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**Heat stress in dairy calves:
Insights on importance, methodology and abatement**

PhD Thesis

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1. Summary

The topic of heat stress in calves is a field of research that has come more into focus only recently, recognising the impact of hot summer weather on all livestock species. We aimed to provide results that are practically relevant, understandable and applicable for dairymen working with calves. We also wished to explore the importance of heat stress in dairy calf, and possible solutions for detection and relief. The importance of the issue was confirmed by analyzing data on calf mortality rates from a large-scale dairy farm covering a period of 25-years. It showed that summer weather could be just as detrimental to the health of young calves as the winter cold. The monthly distribution of calf deaths differed between the 0-14 and 15-60 day age groups. The mortality risk ratio was highest in July (6.92). The mortality risk in the 0-14 day age group was twice as high in three-day periods with an average temperature above 22 °C than in periods of thermoneutrality (mean temperature between 5-18°C). More extended periods of high ambient temperature increased the risk of mortality by up to three times. In the following study, we investigated the usefulness of no-contact thermometry to predict core body temperature and identify animals at risk of hyperthermia. Due to the weak correlation between core body temperature and surface temperature, infrared thermometry has limited reliability in assessing the thermal status. The study on the effect of hutch entrance orientation on hutch microclimate and heat stress responses of calves lead to the conclusion that in outdoor studies, the black globe temperature describes thermal environment better than the dry-bulb temperature. Based on the environmental and animal-based parameters, we concluded that the hutch entrance facing towards east or north in summer has some advantages. The differences in heat load between the most and least favourable microclimates are so low that hutch positioning may address only acute heat stress effects. The following study assessed the extent to which calf hutches offer protection against intense solar radiation during the summer and whether this is improved by covering with heat-reflecting cover, shielding mesh or built-in insulated roof. Based on differences in microclimate, respiratory frequency and behavioural thermoregulation, that reflective covers and mesh shading had advantages but were less durable and needed regular maintenance. Thermally insulated roofs were considered to be most effective in terms of heat stress abatement in dairy calves.

2. Introduction and objectives

In Hungary, as in Europe and North America (Roland et al., 2016), calves are kept predominantly in unshielded, individual calf cages from birth to weaning. Free, separate calf cages consist of a hutch and a grid-bound outdoor exercise area (Ballásch, 1995). Though the hutches provide some shielding, the calves kept in these hutches are prone to the impacts of the actual weather. Cold stress and its effects are well researched in calves, lower critical temperatures are established, and management strategies in cold weather are worked out (Silva and Bittar, 2019). On the other hand, the effects of heat stress came into the front during the last decades. Although possible management practices exist to decrease the exposure of the calves to hot weather, their practical implementation on the farms is scarce. Despite the apparent animal welfare concerns of heat stress, scientific evidence about its economic effects is still missing.

The aims of this thesis therefore were:

- Collecting the current knowledge on the effects of heat stress and on the possible methods of decreasing heat load in dairy calves.
- Examining the effects of hot summer weather) during 25 years on hutch reared dairy calves on a large-scale dairy farm, where no shading or other heat abatement strategies were used.
- Finding a feasible method (body surface temperature) to monitor the heat stress level of the calves without disturbing them with rectal temperature measurements.
- Measuring the effectiveness of existing heat abatement strategies, including the simplest (changing the compass-direction of the hutches) and the most expensive (shading by roofs).

3. Literature review¹

Heat stress is one of the main challenges facing the dairy production industry. Physiological and behavioural coping mechanisms of lactating dairy cows are well documented (Polsky and von Keyserlingk, 2017). However, the thermal status of hutch reared calves receives less attention from a scientific (Roland et al., 2016) and even less so from a management standpoint. This literature review aims to gather current knowledge about the effects of heat stress and the methods of heat alleviation in preweaned Holstein Friesian dairy calves. Biological and environmental indicators of heat stress and methods of heat abatement are discussed.

Indicators of heat stress in the prenatal period

There is growing evidence that the uterine environment of dry cows can convey an indirect effect of environmental stress and evoke adaptive mechanisms in the calf foetus. Signs of adaptation are present also in the postnatal period, which lead to the concept often called 'foetal programming'. Earlier studies have observed that sensitivity to thermal stress is higher in periods of reproduction and neonatal life as compared to other phases of the life cycle (Collier et al., 1982). Effects of maternal heat stress on the growing foetus have been extensively studied by researchers at the Calf Unit of the University of Florida (Gainesville, USA). In the past years, the adaptive responses of the foetus have been elucidated in more detail.

Lower birth weight and adult height

Foetal growth is compromised due to hyperthermia-induced placental insufficiency. Reduced placenta size and function limit the maternal-foetal exchange of oxygen and nutrients. Even a few days shortening of gestation length, which often occurs in times of heat stress (Dahl et al., 2016), shortens the period of rapid fetal growth resulting in reduced birth weight. Calves born from dams exposed to heat stress had lower weaning weight than calves from cooled dams. However, pre-weaning weight gain and body weight in the

1 Bakony & Jurkovich: Journal of Dairy Research, 2020. 87(S1): 53-59.

prepubertal period were not different (Tao et al., 2012; Monteiro et al., 2014). Despite the postpubertal rebound in weight gain, the adult height of calves born from heat-stressed dams did not reach that of calves born from dams cooled in the dry period (Monteiro et al., 2014).

Metabolic shift

Heat stress impairs not only the uterine supply of nutrients but also the heat exchange between the dam and the foetus. The foetus has double the metabolic rate as the mother, that is why a narrower temperature gradient can result in foetal hyperthermia. As seen in the sheep model, the foetus can develop adaptive mechanisms at the expense of growth. These include reduced protein accretion in favour of hepatic gluconeogenesis as well as an increased level of catabolic and reduced level of anabolic hormones. The same diet has induced higher insulin concentrations in calves born to heat-stressed dams than those born to cooled cows. The increased insulin response suggests a carryover effect of maternal heat stress (Tao and Dahl, 2013). Calves born from cows not cooled in the dry period showed similar pancreatic insulin sensitivity and systemic insulin clearance at weaning age to that of calves born from cows cooled in the dry period, but a more rapid glucose clearance during both a glucose tolerance test and an insulin challenge (Tao et al., 2014). Dahl et al. (2016) concluded that calves experiencing heat stress in utero are prone to develop a smaller mature body size and more fat reserves than counterparts in thermoneutrality.

