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Effect of summer conditions and shade on the production and metabolism of Holstein dairy cows on pasture in temperate climate

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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 cloths (80% reduction in solar radiation). A weather station registered the local climatic conditions on open pasture, from which we calculated daily average Heat Load Index (HLI) values. The effects 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 were investigated. RT increased with increasing HLI, but was less for S cows than for NS cows (by 0.02°C and 0.03°C increase per unit increase of HLI, respectively). Hyperchloraemia (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, 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, hyperchloraemia 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 2 days, the milk yield decreased with increasing HLI. For S cows, the milk yield was unaffected by HLI and its quadratic factor. The milk concentrations 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 effects of high HLI on RT, hyperchloraemia and a blood plasma indicator of changing energy metabolism (generally) as well as prevented the decrease in milk yield observed in cows without access to shade.

Keywords: heat stress, dairy cattle, temperate climate, milk, metabolism

Implications

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 preventing this. In this study, the effect of Belgian summer weather on dairy cows with and without access to shade was investigated. Hot summer conditions affected body temperature, metabolism and milk yield and composition. Some effects (the increase in body temperature and the decrease in milk yield, among others) were reduced by access to shade, provided by trees combined with shade cloth.

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Introduction

During summer, in most temperate regions, dairy cattle are kept on pasture for at least few hours a 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 – for example, when high temperatures occur in combination with intense solar radiation. Such conditions can have substantial detrimental effects on the cows' comfort level, feed intake, metabolism and productivity, as illustrated by ample studies 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 mobilise reserves from adipose tissue and skeletal muscles (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 (mobilised from skeletal muscle tissue) and consequently an increased plasma urea or plasma urea nitrogen concentration has been reported in several heat stress studies (Shwartz *et al*., 2009; Baumgard and Rhoads, 2012). Plasma creatinine, another indicator of skeletal muscle breakdown, has been shown to increase due to heat stress as well (Schneider *et al*., 1988; Abeni *et al*., 2007). A more general indicator of alterations in energy metabolism in the liver is the blood plasma concentration of ALP (alkaline phosphatase). This enzyme is involved in the regulation of energy metabolism by the liver, and is known to decrease in response to heat stress (Toharmat and Kume, 1997; Abeni *et al*., 2007). Hyperventilation due to heat stress can cause hyperchloraemia – that is, an increase in blood plasma chlorine (Cl^-), through increased elimination of bicarbonate from the blood in exchange for Cl^-. This exchange takes place in the lung tissue as a direct consequence of increased removal of carbon dioxide via respiration (Afzaal *et al*., 2004). Bicarbonate can also be eliminated in 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*., 1982; West, 2003). Hot summer conditions may, therefore, not only affect cattle comfort and welfare but also dairy producers' income by reducing the milk yield quantitatively and also by reducing the milk quality. In the United States (Bailey *et al*., 2005) as well as the EU, multiple component pricing systems are used to calculate milk payments on the basis of milk fat and protein contents. In the EU, the basic milk price is adjusted according to the actual fat and protein contents (i.e. whether the milk meets fat and protein content standards 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 yields 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 and whether 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). These validation studies were carried out primarily in (sub) tropical and arid regions, and they were carried out on less-productive dairy cows rather than those generally reared nowadays (Zimbelman *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 per 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 great the benefits of shade on pasture are in temperate climatic conditions. 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 with those without, but milk composition was not affected by shade treatment (Kendall *et al*., 2006).

The aim of this study was to assess whether and to what extent rectal temperature (RT), hyperchloraemia, metabolic parameters (cholesterol, urea, creatinine and ALP), milk yield and milk composition are affected by hot climatic 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.

Material and methods

Experimental setup

This study was carried out during three subsequent summers (2011, 2012 and 2013; Table 1), and was approved by the Animal Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO; application no. 2011/151 and 2011/151bis). The experiment was carried out on the pastures of the institute's experimental farm (latitude 50°59'1"N, longitude 3°46"49"E). The Holstein dairy cows were rotated between four (in 2011) or two (in 2012 and 2013) pastures. Each pasture was adjacent to a shaded area surrounded by an electric fence. This shaded area could be accessed from either of the two adjacent pastures through a 3- to 5-m wide passage. Shade was provided by young trees and shade cloths (shading percentage = 80%, Duranet bvba, Ostend, Belgium) that were spanned between the trees (see Supplementary Figure S1). The two shaded areas

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 ± s.e.	Date	HLI[1]	Mean ± s.e.	Date	HLI[1]	Mean ± s.e.
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	

[1]Daily average HLI on this day.

