatments) were pooled, for the three cattle types, more than 50% of the animals under shade retained normal PS and RR, whereas normal RR and PS were significantly less prevalent for animals outside shade. In addition, for adult cows (dairy and beef), the use of shade generally reduced the prevalence of elevated and strongly elevated RR and PS. Thus, we illustrated at least a modestly beneficial effect of shade use on behavioural indicators of thermal discomfort in the three cattle types under study, even during the temperate Belgian summers. This is in line with findings from New Zealand during summer (Schütz et al., 2010; Schütz et al., 2014).

4.3. RESPIRATION RATE (RR) AND PANTING SCORE (PS) AS INDICATORS OF THERMAL DISCOMFORT

The PS is a proven convenient and suitable method to assess thermal discomfort in feedlot cattle (e.g. Brown-Brandl et al., 2006; Gaughan et al., 2010). However, to our knowledge, it has not been used for this purpose Belgian Blue cattle, and it has been used in only one other study on Holstein dairy cattle (Schütz et al., 2014). RR is more commonly used as a measure of thermal discomfort in cattle, especially dairy cattle (e.g. Schütz et al., 2010).

Classification of RRs into classes in accordance to PS classes suggested by Meat and Livestock Australia, were based on research on feedlot steers, mainly of the Angus breed (Gaughan et al. 2010; Gaughan et al. 2008). As pointed out in the introduction, however, the cattle in our study may have had a different heat stress susceptibility, due to their different genetics and their different degree of adaptation.

In order to assess if this was indeed the case, Fig. 5 compares the prevalence of various PS categories in thermoneutral, warm, hot and very hot conditions for the unshaded Belgian Blue beef cows and calves and the Holstein cows in our study with those of Angus steers and steers of other *Bos taurus* breeds as reported by Gaughan et al. (2010) (Fig. 5). It shows that our Belgian Blue beef cows and calves and Holstein cows had stronger PS reactions to hot summer conditions than the Angus x Charolais crossbreds or Hereford x Shorthorn crossbreds of Gaughan et al. (2010) (Fig. 5). The heat-associated changes in PS of our Belgian Blue and our Holstein cattle were most comparable with that of the Hereford cattle and less marked than in the Angus cattle.

Although the reduced pulmonary and cardiac function (Amory et al., 1992; Gustin et al., 1988) might increase the heat stress susceptibility of the Belgian Blue breed, this breed does have a predominantly white or light-coloured coat in comparison to the black-coated Angus. Gaughan et al. (2008) determined that a white coat colour increases the heat stress threshold in terms of the Heat Load Index by three units, in comparison with the black-coated Angus reference. A red coat colour increases the heat stress threshold by one unit. Hereford cattle have a mixed red and white coat. Therefore, it is logical that the predominantly white Belgian Blue cattle and the mixed black and white Holstein cattle in our study, had a similar heat stress tolerance as the Hereford cattle.

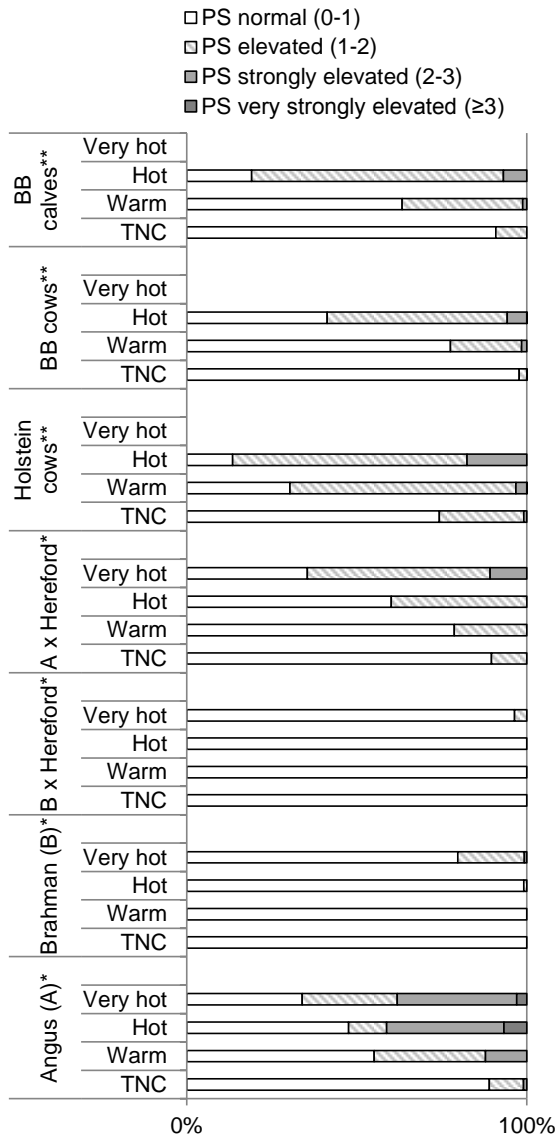


FIGURE 5: Percentage of unshaded animals of different cattle breeds exhibiting normal (0-1), elevated (1-2), strongly elevated (2-3) and very strongly elevated (≥ 3) PS under thermoneutral (TNC), warm, hot and very hot climatic conditions. BB= Belgian Blue. *Data were derived from Gaughan et al. (2010). In this study, Heat Load Index thresholds of 70, 77, 86 and 96 were used to define warm, hot, very hot and extreme conditions, respectively. **Data from own research, black globe temperature (T_{bg}) thresholds of 25°C, 30°C, 40°C were used to define warm, hot and very hot conditions, respectively. However, very hot conditions and extreme conditions did not occur in this study.

5. CONCLUSION

This study suggests that Holstein dairy cows and Belgian Blue beef cows and calves on pasture during Belgian (temperate) summers had to overcome a relatively high threshold before they started to use shade. However, once the threshold was overcome, the probability of shade use increased with increasing degree of heat, to reach an average of $\pm 30 - 40\%$ at the highest observed heat-levels. In addition, thermal discomfort in unshaded cattle of the three types was evident from the increasing RR and PS with increasing degree of heat. We observed at least a modest beneficial effect of shade use on the RR and PS in all three cattle types. The increase in RR in Holstein dairy cows and Belgian Blue beef calves with increasing degree of heat was less pronounced when the animals were in the shade. In addition, under hot conditions, shade use led to normal RR and PS for the majority (>50%) of the three cattle types, whereas the proportion of normal RR and PS was significantly lower for animals outside shade. Thus, shade as provided in the present study appears to alleviate thermal discomfort of Holstein dairy cows and Belgian Blue beef cows and calves kept on pasture during temperate summers.

6. ACKNOWLEDGEMENTS

The authors thank the many people who helped to accomplish this study, including the animal caretakers and technicians (especially Seppe Holemans, Sara Van Lembergen, Piet Van Laere and Thijs Decroos) from ILVO Animal Sciences Unit and master students Evelien Van de Keere and Mathias Roegiers. This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RT 10/13 Pastress). The experiment was approved by ILVO's Ethical Committee (EC 2011/151 and EC 2011/151bis).

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8. SUPPLEMENTAL FIGURE

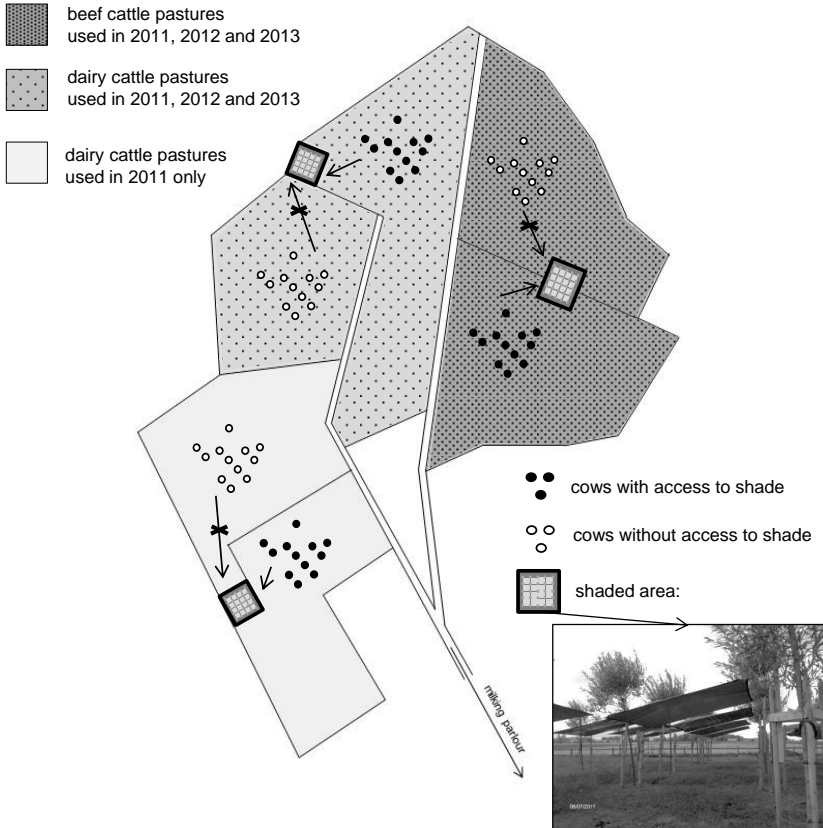


FIGURE S1. Experimental setup in 2011 and 2013. The S group and NS group were kept on separate parcels, with and without access to a fenced shade area, respectively. To exclude effects of allotment on the response variables, the experimental groups and the passage to the shaded area alternated regularly between allotments (daily for dairy cows, weekly for beef cows and calves). The shaded area consisted of a part of the pasture (25m*25m for dairy cattle, two connected pieces of 15m*30m for beef cattle) with evenly spaced (5m) young willow (*Salix alba*), poplar (*Populus alba*) and alder (*Alnus glutinosa*) trees and shade cloth (shading percentage = 80%) spanned between them.

CHAPTER 6

EFFECT OF HEAT LOAD AND SHADE ON PRODUCTION AND METABOLISM OF HOLSTEIN DAIRY COWS ON PASTURE IN TEMPERATE CLIMATE

Adapted from: EFFECT OF HEAT LOAD AND SHADE ON PRODUCTION AND
METABOLISM OF HOLSTEIN DAIRY COWS ON PASTURE IN TEMPERATE
CLIMATE

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ABSTRACT

For dairy cattle on pasture in temperate regions, it is largely unknown to what degree hot summer conditions impact energy metabolism, milk yield and milk composition and how effective shade is in reducing these negative effects. During the summer of 2012, a herd of Holstein cows was kept on pasture without access to shade (treatment NS). During the summers of 2011 and 2013 the herd was divided into a group with (treatment S) and a group without (treatment NS) access to shade. Shade was provided by young trees combined with shade cloth (80% reduction in solar radiation). A weather station registered local climatic conditions on open pasture, from which we calculated daily average Heat Load Index (HLI) values. The effect of HLI and shade on rectal temperature (RT), blood plasma indicators of hyperventilation and metabolic changes due to heat stress, milk yield and milk composition was investigated. RT increased with increasing HLI, but less for S cows than for NS cows (by 0.02 °C and 0.03 °C increase per unit increase of HLI, respectively). Hyperchloremia (an increased blood plasma concentration of Cl⁻, a sign of hyperventilation, increased for NS cows, but not for S cows. The plasma concentration of alkaline phosphatase (ALP), a regulator of energy metabolism in the liver, decreased with increasing HLI for NS cows only. Access to shade thus reduced the effect of HLI on RT, hyperchloremia and the regulation of metabolism by the liver. As HLI increased, the plasma concentration of cholesterol decreased (indicating increased lipolysis) and the plasma concentration of creatinine increased (indicating increased protein catabolism). These effects did not differ between S and NS cows. For NS cows, after a lag-time of two days, the milk yield decreased with increasing HLI. For S cows, the milk yield was unaffected by HLI and its quadratic factor. The milk content of lactose, protein and fat decreased as HLI increased, but only the effect on milk protein content was remediated by shade. In conclusion, access to shade tempered the negative effect of high HLI on RT, hyperchloremia and a blood plasma indicator of changing energy metabolism (generally) and prevented the decrease in milk yield observed in cows without access to shade.

KEYWORDS: Heat stress, dairy cattle, temperate climate, milk, metabolism

1. INTRODUCTION

During summer, in most temperate regions, dairy cattle are kept on pasture for at least a part of the day. Pasturing has some important benefits for animal health and welfare, and improves the public perception of the dairy sector (van den Pol-van Dasselaar, 2005). On the other hand, cattle on pasture may be exposed to aversive climatic conditions, e.g. when high temperatures occur in combination with intense solar radiation. Such conditions can have substantial detrimental effects on the cows' comfort, feed intake, metabolism and productivity, as illustrated by ample research in hot climates. Especially highly productive dairy cows are highly susceptible to heat stress, due to their high metabolic rate, which results in the production of considerable metabolic heat (Kadzere et al., 2002). In addition, their high energy-expenditure increases the possibility that the decreased energy (feed) intake and increased energy requirements during heat stress triggers a state of 'negative energy balance'. Under such conditions, cows must mobilize reserves from adipose tissue and skeletal muscle (Bernabucci et al., 2010).

This altered metabolic state may be reflected by changes in blood plasma concentrations of several 'metabolic heat stress' indicators. Plasma cholesterol concentration has been shown to decrease in response to heat stress, presumably due to increased lipolysis in peripheral tissues (Abeni et al., 2007). Enhanced breakdown of amino acids (mobilized from skeletal muscle tissue) and consequently an increased plasma urea or plasma urea nitrogen concentration has been reported in several heat stress studies (Baumgard and Rhoads, 2007; Shwartz et al., 2009). Plasma creatinine, another indicator of skeletal muscle breakdown, has been shown to increase due to heat stress as well (Abeni et al., 2007; Schneider et al., 1988). A more general indicator of alteration in energy metabolism in the liver is the blood plasma concentration of ALP (alkaline phosphatase). This enzyme is involved in the regulation of the energy metabolism by the liver, and is known to decrease in response to heat stress (Abeni et al., 2007; Toharmat and Kume, 1997). Hyperventilation due to heat stress can cause hyperchloremia, i.e. an increase in blood plasma chlorine (Cl⁻), through an increased elimination of bicarbonate from the blood by exchange for Cl⁻. This exchange takes place in the lung tissue, as a direct consequence of increased removal of carbon dioxide via the respiration (Afzaal et al., 2004). Bicarbonate can also be eliminated by exchange for Cl⁻ in the renal tissue, as a consequence of respiratory alkalosis, a secondary effect of hyperventilation (Afzaal et al., 2004; Calamari et al., 2007; Smith 2009).

Ultimately, a negative energy balance due to heat stress can reduce milk yield and alter milk composition (Collier et al., 1982b; Gwazdauskas, 1985; West, 2003). Thus high heat load may not only affect cattle comfort and welfare, but also dairy producers' income, by reducing the milk yield quantitatively, but also by reducing the milk quality. In the US (Bailey 2005) as well as the EU, multiple component pricing systems are used, that pay dairy producers on the basis of milk fat and protein content. In the EU, the basic milk price is adjusted according to the actual fat and protein content (meeting standard fat and protein content or not) (LEI, 2012).

Provision of shade is regarded as one of the most cost-efficient heat stress mitigation strategies on pasture (Blackshaw and Blackshaw, 1994). Many studies have illustrated the beneficial effects of providing shade to heat-stressed cattle in hot climates, in terms of physiology (Ingraham, 1979; Valtorta et al., 1997), as well as performance. For example, milk yield, and milk fat and lactose yield are known to increase when shade is provided (e.g. Davison et al., 1988). For cattle on pasture in temperate regions, however, it is largely unknown what the capital investment would be to provide shade on pasture is, and if the benefits would outweigh the cost.

First, in temperate climate, it is uncertain how severely milk yield and milk are impacted by summer conditions. Traditionally, negative effects on production have been assumed to start at a Temperature Humidity Index (THI) (as defined by Thom, 1959) value of 72 or even 74 (Hahn et al., 2003), but these threshold values are outdated (Van laer et al., 2014). The validation studies were carried out in primarily (sub)tropical and arid regions; and they were carried out on less productive dairy cows than generally kept nowadays (Zimelman et al., 2009), especially in temperate regions. On the other hand, Brügemann et al. (2011) identified a lower daily average THI value of 60 as the threshold for declining milk protein content in German Holstein cows. Hammami et al. (2013) proposed a daily average THI value of 62 as a new threshold for Western European Holstein cows, above which milk yield was found to decline with 0.164 kg/day/cow. However, the studies on which these thresholds were based, were performed in undefined housing systems. Indoor housed cows were most likely included (especially during hot conditions). Specifically for cattle on pasture in temperate climate, no such thresholds are available.

Second, it is not very clear how big the benefits of shade on pasture are in a temperate climate. The benefits depend predominantly on how effective shade is in reducing the negative heat stress effects. Only limited research on this topic is available for temperate regions (Van laer et al., 2014).