Impaired immune function

In the first 28 days of life, serum IgG concentrations and apparent efficiency of IgG absorption were lower in calves born from heat-stressed dams relative to calves born from cooled dams. Heat stress in late gestation has no evident effect on IgG content of colostrum. It suggests that impaired IgG absorption is presumably due to the deficiency of passive transfer (Tao et al., 2012; Monteiro et al., 2014). However, acute brief heat stress during late gestation did not alter passive antibody transfer capacity in calves (Strong et al., 2015). The proliferation rate of mononuclear cells was lower in calves born from heat-stressed dams than the offspring of cooled dams. However, antibody production in an ovalbumin challenge at 28 days of age was similar in both groups. Both humoral immune response and cell-mediated immune function seem to be altered by heat stress.

Remarks

The key findings of research studies on heat stress in utero bring deserved attention to dry cow management and urge active cooling throughout the nonlactating period. Given that environmental stressors can induce the compensatory hypervascularization of the uterine horn and the alteration of ovarian activity (Collier et al., 1982), further studies are needed to distinguish the maternal and foetal components of hyperthermia-induced intrauterine growth retardation. The metabolic shift in calves heat-stressed in utero makes them prone to preserve energy and acquire less lean tissue. Such a phenomenon is also observed in heat-stressed lactating cows, where increased insulin action is linked to the ‘leaky gut’ syndrome. It is worth investigating in which ways are the two mechanisms different. A clear distinction between the effects of prenatal and perinatal stress, if possible, would also contribute to improved newborn calf management practices.

Indicators of heat stress in the postnatal period

Just as subtle differences in the uterine environment of cooled and noncooled pregnant cows can induce prolonged effects in the calf foetus, severe heat load experienced after birth may also affect performance in the rearing period. However, the term heat stress is used quite loosely. Accurately assessing the amount of strain environmental conditions impose on dairy calves is challenging. As opposed to dairy cows, no clearly defined thresholds of biological or environmental indicators are commonly accepted for dairy calves that would reliably pinpoint the onset of production losses and thus necessitate cooling interventions. The animal-based indices of assessing thermal status proposed in the literature are discussed below.

Acute stress response parameters

Heart rate variability analysis confirmed that calves exposed to solar radiation had a higher sympathetic tone than shaded calves (Kovács et al., 2018c). Endocrine changes also suggest an increased level of stress due to heat exposure. In a study on preweaned calves exposed to heat load, salivary and plasma cortisol concentrations were elevated, indicating an increased

level of stress (López et al., 2018; Kovács et al., 2019). Plasma concentrations of thyroid hormones T3 and T4 were lower in heat stress (López et al., 2018).

Behavioural responses

Altered behaviour is the first sign of thermal discomfort. Calves seek shade, change posture, move less during the hottest hours of the day and bunch to provide shade for each other (Roland et al., 2016). The frequency of changing posture is reduced in hot conditions as a sign of discomfort (Kovács et al., 2018a), similarly to cows (Allen et al., 2015). Hutch vs pen preference or lying vs standing provides valuable information about the heat-absorbing nature of hutch material or thermal conductive properties of the bedding.

Increased respiratory rate

Increased respiratory frequency promotes evaporative heat loss. Textbooks, publications and online guides describe rates of 20-40 to even 50-70 breaths/min as physiological (Rosenberg, 1979; Piccione et al., 2003). Studies on adaptive responses of calves in neutral/shaded vs hot/noncooled thermal environments have reported an approx. 50% increase in average respiratory rates as a sign of increased evaporative cooling efforts (from 47 to 53 [Lima et al., 2013], from 50-78 to 73-105 [Peña et al., 2016] or from 30-50 to 70-140 [Kovács et al., 2018b]). Heavier breathing is induced by an increase in ambient and, consequently, body surface temperature. The elevation of respiratory frequency thus precedes the rise in core body temperature, which must be taken into account when assessing heat stress status.

Elevated rectal temperature

In thermoneutrality, mammals can maintain their physiological body temperature without increased efforts of heat dissipation or heat production. Most sources consider 38,5 – 39,1(39,5) °C as the range of healthy body temperature in calves (Rosenberg, 1979; Piccione et al., 2003). The study of Piccione et al. (2003) has demonstrated that calves in the first 60 days of life acquire a steady daily rhythm of body temperature changes, with an average of 38.3 and an excursion of 1.4 °C. The maximum of body temperature of dairy calves was shown to be on average 39.0 °C. Consistently, studies on calves exposed to high ambient

temperatures report on maximal body temperatures of 39,7 °C (Lima et al., 2013), 40,1 °C (Peña et al., 2016), 40,4 °C (Kovács et al., 2018b), and 39,8 °C (Hill et al., 2016).

Water consumption

Water requirement is elevated in hot weather (Broucek et al., 2009), as calves may lose water via increased respiration and sweating. As the ambient temperature rises from 0 to 35 °C, water intake increases almost 4-fold, from 1.4 L/day to around 4 L/day, in addition to the amount of milk replacer (Quigley, 2001). Making sure each calf is aware that water is available is crucial in preventing dehydration. Moreover, Wiedmeier et al. (2006) showed that increased frequency of changing and rinsing water buckets resulted in a higher average daily gain in the preweaning period.