(625 m^2 each) for dairy cattle were used by a maximum 60 dairy cows on adjacent pastures, thus providing at least 10.5 m^2 of shade per cow.

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 included. In 2011, the study included 125 dairy cows. At the beginning of the experiment (10 June 2011), they were of an average parity of 2.0 ± 1.6 (mean ± s.d.), were 169.1 ± 132.4 days in milk (DIM; mean ± s.d.) and yielded 26.9 ± 11.7 l of milk per day (mean ± s.d.). In 2012, 66 dairy cows were included. 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 June 2012). In 2013, 96 dairy cows were included, 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 June 2013). All the cows were milked twice daily (starting around 0530 and around 1530 h). During each milking session, they received half of the daily portion of concentrates. After milking, they were fed the daily mixed ration of mainly corn silage (49% to 76%, 60% on average) and pre-wilted grass silage (9% to 29%, 21% on average), supplemented with a protein source (soyabean meal or protected soyabean meal) and wheat or corn cob mix. In addition, during some periods, the ration was completed with pressed beet pulp (0% to 25%, 9% on average) and/or by-products from the bio-ethanol or starch industry. 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. In order to exclude potential confounding effects (e.g. from pasture productivity or composition, location of drinking troughs) on the cows' behaviour or productivity due to pasture allotment, NS and S groups were switched daily between allotments. 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 in order to investigate the effect of climatic conditions.

Climatic data

A custom-built Campbell Scientific BWS200 weather station (Campbell Scientific Inc., Logan, UT, USA) located in open pasture, within 500 m of all pastures used in the trial, registered the average air temperature (Ta, in °C), air humidity (RH, in %), solar radiation (Rad, in W/m^2) and wind speed (WS, in m/s) every 15 min. Based on these measurements, 15-min values of the Heat Load Index (HLI; Gaughan *et al.*, 2008) were calculated. When Tbg > 25, $HLI = 8.62 + 0.38 \times RH + 1.55 \times Tbg – 0.5 \times WS + e^{(2.4 - WS)}$. When Tbg < 25, $HLI = 10.66 + 0.28 \times RH + 1.3 \times Tbg – WS$. Tbg stands for black globe temperature (in °C) and is calculated as $Tbg = 1.33 \times Ta - 2.65 \times Ta^{0.5} + 3.21 \times \log(Rad + 1) + 3.5$ (Hahn *et al.*, 2003).

The HLI was used to quantify hot conditions, as this climatic heat stress index incorporates all the relevant climatic variables contributing to thermal (dis)comfort on pasture – that is, 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 (Van laer *et al.*, 2015).

In order to evaluate the effect of shade on microclimate, additional measurements of Tbg were carried out, with Testo 400's Wet Bulb Globe Temperature probe (Testo AG Inc., Lenzkirch, Germany), under shade and without shade. During the nine measurement sessions, performed on 8 days of medium to high heat load (weather forecast predictions of daily maximum temperatures ⩾25°C), for each of the shaded areas, Tbg was measured at 1.5 m height under shade and in an open area nearby (i.e. on three locations 20 to 50 m away from each shaded area). Three measurement sessions took place between 1000 and 1230 h, two sessions between 1200 and 1430 h and four sessions between 1330 and 1600 h. During each measurement session, three instantaneous measurements were taken inside and outside of each shaded area.

Physiological measurements

Physiological measurements took place on 11 days (Table 1). The daily average air humidity ranged between 46.9% and 84.9%, the daily average wind speed was between 0.8 and 6.8 m/s, solar radiation intensity was between 28.2 and 74.3 W/m^2, the daily average air temperature was between 16.0°C and 30.2°C, the daily average Tbg was between 19.7°C and 34.4°C and the daily average HLI value was between 50.8 and 85.5. 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 session (around 1500 h). 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, RT were determined with a digital thermometer (with an accuracy of 0.1°C), and blood samples were obtained; 9 ml blood samples were collected (in lithium–heparin-coated tubes) by punction 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, IN, 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.