In New Zealand (temperate) summer conditions, milk production was 0.5 l/day higher in cows that had access to shade compared to those without, but milk composition was not affected by shade treatment (Kendall et al., 2006).

The aim of this research was to assess whether and to what extent rectal temperature, hyperchloremia, metabolic parameters (cholesterol, urea, creatinine and ALP), milk yield and milk composition are affected by hot conditions, specifically for dairy cows on pasture in temperate summers. In addition, the effectiveness of shade was evaluated by investigating the degree to which shade reduced or prevented these negative effects.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL SETUP

The study took place during three subsequent summers (2011, 2012, 2013; Table 1), and used the same experimental setup that is described in **paragraph 2.1 of Chapter 5**. The experiment was approved by the Animal Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (application nr. 2011/151 and 2011/151bis). The current study focuses on the Holstein dairy cows only. These were rotated between four (in 2011) or two (in 2012 and 2013) pastures, that were all adjacent to a shaded area with young trees and shade cloth spanned between the trees (see **Supplementary Figure S1 in Chapter 5**). The two shaded areas (625 m² each) for dairy cattle, were used by maximum 60 dairy cows on the adjacent pastures, thus providing at least 10.5 m² of shade per cow.

2.2. ANIMALS, MANAGEMENT AND EXPERIMENTAL TREATMENTS

The number of lactating Holstein-Friesian dairy cows used in this experiment varied between 60 and 125, as dry cows left the herd and cows and heifers nearing parturition were regularly added. In 2011, the study used 125 dairy cows. At the beginning of the experiment (10/6/2011) they were of an average parity of 2.0 ± 1.6 (mean \pm SD), were 169.1 ± 132.4 days in milk (DIM; mean \pm SD) and yielded 26.9 ± 11.7 l of milk per day (mean \pm SD). In 2012, 66 dairy cows were used. This group had an average parity of 2.9 ± 1.1 , an average DIM of 180.9 ± 123.4 and milk yield of 30.9 ± 7.3 l/day, at the beginning of the experiment (1/6/12). In 2013, 96 dairy cows were used, with an average parity of 2.0 ± 1.2 , an average DIM of 178.9 ± 117.5 and milk yield of 26.3 ± 7.2 l/day, at the beginning of the experiment (7/6/13). All cows were milked twice daily (starting around 0530 h and starting around 1530 h).

During each milking they received half of the daily portion concentrates. After milking they were fed the daily mixed ration, of which the composition is described in **paragraph 2.2 of Chapter 5**. During the entire study period, the dairy cows were kept on pasture where they could graze ad libitum, except for during milking.

During the summers of 2011 and 2013, the dairy herd was divided into two groups of equal size which were as comparable as possible with regard to traits known to affect susceptibility to heat stress (productivity, parity, age and percentage of black coat). During the summers of 2011 and 2013, one group (the S treatment) was always granted access to the shaded area, whereas the other group (the NS treatment) never had access to shade when on pasture (see also Supplementary **Figure S1 in Chapter 5**). During the summer of 2012, the cows were kept on the same pastures as those used in 2013, but none of the animals had access to shade (NS treatment). The same animal observations were made and samples were taken as in 2011 and 2013 and these data were pooled with those from the NS treatment in 2011 and 2013, to investigate the effect of climatic conditions.

2.3. CLIMATIC DATA

A custom-built Campbell Scientific BWS200 weather station (Campbell Scientific Inc., Logan, Utah, US) located in open pasture, within 500m of all pastures used in the trial, registered the average air temperature (T_a , in °C), air humidity (RH, in %), solar radiation (Rad, in W/m^2) and wind speed (WS, in m/sec) every 15 minutes. Based on these measurements, 15 minute values of the Heat Load Index (HLI) (Gaughan et al., 2008) were calculated. For the formula for calculation of the HLI, we refer to **Table 1 of Chapter 5**.

The HLI was used to quantify hot conditions, because this climatic heat stress index incorporates all relevant climatic variables contributing to thermal (dis)comfort on pasture, i.e. air temperature, air humidity, solar radiation and wind speed. In addition, it was already proven to be the best predictor (out of six climatic heat stress indices) for increasing shade use by Holstein dairy cattle on pasture (see **paragraph 2.5.2 of Chapter 5**).

In order to evaluate the effect of shade on microclimate, additional measurements of T_{bg} were conducted, with Testo 400's Wet Bulb Globe Temperature probe (Testo AG Inc., Lenzkirch, Germany), under shade and outside of shade. During nine measurement sessions, performed on eight days of medium to high heat load (weather forecast predictions of daily maximum temperatures $\geq 25^\circ C$), for each of the shaded areas T_{bg} was measured at 1.5 m height, under shade and in open area nearby (i.e., on three

locations 20-50m away from each shaded area). Three measurement sessions took place between 1000h and 1230h, two sessions between 1200h and 1430h and four sessions between 1330h and 1600h. During each measurement session, three instantaneous measurements were taken inside and outside of each shaded area.

2.4. PHYSIOLOGICAL MEASUREMENTS

Physiological measurements took place on 11 days (Table 1). Daily average air humidity ranged between 46.9 % and 84.9 %, daily average wind speed between 0.8 and 6.8 m/sec, solar radiation intensity between 28.2 W/m² and 74.3 W/m², daily average air temperature between 16.0 °C to 30.2°C, daily average T_{bg} between 19.7 °C and 34.4 °C, and daily average HLI value between 50.8 and 85.5.

TABLE 1. Overview of the daily average Heat Load Index (HLI) on the days of blood sampling and measurement of rectal temperatures (RT).

2011 (11 days)			2012 (8 days)			2013 (13 days)		
date	HLI ¹	mean ± SE	date	HLI ¹	mean ± SE	date	HLI ¹	mean ± SE
8 June	50.8	66.9 ± 3.1	11 July	53.7	69.5 ± 3.9	19 June	72.2	71.5 ± 3.0
15 June	63.3		18 July	53.7		26 June	54.5	
23 June	53.0		24 July	73.8		4 June	59.4	
27 June	82.1		26 July	80.0		8 July	77.1	
11 July	66.6		1 Aug.	74.9		15 July	76.8	
19 July	56.7		9 Aug.	68.1		18 July	82.0	
4 Aug.	72.2		12 Aug.	68.3		23 July	84.6	
17 Aug.	69.1		19 Aug.	83.2		31 July	66.4	
25 Aug.	69.0					2 Aug.	85.5	
2 Sep.	73.9					13 Aug.	56.7	
10 Sep.	78.8					23 Aug.	75.2	
						30 Aug.	62.0	
						4 Sep.	77.0	

¹Daily average HLI on this day.

At the end of these 11 observation days, 20 ‘focal animals’ were separated from the herd before entering the milking parlour for the evening milking (around 1500h). In 2011 and 2013, always the same 10 ‘matched’ pairs (as comparable as possible in terms of productivity, parity, age and percentage of black coat) with one pair member in each experimental group (NS or S) were sampled. From these 20 focal cows, rectal temperatures were determined with a digital thermometer (with an accuracy of 0.1°C) and blood samples were obtained. 9ml blood samples were collected (in lithium-heparin coated tubes) by puncture of the tail vein. The samples were cooled immediately, plasma was centrifuged and frozen at -20°C until analysis.

An automatic clinical chemistry analyser (Cobas 8000 Modular Analyser; Roche Diagnostics; Indianapolis, USA) was used to determine the concentrations of several blood plasma indicators of metabolic changes that are known to be related to heat stress (i.e. the concentrations of cholesterol, urea, creatinine, and ALP) and the concentration of Cl^- (a sign of hyperventilation) in the blood plasma.

2.6. MILK YIELD & MILK COMPOSITION

Within each trial-period (each summer), milk yields from each cow were saved by an automated registration system, except for a period between 26/07/2013 and 22/08/2013, due to a defect in the registration system. The automated system summed the milk yield from each morning with that of the evening before, to obtain individual daily milk yield (MYX) data. MYX was coupled to climatic data from one day, two days and three days before. Thus the milk yield dataset contained milk yields from 98 days in 2011 (10/6/2011 – 15/9/2011), 117 days in 2012 (1/6/2012 – 25/09/2012), and 105 days in 2013 (7/6/2013 - 19/9/2013). These data were coupled to the daily average HLI's, which ranged between 46.1 and 86.7 (mean \pm SE= 61.6 \pm 0.5) on the day before, between 46.1 and 86.7 (mean \pm SE= 61.6 \pm 0.5) two days before and between 46.7 and 86.7 (mean \pm SE= 61.7 \pm 0.5) three days before.

In addition, data from monthly determinations of milk composition, with a mid-infrared spectrophotometer, by the Flemish milk monitoring service (Melk Controle Centrum Vlaanderen, <http://www.mcc-vlaanderen.be>) were obtained. These determinations were carried out every five weeks, unrelated to weather conditions. Content of fat, protein, lactose and urea were also coupled to climatic data from one day, two days and three days before. Thus the milk composition dataset contained data from three days in 2011, three days in 2012 and four days in 2013, and coupled to it daily average HLI's that ranged between 50.6 and 76.3 (mean \pm SE = 59.4 \pm 2.5) on the day before, between 46.1 and 75.7 (mean \pm SE = 57.6 \pm 2.5) two days before, and between 48.6 and 67.8 (mean \pm SE =56.2 \pm 1.7) three days before.

2.7. DATA ANALYSIS

2.7.1. EFFECT OF SHADE ON MICROCLIMATE

The difference in T_a and T_{bg} measured in open area and under shade was modelled using a linear mixed model ANOVA (proc mixed, in SAS 9.3). Day of measurement and shade area were included as random factors.

2.7.2. PHYSIOLOGICAL MEASUREMENTS

The effect of HLI and treatment on rectal temperature (RT) and blood plasma concentrations of ALP, cholesterol, creatinine, urea and Cl⁻ was investigated by means of mixed linear regressions (proc mixed, in SAS 9.4), which also took the productivity of the cow into account (as a fixed effect).

Both linear and quadratic models were tested to determine the effect of HLI on several dependent variables. In the linear models, the fixed effects were: (1) the effect of the productivity of the cow, i.e. the summed milk yield of the morning of the same day and the evening before, centred over the dataset (the overall average daily milk yield was subtracted from the individual value), (2) the effect of treatment (NS or S), and (3) the interaction of (2) with the daily average HLI. In the quadratic models, the fixed effects were (1), (2) and (3), and additionally, the interaction of (2) with the square of the daily average HLI (for model equations, we refer to Appendix A1).

Generally, the strictly linear models yielded the best fit, i.e. the lowest AICC-value (Corrected Akaike Information Criterion). Therefore, only results from the strictly linear models are reported.

2.7.3. MILK YIELD

To investigate the effect of HLI and access to shade on milk yield, the daily milk yield data (the number of litres/day) from each cow in the herd, over the entire experimental periods, were used. Mixed linear regressions (proc mixed in SAS 9.4) were carried out, which also took the lactation stage of the cow into account (as a fixed effect). Irrespective of the potential effect of HLI, the milk yield can be assumed to decrease linearly between peak lactation and late lactation, i.e. between 42 and 305 DIM (Adediran et al., 2012). Data from <42 DIM and >305 DIM were omitted from the dataset. The effect of HLI was included in the models as a linear factor as well as a quadratic factor, to detect non-linear effects. The daily milk yield was thus modelled as a function of (1) the effect of the lactation stage (DIM), (2) the effect of treatment (NS or S), (3) the interaction of (2) with the daily average HLI one, two, or three days before sampling, and (4) the interaction of (3) with the square of the daily average HLI (for model equations, we refer to Appendix A1).

2.7.4. MILK COMPOSITION

The effect of HLI and treatment (S or NS) on the milk content of fat, protein, lactose and urea, was investigated by means of linear mixed regressions (proc mixed in SAS 9.4), which also took the milk yield (quantity) into account, as a fixed effect. Milk protein content and fat content, for example, are known to co-vary greatly with milk yield (Welper and Freeman, 1992).

For the milk composition, again, both models with and without a squared HLI factor were tested. In the linear models, the fixed effects were: (1) the effect of the milk quantity, i.e. the milk yield on the day of sampling, centred over the dataset (the overall average milk yield, 26.9 kg/day, was subtracted from the individual value), (2) the effect of treatment (NS or S), (3) the daily average HLI, and (4) the interaction of (2) and (3). In the quadratic models, the fixed effects were (1), (2), (3) and (4), and additionally, the interaction of (3) with the square of the daily average HLI and the interaction of (4) with the square of the daily average HLI (for model equations, we refer to Appendix A1).

The models without the quadratic HLI factor always yielded the best fit (the lowest AICC-value). Consequently, only results from these strictly linear models are reported here. For each composition variable, the model that yielded the lowest AICC value, i.e. the model with the HLI of either one, either two or either three days before sampling (Table 2), was considered to be the best fitting model. Only the results of these models are discussed.

TABLE 2. AICC¹-values of models for milk composition variables in function treatment (NS or S) and its interaction with the Heat Load Index (HLI)².

	X = HLI ²		
	1 day	2 days	3 days
	<u>before sampling</u>	<u>before sampling</u>	<u>before sampling</u>
Y = milk [urea]	2927	2929	2942
Y = milk [lactose]	-233	-262	-286
Y = milk [protein]	80	66	50
Y = milk [fat]	887	891	886

¹AICC=Corrected Akaike Information Criterium. ²Daily average of one day, two days and three days before sampling.

In all mixed linear regression models, a random individual effect, nested within the experimental year, was included to correct for repeated measurements per individual, within year. Because of computational limitations of the analysis software, observations of the same individual in different years were regarded as independent. All analysis were performed using proc MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC).

3. RESULTS

3.1. EFFECT OF SHADE ON MICROCLIMATE

In comparison with a nearby open area, shade lowered T_{bg} by 4.5 °C (P < 0.0001); the mean T_{bg} (± SE) was 30.3 ± 2.0 °C outside shade and 25.8 ± 2.0 °C under shade.

3.2. RECTAL TEMPERATURE (RT) AND BLOOD PLASMA INDICATORS OF METABOLIC ALTERATIONS

The RT and all blood plasma variables were significantly affected by HLI (P < 0.0001 for RT, Cl⁻, cholesterol and creatinine, P = 0.0003 for urea, P = 0.015 for ALP). The RT and the blood plasma concentration of Cl⁻ and ALP were also significantly influenced by the interaction between HLI and treatment (P = 0.0002 for RT, P = 0.0065 for Cl⁻ and P = 0.026 for ALP, whereas P = 0.700 for cholesterol, P = 0.313 for urea, P = 0.293 for creatinine).

RT increased with increasing HLI for both treatments, however the response was less pronounced in the cows with access to shade (Fig. 1 and Table 3). The mean RT was 39.5 ± 0.1 °C for cows without access to shade and 39.2 ± 0.1 °C for cows with access to shade (difference: P=0.0011), at the highest observed daily average HLI (i.e. 85).

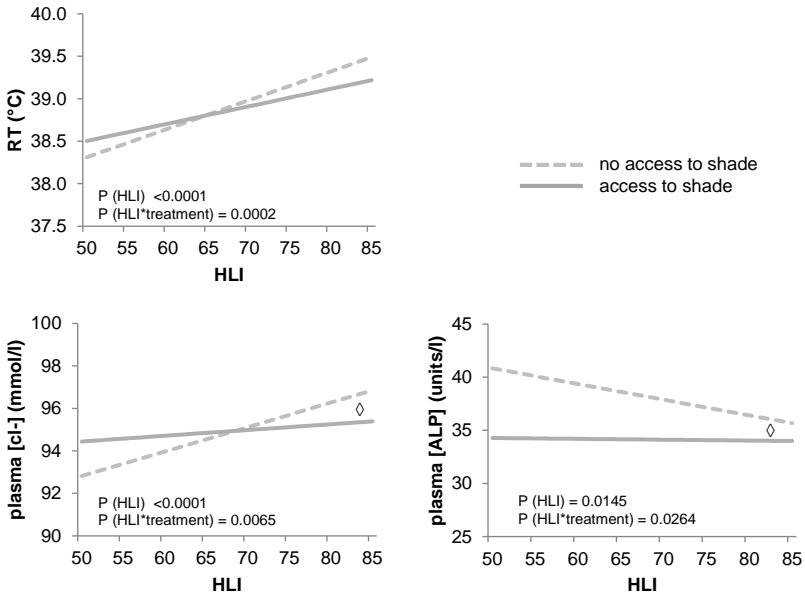


FIGURE 1. Effect of the daily average Heat Load Index (HLI) and treatment (NS or S) on the rectal temperature (RT) and blood plasma [Cl⁻] and [ALP]. ◊ Blood plasma [Cl⁻] and [ALP] are not significantly influenced by HLI for animals with access to shade (Table 3).

The plasma concentration of Cl⁻ increased along with increasing HLI for cows without access to shade. However, for cows with access to shade, plasma Cl⁻ was not significantly affected by HLI (Fig. 1, Table 3). The plasma concentration of ALP decreased with increasing HLI, for cows without access to shade only. For cows with access to shade, ALP was unaffected by HLI (Fig. 1, Table 3). The plasma concentration of cholesterol and urea decreased whereas that of creatinine increased with increasing HLI, irrespective of access to shade (Table 3).