Early mortality

The biological cost of adaptation to prolonged heat exposure can impact calf welfare and the profitability of rearing. High ambient temperature, especially in calves housed outdoors, proved to be a risk factor for early calf mortality in veal calves (Renaud et al., 2018). Extreme heatwaves can cause excess death of different cattle subpopulations, including dairy calves (Morignat et al., 2014). There is also evidence that mortality of hutch-housed calves increases in the summer months (Martin et al., 1975; Stull et al., 2008). Research data are, however, inconsistent, as Mellado et al. (2014) showed mortality of 1-21 day old Holstein calves to be higher in moderate conditions than in the hot season (Mellado et al., 2014). Others also found calf mortality not to be associated with hot weather (Wells et al., 1997; Urie et al., 2018). The term 'early mortality' is rather unspecific, though. Death occurring in the preweaning period has multiple causes. Further data on the prevalence of different causes of death could highlight areas that need more attention in periods of hot weather.

Reduced feed intake and weight gain

The small number of studies on seasonal effects of growth in dairy calves all agree on a lower average daily weight gain in seasons with higher ambient temperature (Donovan et al., 1998; Broucek et al., 2009; López et al., 2018). Stress, including heat stress, has a biological cost, namely, the amount of energy shifted away from growth or production to

adaptative mechanisms (Moberg, 2000). Indeed, the average daily preweaning weight gain of hutch-reared calves was shown to be lower in seasons with high average temperatures (López et al., 2018). Prolonged inactivity and discomfort decrease starter intake (Bateman et al., 2012; Holt, 2014), which is held accountable for the reduced growth rate in the hottest periods of the year, rather than the consequences of maternal heat stress. Given that dry cow heat abatement is an overlooked area in dairy farming, it is tempting to speculate that prenatal heat stress effects possibly mask postnatal heat stress responses. However, it was shown that postnatal thermal conditions (cooling vs no cooling) dominate calf welfare and performance in the preweaning period, irrespective of prenatal thermal status (cooled vs not cooled dams) (Dado-Senn et al., 2020).

Remarks

Heat stress affects many physiological and production indicators in calves. It is essential to determine which of these is considered valid for defining heat stress. Animal-based indices are the primary measures of animal welfare. The physiological thresholds (if not already available) for defining heat stress should be determined. Production indicators are also relevant; however, the authors believe that importance is only secondary to animal-based indicators. We believe that the principles of animal welfare should prevail and that this will be economical in the long term.

Thermoneutral zone and measurements of environmental heat load

Heat stress abatement of hutch-reared dairy calves is largely ignored in dairy management. However, maintaining constant body temperature in conditions of high ambient temperature, intense solar radiation and high relative humidity is not possible without expending extra energy. Knowing the factors that affect the thermoregulation of the calf promotes housing and environmental modifications that could save energy for growth and health.

Thermoneutral zone not clearly defined

There is far more information on the effects of cold on welfare than on the upper critical temperature of dairy calves. Several different ambient temperatures were reported as set

points of increased evaporative heat dissipation. Gebremedhin et al. (1981) observed increased respiration as temperature exceeded 20 °C. Other researchers agreed on 26 °C as the upper critical temperature of preweaned calves (Spain and Spiers, 1996; Holt, 2014; Collier et al., 2019). Neuwirth et al. (1979) observed the first signs of heat stress at 32°C, 60% relative humidity.

Environmental indicators of heat stress

The dry-bulb temperature (DBT, also termed ‘ambient temperature’ or ‘air temperature’) is accepted to be the sole reliable indicator of the thermal environment of calves in most heat stress studies. In dairy cows, the effect of relative humidity on heat dissipation capacity is well documented, and that knowledge has been incorporated in the temperature-humidity index (THI), the weighted estimator of environmental heat load. It shows a strong correlation to biomarkers of heat stress (Bouraoui et al., 2002; Dikmen and Hansen, 2009; Bernabucci et al., 2010). Attempts have been made to adopt the THI in calf studies (Pena et al., 2016; Manriquez et al., 2018); however, its reliability is limited. Little is known about how relative humidity affects the heat dissipation of dairy calves. THI formulas and thresholds were initially developed for humans and later used in animal studies, mainly in lactating dairy cows in stabled environments. Currently, several different equations exist for calculating the THI (Bohmanova et al. 2007). We have no accurate knowledge on how relative humidity affects the thermal perception of preweaning calves, therefore what weighting factor should be given. Second, it was earlier shown that ambient temperature shows a stronger correlation to the heat stress response of dairy calves than the most used THIs for the assessment of thermal stress in preweaning calves (Kovács et al., 2018b).

Quantifying radiant heat

In outdoor conditions, radiant heat and wind speed are determining factors in the operative temperature (**Picture 1**), that is, the temperature perceived by the animal. The black globe temperature (BGT) is commonly used to measure how thermal radiation modifies the sensible heat content of the environment. It is measured by a dry bulb thermometer placed in the centre of a dark-coloured hollow metal sphere, and thereby the measured temperature integrates the amount of radiant heat absorbed by the shell. In conditions where the latent heat content of the animal's environment (in particular, solar radiation) is expected to modify

total heat exchange, the BGT is advised to be used instead of ambient temperature (Hahn et al. 2009). The THI incorrectly estimates the environmental heat load in hutch reared calves, as it does not incorporate the radiant temperature and airspeed. The use of complex environmental indices was proposed for outdoor measurements by several reports (Gaughan et al., 2008; Mader et al., 2010; Hammami et al., 2013); however, it has not yet been adopted in studies on dairy calves.



Picture 1 Thermal energy exchange between the animal and the outside unbuffered environment (after DeShazer et al., 2009)

Remarks

The environmental thresholds to decide between thermoneutrality or heat stress in dairy calves have not been sufficiently defined. Adequate definitions and limits are needed to judge the real effects of heat stress and to measure the efficiency of heat stress control.

Techniques to decrease heat load in calves

From birth until weaning, most calves are kept outdoors, in individual hutches with small, fenced exercise pens. In summer, the microclimate of polyethylene hutches, even if placed under shade, is worse than that of plywood hutches (Lammers et al., 1996; Peña et al., 2016).