Milk yield and 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 July 2013 and 22 August 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 (MY^X) data. MY^X was coupled with climatic data from 1, 2 and 3 days before. Thus, the milk yield data set contained milk yields from 98 days in 2011 (10 June 2011 to 15 September 2011), 117 days in 2012 (1 June 2012 to 25 September 2012) and 105 days in 2013 (7 June 2013 to 19 September 2013). These data were coupled with the daily average HLIs, which ranged between 46.1 and 86.7 (mean ± s.e. = 61.6 ± 0.5) on the day before, between 46.1 and 86.7 (mean ± s.e. = 61.6 ± 0.5) 2 days before and between 46.7 and 86.7 (mean ± s.e. = 61.7 ± 0.5) 3 days before.

In addition, data from monthly determinations of milk composition, with a mid-IR spectrophotometer, by the Flemish milk monitoring service (Melk Controle Centrum Vlaanderen, http://www.mcc-vlaanderen.be) were obtained. These determinations were carried out every 5 weeks, unrelated to weather conditions. Contents of fat, protein, lactose and urea were also coupled with climatic data from 1, 2 and 3 days before. Thus, the milk composition data set contained data from 3 days in 2011, 3 days in 2012 and 4 days in 2013, and coupled to it daily average HLIs that ranged between 50.6 and 76.3 (mean ± s.e. = 59.4 ± 2.5) on the day before, between 46.1 and 75.7 (mean ± s.e. = 57.6 ± 2.5) 2 days before and between 48.6 and 67.8 (mean ± s.e. = 56.2 ± 1.7) 3 days before.

Data analysis

Effect of shade on microclimate. The difference in Ta and Tbg measured in open area and under shade was modelled using a linear mixed model ANOVA (proc mixed, in SAS 9.3). Measurement session and shade area were included as random factors.

Physiological measurements. The effect of HLI and treatment on RT and blood plasma concentrations of ALP, cholesterol, creatinine, urea and Cl^- were investigated using 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 as follows: (1) the effect of the productivity of the cow – that is, the summed milk yield of the morning of the same day and the evening before, centred over the data set (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), as well as the interaction of (2) with the square of the daily average HLI (for model equations, refer Supplementary Material S1). Generally, the strictly linear models yielded the best fit – that is, the lowest AICC value (Corrected Akaike Information Criterion). Therefore, only results from the strictly linear models are reported.

Milk yield. In order 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 period, 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 – that is, between 42 and 305 DIM (Adediran *et al*., 2012).

Table 2 *AICC values of models for milk composition variables in function treatment (NS or S) and their interaction with the Heat Load Index (HLI)*[1]

	X = HLI[1]		
	1 day before sampling	2 days before sampling	3 days before sampling
Y = milk (urea)	2927	2929	2942
Y = milk (lactose)	−233	−262	−286
Y = milk (protein)	80	66	50
Y = milk (fat)	887	891	886

AICC = Corrected Akaike Information Criterion.
[1]Daily average of 1, 2 and 3 days before sampling.

Data from <42 and >305 DIM were omitted from the data set. 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 1, 3 or 3 days before sampling and (4) the interaction of (3) with the square of the daily average HLI (for model equations, refer Supplementary Material S1).

Milk composition. The effects of HLI and treatment (S or NS) on the milk contents of fat, protein, lactose and urea were investigated using 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 as follows: (1) the effect of the milk quantity – that is, the milk yield on the day of sampling, centred over the data set (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 between (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, refer Supplementary Material S1). 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 (Table 2) – that is, the model with the HLI of either 1, 2 or 3 days before sampling – was considered to be the best fitting model. Only the results of these models are discussed.

All mixed linear regressions included a random factor to correct for repeated measurements per cow and a random factor to correct for potential year effects.

Results

Effect of shade on microclimate

In comparison with a nearby open area, shade lowered Tbg by 4.5°C ($P < 0.0001$); the mean Tbg (± s.e.) was 30.3 ±2.0°C outside shade and 25.8 ± 2.0°C under shade.