TABLE 3. Effects of the daily average Heat Load Index (HLI) and treatment (NS or S)¹ on the rectal temperature and blood plasma indicators of metabolic alterations.

Y=	Effect	Estimate	SE	P-value
rectal temperature (°C)	Intercept	37.48	0.22	<0.0001
	milk yield (average)	0.003	0.003	0.2261
	treatment=NS	-0.85	0.25	0.0008
	treatment=S	.	.	.
	HLI*treatment=NS	0.03	0.002	<0.0001
	HLI*treatment=S	0.02	0.003	<0.0001
plasma [cholesterol] (g/l)	Intercept	2018.30	126.38	<0.0001
	milk yield (average)	8.68	1.57	<0.0001
	treatment=NS	-79.19	153.67	0.6068
	treatment=S	.	.	.
	HLI*treatment=NS	-5.32	1.10	<0.0001
	HLI*treatment=S	-6.03	1.48	<0.0001
plasma [urea] (g/l)	Intercept	347.69	36.04	<0.0001
	milk yield (average)	0.47	0.46	0.3051
	treatment=NS	-50.30	42.42	0.2362
	treatment=S	.	.	.
	HLI*treatment=NS	-0.78	0.35	0.025
	HLI*treatment=S	-1.37	0.47	0.0039
plasma [ALP ²] (units/l)	Intercept	34.68	4.43	<0.0001
	milk yield (average)	0.05	0.05	0.4025
	treatment=NS	13.52	5.46	0.0138
	treatment=S	.	.	.
	HLI*treatment=NS	-0.15	0.04	<0.0001
	HLI*treatment=S	-0.01	0.05	0.8727
plasma [creatinine] (g/l)	Intercept	5.86	0.57	<0.0001
	milk yield (average)	-0.01	0.01	0.0445
	treatment=NS	-0.92	0.67	0.1697
	treatment=S	.	.	.
	HLI*treatment=NS	0.06	0.01	<0.0001
	HLI*treatment=S	0.05	0.01	<0.0001
plasma [Cl ⁻] (mmol/l)	Intercept	93.09	1.97	<0.0001
	milk yield (average)	0.001	0.02	0.8354
	treatment=NS	-6.01	2.28	0.0087
	treatment=S	.	.	.
	HLI*treatment=NS	0.12	0.02	<0.0001
	HLI*treatment=S	0.03	0.03	0.2976

¹NS= no access to shade, S= access to shade. ²ALP= alkaline phosphatase.

3.3. MILK YIELD

For cows with access to shade, the milk yield was not significantly affected by the HLI (or its quadratic factor) on one, two and three days before (Table 4). The milk yield for animals without access to shade, however, was affected by the HLI (and its quadratic factor) two days before. For example, when the daily average HLI value increased from 65 to 85, the milk yield (for cows with an average number of DIM) decreased from 25.1 l/day to 24.1 l/day (Fig. 2, Table 4). For the cows with access to shade, the daily milk yield did not significantly decrease with increasing HLI (Fig. 2, Table 4).

TABLE 4. Effects of lactation stage¹, treatment (NS or S)² and its interaction with the daily average heat load index (HLI)³ and its quadrat (HLI²)³ on the daily milk yield.

X=	Effect	Estimate	SE	P-value
HLI	Intercept	33.62	4.22	<0.0001
1 day before sampling	DIM	-0.06	0.00	<0.0001
	treatment=NS	2.04	4.71	0.6653
	treatment=S	.	.	.
	HLI*treatment=NS	0.08	0.08	0.3233
	HLI*treatment=S	0.16	0.12	0.1882
	HLI ² *treatment=NS	-0.001	0.001	0.2377
	HLI ² *treatment=S	-0.001	0.001	0.1308
HLI	Intercept	42.10	4.23	<0.0001
2 days before sampling	DIM	-0.06	0.0001	<0.0001
	treatment=NS	-9.01	4.72	0.0561
	treatment=S	.	.	.
	HLI*treatment=NS	0.16	0.08	0.0482
	HLI*treatment=S	-0.11	0.12	0.3641
	HLI ² *treatment=NS	-0.001	0.001	0.0282
	HLI ² *treatment=S	0.001	0.001	0.4720
HLI	Intercept	42.16	4.20	<0.0001
3 days before sampling	DIM	-0.06	0.00	<0.0001
	treatment=NS	-2.78	4.71	0.5551
	treatment=S	.	.	.
	HLI*treatment=NS	-0.03	0.08	0.7216
	HLI*treatment=S	-0.11	0.12	0.3475
	HLI ² *treatment=NS	0.00001	0.001	0.9917
	HLI ² *treatment=S	0.001	0.001	0.4714

¹DIM=days in milk (lactation stage). ²NS= no access to shade, S= access to shade.

³Daily average of one day, two days and three days before sampling.

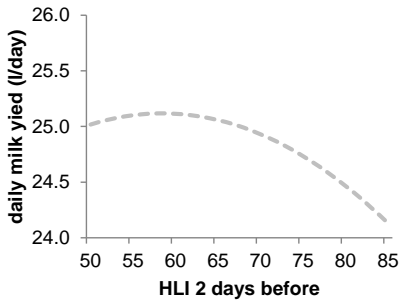


FIGURE 2. Daily average milk yield for cows without access to shade (NS) (and with an average number of DIM, i.e. 202) in function of the daily average Heat Load Index (HLI) two days before.

The model for cows without access to shade, was used to assess the annual decrease in milk yield (in litres per cow) due to the lack of shade, for the years 2012 and 2013. This assessment was based on the modelled decrease in milk yield per five unit increase in HLI and the occurrence of the corresponding HLI levels in 2012 and 2013 (Table 5). This assessment was not made for 2011, because no climatic measurements were available before 8/06/2011. The results of the assessment indicate that, in 2012, the milk yield for cows without access to shade declined by 8.0 l/year/cow due to daily average HLI values above 70 (total n=23) (Table 5). In 2013, there were 31 days with daily average HLI above 70. Consequently the milk yield for cows without access to shade declined by 13.0 l/year/cow (Table 5).

TABLE 5. Assessment of the potential loss in yearly milk yield per cow, due to lack of shade in the Belgian summers of 2012 and 2013.

HLI	Milk yield (l/day/cow)	Decline relative to HLI=60 (l/day/cow) ¹	HLI class	Number of days in 2012 ²	Resulting loss in 2012 (l/day/cow) ³	Number of days in 2013 ²	Resulting loss in 2013 (l/day/cow) ³
60	25.1	0.0	60-65				
65	25.1	0.0	65-70				
70	24.9	0.2	70-75	10	2.0	9	1.8
75	24.7	0.4	75-80	9	3.6	14	5.6
80	24.5	0.6	80-85	4	2.4	6	3.6
85	24.1	1.0	85-90	0	0.0	2	2.0
total milk yield loss per year ⁴ :				2012: 8.0 l/year/cow		2013: 13.0 l/year/cow	

¹The degree of decline in milk yield with every five unit increase in HLI above the HLI that gave the maximum milk yield (HLI=60). ²The occurrence of the number of days with these HLI levels in 2012 and 2013, based on the data from the weather station on our experimental pastures. ³The resulting milk yield loss per HLI level, for both years. ⁴The total milk yield loss is the sum of milk yield loss per HLI level, for both years.

3.4. MILK COMPOSITION

The milk urea content was best explained by the HLI one day before sampling (Table 2). For cows with access to shade, the milk urea content was unaffected by HLI. For cows without access to shade the milk urea content decreased with increasing HLI (Fig.3, Table 6; $P(\text{HLI}*\text{treatment})=0.0027$). The milk content of lactose, protein, fat were best explained by the HLI three days before sampling (Table 2). The milk lactose content decreased with increasing HLI, irrespective of access to shade (Table 6; $P(\text{HLI}*\text{treatment})=0.1248$). The milk protein content decreased with increasing HLI, but the decline was less marked for cows with versus without access to shade (Fig.3, Table 6; $P(\text{HLI}*\text{treatment})=0.0465$). The milk fat content was unaffected for animals without access to shade but decreased with increasing HLI for cows with access to shade (Fig.3, Table 6; $P(\text{HLI}*\text{treatment})=0.0300$).

TABLE 6. Effects of treatment (NS or S)¹ and its interaction with the Heat Load Index (HLI)² on the milk composition variables.

Y	X	Effect	Estimate	SE	P-value
milk [urea]	HLI 1 day before sampling	Intercept	26.21	4.70	<0.0001
		milk yield (average)	0.06	0.05	0.1876
		treatment=NS	18.33	5.11	0.0004
		treatment=S	.	.	.
		HLI*treatment=NS	-0.22	0.04	<0.0001
		HLI*treatment=S	0.06	0.08	0.4946
milk [lactose]	HLI 3 days before sampling	Intercept	5.46	0.15	<0.0001
		milk yield (average)	0.01	0.00	<0.0001
		treatment=NS	-0.22	0.16	0.1739
		treatment=S	.	.	.
		HLI*treatment=NS	-0.01	0.002	<0.0001
		HLI*treatment=S	-0.01	0.003	<0.0001
milk [protein]	HLI 3 days before sampling	Intercept	4.07	0.23	<0.0001
		milk yield (average)	-0.03	0.00	<0.0001
		treatment=NS	0.51	0.25	0.0419
		treatment=S	.	.	.
		HLI*treatment=NS	-0.02	0.002	<0.0001
		HLI*treatment=S	-0.01	0.004	0.0056
milk [fat]	HLI 3 days before sampling	Intercept	5.30	0.59	<0.0001
		milk yield (average)	-0.03	0.00	<0.0001
		treatment=NS	-1.40	0.66	0.0354
		treatment=S	.	.	.
		HLI*treatment=NS	0.0001	0.01	0.9851
		HLI*treatment=S	-0.03	0.01	0.0134

¹NS= no access to shade, S= access to shade. ²Daily average HLI of one day, two days and three days before sampling, depending on which day provided the best fitting model (model with the lowest AICC value, Table 2).

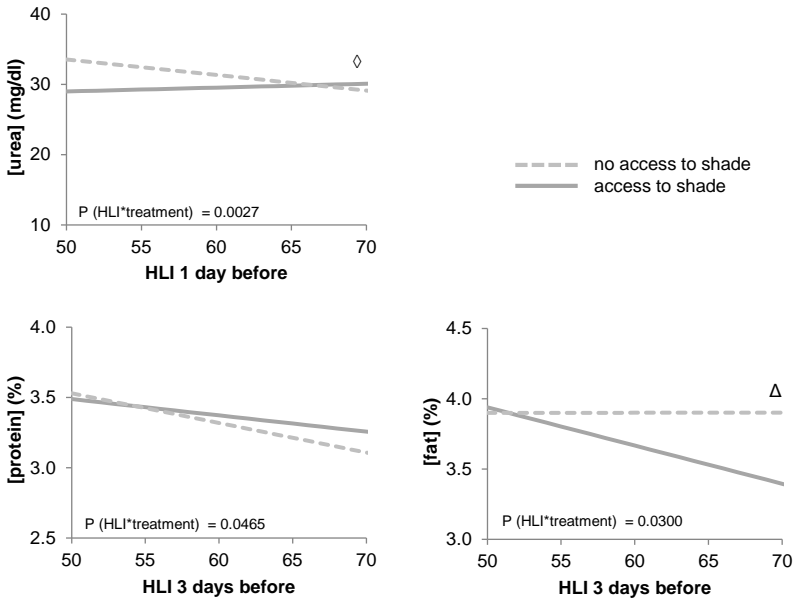


FIGURE 3. Effect of the daily average Heat Load Index (HLI) - one day before or three days before, depending on what day gave the best fit (lowest AICC, see table 4) - and treatment on milk urea, protein and fat content. P-values for the interaction between HLI and treatment are given, \diamond : the milk composition variable is not significantly influenced by HLI for animals with access to shade, Δ the milk composition variable is not significantly influenced by HLI for animals without access to shade (Table 6).

4. DISCUSSION

4.1. HOT SUMMER CONDITIONS AFFECTED RECTAL

TEMPERATURE, HYPERCHLOREMIA AND ENERGY METABOLISM

The rectal temperature and the energy metabolism of cows without access to shade was substantially affected by hot summer conditions. At HLI=85 (\pm the highest observed daily average value in the RT dataset), the RT of cows without access to shade was on average 39.5 °C. This result is comparable to the RTs around 39.5 °C that Muller et al. (1994a) observed at air temperatures around 35 °C, for unshaded Holstein cows in South Africa. Also daily maximum body temperatures (as measured vaginally) were comparable for Holstein cows in New Zealand summers (Kendall et al., 2006; Tucker et al., 2008). The increase of RT was tempered by having access to shade. This is in line with the results of Kendall et al. (2006).

In line with previous research, the plasma concentration of Cl⁻ increased with increasing degree of heat (Calamari et al., 2007), for cows without access to shade. This hyperchloremia is likely a sign of hyperventilation (Afzaal et al., 2004; Smith 2009). In our research parallel to the current study, respiration rates indeed regularly exceeded 120 breaths per minute (>20% of the observations of dairy cows outside shade; Van laer et al., submitted data). Access to shade, however, seemed to prevent hyperventilation, given that the Cl⁻ concentration did not increase for animals with access to shade. This is consistent with the finding that the prevalence of respiration rates ≥ 120 breaths per minute was reduced by use of shade (Van laer et al., submitted).

Increasing HLI was associated with a decrease in plasma ALP, indicating a general alteration in energy metabolism, but this was prevented by access to shade. Increasing HLI was also associated with a decrease in the plasma concentration of cholesterol. A similar finding was reported by Toharmat and Kurne (1997). The change may be due to decreased liver activity and increased lipolysis in peripheral tissues (Abeni et al., 2007). Increased skeletal muscle breakdown was established, as indicated by the increase in plasma creatinine concentration, also reported by Abeni et al. (2007) and Schneider et al. (1988). The effect of hot summer conditions on lipolysis and amino acid breakdown was not reduced by access to shade, however.

Our findings on urea concentrations in blood as well as in the milk were unexpected. An increased breakdown of amino acids in response to hot summer conditions was expected to increase blood plasma urea (Shwartz et al., 2009), and milk urea content (Gallardo et al., 2005). On the other hand, also Abeni et al. (2007) found a decrease in blood plasma urea concentration during two hot periods under Italian summer conditions. In cattle, plasma and milk urea concentrations are very much determined by the rumen degradable protein balance: a largely positive balance leads to higher NH₃ production in the rumen, which results in higher urea concentrations in blood and milk. In the current study, the observed decrease in the urea concentration might thus be due to a shift in feeding behaviour. From previous research it is known that during hot days cows reduce their feed intake during the hottest part of the day (Silanikove 2000). Although data on individual feed intake are unavailable for this study, hot summer conditions may have reduced the intake of grass on pasture, which is an important source of degradable protein. This possibly reduced the availability of NH₃ in the rumen and thus the urea levels in blood and milk. For cows with access to shade, hot summer conditions did not reduce milk urea content, which suggests that changes in rumen degradable protein balance were less pronounced in this treatment group.

However, completely unravelling the effects of hot conditions (in temperate summers) on feed intake, energy intake and protein intake was well beyond the scope of this study.

Nevertheless, our findings with respect to the various blood plasma indicators of metabolic changes indicate that the absence of shade, even in a temperate region such as Belgium, under hot summer conditions, is able to trigger at least some degree of 'negative energy balance' in Holstein dairy cows kept on pasture. In addition, the observed hyperchloremia suggests that cows without access to shade suffered from hyperventilation under hot summer conditions. This can be assumed to also reflect substantial thermal discomfort (see Van laer et al., submitted data). Furthermore, this study indicates that, even in a temperate climate, the negative energy balance due to heat stress ultimately reduces milk yield and alters milk composition.

4.2. HOT SUMMER CONDITIONS DECREASED MILK YIELD FOR COWS WITHOUT ACCESS TO SHADE

Increasing HLI was associated with a decreasing milk yield for unshaded cows. After a lag-effect of two days, their milk yield declined, starting at a daily average HLI around 65. The higher the HLI increased, the steeper the milk yield decline became. At HLI = 85, the milk yield was 4.2% lower than at HLI = 65 (24.1 l/day/cow versus 25.1 l/day/cow). Another study which related the milk yield of Holstein dairy cattle in temperate climate to HLI (Hammami et al., 2013), found a reduction in milk yield with 0.1% per unit increase of the HLI, but above the threshold of 80 only (decline of 0.12 kg/day/cow versus yield of 23.8 kg/day/cow under thermoneutral conditions). The data for that study were obtained from cows in unspecified housing systems, however. Indoor housed cows were probably included (especially during hot summer conditions), which might explain the higher threshold for milk yield decline. In contrast, a study on cows on pasture in New Zealand, found no relationship between the daily maximum HLI and daily total milk production (Kendall et al., 2006). Furthermore, no studies relating milk yield to the HLI are known.