Rectal temperature and respiration rates were higher in calves housed in plastic hutches as compared to plywood, however, no differences in weight gain or general health status were observed (Lammers et al., 1996; Peña et al., 2016). The practicality of durable and more hygienic plastic and fibreglass hutches make them the most popular type of housing for outdoor reared calves worldwide. Plastic hutches provide reasonably good protection against cold but offer little protection against the heat load of direct solar radiation. Solar radiation on the ground level is influenced by the movement of the sun. It resulted in greater solar radiation in the north-south orientation as opposed to the east-west in a greenhouse tunnel study (Wang and Boulard, 2000). Solar irradiation and the incidence angle can increase the temperature of all kinds of materials, including metals (Kordun, 2015), wood (Castenmiller, 2004), glass or plastic (Santos and Roriz, 2012; Wong and Eames, 2015). The thermal conductivity of certain types of artificial polymers is so high that it makes them available to substitute for metal in solar collectors (Ariyawiriyanan et al., 2013). Of course, plastics used for blow moulding are not thermoplastics; however, there is great variability in their thermal conductivity (Yang, 2007). The thermal efficiency of plastics is influenced by the solar incidence angle, achieving the highest efficiency when the panel is oriented to the south and tilted at a low angle (Ariyawiriyanan et al., 2013). The thermal properties of the plastic used during the manufacturing of hutches are improving, however, it is still necessary to reduce heat load and heat absorption of plastic and fibreglass hutches in summer using additional shade (Andrews and Davison, 2002).

Increasing airflow

Increasing airspeed could help heat dissipation of the calves. Elevation of the rear side of the hutches is showed to increase airspeed and decrease CO₂ concentration inside the hutch, making it apparently more comfortable for the indwelling calf (44 vs 58 breaths/min, compared to the control) (Moore et al., 2012). The specific design of hutches to maximize ventilation – including ridge-top vents and adjustable vent doors – are practical alternatives to labour-intensive manipulation of hutches. The use of fans provides a favourable microclimate (Hill et al., 2011; Dado-Senn et al., 2020), but this is limited to indoor conditions and thus not widespread.

Orientation of hutches

The orientation of calf hutches affected the inner microclimate and, consequently, the heat load of calves on sunny days (Bakony et al., 2019, see also chapter 4.3). Respiration rate was elevated in all four groups, being highest in the south and west-facing hutches. The probabilities of a calf lying, being inside the hutch or seeking shade at the time of observation, respectively, were highest in the group of hutches facing south. The level of heat stress in south-facing hutches is attributed to exposure to the most intense solar radiation and the least amount of shade during the day. Individual calf hutches should be positioned to face north or east in the summer period. Oriented alignment of calf hutches could serve as a no-cost measure of improving calf welfare.

Reflective covers

Friend et al. (2014) tested different radiant barriers. The silver painting was practically ineffective, while laminates and aluminized plastic covers decreased black globe temperature by 2-4 °C in empty hutches. Carter et al. (2014) found that reflective covers provided a more favourable inner climate at low and high ambient THI. The increase in respiration rate and ear canal temperature of the calves, relative to THI, were moderate in insulated hutches. Average daily gain did not differ between calves housed in covered or uncovered hutches. Other studies doubt the advantages of reflective covers. Manriquez et al. (2018) found that average THI and ambient temperature were higher (68.6 vs 67.6, and 23.2 vs 22.8 °C, respectively) in the hutches covered with aluminized plastic material. However, rectal temperature and respiratory rate were not different in control and experimental calves. The authors supposed that reflective covers impede the cooling of the hutch material in the evening hours.

Shading structures

Shading is evidentially more effective in decreasing exposure to solar radiation than reflective covers. Shading reduces the temperature both inside and outside the hutch (Coleman et al., 1996; Spain and Spiers, 1996; Gu et al., 2016; Kovács et al., 2019). The respiratory rate of calves is usually lower under shade (Spain and Spiers, 1996; Gu et al., 2016), and calves spend more time lying in shaded areas (Gu et al., 2016). Shading also

provides more comfortable conditions for caretakers in all seasons (Coleman et al., 1996). In practice, greenhouse shade nets (80-85% shade rate) installed at the height of 2m are one way of providing shade (Coleman et al., 1996; Spain and Spiers, 1996; Kovács et al., 2019). Thatch shading or well-grown trees can also be effective (Kamal et al., 2014). A built roof is a bigger investment for dairy operations; however, it offers protection from precipitation and build-up of radiant heat while maintaining adequate airflow. Calf mortality rates were observed to drop after the installation of a roof (personal observation).

Remarks

Of the techniques used to reduce the heat load, shading is the most effective, however, not widespread. The low-cost measures are flexible but rarely hard-wearing. A permanent solution is, still, a more considerable financial investment that requires proven results on the favourable effects. In the authors' opinion, roof construction is certainly a desirable goal in protecting the calves against solar radiation. Longitudinal studies on the impacts of shading would support the economic feasibility of roof installations.

Nutritional management in heat stress

We have shown that increased energy demands of heat dissipation coupled with reduced starter intake often result in the reduced growth rate of calves in the summer months. In support of weight gain, increasing the plane of nutrition or using different feed additives are the main strategies for nutritional interventions.