Physiological measurements

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 concentrations 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 and $P = 0.293$ for creatinine). RT increased with increasing HLI for both treatments; however, the response was less pronounced in cows with access to shade (Figure 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).

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 (Figure 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 (Figure 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).

Milk yield

For cows with access to shade, the milk yield was not significantly affected by the HLI (or its quadratic factor) on 1, 2 and 3 days before (Table 4).The milk yield for animals without access to shade, however, was affected by the HLI (and its quadratic factor) 2 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 to 24.1 l/day (Figure 2, Table 4). For the cows with access to shade, the daily milk yield did not significantly decrease with increasing HLI (Figure 2, Table 4). 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 5 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 May 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 per 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 per cow (Table 5).

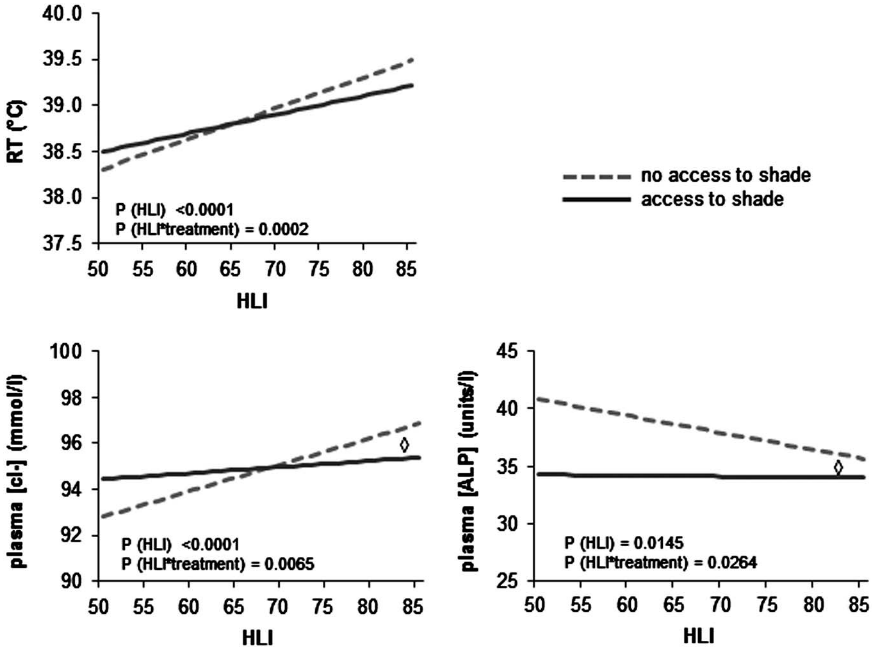

Figure 1 Effect of the daily average Heat Load Index (HLI) and treatment (NS or S) on rectal temperature (RT) and blood plasma (Cl^-) and alkaline phosphatase (ALP). ◊ Blood plasma (Cl^-) and (ALP) are not significantly influenced by HLI for animals with access to shade (Table 3).

Milk composition

The milk urea content was best explained by the HLI 1 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 (Figure 3, Table 6; $P(\text{HLI} \times \text{treatment}) = 0.0027$). The milk contents of lactose, protein and fat were best explained by the HLI 3 days before sampling (Table 2). The milk lactose content decreased with increasing HLI, irrespective of access to shade (Table 6; $P(\text{HLI} \times \text{treatment}) = 0.1248$). The milk protein content decreased with increasing HLI, but the decline was less marked for cows with *v.* without access to shade (Figure 3, Table 6; $P(\text{HLI} \times \text{treatment}) = 0.0465$). The milk fat content was unaffected for animals without access to shade, but decreased with increasing HLI for cows with access to shade (Figure 3, Table 6; $P(\text{HLI} \times \text{treatment}) = 0.0300$).