The decline in milk yield in our study in a temperate climate was less marked than the declines reported in (sub)tropical or arid climates. For example, a large scale study in Arizona, which is characterized by a desert climate, reported a significant decline in milk yield (about 6.8%) when daily minimum THI increased from 65 to 73 (decline of 2.2 kg/day/cow versus yield of 32.2 kg/d/cow at THI = 65) (Zimbelman et al., 2009).

In the present study, the decrease in milk yield coupled to increasing HLI did not occur when cows had access to shade. The milk yield also benefited from access to shade in New Zealand summers - with a difference of 0.5 l/day/cow (Kendall et al., 2006) – and in South-African summers -with a 5.5% difference (Muller et al., 1994b).

4.3. HOT SUMMER CONDITIONS ALTERED MILK COMPOSITION

The milk content of lactose, protein and fat was significantly affected by hot summer conditions. As HLI increased, after a lag-time of three days, the milk lactose production decreased by about 0.02 % and the protein content decreased by about 0.01 % per unit increase of HLI. This decline in protein content is less than the 0.06% decline reported by Gantner et al. (2011) for cows in free stall barns in Croatia.

We demonstrated no unambiguous effect of shade on the relationship between HLI and milk composition. Contrary to expectations, the milk fat content was unaffected for cows without access to shade, but did decrease by about 0.03 % per unit increase of HLI for cows with access to shade. For a cow with access to shade the daily fat yield would thus decrease by 8g/day per unit increase of HLI. This decline is comparable with the decline of 10g/day per unit increase of the HLI above 80 that Hammami et al. (2013) found, but not as steep as the decline of 0.07% found by Gantner et al. (2011) at daily THI \geq 72. On the other hand, cows with access to shade showed a less marked decrease of the milk protein content.

In conclusion, hot summer conditions may affect dairy producers' income, due to reduced quantity as well as quality of the milk produced. However the heat stress remediating effect of shade on the milk composition remains unclear. This might be due to the relatively low number of milk composition samples from periods of high HLI. The milk composition dataset contained data from only 10 days in total, with daily average HLI 's up to only 76.3 (mean \pm SE = 59.4 \pm 2.5) on the day before, and only 67.8 (mean \pm SE = 56.2 \pm 1.7) three days before. Therefore, further research would be useful to determine to what degree shade can reduce the negative effect of heat stress on milk composition, specifically for dairy cows on pasture in temperate summers.

4.4. OTHER ASPECTS OF PROVISION OF SHADE ON PASTURE

The current study showed that absence of shade on pasture during hot summer conditions can reduce dairy producers' income. In a study parallel with the current study, we also demonstrated that shade improves thermal comfort for cows (Van laer et al., submitted data). However, potential effects of hot summer conditions and shade on veterinary costs, feed intake, pasture productivity, etc. remain unknown.

In addition, the cost for provision of shade on pasture depend greatly on the design and size of the shading structure. As a minimum, generally 3.5 m² to 6.5 m² shade per cow is recommended (Armstrong, 1994). Yet, Schütz et al. (2010) demonstrated that 9.6 m² of shade per cow elicited twice as much shade use and more simultaneous shade use by several cows, fewer aggressive interactions and lower respiration rates than 2.4 m² of shade per cow. In a field study on commercial farms, the same authors found the prevalence of high Panting Scores (≥ 2) to decrease by 0.3% with every additional 1m² of shade per cow, (Schütz et al., 2014).

Trampling and manure deposition in shaded resting areas may also be reduced by high individual space allowance, or by using movable structures (Armstrong, 1994). Movable structures are also suitable for rotational grazing systems. Shade cloth is ideal for the construction of lightweight movable structures, which are being commercialized in for example the US (Dr. T. Brown-Brandl, pers. comm.). On the other hand, shade provision by trees on pasture creates a more natural landscape, greater biodiversity and landscape connectivity. Other points of attention regarding natural or artificial shade on pasture, are discussed in Van laer et al. (2014).

5. CONCLUSIONS

The first aim of our research was to assess the degree of negative impact of hot summer conditions occurring in temperate summers, on rectal temperature, metabolic parameters, milk yield and milk composition. Increasing HLI increased RT, a sign of hyperventilation, signs of lipolysis and skeletal muscle amino acid catabolism, whereas it decreased the milk content of lactose, protein and fat. For cows without access to shade, the milk yield, after a lag-period of two days, also decreased notably with increasing HLI. The higher HLI increased, the steeper the milk yield decline became. At daily average HLI = 85, the milk yield two days later was 1.0 l/day/cow lower than

at daily average HLI = 65. The second aim was to evaluate the effectiveness of shade in preventing the abovementioned negative effects. The effect of hot summer conditions on lipolysis and amino acid breakdown (as assessed by blood plasma concentrations of cholesterol and creatinine) was not tempered by having access to shade. The effects of hot summer conditions on milk composition was not unambiguously ameliorated by shade either. But the increase of RT, hyperchloremia (a sign of hyperventilation) and the decrease of ALP (a regulator of metabolism in the liver) in the blood plasma was ameliorated by shade. Access to shade prevented the decrease in milk yield that was observed in cows without access to shade. Additional research would be useful to investigate other potential benefits of shade, aspects of optimal shade area design and size (e.g. to prevent excessive trampling of the grass and excessive manure deposition) and the cost of an adequate shade area, in order to allow a cost-benefit analysis for provision of shade on pasture in temperate climate.

6. ACKNOWLEDGEMENTS

This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RT 10/13 Pastress). The experiment was approved by ILVO's Ethical Committee (EC 2011/151 and EC 2011/151bis). The authors thank the many people who helped to accomplish this study, including the animal caretakers and technicians (especially Seppe Holemans, Sara Van Lembergen and Thijs Decroos) from ILVO Animal Sciences Unit and master student Evelien Van de Keere. We also thank Miriam Levenson for English language editing.

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APPENDIX A1.

Mixed Model equations used to determine the effect of HLI and treatment (S or NS) on physiological measurements, milk yield and milk composition variables:

- Y= rectal temperature / [cholesterol] / [urea] / [creatinine] / [ALP] / [Cl-]:

- Linear models:

$$Y = \beta_0 + \beta_1 * x_1 + \beta_2 * x_2 + \beta_3 * x_2 * x_3 + c + y + \epsilon$$

- Quadratic models:

$$Y = \beta_0 + \beta_1 * x_1 + \beta_2 * x_2 + \beta_3 * x_2 * x_3 + \beta_4 * x_2 * x_3^2 + c + y + \epsilon$$

where x_1 = the effect of the productivity of the cow: daily milk yield, centred over the dataset (overall average daily milk yield was subtracted from the individual value), x_2 = the effect of treatment (NS or S), x_3 = the effect of the HLI, c = random cow (nested within year) effect, y = random year effect

- Y = daily milk yield:

$$Y = \beta_0 + \beta_1 * x_1 + \beta_2 * x_2 + \beta_3 * x_2 * x_3 + \beta_4 * x_2 * x_3^2 + c + y + \epsilon$$

where x_1 = the effect of the lactation stage (DIM), x_2 = the effect of treatment (NS or S), x_3 = the effect of the HLI one, two, or three days before sampling, c = random cow (nested within year) effect, y = random year effect

- Y= urea content (mg/dl) / % lactose / % protein / % fat:

- Linear models:

$$Y = \beta_0 + \beta_1 * x_1 + \beta_2 * x_2 + \beta_3 * x_3 + \beta_4 * x_2 * x_3 + c + y + \epsilon$$

- Quadratic models:

$$Y = \beta_0 + \beta_1 * x_1 + \beta_2 * x_2 + \beta_3 * x_3 + \beta_4 * x_2 * x_3 + \beta_5 * x_2 * x_3^2 + c + y + \epsilon$$

where x_1 = the effect of the milk quantity, i.e. the milk yield on the day of sampling centred over the dataset (overall average - 26.9 kg - was subtracted from the individual value), x_2 = the effect of treatment (NS or S), x_3 = the effect of the HLI one, two or three days before sampling, c = random cow (nested within year) effect, y = random year effect

CHAPTER 7

GENERAL DISCUSSION



The doctoral research described in this dissertation, investigated the impact of cold and hot conditions in Belgium (a country with a typical temperate climate) on thermal comfort of cattle kept outdoor, and the value of several types of shelter as protection against cold and heat stress.

Firstly, we conducted a literature review and studied historic Belgian weather data to (roughly) assess whether aversive weather conditions occur frequently enough and can be severe enough to recommend the provision of shelter to reduce thermal discomfort, and its' consequences, in cattle kept outdoors (**Chapter 1**). Recent literature concerning highly producing cows in temperate climatic regions was studied. This demonstrated that the earliest heat stress threshold values for pre-defined climatic indices are outdated and too general to evaluate heat stress in highly producing dairy cattle and the existing variety of other cattle types. Recent literature suggested a daily average THI of 68 (Zimbelmann et al., 2009) or even 62 (Hammami et al., 2013) as heat stress threshold for declining milk yield in highly producing dairy cows.

We used the THI threshold values of 68 and 62 in our assessment of the prevalence of 'potential heat stress conditions' in Belgium (**Chapter 1**), based on daily climatic data from the period between February 1994 and May 2005. The results indicated that the THI was greater than 62 on 15% of the days and greater than 68 on 3% of the days. This suggested that weather conditions in Belgium are such that the risk for heat stress in unprotected outdoor cattle are not exceptional. In our subsequent research we investigated whether such 'assumed heat stress conditions' indeed influenced the sheltering behaviour of non-commercial (without milk or meat production aim) cattle in eight Belgian nature reserves (**Chapter 2**) and of dairy and beef cattle on pasture (**Chapter 5**). For the dairy and beef cattle on pasture we also investigated the effect of heat load and shade on the occurrence and severity of thermal discomfort (**Chapter 5**) and, for dairy cows, some of its potential consequences (**Chapter 6**).

Cold stress, as opposed to heat stress, is assumedly a lesser problem for commercial cattle in temperate regions, because these are generally kept indoors during winter (e.g. Krohn et al., 1992). Cattle used in year-round grazing management of nature reserves, however, do stay outdoors during winter. Evaluating the occurrence of cold conditions in Belgium (Melle) between February 1994 and May 2005 (**Chapter 1**), we found that the daily average WCI fell below zero on 20% of the days in these 11 years. The daily minimum WCI reached below -10 on 14% of these days. Based on literature, such outdoor winter conditions were assumed to pose little risk for severe cold stress (with obvious health-effects) to healthy adult cattle. Still, such

conditions might temporarily cause discomfort, and thus motivate outdoor housed cattle to seek shelter. We investigated whether this expectation was met, in **Chapter 3**. More specifically, we investigated whether cold winter conditions increased the use of natural and/or artificial shelter, as indication of thermal discomfort in unsheltered locations, by cattle in eight Belgian nature reserves.

The current chapter describes (separately for the studies on the cattle in nature reserves and the studies on dairy and beef cattle on pasture) the limitations of our research, along with suggestions for future research, our main findings and their implications. In addition, we provide practical advice, for cattle keepers, animal welfare organisations and policy makers, concerning the prevention and detection of heat stress in cattle.

1. THE USE OF NATURAL AND ARTIFICIAL SHELTER BY CATTLE IN NATURE RESERVES

The field study described in **Chapter 3** investigated the effect of summer climatic conditions on the sheltering behaviour of cattle in eight Belgian nature reserves. In the same reserves, we also investigated the effect of cold winter conditions on sheltering behaviour, as described in **Chapter 4**.

1.1. LIMITATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

As any scientific study, this doctoral research was necessarily limited in time, scope and resources. Therefore, it is subject to a number of limitations that should be kept in mind when drawing conclusions from the study results. In addition, the limitations indicate where the opportunities lie or scope should lie for further research.

For example, the limitation in time raises the question if the range of climatic conditions comprised in the study periods are representative (enough) for the range of climatic conditions that are known to occur in the research area (Belgium). Only when this is sufficiently the case, we can confidently extrapolate our research results from three winters and two summers to Belgian winters and summers in general. When the range of climatic conditions covered were too small in comparison with previous winters and summers, it would be recommendable to continue the study over subsequent years.

The summer climatic conditions during this study were fairly representative for recent Belgian summers. The maximum THI registered in the eight study reserves was about 85 (**Chapter 3**). In the period between February 1994 and May 2005, in Melle (central Belgium), daily maximum THI values above 85 were also rare. The daily maximum THI exceeded 85 on only 10 out of 5057 days (thus 0.2%), and never exceeded 88 (**Chapter 1**). In addition, **Fig. 1a** shows that the mean of the daily maximum THI's registered in our study reserves in the summers of 2012 and 2013, was often very close to that registered during the summer months of 1994-2005 in Melle. When this was the case, the 95% percentile and the 5% percentile of the daily maximum THI's in the nature reserves were, respectively, higher and lower than those between 1994 and 2005. This indicates that the summers included in our study covered a relatively range of heat load degrees in comparison to the historic data from 1994 to 2005.

In the summer of 2003, Western Europe was struck by a heat wave. Especially in the first two weeks of August 2003, in comparison with previous years, exceptionally high temperatures were measured in Mediterranean regions and France, but in southern England, Germany, the Netherlands and Belgium as well (García-Herrera et al., 2010; Trigo et al., 2005). By comparing the daily maximum THI registered in Melle during this summer to the daily maximum THI's registered in our study reserves in 2013 (**Fig. 1a**), we deduce that the summer of 2013 was almost as hot as that of 2003. This suggests that, currently, we can confidently extrapolate our summer research results to Belgian summers in general.

In the future, however, the occurrence of extreme weather conditions is expected to increase even further with global warming, as already discussed in **Chapter 1**. As such, it is possible that, in future summers, cattle in nature reserves would make even more use of summertime shelter, in comparison with the current study.

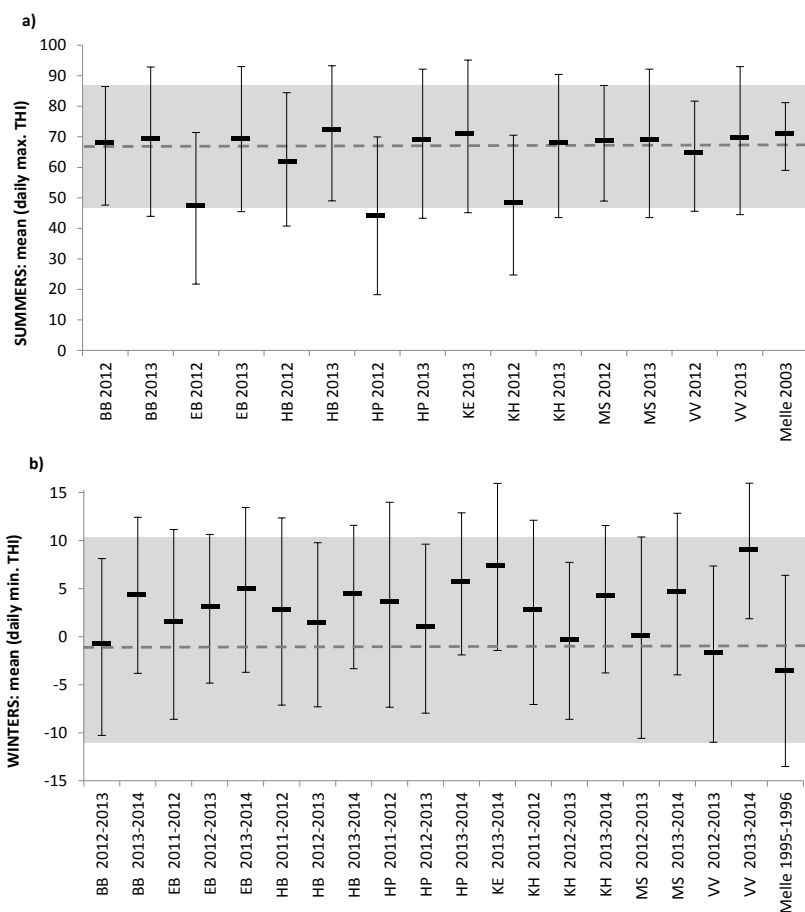


FIGURE 1. The daily maximum THI (a) and the daily minimum WCI (b) during the studies in the nature reserves, in comparison with the mean (dashed lines) and the 5% percentile and the 95% percentile (edges of the shaded areas) during the summers and winters of 1994-2005, based on daily data from another weather station located in Melle. From between 1994 and 2005, only daily data were available. Thus daily max. THI values were based on the daily max. air temperature and the daily mean air humidity. The daily min. WCI values were based on the daily min. air temperature and the daily average wind speed. The righthand bar of (a) shows the mean daily max. THI value during the warm summer of 2003, in Melle. The righthand bar of (b) shows the mean daily min. WCI value during the cold winter of 1995-1996, in Melle.