Preference for liquid feed and water

Considering that calves prefer liquid feed over solids in hot weather, a promising approach is to increase the energy content of milk replacer. Increased feeding rate of milk replacer (0.66 kg vs 0.44 kg dry matter/day, 21% crude protein, 21% fat) increased average daily gain and hip width in calves raised in summer (Hill et al.; 2012). An accelerated milk replacer feeding program (0.66 or 0.77 kg dry matter of milk replacer daily, 26% crude protein, 17% fat) improved energy intake and weight gain in 3-56 day-old calves during summer (Orellana Rivas et al., 2020). Increasing dietary fat content of the milk replacer from 10 to 20 %

(besides 20% crude protein) yielded higher body weight body size of weaned calves (Blair, 2015). Adding water to dry calf starter was also shown to increase palatability. Starter intake and average daily gain of calves increased when feeding 75% or 50% dry matter vs 90% dry matter diets (Beiranvand et al., 2015). As mentioned in an earlier chapter, merely the regular provision of fresh, clean water yielded higher weight gain in hutch-reared calves (Wiedmeier et al., 2006)

Vitamins, minerals, yeasts

Trials on the use of different feed additives yielded varying results. Supplementation with a general health-promoting feed additive (CalfBoost®) containing fat-soluble and B-vitamins, omega 3 and 6 fatty acids, electrolytes, calcium, magnesium, and selenium did not provide an additional benefit to summer reared calves (Blair, 2015). A combination of vitamins A and E and microelements increased growth performance post-weaning, enhanced immune functions and antioxidant capacity (Bordignon et al., 2019). An exciting approach was adding *Saccharomyces boulardii* to milk replacer to calves between 1-28 days of age (Lee et al., 2019). It resulted in higher dry matter intake and improved gut health during thermoneutrality and higher dry matter intake, but lower rectal temperature and cortisol levels in experimental heat stress than that of untreated controls (Lee et al., 2019). The authors explained the results with the balancing effect of yeast supplementation on intestinal flora that lowered lipopolysaccharide absorption due to the leaky gut syndrome in times of heat stress.

Similarly to dairy cows, the effects of chromium supplementation was also studied in dairy calves. It is known to potentiate insulin action and enhance glucose metabolism. In the study of Kargar et al. (2018), oral supplementation with a chromium-methionine complex in a dose of 0.05 mg/ kg body weight was associated with greater meal sizes and longer meal durations. The respiratory rates of supplemented calves were lower than that of unsupplemented calves in high ambient temperatures. Overall average daily gain and body weight at weaning was higher in Cr supplemented calves; however, the difference gradually diminished after weaning.

Remarks

Strategies for nutritional alleviation of heat stress are, at present, not numerous in calves, contrary to that in dairy cows. However, results are encouraging since certain cost-effective adjustments in the feeding regime can promote appetite or provide extra energy in times of increased maintenance requirements. While, in dairy cows, nutritional interventions can only be secondary to adequate cooling technologies, seasonal adjustments in the feeding protocol are advisable in calf rearing. The physiological chromium requirements of ruminants are not precisely known. Thus, the growth-promoting effects of chromium supplementation can partially be due to satisfying by chromium-deficiency of control animals; however, this hypothesis needs further testing. Despite the promising results, trivalent chromium is not authorized as a feed additive in the European Union, based on the scientific opinion of the European Food Safety Authority (EFSA, 2009).

4. Own examinations

4.1. Seasonal pattern in the incidence rate of preweaning calf mortality in a large-scale Hungarian dairy herd: a retrospective study of 25 years²

Objectives

In view of contradicting results of whether mortality rate is a valid indicator of heat stress in dairy calves, we aimed to investigate in a retrospective study how mortality rates of preweaning calves were influenced by season on a commercial dairy farm. We hypothesized that season (calendar month) is in association with the preweaning mortality rate. We tried to quantify the differences in mortality rates between periods of presumed heat stress and thermoneutrality. We have narrowed down our investigation to a single industrial-scale dairy farm, to hold the management factors which could also influence calf mortality (Mee et al., 2013; Santman-Berends et al., 2014) as constant as possible.

Materials and methods

The farm management data of Enyingi Agricultural Ltd. (Kiscséripuszta, Hungary, 47°02'12.5"N 18°21'30.1"E) from 1991 to 2015 were used in the analysis. The farm had an average animal population of 1500-1800 Holstein Friesian cows and their offspring in the studied period. The calves were housed in individual wooden hutches with slate roofs from birth until weaning (around 60 days of age). The location and the type of calf hutches did not change throughout the study period. At the time of necessary repurchase of new calf hutches, the same type of hutches were purchased. The inevitable changes that occurred in the vaccination protocol, feeding regime and milk replacer throughout the study period were considered not to be related to season or weather that would interfere with the effect of temperature conditions on mortality. In the studied period, 46,899 calves were born, out of which 2,155 died at the farm before weaning. The mortality rate in the preweaning period is age-specific (Santman-Berends et al., 2019). High temperatures may be particularly challenging for newborn calves due to their immature thermoregulation and innate immunity

2 Bakony et al.: Scientific Reports, under review

(Hulbert and Moisés, 2016; Dahl et al., 2020). Therefore, we distinguished between the age groups of 0-14 days and 15-60 days (Santman-Berends et al., 2019). We collected meteorology data from the National Centers for Environmental Information (Asheville, NC, USA; <https://www1.ncdc.noaa.gov/pub/data/g sod/>), using the data from the Hungarian Meteorological Service station nearest to the farm (Siófok, Hungary, 46°54'35.1"N 18°02'41.2"E). Weather data included daily mean, minimum and maximum of hourly dry bulb temperature measurements. First, we compared average daily mortality rates in each calendar month between the 0-14 days age group and the 15-60 days age group. The number of births and the number of deaths were available for each day from 1 Jan 1991 to 31 Dec 2015. Stillbirths and deaths occurring within 24 hours after birth are also included in the mortality data. Since the farm population was an open population, that is, the number of animals varied day by day due to births, deaths or sales, we defined the number of calves at risk for a given period via calf-days (Stevenson, 2008). According to this, the average daily mortality rate in a given period was calculated by dividing the number of deaths by the sum of calf-days in that period. In the first analysis, we calculated the average daily mortality rates for calendar months and applied the chi-squared test to compare the annual distribution of mortality in the two age groups (0 to 14 vs 15 to 60 days). The adjusted standardized residuals were computed to explore which months contributed most to the difference (MacDonald and Gardner, 2000). The p-values were corrected for multiplicity by the Bonferroni-Holm method (Holm, 1979). Second, we determined the average mortality rates of the first age group (0-14 days) in periods of heat stress and thermoneutral periods and compared them by Fisher's exact test. For this purpose, the study period was divided into consecutive 3-day blocks, and those in which the mean temperature was at least 22 °C on each day were considered heat stress periods. Blocks with the mean temperature between 5-18°C on each day served as reference. For comparison, we repeated the analysis with 4-day and 5-day periods and with temperature thresholds 23, 24, 25 and 26 °C. Statistical computation was carried out by R 3.5.2 (R Core Team, 2019).