Discussion

Hot summer conditions affected RT, hyperchloraemia and energy metabolism

The RT and the energy metabolism of cows without access to shade were substantially affected by hot summer conditions. At HLI = 85 (± the highest observed daily average value in the RT data set), the RT of cows without access to shade was on average 39.5°C. This result is comparable with 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. Moreover, 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 hyperchloraemia is likely a sign of hyperventilation (Afzaal *et al.*, 2004; Smith 2009). In our research, parallel to the present study, respiration rates indeed regularly exceeded 120 breaths per minute (>20% of the observations of dairy cows outside shade; Van laer *et al.*, 2015). 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 the use of shade (Van laer *et al.*, 2015). 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 Kume (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, and was 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 concentrations (Shwartz *et al.*, 2009) and milk urea content (Gallardo *et al.*, 2005). On the other hand,

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	s.e.	*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; 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

ALP = alkaline phosphatase.
[1]NS = no access to shade; S = access to shade.

Abeni *et al.* (2007) also found a decrease in blood plasma urea concentrations 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 NH_3 production in the rumen, which results in higher urea concentrations in blood and milk. In the present study, the observed decrease in the urea concentration might, therefore, 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 NH_3 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 hyperchloraemia suggests that cows without access to shade can suffer from hyperventilation under hot summer conditions. This can be assumed to reflect substantial thermal

Table 4 *Effects of lactation stage[1], treatment (NS or S)[2] and its interaction with the daily average Heat Load Index (HLI)[3] and its quadrat (HLI^2)[3] on the daily milk yield*

X	Effect	Estimate	s.e.	*P*-value
HLI: 1 day before sampling	Intercept	33.62	4.22	<0.0001
	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: 2 days before sampling	Intercept	42.10	4.23	<0.0001
	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: 3 days before sampling	Intercept	42.16	4.20	<0.0001
	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

[1]DIM = days in milk (lactation stage).
[2]NS = no access to shade; S = access to shade.
[3]Daily average of 1, 2 and 3 days before sampling.

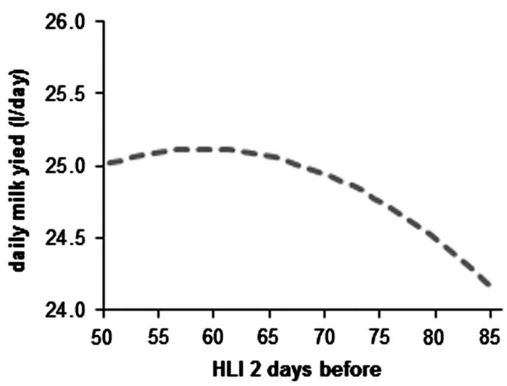

Figure 2 Daily average milk yield for cows without access to shade (NS) (and with an average number of days in milk (DIM), i.e. 202) in function of the daily average Heat Load Index (HLI) 2 days before.

discomfort as well (see Van laer *et al.*, 2015). 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.

Hot summer conditions decreased the milk yield of cows without access to shade

Increasing HLI was associated with decreasing milk yield for unshaded cows. After a lag-effect of 2 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 per cow *v.* 25.1 l/day per cow). Another study that 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 per cow *v.* yield of 23.8 kg/day per 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, at present.

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

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

[1]The degree of decline in milk yield with every 5 unit increase in HLI above the HLI that gave the maximum milk yield (HLI = 60).
[2]The occurrence of the number of days with these HLI levels in 2012 and 2013, based on data from the weather station on our experimental pastures.
[3]The resulting milk yield loss per HLI level, for both years.
[4]The total milk yield loss is the sum of milk yield loss per HLI level, for both years.