In the three winters (between October 1st and April 14th) of the study investigating cold conditions, the minimum WCI registered was about -17 °C (**Chapter 4**). In comparison, between October 1st and April 14th of the 11 years between 1994 and 2005, the daily minimum WCI, based on the daily minimum air temperature and daily average wind speed, in Melle reached below - 17 °C on 0.3% of the days. In addition, **Fig. 1b** shows that the mean daily minimum WCI registered during the winter of 2012-2013, in most reserves was close to the mean of the daily minimum WCI in the winters of 1994-2005 (in Melle), which -1.3 °C, based on the daily minimum air temperature and daily average wind speed. During the winter of 2012-2013, the 95% percentiles and the 5% percentiles for the nature reserves correspond relatively well with those for 1994 – 2005. The winter of 2011-2012 was (averaged over the four reserves studied in this winter) about 4°C warmer than the average winter between 1994 and 2005. The winter of 2013-2014, was even warmer in comparison with the average between 1994 and 2005.

However, even colder winters have occurred in Belgium 1994 and 2005. For example, the right-hand bar of Fig. 1b shows that, during the cold Belgian winter of 1995-1996, the mean daily minimum WCI was -3.6 °C, and the corresponding 5% percentile was -13.5 °C. Thus, the winter weather conditions in which we studied the effect of cold on the sheltering behaviour of cattle in nature reserves, were relatively warm in comparison with the coldest of Belgian winters of 10 to 20 years ago. In our study, very cold conditions were thus less frequent and potentially shorter than before.

In **Chapter 1** we already discussed that, in Europe and other high-latitude areas, global warming is expected to decrease incidence of very low temperatures in the future. On the other hand, there is no clear-cut ongoing or predicted change in the frequency or intensity of storm events, which can also contribute to the risk of thermal discomfort, and thus the motivation of cattle to use shelter in cold winter conditions. As such, there is insufficient scientific basis to hypothesise whether cattle in nature reserves would make more or less use of wintertime shelter in the future, in comparison with the current study.

A second limitation of the current study is that the effect of precipitation on cattle sheltering behaviour was not investigated in the current research. Rain and snow may have a substantial impact on thermal comfort, as discussed in **Chapter 1**, and thus sheltering behaviour (Vandenheede et al., 1994; Graunke et al., 2011). In our study, measurements of precipitation intensity were made, but they were of questionable reliability, due to unforeseen logistical issues.

A third limitation lies in the relatively low number of reserves in the study. Behavioral data were obtained from one animal per reserve, limiting the dimension of the study to eight independent experimental units, i.e. eight animals. Within each of these experimental units, a repeated measures design was used, given that animal locations were registered every 30 minutes, on many subsequent days.

Naturally, the statistical analysis of the use of open area (binomially distributed) was adjusted to this experimental design, by using mixed models (more specifically, mixed model logistic regressions), so that random effects could correct for the effect of repeated measurements per day, nested within the experimental unit, i.e. individual animal or reserve. The random time factor accounted for the fact that different observations (animal locations) are more dependent on each other when taken in close succession, and become less dependent on each other as they are further separated in time. This was modelled by an autoregressive covariance structure. Nesting this time factor within the individual animal, accounted for the fact that different observations (locations) of the same individual are not independent of each other, while they are independent of observations (locations) of the other seven animals, in the other seven reserves. This was judged necessary because of many potential differences between the experimental units, that would otherwise constitute potential confounding factors. Such confounding factors could be associated with the animal that was followed as well as the reserve in which it resided.

First, the heat and cold stress susceptibility of the monitored cattle may have differed because of differences in breed, colour and depth of the coat, body volume and body condition, health status and degree of acclimatisation (see also paragraph 3 of Chapter 1). Secondly, the cattle's proneness to use open area, natural shelter and the artificial shelter could have been influenced by several reserve specific factors. The effect of the amount of natural shelter and the spatial distribution of shelter were taken into account as fixed effects in the logistic models. But, as also discussed in paragraph 4 of Chapter 3 and paragraph 4 of Chapter 4, the cattle's terrain use could also have been influenced by the location of water and feed sources and the presence and location of physical barriers to animal movement, and other unknown factors. Naturally, it is impossible to analyse the effect of all these factors. But this is exactly the reason for the use of random factors in mixed models, which we gratefully used to analyse the effect of climatic conditions on the use of open area.

Despite the many differences between the eight experimental units, our research did demonstrate one same, robust trend across them, i.e. that increasing heat load in summer and decreasing apparent temperatures below 0 °C made cattle avoid open area and increase their use of the available (natural or artificial) shelter. Therefore, we are rather confident that the same conclusion would have emerged when we would have studied other or more reserves grazed (year-round) by adult cattle from adult and assumingly healthy individuals of the studied breeds.

On the other hand, the results of our study on adult and assumingly healthy individuals of the studied breeds, cannot simply be generalised to 'cattle in general', given that heat and cold stress susceptibility can differ substantially according to cattle condition, breed and age (and other individual characteristics: **Chapter 1**). Juvenile animals were not studied, but it is known that their 'lower critical temperature' is higher than for adults (**Chapter 1**), so possibly they seek shelter at higher apparent temperatures already, in comparison with the adult cattle we studied. In addition, cattle in suboptimal health condition may have different needs for shelter - in terms of thresholds and preferences for shelter location - than healthy cattle.

Another limitation is posed by the specific breeds of cattle that were used in this study, which might or might not be characterized by a different cold or heat stress susceptibility. Most of the cattle we studied belonged to the Galloway breed (in four out of eight reserves) or the related Aberdeen-Angus breed (in four out of eight reserves). Both are beef breeds developed in Scotland in the nineteenth century (www.livestockconservancy.org). They are early maturing and have a lower energy demand than later maturing beef breeds (Wallis de Vries 1994). Consequently, the Galloway and Aberdeen-Angus accumulate fat easily, even on low-energy forage in a cold, wet climate, which gives them a low cold stress susceptibility. On the other hand, the Galloway and Aberdeen-Angus might be less tolerant to heat load than other cattle breeds, due to their thick hair coat, which may hinder the transfer of excessive heat to the environment (Finch et al. 1984; Yeates et al. 1955). A thinner and sleek coat has indeed been found to favour heat dissipation (Turner and Schleger 1960). One of the other breeds of cattle in this study was the 'Oost-Vlaams Roodbont' or 'Oost-Vlaams Wit-Rood' breed. This is a traditional Flemish dual-purpose breed, similar to the Dutch 'Maas-Rijn-IJssel' breed that is classified as late-maturing by Wallis de Vries (1994), and has a higher energy demand than the Galloway and Aberdeen-Angus. Its coat is less thick than that of the Galloway and Aberdeen-Angus, and thus a higher cold susceptibility and lesser heat susceptibility could have been expected.

However, our study does not allow a proper comparison between breeds. First, none of the reserves were populated by different breeds. This implies that effects of breed on sheltering behaviour are always interwoven with the effects of the characteristics of the reserves, such as the location of drinking places and preferred grazing spots. Secondly, for a proper comparison between breeds, we had an insufficient number of replicates per breed, given that only two of our study reserves were grazed by Angus-Aberdeens, only one reserve was grazed by Heck cattle and only one reserve was grazed by 'Oost Vlaams Wit-Rood' cattle) per breed. On the other hand four of our study reserves were grazed by Galloways.

This is a consequence of the fact that in Flanders (the Northern part of Belgium), year-round grazing is mostly realised by robust, foreign cattle breeds, such as the Galloway and Aberdeen-Angus and, less frequently, Scottish Highland cattle. The two largest nature conservation organisations in Belgium performing year-round grazing management with cattle are Natuurpunt vzw and the Government of Flanders' Agency for Nature and Forest (ANB). For year-round grazing, Natuurpunt vzw uses their own cattle, most being Galloway or Aberdeen-Angus, and some 'Oost Vlaams Wit-Rood' (http://www.natuurpunt.be/nl/natuurbehoud/natuurbeheer/begrazing_1920.aspx). ANB also uses Scottish Highland cattle, e.g. in 'De Panne' (Neels, 2002; Meert, 2002; Cosyns, 2013) and 'De Zwinduinien' (<http://www.natuurenbos.be/~media/Files/Domeinen/West-Vlaanderen/zwinduinien/zwinduinien%20EN.pdf>).

Most likely, these robust, Scottish breeds are often used because of their (presumed) better adaptation to harsh climatic conditions and low qualitative feed availability. In an inquiry of 130 managers of Flemish grazed nature reserves (Tilkin, 2014), most managers stated to prefer robust breeds: 53% of the respondents strongly preferred a breed that requires no shelter and 50% strongly preferred a breed that requires no supplementary feeding in winter. Most managers believed that these characteristics were better represented in foreign versus local breeds (43% of the respondents rather agreed and 20% completely agreed with this statement). Tilkin (2014) also evaluated the theoretical suitability of different species and breeds of large grazers for the management of typical western European nature reserves, based on a literature study into their characteristics relevant for grazing management, such as 'winter hardiness', disease susceptibility, habitat and feed preferences, manageability and aggressiveness. In this evaluation, local breeds did not score worse than foreign breeds (such as Konik horses or Galloway, Scottish Highland or Limousin cattle), in general and for the specific characteristics of 'need for feed supplementation' and 'winter hardiness'.

However, to our knowledge, the susceptibility to cold and heat stress of these robust, foreign breeds versus other (e.g. local) breeds has not been investigated scientifically with field experiments. Future large-scale field studies into this question would thus be useful, and could e.g. investigate if cattle of different breeds (e.g. foreign versus local breeds) start to seek shelter at different cold stress levels in winter or different heat load levels in summer.

Up to now, heat and cold stress thresholds (in terms of apparent temperatures or other climatic indices) have only been validated for dairy and beef cattle in farming settings. For cattle breeds typically used in year-round grazing management of nature reserves (such as the Galloway or the Scottish Highlander) no thresholds have been developed yet, that could be used to assess the need for shelter based on climatic measurements.

However, using sheltering behaviour as the only response variable, as we did in the current research, does not suffice to decide whether cattle welfare is impaired in absence of shelter. Such a conclusion would require an assessment of 'welfare' or 'suffering' in absence of shelter. This was beyond the scope of our study and would require a very different methodology. Additional indications of heat and cold stress - such as Panting Scores, body temperatures or other physiological measurements - would have to be used to evaluate welfare in the presence versus absence of shelter. Other physiological indicators of discomfort or stress, such as systemic glucocorticoid levels as a measure of HPA axis activity (Mormède et al., 2007), can also be used. However, the collection of samples might pose a challenge because cattle grazing free in nature reserves might be difficult to approach.

On the other hand, if already a merely observational study like ours would have found no difference in sheltering behaviour between different climatic conditions, this would have been an indication that the Belgian climatic conditions are not severe enough to initiate thermal discomfort in the studied cattle in nature reserves. Yet, the finding that the cattle did seek shelter from heat and cold, suggests that they used this strategy to avoid existing thermal discomfort. Further research would be useful to assess whether seeking natural and/or artificial shelter indeed successfully reduced behavioural indications and/or physiological consequences of thermal discomfort. Until conclusive scientific evidence for such a more objectively evaluated 'need' for shelter becomes available, the 'precautionary principle' - the view that the lack of full scientific certainty cannot be used as a reason for postponing measures to prevent potential negative effects on animal welfare (Croney and Millman, 2007) - can be used to argue in favour of providing at least some form of additional shelter in open reserves containing little natural shelter.

1.2. MAIN FINDINGS

Despite the above limitations, our research demonstrated that, even in a temperate region such as Belgium, cattle in the nature reserves increasingly avoid open area and thus increase their use of shelter with increasing heat load in summer and with the apparent temperature (WCI) decreasing below 0°C in winter. The strength of these responses differed between nature reserves and was associated with the amount and spatial distribution of natural shelter in the reserve.

Artificial shelter, with one open side and three closed walls, generally offered better protection against "wind chill" than natural shelter, as indicated by our micro-climatological measurements. Nevertheless, the cattle in almost all reserves used natural rather than artificial shelter as protection against the cold, with the exception of one reserve where natural shelter was sparse and one reserve where the cattle exceptionally received additional feed inside the artificial shelter. Also in summer, artificial shelter was rarely used and cattle rather used natural shelter during high heat load, as long as the latter was sufficiently available.

Only in the one reserve where little and non-dense natural shelter was present, cattle rather used artificial than natural shelter during high heat load. Sufficiently dense natural shelter blocked solar radiation as well as the artificial shelters. Additionally it allowed more evaporative cooling than the artificial shelters. Thus, in Belgian climate, additional artificial shelter (of the type used in this study) does not seem to have a lot of added value for the thermal comfort of the (adult and assumedly healthy) cattle of the studied breeds as long as there is adequate natural shelter.

1.3. IMPLICATIONS

In all studied reserves, the use of shelter (natural or artificial, depending on the availability) increased with increasing heat load in summer and cold in winter. This suggests that the cattle aimed to avoid existing thermal discomfort in open area. Only when natural shelter was sparse, the artificial shelter was rather used as protection against cold and heat. Consequently, in open reserves where natural shelter is sparse, the 'precautionary principle' can be used to argue in favour of providing at least some form of additional shelter.

Our study indicates that, when managers of reserves containing little natural shelter indeed decide to provide extra shelter, cattle would prefer extra trees and shrubs. On the other hand, one could also argue that - from the viewpoint of animal - it would be even better to simply always provide the grazers in the nature reserves with the choice between artificial shelter(s) and natural shelter. This argument is also strengthened by the finding that artificial shelter was more effective than natural shelter in cold conditions. In addition the completely closed roof of an artificial shelter can be assumed to provide better protection from precipitation (e.g. rain) than tree cover.

When choosing the type (natural and/or artificial) as well the location of shelter, conflicts may arise between animal welfare concerns on the one hand and vegetation and landscape management objectives on the other hand. For example, an argument against additional natural shelter in open reserves, is the fact that in such reserves the management often precisely aims at the preservation of an open landscape and plant species typical of such landscapes, e.g. by grazing management. In such cases, artificial shelter may be a better option. On the other hand, an artificial shelter is considered by some as a disturbance of the (semi-natural) landscape. When choosing a location for additional shelter, another concern is the prevention of excessive trampling and excessive manure deposition in and around the shelter. 'Point source pollution' in biologically valuable vegetation patches aimed to be preserved, is clearly unwanted, especially in vegetation types specific for nutrient-poor soils which are already rare in areas (formerly) dominated by agricultural activities, as in large parts of Belgium. The shelter location should thus be carefully chosen, in order to meet the demands of both the grazers and the reserve managers. Presumably grazers will make use of the shelter more easily when it is not segregated from the rest of the terrain by physical barriers, such as steep slopes, impenetrable vegetation, or (hard to cross) water courses and when it is located close to frequently used locations, e.g. locations that are preferred for feeding, drinking or resting.

Yet, the demands and concerns of grazers and reserve managers need not always oppose each other, at all. As also pointed out by Bailey (2004), strategic placement of 'resources' valuable to grazers (e.g. shelter) can also be used to 'lure' them to underutilized parts of the reserve, e.g. in order to graze over-abundant plant species and to 'lure them away' from over-grazed vegetation types.

2. SHADE AS PROTECTION AGAINST HEAT LOAD FOR DAIRY AND BEEF COWS ON PASTURE

The second part of this doctoral study focused on cattle in the context of livestock production: Holstein dairy and Belgian Blue beef cattle on pasture.

2.1. LIMITATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

Although this part of the doctoral research comprised an experimental study, in which a better control of confounding factors is possible in comparison with observational studies, it still is subject to several limitations, of which we discuss the most relevant ones in the subsequent paragraph.

The first limitation is inherent to the statistical models that were chosen to analyse the data that were collected over the course of three summers. Among the three years, the composition of the studied cattle herds changed, due to which some cows were studied in one year only, whereas other cows were studied in two or three years. In all analyses, a random factor was included to correct for repeated measurements (per individual) within the same experimental year. Observations of the same individual in different years, however, were necessarily treated as independent. From a biological point of view, this constitutes a limitation, because an individual's thermal tolerance is influenced by certain unchangeable animal characteristics (see **Chapter 1, paragraph 3**) that exert the same influence in every experimental year. An ideal statistical analysis would thus correct for the possibility that an individual's response to a certain degree of heat load is not independent of the same individual's response in another year. However, this was impossible due to computational limitations of the analysis software.

Secondly, during the three experimental summers the climatic conditions might or might not have been representative (enough) for the range of summer climatic conditions that are known to occur in Belgium. The daily maximum THI registered in our study (located in Melle) exceeded 79 on 1% of the days between 10/6/2011 and 19/09/2013. The maximum daily maximum THI registered in our study was 83. This is largely consistent with the summertime measurements of another weather station, located in Melle, between February 1994 and May 2005. In **Chapter 1** we demonstrated that the daily maximum THI calculated on the basis of the measurements of this weather station exceeded 79 on 3% of all days between February 1994 and May 2005. The daily maximum THI exceeded 85 on 26 out of 5057 days, thus 0.5% of the days, only. In addition, **Fig. 2** shows that the highest daily maximum HLI's registered in 2013 approached the highest peaks in the 95%

percentile of the daily maximum THI registered during the summer months of 1994-2005, by another weather station located in Melle. Therefore, we conclude that the climatic conditions in which we studied the effect of heat load on the dairy and beef cows and calves, were fairly representative for Belgian summers, and thus (currently) we can extrapolate our research results to Belgian summers in general. However, it must be mentioned here again, that the importance of summertime shelter for cattle on pasture in temperate, midlatitudinal regions can be expected to increase even further in the future, due to ongoing global warming (see **Chapter 1**).