Results

In the studied 25-year period, the average daily mortality rate of calves younger than two months was 9.64 per ten thousand, exhibiting elevated mortality rates in the winter and summer months (**Table 1**). The mortality risk ratio of the age group 0 to 14 days compared

to the rest (15 to 60 days) was above 2 throughout the year (**Table 1**). It was highest in July (6.92), the hottest month in Hungary, and lowest in January (2.37). Monthly average, maximum and minimum temperatures and cumulative incidence of calf deaths in 0-14 day and 15-60 day age groups are summed up in **Figure 1**. A significant difference was found between the monthly cumulative incidence numbers in the two age groups by a chi-square homogeneity test ($p < 0.0001$). The Bonferroni-Holm-corrected adjusted standardized residuals detected the difference as significant in 3 months, namely in the coldest (January, February; $p < 0.001$) and hottest (July, $p = 0.0018$) month of the year (**Figure 1**). In accordance with the mortality rates, cumulative incidence proved to be highest in July (165) and August (177) in the age group of 0 to 14 days (**Figure 1**). In contrast, it was highest in the winter months among older calves.

The average mortality risk and odds ratios in the 0-14 day age group are displayed in **Table 2**, along with the defining parameters. The risk ratios were calculated by dividing the mortality rate in the risk period with the mortality rate in the reference periods, thereby informing about the effect of heat stress periods. The mortality risk in the risk periods was at least twice as high as in the reference periods, as shown by risk ratios around 2 (see **Table 1**, column “Risk ratio”). With a daily mean temperature of 25°C or more (heatwaves), the risks were three times as high as in the reference period. Varying the length of the reference and risk periods did not substantially change the calculated measures of association.

Table 1 Average daily mortality rate of calves (per ten thousand) around the year in total and by age groups

Age group	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
0-60 days	12.4	12.5	9.4	7.2	6.8	8.7	11.1	11.9	9.5	8.4	7.1	10.7
0 to 14 days	20.7	22.1	20.3	16.2	15.9	17.5	26.3	28.5	22.3	19.6	16.2	21.8
15 to 60 days	8.7	8.5	5	3.4	2.5	4.5	3.8	4.6	4.2	3.6	2.9	5.7
^a RR _{inc.rate}	2.37	2.60	4.06	4.76	6.36	3.88	6.92	6.19	5.31	5.44	5.58	3.82

^aRisk ratio (RR_{inc.rate}) is the ratio of the incidence rate of the 1st age group divided by that of the 2nd one.

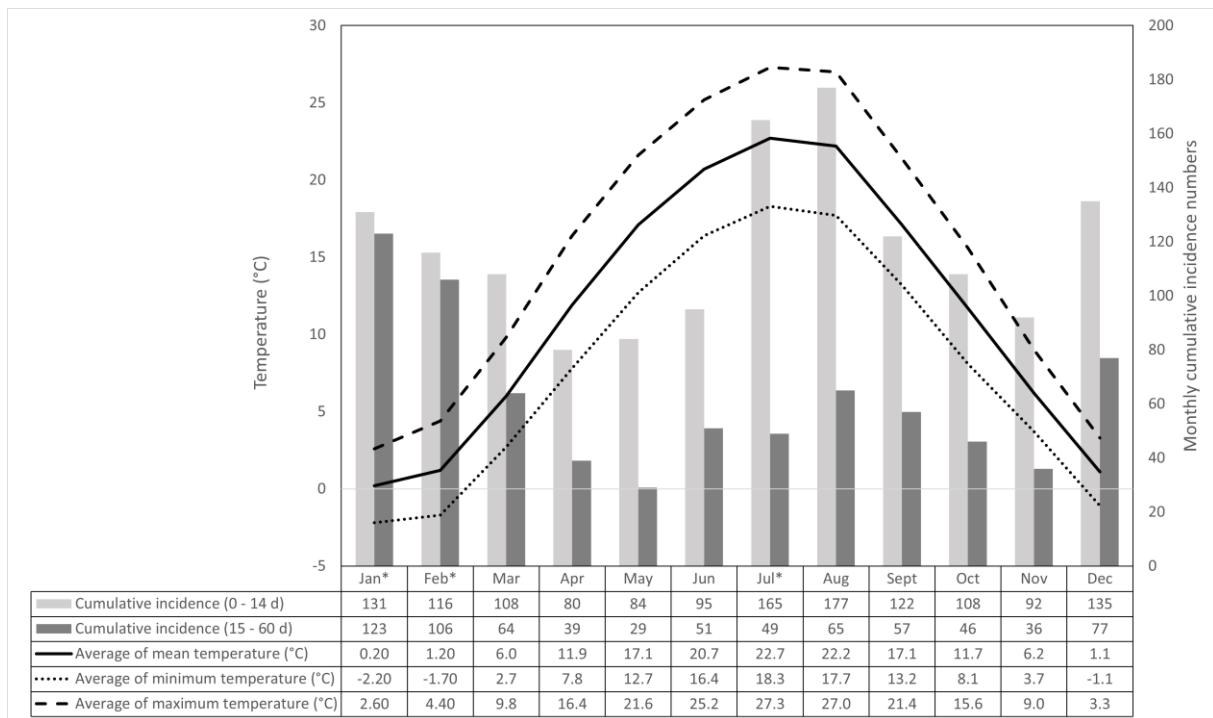


Figure 1 Monthly cumulative incidence number of calf deaths in the two age groups and monthly averages of mean, minimum and maximum ambient temperatures. Asterisks indicate months that contributed significantly to the difference in the annual distribution