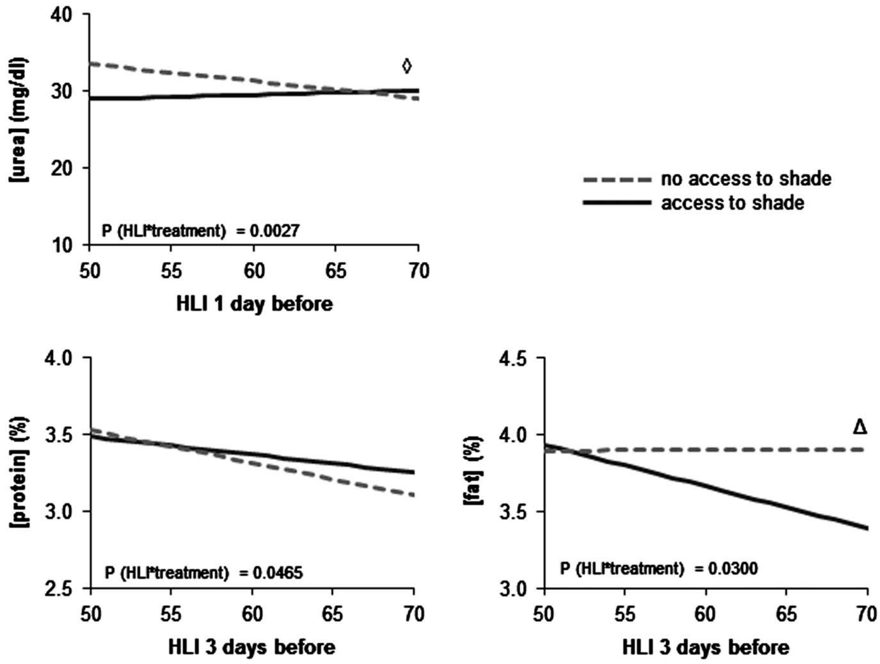

Figure 3 Effect of the daily average Heat Load Index (HLI) – 1 day before or 3 days before, depending on what day gave the best fit (lowest AICC (Corrected Akaike Information Criterion), see Table 4) – and treatment on milk urea, protein and fat content. *P*-values for the interaction between HLI and treatment are given, ◊ 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).

study, the decrease in milk yield coupled with 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 per cow (Kendall *et al.*, 2006) – and in South-African summers – with a 5.5% difference (Muller *et al.*, 1994b).

Hot summer conditions altered milk composition

The milk contents of lactose, protein and fat were significantly affected by hot summer conditions. As HLI increased, after a lag-time of 3 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.* (2012) 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 8 g/day per unit increase of HLI. This decline is comparable with the decline of 10 g/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.* (2012) at daily THI ⩾ 72. On the other hand, cows with access to shade showed a less marked decrease of the milk protein content.

Table 6 *Effects of treatment (NS or S)*[1] *and their interaction with the Heat Load Index (HLI)*[2] *on the milk composition variables*

Y	*X*	Effect	Estimate	s.e.	*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

AICC = Corrected Akaike Information Criterion.
[1]NS = no access to shade; S = access to shade.
[2]Daily average HLI of 1, 2 and 3 days before sampling, depending on which day provided the best fitting model (model with the lowest AICC value, Table 2).

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 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 data set contained data from only 10 days in total, with daily average HLIs up to only 76.3 (mean ± s.e. = 59.4 ± 2.5) on the day before and only 67.8 (mean ± s.e. = 56.2 ± 1.7) 3 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.

Other aspects of provision of shade on pasture

The present study showed that the absence of shade on pasture during hot summer conditions can reduce dairy producers' income. In a study parallel to the present study, we also demonstrated that shade improves thermal comfort for cows (Van laer *et al.,* 2015). 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 depends greatly on the design and size of the shading structure. As a minimum, generally 3.5 to 6.5 m^2 shade per cow is recommended (Armstrong, 1994). However, Schütz *et al.* (2010) demonstrated that 9.6 m^2 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 compared with 2.4 m^2 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 1 m^2 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 commercialised in, for example, the United States (Dr T. Brown-Brandl, personal communication). 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 the study by Van laer *et al.* (2014).

Conclusions

The first aim of our study was to assess the degree of negative impact of hot summer conditions, occurring in temperate summers, on RT, 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 contents of lactose, protein and fat. In cows without access to shade, the milk yield, after a lag-period of two days, also decreased notably with increasing HLI. The higher the HLI increased, the steeper the milk yield decline became. At daily average HLI = 85, the milk yield 2 days later was 1.0 l/day per cow lower than at daily average HLI = 65. The second aim was to evaluate the effectiveness of shade in preventing the above-mentioned 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 were not unambiguously ameliorated by shade either. However, the increase of RT, hyperchloraemia (a sign of hyperventilation) and the decrease of ALP (a regulator of metabolism in the liver) in the blood plasma were 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.

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Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1751731115000816

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