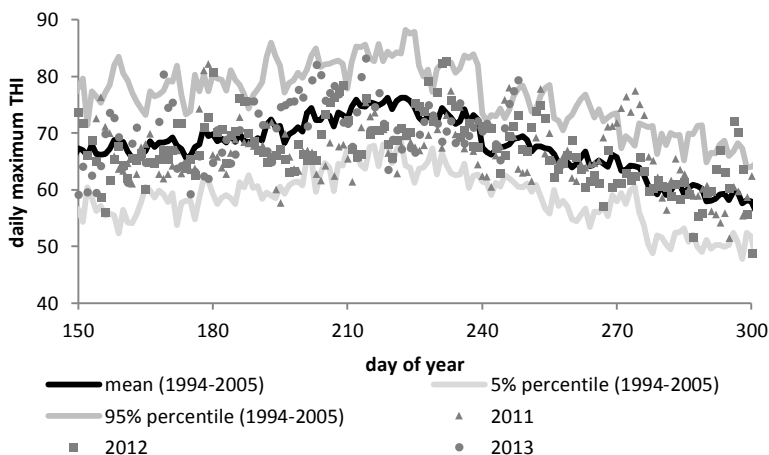


FIGURE 2. The daily maximum THI per day of the summer months (29 May = day 150 - 26 October = day 300) of the years in which our study took place (2011, 2012, and 2013), in comparison with the mean, the 5% percentile and the 95% percentile of the daily maximum THI registered during the summer months of 1994-2005, by another weather station located in Melle.

Although the study includes data from three summers which seem quite representative for Belgian summers in general, it was carried out at one specific farm only. In addition, the experimental setup with shade provided by a combination of young trees and shade cloth, – to our knowledge - was unique and not comparable with general practice in the (Belgian) cattle industry. We spanned shade cloth between the young trees, to make sure sufficient shade was provided per shade area (for 50 to 60 cows, half of the maximum number of cows in the ILVO’s dairy herd), given that the young trees did not yet provide very much shade at the start of the experiment. It would be interesting to check if similar research on several commercial farms (with shade provided by either trees only or shade cloth only) would confirm the magnitude of heat load and shade effects on cattle comfort and productivity found in the current study.

To our knowledge, there is only one field study, with natural shade, i.e. trees, hedges, and shrubs, on several commercial farms located in a temperate climatic region. That study was recently carried out by Schütz et al. (2014) in New Zealand. The authors investigated the effect of different amounts of shade on the use of shade, respiration rate (RR) and Panting Score (PS) using observation methods similar to ours (**Chapter 5**). They reported that, on average, 27% of animals in the herd used shade during observation days (10.00h-15.30h) during which the HLI ranged between 70 and 91, but for every 1 m² increase in shade availability per cow, the shade use increased by 3.1%. Although our doctoral research did not study the effect of different amounts of shade, similarly to Schütz et al. (2014), we also found that in 2013 (when cows were used to the shade structure and water was available in the shaded part of the pasture), an increase in HLI from 70 to 90 corresponded with an increase in shade use from about 5% to 50% for dairy cows and from about 10% to 40% for beef cows. Moreover, Schütz et al. (2014) found that access to shade lowered the proportion of the herd that had a high Panting Score (PS ≥2), similarly to our results. Schütz et al. (2014) found that shade reduced PS ≥2 from 6% to 2% for dairy cows, whereas we found a reduction from 13% to 0% for dairy cows and a reduction from 5% to 0% for beef cows (**Chapter 5**).

To our knowledge, no other field studies investigated the effect of heat load and shade on metabolism, milk yield and milk composition of dairy cows, specifically on commercial farms with pastured cows in temperate summers. Hammami et al. (2013) have used a large dataset from many commercial dairy farms in the Grand Duchy of Luxembourg to investigate the effect of heat load on milk yield and milk composition. However, the data did not specifically concern cows on pasture, i.e. the housing system was unspecified. In our study, a smaller but still fairly large dataset was used to demonstrate that shade can prevent the decrease in milk yield that heat load can cause in absence of shade. However, we did not demonstrate an unambiguously heat stress remediating effect of shade on the milk composition, probably due to the relatively low number of samples from periods of high heat load (**Chapter 6**). More specific and large scale research would thus be useful to investigate if and to what degree shade can reduce the negative effect of high heat load on milk composition, specifically for cows on pasture in temperate area. In addition, more research would be useful to investigate potential disadvantages of shade, aspects of optimal shade area design and size, e.g. to prevent excessive trampling and manure build-up, a risk factor for mastitis (Gregory, 1995), and the resulting cost of an adequate shade area, in order to allow a cost-benefit analysis for provision of shade on pasture in temperate climate.

2.2. MAIN FINDINGS

Despite the above limitations, we did demonstrate that heat load affected the voluntary use of shade on pasture and several indications of thermal discomfort differed between cattle with and without access to shade and between inside and outside shade (**Chapter 5 and 6**). For Holstein dairy cows and Belgian Blue beef cows and their suckling calves, increasing heat load was associated with an increase in the voluntary use of shade and an increase in visual indicators of thermal discomfort, i.e. an increase in respiration rate and Panting Score. The increase in respiration rate and the prevalence of highly elevated respiration rates and Panting Scores were reduced by using shade (**Chapter 5**). Thus, shade improved cattle's thermal comfort in warm conditions.

In the same experimental setting, for Holstein dairy cows we also aimed to estimate the effect of temperate summer heat load and shade on body temperature and responses with a more direct economical relevance (**Chapter 6**), i.e. energy metabolism, milk yield and milk composition. Increasing heat load was associated with an increase in body temperature, indications of changes in energy metabolism (in general, and indications of increased fat and protein catabolism), signs of hyperventilation, decreasing milk yield and a decrease in the milk lactose, protein and fat content. The increase in body temperature was tempered by having access to shade. The indications of hyperventilation and the decrease in milk yield were inhibited by having access to shade. A rough simulation of the resulting potential loss in yearly milk yield (quantity only) indicated that the lack of shade would cause an average loss of 13 litres of milk per cow, in a summer like that of 2013, with 31 days with a daily average HLI > 70.

2.3. IMPLICATIONS

In conclusion, our study suggests that the provision of shade on pasture improves thermal comfort of dairy cows, adult beef cows and calves and productivity of dairy cows in hot conditions that are not exceptional during summertime in temperate climatic regions, such as Belgium.

This implies that it is recommendable, in the cattle's interest as well as in the cattle keepers' interest, to provide cattle that are kept on pasture in Belgian summers with shade. Alternatively, cattle can be kept indoors during heat stress conditions, to avert the (financial and labour) investment in an adequate shade construction or trees on pasture.

On the one hand, this would suit with the trend towards decreased pasturing in the dairy sector. In the Netherlands, in 2002, a survey among 500 dairy farmers indicated that dairy cows had unrestricted access to pasture on about 50% of the farms (Van den Pol-van Dasselaar, 2002). The authors expected grazing to decrease in most European countries, and in 2005 they indeed reported that in the UK, Denmark and Germany – the only three countries from which data were reported - grazing had decreased (Van den Pol-van Dasselaar, 2005). Indoor housing is often preferred by cattle keepers, because it allows a more stable feeding regime, a more controlled environment and less labour investment, and thus higher economic gains. On the other hand, consumers indicate that they value pasturing as an important (for cattle welfare, nature and landscape) aspect of dairy farming (Van den Pol-van Dasselaar, 2002). This demand has been picked up by the dairy retail sector (in Belgium and the Netherlands, e.g. Milcobel, Friesland-Campina), which currently (2014) rewards dairy producers for pasturing by paying premiums for milk from pastured cows.

In the beef cattle sector summer pasturing is still more common than in the dairy sector, especially for (suckler) cows and calves. Beef cows, unlike dairy cows, do not need to come inside to be milked and pasture (grass) is regarded as a suitable, economic and sustainable feed source for beef cattle (Dillon et al., 2005; Peyraud et al., 2010). For beef cattle keepers, it would thus rather constitute a deviation from common practice to keep their cows and calves indoor during summer. In addition, keeping cattle inside will only benefit their comfort if the cattle houses effectively provides a cooler environment than the outdoors, by means of adequate ventilation and/or other cooling mechanisms.

Moreover, from the perspective of the cow, it might be better to provide the free choice between inside and outside. In such cases, however, it is recommendable to provide shade on pasture in order to ensure thermal comfort. For additional points of attention regarding shade on pasture (e.g. matters of design, materials and space allowance) we refer to **Chapter 7**, paragraph 4.4.

Even when adequate shade is available on pasture, we still advise to monitor the 'ad hoc' heat stress risk and prevalence under hot conditions. A practical guide for this purpose is provided in the next paragraph.

3. PRACTICAL ADVICE FOR THE PREVENTION AND ASSESSMENT OF HEAT STRESS IN CATTLE

Given that, until recently, cattle heat stress has long been an ‘underexposed’ phenomenon in temperate regions (like Belgium), there is a lack of practical but scientifically validated guides for prevention and especially for detection of heat stress in cattle. This chapter aims to fulfil the need of such a guide, based on the results described in the previous chapters. The current doctoral research has validated (1) a method to assess the risk of heat stress in cattle, based on the measurement of climatic conditions and (2) a complementary animal-based method to assess the actual occurrence and severity of thermal discomfort due to heat load in Holstein dairy cows and Belgian Blue beef cattle. When the weather forecast predicts warm conditions, cattle keepers should take heat stress prevention measures, i.e. prepare to keep their cattle inside or make sure sufficient shelter (e.g. shade) is available on pasture. In addition to the prevention measures, under hot conditions it is advisable to monitor the ‘ad hoc’ heat stress risk and prevalence.

First, a risk assessment can be made, based on the instantaneous value of a climatic heat stress index, such as the Heat Load Index (HLI). The HLI incorporates the effects of four climatic variables that determine thermal comfort. It includes the effect of air humidity and wind speed, and it accounts for both air temperature (T_a , in °C) and the intensity of solar radiation (Rad , in W/m^2), via the black globe temperature (T_{bg} , in °C). T_{bg} can either be measured by a black globe thermometer or either be calculated as: $T_{bg} = 1.33 * T_a - 2.65 * T_a^{0.5} + 3.21 * \log(Rad+1) + 3.5$. As cattle keepers seldom have measurements of T_{bg} or Rad at hand at the farm, they can assess the value of T_{bg} by means of a chart (Table 1) and knowing that, in Belgian summers, Rad is maximally about $100 W/m^2$ and during the day about $40-50 W/m^2$ on average. $Rad \leq 2 W/m^2$ occurs almost exclusively at night. Subsequently, HLI can be calculated based on T_{bg} , relative humidity (RH, in %) and wind speed (WS, in m/sec): $HLI = 10.66 + 0.28 + 1.3 * RH * T_{bg} - WS$ when $T_{bg} \leq 25$ °C and $HLI = 8.62 + 0.38 * RV + 1.55 * ZBT - 0.5 * WS + e^{(2.4 - WS)}$, when $ZBT > 25$ °C.

Alternatively, when exact measurements of RH and WS are lacking, a second chart (Table 2) can be used to estimate HLI at a fixed humidity of 70% (average during Belgian summer) and with increasing black globe temperature and decreasing wind speed. The colour gradient in the table indicates how strongly these conditions affect the thermal comfort of cattle, based on Gaughan et al. (2008) and our own research on Holstein dairy cows and Belgian Blue beef cows and calves.

TABLE 1. Chart for determination of the black globe temperature (T_{bg}, in °C) based on air temperature (T_a, in °C) and the intensity of solar radiation (Rad, in W/m²).

RAD in W/m ²	T _a in °C																				
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
2	20	21	22	23	24	25	26	27	28	29	30	32	33	34	35	36	37	38	39	40	41
10	22	23	24	25	26	27	28	29	30	31	32	33	34	36	37	38	39	40	41	42	43
20	22	24	25	26	27	28	29	30	31	32	33	34	35	36	38	39	40	41	42	43	44
40	23	24	26	27	28	29	30	31	32	33	34	35	36	37	38	40	41	42	43	44	45
60	24	25	26	27	28	29	30	31	32	34	35	36	37	38	39	40	41	42	43	45	46
80	24	25	26	28	29	30	31	32	33	34	35	36	37	38	39	40	42	43	44	45	46
100	25	26	27	28	29	30	31	32	33	34	35	36	38	39	40	41	42	43	44	45	46

TABLE 2. Chart for determination of the Heat Load Index (HLI) based on the black globe temperature (T_{bg}, in °C) and the wind speed (WS), at a fixed value of 70% for the relative air humidity (RH).

WS		T _{bg} in °C																				
km/h	m/sec	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
54	15	41	43	44	45	46	48	68	70	71	73	74	76	77	79	80	82	84	85	87	88	90
36	10	46	48	49	50	51	53	71	72	74	75	77	78	80	81	83	84	86	88	89	91	92
18	5	51	53	54	55	56	58	73	75	76	78	79	81	82	84	85	87	89	90	92	93	95
14	4	52	54	55	56	57	59	74	75	77	78	80	81	83	85	86	88	89	91	92	94	95
11	3	53	55	56	57	58	60	75	76	78	79	81	82	84	85	87	89	90	92	93	95	96
7	2	54	56	57	58	59	61	76	78	79	81	82	84	85	87	88	90	92	93	95	96	98
4	1	55	57	58	59	60	62	79	81	82	84	85	87	88	90	91	93	95	96	98	99	101
0	0	56	58	59	60	61	63	87	88	90	91	93	94	96	97	99	100	102	104	105	107	108
Correspondent heat stress risk (Gaughan et al., 2008)																						
HLI < 70	Thermoneutral conditions → no heat stress	70 ≤ HLI < 77	Warm conditions → mild heat stress				77 ≤ HLI < 86	Hot conditions → moderate heat stress				HLI ≥ 86	Very hot conditions → severe heat stress									

In Table 2, the lightest shading (HLI < 70) indicates thermoneutral conditions, in which we indeed observed very little shade use (< 10% probability of shade use, **Chapter 5**). At black globe temperatures below 25°C, and thus HLI values always below 70 (Table 2), the average respiration rate (RR) remained below 90 BPM and the average Panting Score (PS) remained below 1 for the three cattle types, even when they had no access to shade (**Chapter 5**).

The second lightest shading (HLI between 70 and 77) indicates warm conditions and thus mild heat stress. In these conditions we observed a substantial increase in the use of shade ($\geq 10\%$ probability of shade use, **Chapter 5**) when cattle were well acquainted with the shading structure and a drinking trough was present in the shaded area (as in 2013, **Chapter 5**). When Tbg was above 30 °C, and thus HLI > 70 (unless wind speed > 20 m/s, which is very unlikely), shade improved the thermal comfort of the three cattle types, as indicated by a higher proportion of normal RR and PS (**Chapter 5**). When the daily average HLI increased from 70 to 77, we observed a milk yield decline of 0.3 l/day/cow (after a lag period of two days) for an average Holstein dairy cow without access to shade (**Chapter 6**), whereas the milk yield of dairy cows with access to shade was not significantly influenced.

The second darkest shading (HLI between 77 and 85, and thus Tbg > 25°C) indicates hot conditions and thus moderate heat stress. In these conditions we observed a substantial increase in the use of shade ($\geq 10\%$ probability of shade use, **Chapter 5**), even when cattle were not very well acquainted (less than three months) with the shading structure and no water was available in the shaded area (as in 2011, **Chapter 5**).

The darkest shading (HLI ≥ 86) indicates very hot conditions and thus severe heat stress. Over the course of the three years of the current study, HLI reached the threshold of 86 on 17 out of 832 days (thus, 2% of the days). On four of these 17 days, the HLI was over 86 during more than six hours. Five times the HLI was over 86 on two consecutive days (Table 3).

TABLE 3. Occurrence and duration of ‘severe heat stress events’ – defined as HLI ≥ 86 - in the entire study period (10/6/11 till 19/9/2013, approximately 20 000 hours).