Table 2 Reference and heat stress periods and risk ratios

Length (days)	No. of reference periods ¹ (No. of days in ref. periods)	Mortality risk in the reference periods (per 1000 calf-days)	Minimum daily mean temperature in the risk periods (°C)	No. of risk periods (No. of days in risk periods)	Risk Ratio	Odds Ratio	Odds Ratio Confidence interval	p-value
3	943 (2829)	1.76	22	248 (744)	2.07	2.07	1.74; 2.46	< 0.0001
			23	165 (495)	2.32	2.33	1.92; 2.81	
			24	109 (327)	2.53	2.54	2.04; 3.15	
			25	63 (189)	3.00	3.01	2.32; 3.86	
			26	32 (96)	3.54	3.56	2.56; 4.84	
			22	152 (608)	2.05	2.05	1.69; 2.48	
4	625 (2500)	1.72	23	102 (408)	2.28	2.28	1.84; 2.81	< 0.0001
			24	64 (256)	2.65	2.66	2.08; 3.37	
			25	34 (136)	3.39	3.40	2.54; 4.49	
			26	19 (78)	3.15	3.16	2.12; 4.57	
			22	102 (510)	1.94	1.94	1.58; 2.38	
			23	64 (320)	2.20	2.21	1.75; 2.77	
5	461 (2305)	1.80	24	32 (160)	2.58	2.59	1.92; 3.44	< 0.0001
			25	15 (75)	3.50	3.52	2.43; 4.97	
			26	7 (35)	3.37	3.38	1.94; 5.52	
			22	102 (510)	1.94	1.94	1.58; 2.38	

¹Minimum and maximum daily mean temperature in the reference periods were between 5°C and 18°C.

Discussion

In Hungary, having a relatively dry continental climate, January is the coldest month with an average temperature around 0 and -1.5 °C, and July is the hottest with an average temperature above 22 °C (**Table 1**). In calves, exposure to temperatures outside the thermoneutral zone may induce thermal stress (Roland et al., 2016; Silva and Bittar, 2019). The housing and management conditions on the studied farm were similar in all seasons throughout the study period. The number of personnel taking care of the calves was equal around the year and not influenced by the holiday or vacation season. Thus, we concluded that the weather conditions affected the observed monthly changes in preweaning calf mortality rates. The highest incidence rate of calf deaths in the 0-14 days age group vs the 15-60 day old age group was observed in summer. It indicates that not only low but also too high temperatures can reduce the survival of newborn calves.

Concerning the second analysis, the annual number of risk and reference periods were even throughout the 25-year study period (data are not displayed). Therefore, we could rule out that exceptionally hot summer of a few of the years would be responsible for increased calf mortality. We did not set the aim of defining a specific threshold for heat stress. Instead, we aimed to justify the need for heat stress abatement by confirming a positive association between mortality rate and high temperatures. Compared to thermoneutrality, an average daily temperature of 22°C or above for 3-5 consecutive days was associated with a 97-107 % increase in calf mortality (Error! Reference source not found.).

Our findings are in accordance with Martin et al. (1975), who showed that ambient temperatures are among the environmental factors that increase calves' mortality rate. In a more recent study (Stull et al., 2008), the data of rendering companies showed that the relationship between mortality in the preweaning period and average temperature follows a U-shaped curve. Higher calf mortality rates were associated with an average daily average temperature above 24 °C (Stull et al., 2008). The arrival of calves to a veal facility in summer was also a risk factor for increased mortality (Renaud et al., 2018). The 2003 and 2006 heatwaves in France were associated with an increase in the mortality rate of both 0-7 day-old and 8-60 day-old dairy calves (Morignat et al., 2014).

The above authors explained the observed increases in mortality with the thermal stress that exhaust the adaptive capacity of the animal body. Heat stress can compromise both the humoral and cellular immune response across the life cycle in dairy cattle, as reviewed by

Dahl et al. (2020). Indeed, the incidence of respiratory diseases increased in the summer months (Louie et al., 2018). Lower feed intake in association with inactivity during the hottest hours of the day reduce energy intake. Increased respiration and perspiration increase water loss and may worsen the status of calves with diarrhoea.

Thermal stress challenges calves' resilience and contributes to higher susceptibility to disease and potentially death. However, it has also been reported that the first and last quarter of the year take a higher toll on calf health across US dairies (Wells et al., 1997). We found our study design not directly comparable to that of Wells et al. (1997). They investigated the average death incidence rate in the quarters of the year. Months within each quarter can have a substantially different impact on calf mortality. Furthermore, a possible interaction between the season of calf birth and the dairies' geographic region was not investigated, which could have influenced the results. Urie et al. (2018) also found that the preweaning calf mortality rate negatively correlated with the average temperature-humidity index. However, their study involved only a one-year period, in which weather events or other conditions could differ from the usual and confound the results.

High temperatures at birth usually go together with high temperatures in the last phase of gestation. In the present study, July calvings are preceded by early or midsummer. Consequently, the direct effects of hot weather on the newborn calf may be coupled with the carryover effect of maternal heat stress. Heat stress in utero can lead to intrauterine growth retardation, which adversely affects the adaptive skills of the newborn (Dahl et al., 2019; Dado-Senn et al., 2020). A reduction in gestation length (Dahl et al., 2016) also affects calf viability. Heat stress makes dry cows potentially more prone to dystocia, which increases the risk of stillbirth and death before 120 days of age (Lombard et al., 2007; Arnott et al., 2012).