2011		2012		2013	
date	duration (h:m)	date	duration (h:m)	date	duration (h:m)
27/June	2:30	20/June	0:15	18/June	7:45
28/June	9:15	28/June	0:15	22/July	9:30
3/Sept	1:00	26/July	1:00	23/ July	4:00
29/Sept	0:30	18/Aug	2:45	1/Aug	0:45
1/Oct	0:30	19/Aug	5:00	2/Aug	8:30
2/Oct	0:15			5/Aug	1:15

Thus, we observed relatively few ‘severe heat stress events’ with HLI \geq 86. On the other hand, when such high heat loads occur, they do exert substantial negative effects on cattle comfort and productivity. Our research demonstrated that an instantaneous HLI value \geq 86 caused substantial discomfort, as indicated by high respiration rates and Panting Scores (**Chapter 5**). A daily average HLI value \geq 86 also decreased the milk yield for dairy cows without access to shade, as demonstrated in **Chapter 6**. We demonstrated a decrease by 1 l/day/cow (from 25.1 l/day to 24.1 l/day per cow), after a lag period of two days, as the daily average HLI increased from 65 to 85.

As discussed in Chapter 1, the duration of exposure to excessive heat load will greatly influence cattle’s responses, but this effect is not easily quantified. Climatic conditions in the ‘severe heat stress category’ will require less time to elicit substantial negative effects in cattle, whereas conditions in the ‘mild heat stress’ category must have a longer duration to attain the same effect. Gaughan et al. (2008) have touched upon this issue by developing the ‘Accumulated Heat Load’ (AHL) model. The AHL is a measure of the animal’s heat load balance, determined by the duration of exposure above the (singular) ‘heat stress’ threshold HLI for the relevant cattle type. This threshold is HLI = 77 for a healthy, unshaded Angus steer, but correction to this value can be made in function of e.g. breed, colour, availability of shade and drinking water. This AHL-measure proved to predict the tympanic temperature of heat exposed cattle better than the average HLI over the previous 24h (Gaughan et al., 2008). Further research would thus be useful to identify and further validate a threshold HLI specifically for Holstein dairy cattle and Belgian Blue beef cattle, in the Belgian or more general a temperate climate, to use as a basis for the AHL model.

Until such specific thresholds have been validated, and in practice, where exact measurements of T_{bg}, RH and WS are often lacking, HLI charts (such as Table 2) can be used to roughly assess the need for protection against heat stress.

However, this method does not take individual differences in heat stress susceptibility (as also discussed in **Chapter 1**) into account. Therefore, it is advisable to additionally observe the cattle themselves to assess thermal (dis)comfort. As also discussed in **Chapter 1**, behavioural indications of thermal discomfort include:

- decreased activity, but more standing and less lying
- decreased feed intake and less ruminating
- more drinking and grouping around drinking troughs
- seeking shade
- in absence of shade: grouping to try to stand in each other's shade
- panting and drooling

In addition, thermal discomfort has often been assessed based on RR in the past. However, the PS is a more complete assessment method, as it includes more signs of discomfort than increased respiration rate only. Other signs that are included are the 'deepness' versus 'shallowness' of breathing, drooling, breathing with open mouth and extending the neck to 'gasp for air'. The PS is faster and more easily scored from a safe distance from the cattle, without needing any measurement instruments. Therefore, it is a suitable and convenient method for application on (even large) farms. The PS can be scored on the five-point ordinal scale of Gaughan et al. (2008) or on the Visual Analogue Scale that we used in the present study (**Chapter 5**), and a photo-guide is available via <http://www.mla.com.au/files/02daccf7-a8ef-4c2e-9288-9d5900e40fa9/heatload-in-feedlot-cattle.pdf>. This guide also mentions that the transition from PS 2.5 to PS 4.5 can take place in less than two hours under severe heat stress conditions. When $PS \geq 3.5$ for $\geq 10\%$ of the herd, the mortality risk in the herd increases. Consequently, it is highly recommendable to take heat stress prevention measures from $PS \geq 2$ on.

Fatigue by driving and chasing cattle should be avoided during hot conditions. Previous research as well as the current study demonstrated that offering shade is an effective heat stress prevention measure. Other heat stress prevention measures include sprinklers or showers. In any case, sufficient clean and fresh water should be provided at all times, preferably inside the shade.

As a final, additional prevention measure, the feeding regime can be adapted to minimize the consequences of thermal discomfort. Although it was beyond the scope of this doctoral research project to investigate such nutritional strategies, we do provide a short overview of cattle heat stress literature describing such heat stress feeding strategies.

The time of feeding and feed composition can both be adapted to maximise energy intake (Brosh et al., 1998; Davis et al. 2003), while limiting ruminal heat production (West 2003). On the other hand, sufficient roughage in the ration should be maintained to avoid rumen acidosis (Stone, 2004). The ration can be adjusted for mineral-losses due to salivation, sweating and increased urinary excretion balance (West 2003). Yeast culture supplements are thought to enhance ruminal digestion and thus reduce negative effects of heat stress on energy metabolism. However, Schwartz et al. (2009) found that feeding yeast cultures did not prevent the effects of heat stress on dry matter intake and negative energy balance, despite cows fed with yeast culture having slightly reduced rectal temperatures. Furthermore, supplying feed with niacin (vitamin B3) can increase peripheral vasodilation and increase sweating rates to dissipate more body heat during high heat load (Zimbelman, 2013).

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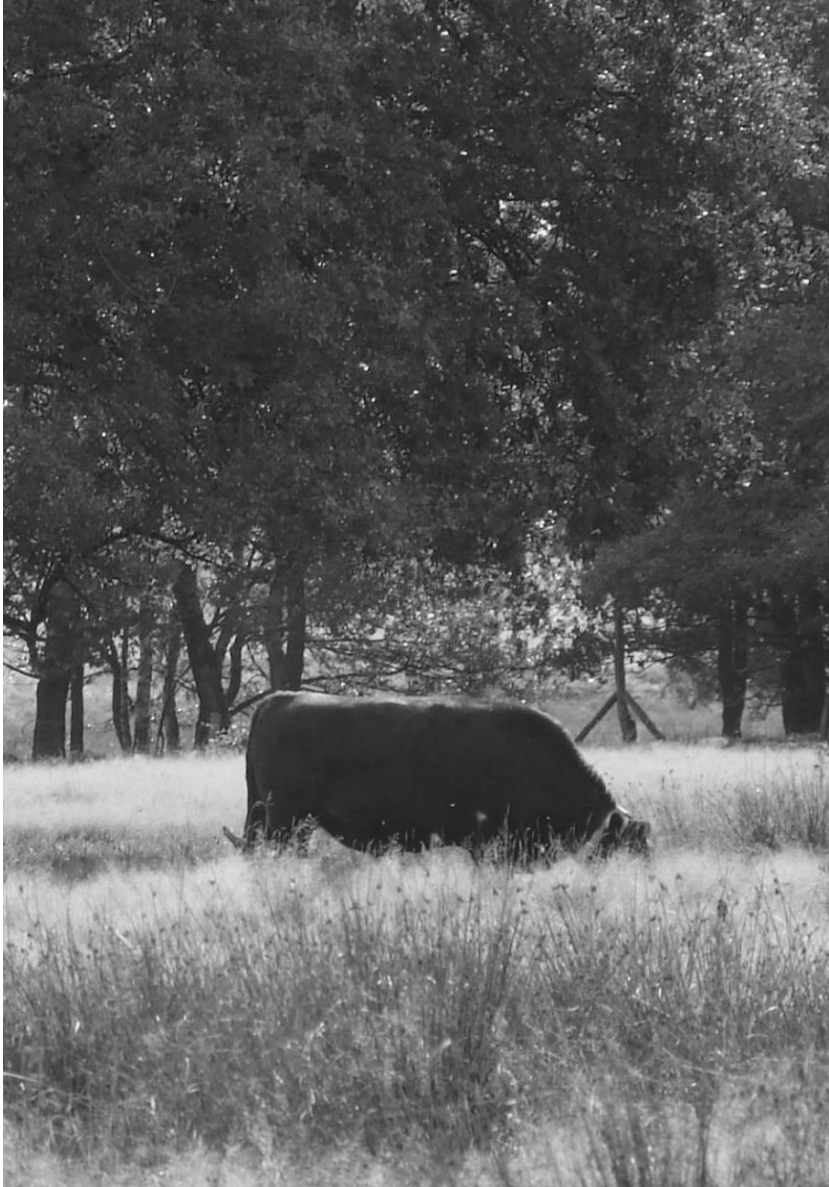
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CHAPTER 8

CONCLUSIONS



Our study on (adult and assumedly healthy) cattle grazing in nature reserves demonstrated that the use of shelter (natural or artificial, depending on a.o. the availability) increased (a) with increasing heat load in summer and (b) with the apparent temperature decreasing below 0°C in winter. In winter as well as summer, artificial shelter was rarely used and cattle rather used natural shelter as protection against cold and heat, as long as it was sufficiently available. Only in one reserve where little and non-dense natural shelter was present, cattle rather used artificial than natural shelter as protection against aversive weather conditions. In winter, artificial shelters with one open side and three closed walls, offered better protection against 'wind chill' than natural shelter. In summer, however, the higher wind speed in the natural shelter was beneficial, because it allowed more evaporative cooling, and thus natural shelter provided better thermal comfort than the artificial shelters. In conclusion, in Belgian climate, an additional artificial shelter of the type that we used (three out of four walls and the roof were closed) has little added value for the thermal comfort of (adult and assumedly healthy) cattle of the studied breeds, as long as there is sufficient natural shelter.

Our study on dairy and beef cattle on pasture in three Belgian summers demonstrated that shade improved the cattle's thermal comfort in warm conditions. For Holstein dairy cows and Belgian Blue beef cows and their suckling calves, increasing heat load was associated with increasing use of shade and increasing indicators of thermal discomfort. However, in hot conditions, the proportion of normal RR and PS was significantly higher for animals that used shade, whereas the proportion of strongly elevated RR and PS was generally lower for animals that used shade. In the same experiment, for Holstein dairy cows, increasing heat load was associated with a lesser increasing body temperature for cows with access to shade, as compared to cows without access to shade. For the dairy cows, increasing heat load was also associated with indications of changes in energy metabolism (in general + indications of increased fat and protein catabolism), indications of hyperventilation, decreasing milk yield and a decrease in the milk lactose, protein and fat content. Having access to shade prevented the indications of hyperventilation and the decrease in milk yield. Thus, our study has confirmed the added value of shade on pasture to improve thermal comfort of dairy cows, adult beef cows and calves during hot conditions in Belgium. In addition, we demonstrated a beneficial effect of shade on the productivity of dairy cows on pasture.

Furthermore, we validated two complementary methods to assess cattle heat stress in practice. When the weather forecast predicts warm conditions, heat stress prevention measures (e.g. provision of shade on pasture or keeping cattle inside) should be taken, but additionally, actual heat stress prevalence should be monitored ad hoc. For this purpose, a rough assessment of the heat stress risk can be based on the instantaneous value of a climatic heat stress index, such as the Heat Load Index (HLI). Additionally, an actual animal based-assessment of thermal discomfort can be made by observing the Panting Score.

SUMMARY

In a temperate climate, i.e. in Belgium, we investigated (1) how to detect thermal discomfort in outdoor kept cattle, (2) how frequently it occurs, (3) what and how severe the consequences are, and (4) how it can be prevented.

In the first part of this thesis, the focus was on cattle in year-round grazing management of nature reserves, and addressed the question whether the cattle would benefit from an artificial shelter in addition to the presence of natural shelter (vegetation) as protection against cold and heat. The sheltering behaviour of cattle was studied in eight Belgian nature reserves. During three winters and two summers the terrain use of one cow per herd was determined every 30 minutes, by means of a GPS collar. All animal locations ($n = 58\ 101$ for the summers, $n = 73\ 371$ for the winters) were plotted on digital maps of the reserves, to determine whether they were recorded (1) in open area, (2) under natural shelter or (3) under artificial shelter. These data were coupled to climatic data recorded by weather stations in the nature reserves.

Despite the relatively long duration of the study and the wide variety of climatic conditions covered (air temperatures varied between $-18.1\text{ }^{\circ}\text{C}$ and $35.8\text{ }^{\circ}\text{C}$), the artificial shelter was used little in most reserves. In summer as well as in winter, in six out of eight reserves cattle spent less than 2% of their time in the artificial shelter. Only in two study reserves, the artificial shelter was used more than 2% of the time. One of these two reserves (KH) was 'exceptional', because it is a small reserve with little and non-dense natural shelter. The second of these reserves (BB) was also exceptional as it was the only one where cattle were provided additional feed (hay) inside the shelter during harsh winter conditions. Consequently, in this reserve, the association with feed probably influenced the use of the artificial shelter.

Summer sheltering behaviour was modelled, separately, as a function of six different climatic 'heat stress indices'. Out of the six indices, the Heat Load Index (HLI) yielded the best explanatory model. The HLI combines the effects of temperature, humidity, wind speed and solar radiation. In addition, the interaction of the HLI with the amount and the spatial distribution of natural shelter influenced sheltering behaviour. An increasing HLI was associated with a decrease in the use of open area, but this decrease was less when shelter was scarce and highly scattered across the reserve.

In six out of the eight study reserves, there were too few observations in the artificial shelter to (reliably) model its use. In the other two reserves, the summertime use of artificial shelter increased with increasing HLI. In one of these reserves (BB), however, the use of natural shelter increased much steeper than the use of artificial shelter. In conclusion, only the smallest reserve with the least natural shelter (KH), artificial shelter rather than natural shelter was used as protection against high heat load.

Winter sheltering behaviour was modelled as a function of apparent temperature. In literature, only two climatic 'cold stress indices' for cattle have been proposed: the Wind Chill Index (WCI) and the Comprehensive Climatic Index (CCI). Both are interpreted as apparent temperature, but the CCI combines the effect of temperature, wind speed, air humidity and solar radiation, whereas the WCI does not take these last two climatic factors into account. The CCI explained changes in sheltering behaviour best. These changes generally started at a CCI of 0°C. As CCI decreased further below 0°C, the use of open area decreased slightly during the day but more strongly during the night, except when natural shelter was scarce and highly scattered. Similarly to summertime, the wintertime use of artificial shelter could only be modelled (reliably) in two reserves (KH and BB). Again, the smallest reserve with the least natural shelter (KH) was the only reserve where artificial shelter rather than natural shelter was used as protection against cold, during the day as well as during the night.

In conclusion, in Belgian climate, additional artificial shelter (of the type used in this study) did not seem to have a lot of added value for the thermal comfort of adult and assumedly healthy cattle of the studied breeds, as long as there was adequate natural shelter. In nature reserves containing little natural shelter, however, the use of the artificial shelter did increase as the climatic conditions became more extreme. The study methods that were used were, however, not appropriate to evaluate if animal welfare would be diminished by not having access to artificial shelter. Until sufficient scientific support is available to make the above evaluation, we can (temporarily) draw from the 'precautionary principle' to recommend additional shelter in nature reserves where natural shelter (vegetation) offers insufficient protection against aversive climatic conditions.

Our study indicates that when one decides to provide additional shelter, cattle would rather use natural shelter than artificial shelter. On the other hand, the choice between natural and artificial shelter also depends on the reserve's vegetation and landscape management objectives, and should thus be carefully considered, in order to meet the demands of both the grazers and the reserve managers.

In the second part of this thesis, the effect of heat load and the effect of shade (as protection against excessive heat load) on the welfare and productivity of Holstein dairy and Belgian Blue beef cattle were investigated. During the summers of 2011 and 2013, the herd of dairy cows and the herd of Belgian Blue suckler cows were divided in a group with and a group without access to shade. Shade was provided by young trees between which shade cloths (with a shading percentage of 80%) were suspended. In 2012, all cattle were kept on pasture without access to shade.

During the three summers, air temperature, air humidity, wind speed and intensity of solar radiation were recorded by a weather station on pasture. From these measurements several climatic 'heat stress indices', such as the Temperature Humidity Index (THI) and the Heat Load Index (HLI), were calculated. During days of low to very high heat load, the use of shade (for animals that had access to it) was monitored (only in 2011 and 2013). Moreover, the respiratory rate and Panting Score (visual assessment of heat stress, based on mainly panting and drooling) were scored hourly for animals inside and outside shade. At the end of these days, rectal temperature was measured and a blood sample was taken, for 10 'focal' dairy cows in each treatment. The blood plasma was analysed for different indicators of metabolic changes due to heat stress. For each dairy cow in the experiment, the daily milk yield was registered. In addition, we collected data from monthly determinations of milk composition. For all of these response variables we examined the effect of heat load and its interaction with shade.

For Holstein dairy cows, Belgian Blue beef cows and their calves, the HLI explained the observed increase in shade use with increasing heat load best. In the three cattle types, an increasing HLI was associated with increasing indications of thermal discomfort (respiration rates and Panting Scores). In hot conditions, the use of shade improved thermal comfort, based on a reduced incidence of (highly) elevated respiration rates and Panting Scores for cattle inside shade. For the dairy cows, increasing daily average HLI values were also associated with increasing body temperature, indications of changes in energy metabolism (general + indications of increased fat and protein catabolism), signs of hyperventilation, decreasing milk yield (after a 'lag' period of two days) and a decrease in the milk lactose, protein and fat content (after a 'lag' period of three days). Having access to shade tempered the increase in body temperature and prevented the signs of hyperventilation and the decrease in milk yield. Thus, our study has confirmed the added value of shade on pasture to increase thermal comfort of dairy cows, adult beef cows and calves and productivity of dairy cows on pasture during hot conditions in temperate climate.

Moreover, this research has validated a method to assess the risk of heat stress and, additionally, the actual degree of thermal discomfort in Holstein dairy cows and Belgian Blue beef cattle. The method combines the measurement of climatic conditions with complementary animal-based observations, i.e. the Panting Score.

SAMENVATTING

In dit doctoraatsproject onderzochten we specifiek in een gematigd klimaat, meer bepaald in België, (1) hoe thermisch ongemak bij in open lucht gehouden runderen kan worden gedetecteerd, (2) hoe frequent het voorkomt, (3) wat de gevolgen zijn en hoe ernstig deze kunnen zijn, en (4) hoe het kan worden voorkomen.

Een eerste deel van het onderzoek focuste op runderen in jaar-rond begrazingsbeheer van natuurgebieden. We onderzochten of runderen gebruik zouden maken van artificiële beschutting (een schuilhok), bovenop de aanwezige natuurlijke beschutting (vegetatie), als bescherming tegen koude en hitte. In acht Vlaamse natuurreservaten werd het terreingebruik van één rund (per gebied) gedurende drie winters en twee zomers opgevolgd, per 30 minuten, door een GPS-halsband. Alle dierlocaties ($n = 58\ 101$ voor de zomers, $n = 73\ 371$ voor de winters) werden uitgezet op de digitale kaarten van de studiegebieden, om te bepalen of ze (1) in open gebied, (2) onder natuurlijke beschutting of (3) onder artificiële beschutting lagen. Deze gegevens werden gekoppeld aan klimatologische gegevens die verzameld werden door weerstations ter plaatse in de natuurgebieden.

Ondanks de relatief lange duur van de studie en de grote variatie aan klimatologische omstandigheden (tijdens de studie varieerde de luchttemperatuur tussen $-18,1\ ^\circ\text{C}$ en $35,8\ ^\circ\text{C}$), werd de artificiële beschutting in de meeste studiegebieden zeer weinig gebruikt. In de zomer zowel als in de winter, werd de artificiële beschutting in zes van de acht studiegebieden minder dan 2% van de tijd gebruikt. In slechts twee studiegebieden werd de artificiële beschutting meer dan 2% van de tijd gebruikt. Een van deze twee studiegebieden (KH) was 'uitzonderlijk' omdat het een erg klein gebied was met weinig en weinig dichte natuurlijke beschutting. Het tweede van deze gebieden (BB) was ook 'uitzonderlijk' omdat dit het enige gebied was waar er tijdens barre winteromstandigheden (hooi) werd bijgevoerd in de artificiële beschutting. In dit gebied werd het gebruik van de artificiële beschutting dus waarschijnlijk beïnvloed door de associatie met voer.

Het zomer-schuilgedrag werd gemodelleerd in functie van zes verschillende klimatologische 'hittestress-indices'. Van deze zes klimatologische indices, gaf de Heat Load Index (HLI) de beste verklaring voor het geobserveerde schuilgedrag. De HLI combineert het effect van temperatuur, luchtvochtigheid, windsnelheid en zonnestraling.

Bovendien werd ook bepaald hoe de interactie van de HLI met de hoeveelheid en de ruimtelijke verdeling van natuurlijke bescherming over het studiegebied het schuilgedrag beïnvloedde. Een toenemende HLI was steeds geassocieerd met een afname in het gebruik van open gebied, maar deze afname was minder sterk wanneer natuurlijke beschutting schaars en sterk verspreid was. In zes van de acht studiegebieden, waren er te weinig waarnemingen in de artificiële beschutting om het gebruik ervan (betrouwbaar) te kunnen modelleren. In de twee overige gebieden, nam het gebruik van artificiële beschutting in de zomer toe met toenemende HLI. In één van deze gebieden (BB), steeg het gebruik van natuurlijke beschutting echter veel sterker dan het gebruik van artificiële beschutting. Dus, enkel in het kleinste en minst begroeide studiegebied (KH), werd de artificiële beschutting eerder dan natuurlijke beschutting gebruikt als bescherming tegen hitte.

Het winter-schuilgedrag werd gemodelleerd als een functie van twee klimatologische koudstress-indices: de Wind Chill Index (WCI) en de Comprehensive Climatic Index (CCI). Beide worden geïnterpreteerd als gevoelstemperatuur, maar de CCI combineert het effect van temperatuur, windsnelheid, luchtvochtigheid en zonnestraling, terwijl de WCI deze laatste twee klimatologische factoren niet in rekening brengt. De CCI gaf dan ook een betere verklaring voor de veranderingen in schuilgedrag waargenomen in deze studie, die in het algemeen startten bij een CCI van 0 °C. Naarmate de CCI verder onder 0 °C daalde, nam ook het gebruik van open gebied af, lichtjes tijdens de dag, maar sterker tijdens de nacht, behalve wanneer natuurlijke beschutting schaars en zeer verspreid was. Net als in de zomer, kon ook in de winter het gebruik van artificiële beschutting enkel in twee studiegebieden (KH en BB) (betrouwbaar) gemodelleerd worden. Ook in de winter werd er enkel in het kleinste en minst begroeide studiegebied (KH), overdag zowel als 's nachts, eerder gebruik gemaakt van artificiële dan natuurlijke beschutting als bescherming tegen koude.

Voor volwassenen en verondersteld gezonde runderen van de onderzochte rassen, lijkt extra artificiële beschutting, van het type gebruikt in deze studie, dus weinig toegevoegde waarde te hebben voor het thermisch comfort. In het Belgische klimaat, zolang er adequate natuurlijke beschutting aanwezig is. In het meest schaars begroeide natuurgebied, daarentegen, werd het schuilhok wel meer gebruikt naarmate de klimatologische omstandigheden extremer werden. Anderzijds lieten de gebruikte studiemethoden niet toe om te evalueren of het welzijn van de dieren verminderd zou zijn bij het ontbreken van een schuilhok.

Tot er voldoende wetenschappelijke basis is om een dergelijke evaluatie te maken, kan er (voorlopig) wel gesteund worden op het 'voorzorgsprincipe' om extra beschutting te adviseren in natuurgebieden waar natuurlijke beschutting (vegetatie) onvoldoende beschutting lijkt te bieden tegen aversieve weersomstandigheden.

Onze studie geeft aan dat runderen in dit geval meer gebruik zouden maken van natuurlijke dan artificiële beschutting. Anderzijds hangt de keuze tussen natuurlijke en artificiële beschutting ook af van de doelstellingen van het beheer wat betreft vegetatieontwikkeling en landschapsbehoud. De keuze voor een bepaald type beschutting moet dus gemaakt worden op een doordachte manier, met het oog op de noden en wensen van de grazers zowel als de natuurbeheerders.

In het tweede deel van de studie onderzochten we het effect van hitte en het effect van schaduw, als bescherming tegen hitte, op het welzijn en de productiviteit van Holstein melkkoeien en Belgisch Wit Blauwe vleesvee runderen. Tijdens de zomers van 2011 en 2013, werden een kudde melkkoeien en een kudde Belgisch Wit Blauwe zoogkoeien beiden verdeeld in een groep met en een groep zonder toegang tot schaduw. Schaduw werd voorzien door jonge bomen waartussen bijkomend schaduwdoeken werden opgehangen, die de zonnestraling reduceerden met 80%. In 2012 werden alle runderen op de weide gehouden zonder toegang tot schaduw. Tijdens de drie zomers werden de luchttemperatuur, de luchtvochtigheid, de windsnelheid en de intensiteit van de zonnestraling geregistreerd door een weerstation op de weide. Uit deze metingen werden meerdere klimatologische 'hittestress indices' berekend, zoals 'Temperature Humidity Index' (THI) en de 'Heat Load Index' (HLI). Gedurende dagen met lage tot zeer hoge graad van hitte, werd het gebruik van schaduw (door de dieren die er toegang tot hadden; alleen in 2011 en 2013) opgevolgd. Bovendien werden ook de ademhalingsfrequentie en de 'Panting Score' (een visuele beoordeling van hittestress, voornamelijk gebaseerd op de mate van hijgen en kwijlen) elk uur gescoord voor dieren binnen en buiten schaduw. Op het einde van dezelfde dagen, werd er bij 10 'focale' melkkoeien in elke behandelings-groep de rectale temperatuur gemeten en een bloedstaal afgenomen. Het bloedplasma werd geanalyseerd op verschillende indicatoren van metabole veranderingen ten gevolge van hittestress. Voor elke melkkoe in het experiment werd ook de dagelijkse melkgift en de gegevens van de maandelijkse bepalingen van melksamenstelling bijgehouden. Voor al deze respons-variabelen onderzochten we het effect van hitte en de interactie met het effect van schaduw.

Voor de melkkoeien en voor de zoogkoeien en hun kalveren nam het gebruik van schaduw toe met toenemende hitte en gaf de HLI de beste verklaring voor dit effect. Voor de drie types runderen was een toenemende HLI ook geassocieerd met toenemende indicaties van thermisch ongemak (ademhalingsfrequentie en 'Panting Scores'). In warme omstandigheden verbeterde het gebruik van schaduw het thermisch comfort, op basis van een verminderd voorkomen van (sterk) verhoogde ademhalingsfrequenties en 'Panting Scores' bij runderen in de schaduw. Voor de melkkoeien was een toename van de HLI ook geassocieerd met een toenemende lichaamstemperatuur, indicaties van veranderingen in het energiemetabolisme (algemeen, plus indicaties van een verhoogd vet- en eiwit-katabolisme), indicaties van hyperventilatie, een daling van de melkgift (met een 'vertraging' van twee dagen) en een daling van het gehalte aan lactose, eiwit en vet in de melk (met een 'vertraging' van drie dagen).

Het toegang hebben tot schaduw reduceerde de stijging van de lichaamstemperatuur, voorkwam de indicaties van hyperventilatie en voorkwam de daling van de melkgift. Onze studie heeft dus bevestigd dat schaduw op de weide tijdens warme omstandigheden inderdaad het thermisch comfort van melkkoeien, volwassen zoogkoeien en kalveren verbetert, en bovendien de productiviteit van de melkkoeien verbetert, zelfs in het Belgisch klimaat.

Bovendien heeft dit onderzoek een methode gevalideerd om bij Holstein melkkoeien en Belgisch Wit Blauw vleesvee (1) op basis van klimatologische metingen het risico op hittestress in te schatten en (2) bijkomend op basis van observaties van het dier zelf (meerbepaald, de Panting Score) ook de werkelijke mate van thermisch ongemak te beoordelen.

CURRICULUM VITAE AND LIST OF PUBLICATIONS

EDUCATION

Doctoral training (2011 - 2015): PhD in Veterinary Science, Ghent University (BE). Doctoral thesis: 'Detection, consequences and prevention of thermal discomfort for cattle kept outdoors in Belgium'. Eva Van laer, Prof. Dr. Frank Tuytens (promotor), Dr. Christel Moons (co-promotor), 2015.

PhD course (November 2012): 'Non-invasive Monitoring of Steroid Hormones', Institute of Biochemistry, University of Veterinary Medicine, Vienna (AU).

Lab Animal Science (October 2011): FELASA Category C, Ghent University (BE).

Master in Biology (2008 tot 2010): majors Ecology and Evolution, minor Scientific Research, Ghent University (BE). Master thesis: 'De invloed van ruimtelijke patronen op het foeragegedrag van runderen in een brakwaterschor'. Eva Van laer, Prof. Dr. Maurice Hoffmann (promotor), Alexander Van Braeckel (co-promotor), 2010.

Bachelor in Biology (2005 -2008), Ghent University (BE).

Secondary education (1999 - 2005): Onze Lieve Vrouw Presentatie Bornem.

Primary education (1993 - 1999): Onze Lieve Vrouw Presentatie Bornem.

CARREER

2010 – 2015: PhD student / Scientific Researcher at the Institute for Agricultural and fisheries Research (ILVO), Animal Sciences Unit, Farm animal Welfare & Behaviour Research Group.

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ACKNOWLEDGEMENTS/DANKWOORD

First, I would like to thank the chairmen (Prof. Dr. Frank Gasthuys and Prof. Dr. Piet Deprez) and the members (Prof. Dr. Lena Lidfors, Dr. Kees van Reenen, Prof. Dr. Maurice Hoffmann, Prof. Dr. Stefaan De Smet and Prof. Dr. Sarne De Vlieghe) of the examination committee for participating in my thesis defence and for their evaluation of this manuscript.

Dan wil ik het ILVO bedanken om me de kans te geven om aan dit doctoraat te beginnen, maar ook en vooral om het af te werken. Hartelijk dank aan Frank Tuytens, om mijn promotor te zijn en in mij en ons project te blijven geloven, doorheen de soms frustrerende tegenslagen met apparatuur, proefopzet en het publiceren. Ook bedankt, Christel Moons, om zo'n steunende copromotor te zijn, ook vooral tijdens de intense periode van schrijven, herschrijven en publiceren. Bedankt om veel van mijn teksten meer verstaanbaar te maken voor de 'outsiders'. Bedankt, Bart Sonck, voor uw steun en om me altijd snel te helpen met dringende vraagstukken, ondanks uw drukke agenda. Bedankt voor de goede samenwerking in het begeleiden van 'onze' masterstudenten. Ook wil ik nog Frank Ödberg, Daniël De Brabander en Leo Fiems bedanken, die betrokken waren bij de opstart van het project, en ook Sam De Campeneere en Leen Van Daele die (ook) later hun schouders onder het project gezet hebben.

Bedankt aan de Federale Overheidsdienst Volksgezondheid, cel contractueel onderzoek, voor de (gedeeltelijke) financiering van het onderzoek (het PASTRESS project, RT10/13). Ook bedankt de aan de partners van KULeuven (Rony Geers en Melissa Snoecks) voor de samenwerking in dit project.

Verder ben ik heel dankbaar voor de samenwerking met Natuurpunt en het Agentschap voor natuur en Bos (ANB), om me mijn onderzoek te laten voeren in hun natuurgebieden. Bedankt aan Karen Helsen en Griet Nijs, en alle conservatoren, werknemers en vrijwilligers voor de leuke samenwerking. Het was altijd fijn om met jullie samen te werken in de mooie natuurgebieden! Bedankt aan Jürgen Vangeyte en het team van ILVO T&V voor het bouwen van de schuilhokken. Ook nog bedankt aan de 'reddende engelen' van INBO voor het helpen opsporen van de verloren GPS halsband in Ename Bos.

Dan zijn er erg veel namen die ik zou kunnen opsommen in de volgende paragraaf. Een beetje té veel, dus zal ik gewoon zeggen: dank aan iedereen (collega's, master-studenten, stage-studenten, ...) die me wat van hun tijd gegeven hebben om te helpen met proefopzet en data verzamelen op het proefbedrijf van ILVO en in 'onze' natuurgebieden. We hebben veel gewerkt,

en we kunnen allemaal bevestigen dat ook mensen onder de bestudeerde weersomstandigheden wel eens last hebben van hitte- en koudestress! Maar heel vaak was het ook gewoon leuk. Een 'dikke merci' aan de technici (in het bijzonder Thijs Decroos, Esther Beeckman, Sara Van Lembergen, Seppe Holemans en Piet Van Laere) en de dierverzorgers van ILVO-Dier, ook om me heel wat praktische zaken bij te brengen over rundveehouderij. Ook bedankt aan de andere collega's bij ILVO (in het bijzonder 'room-mate' Stephanie Buijs en de anderen aan het einde van 'den dierenwelzijnsgang'), voor hun geapprecieerde onderzoeks-advies, steun, ontspannende 'koffie-kletsen' en middagpauzes (soms met sport of Kubb), en natuurlijk de muzikale opvoeding!

Verder ben ik heel erg dankbaar voor mijn goede ouders en 'de klein en eure vent', om mij warm te steunen doorheen mijn studies en al 'onze' andere dromen en ambities. Bedankt aan alle vrienden, die vaak geluisterd hebben naar mijn enthousiasme zowel als geklaag over mijn werk, en die af en toe de dagelijkse routine eens onderbroken hebben. Misschien heb ik jullie niet zo vaak gezien als ik zou gewild hebben, maar het was altijd aangenaam om mijn batterijen eens te kunnen opladen met een toureke door de dreef, een bezoekje met een trappistje, een namiddag op den Boskant, een stevige wandeling, een kampvuur, een brunch of etentje of een weekend in Oppuurs, de Ardennen of Londen. Bedankt, iedereen!

En niet in het minst, een heel erg dikke merci aan Dieter, om een fantastische grafisch ontwerper maar ook het beste lief, de beste vriend en de beste partner te zijn om bij thuis te komen en mee weg te gaan. Bedankt voor je bewonderenswaardige geduld, volharding, steun en optimisme. Ik ben erg blij en trots om bij jou te horen.