4.2. The use of surface temperatures in assessing thermal status in hutch-reared dairy calves

Objectives

Increased mortality in the summer suggests that monitoring of the general health and thermal status of young calves in hot weather is crucial. As described in Chapter 3., elevated rectal temperature is one of the indicators used in assessing the thermal status of calves. However, measuring rectal temperatures requires direct contact with the animals, is time-consuming and does not allow continuous measurements. The duration of handling and restraint, type of thermometer, insertion depth and placement can all have an effect on the results. Methods of no contact thermometry and thermography have been and are currently developed (Godyń et al., 2019; Wijffels et al., 2021). Other technologies, for example, a ruminal bolus may lose efficiency over time or inaccurate in measurements due to the influence of drinking and fermentation. Intravaginal devices provide measurements in strong agreement with rectal temperatures (Suthar et al., 2013), however, it often leads to vaginal irritation. Subcutaneous implants are costly, time-consuming and invasive.

Infrared thermometry is a noninvasive technique that might serve as a simple method for body temperature detection without causing any unnecessary disturbance. Already attempts have been made to establish automated systems for temperature monitoring using infrared thermography for health control in swine (Kammersgaard et al., 2013) and cattle (Hoffmann et al., 2013; Poikalainen et al., 2012).

While contact thermometry is based on conductive heat transfer, infrared thermometry measures the emitted radiation. The temperature of the rectal cavity is integrated into the body core, whereas the surface temperature relates to the body coat, which is in constant heat exchange with the surrounding environment (DeShazer et al., 2009).

Obtaining skin temperatures are feasible without disturbing the animal and could be easily and quickly performed by the stockperson as part of routine daily observations. Therefore, we wished to investigate how informative skin temperature measurements can be in assessing the thermal status of hutch reared preweaning calves. Another aspect of knowing the surface temperatures is that it reflects the temperature of the body shell. A cooler body shell allows heat transfer from the core, while a warmer body shell reduces it, resulting in increasing core temperature. We aimed to assess the temperature gradient between the core and shell besides in relation to ambient temperatures. In summary, we aimed to assess the

usefulness of skin temperature measurements in the following aspects (controlling for shaded/unshaded conditions):

- a. strength of association between rectal and surface temperatures,
- b. the magnitude of difference between skin temperatures and rectal temperature in relation to ambient temperature,
- c. predictive value of skin temperatures in assessing the risk of hyperthermia.

Materials and methods

Animals and measurements

Measurements took place in a commercial dairy in Martonvásár, Hungary (47°17'24.3"N 18°48'46.1"E). The farm has a cow population of 1000 Holstein Friesian cows and their offspring. The calves are housed in individual fibreglass-reinforced polyester hutches outdoors from birth till weaning. The study was carried out during a 5-day period in hot August weather. Altogether 16 calves aged 6-7 weeks were chosen for the study. An agricultural mesh with 80% shielding was stretched over eight calf cages at the height of 2 m from the ground to shield the cages in their entirety, while eight others were left unshaded. Ambient temperature and relative humidity were measured with Voltcraft DL-181THP devices (Conrad Electronic SE, Hirschau, Germany) in 10 min intervals inside and outside one of the hutches in the shaded and unshaded groups during the total length of the study. The rectal temperature of the calves was measured by a digital thermometer (VT 1831, Microlife AG, Widnau, Switzerland) at an insertion depth of 8 cm every 4 hours. Surface temperatures were measured on body parts not exposed to the sun, in the same intervals as rectal temperature with an infrared thermometer with 2-point laser marking (Testo 830 T2, Testo SE & Co. KGaA, Lenzkirch, Germany). Body temperature measurements were in every case carried out outside the hutch. Measuring sites included: leg (metacarpus), nozzle, eye bulb, scapula, ear. The device was about 10-20 cm distance from the body surface during measurement. Other physiological indicators of the stress response were also measured, but they were not used in the analysis of the present study.

The temperature data (both ambient and body temperatures) of the daytime hours (8:00-20:00) of the three hottest days (daily average temperatures between 27.3 °C – 30.5 °C) were used in the analysis.

Statistical analysis

First, we have tested whether the mesh, in fact, provided a more favourable thermal environment for the calves. We did this by estimating the effect of shading on the average daily temperature and the diurnal fluctuation of the ambient temperature measured in the outdoor areas, by fitting a linear mixed model with the time of measurement (time of day: morning, noon, afternoon, evening) and shading as fixed factors and day of measurement as a random factor.

Afterwards, the aims mentioned in the introduction were achieved as follows:

- a. To assess the strength of the association between core, skin and ambient temperatures, the repeated measures correlation method (Bland and Altman, 1995a,b) was used, which accounts for repeated measurements on the same subject.
- b. The heat dissipation capacity is directly proportional to the area of a surface, its thermal conductivity and the temperature gradient between the surface and core temperature. The temperature gradient between body shell and core was estimated by calculating the difference between the rectal and skin temperatures. We aimed to compare the changes in heat dissipation capacity of the different body regions (as represented by temperatures of various sites) with increasing ambient temperature. We have investigated the effect of ambient temperature (independent variable) on the temperature gradients (dependent variable) with linear mixed models, with the calf as a random factor. The coefficients estimated for the ambient temperature inform about the degree of change in the thermal gradient between core and shell - and therefore the heat dissipation capacity - per one unit of increase in ambient temperature. This way, the regions that are most influential in the overall heat dissipation capacity can be identified.
- c. Surface temperatures of body regions identified as most informative about the heat dissipation capacity were then used to predict the risk of hyperthermia (rectal

temperature not lower than 39.5 °C, after Piccione et al., 2003). For this, the CART classification method was used. It works by splitting the data into two groups based on one of the explanatory variables (skin temperatures) to achieve maximal homogeneity of the outcome variable (risk of hyperthermia) within the two groups. Then a measure of association is determined between the explanatory variable and the outcome variable in each of the two groups. The cutting point is chosen so as the difference in association is significantly different between the two separate groups. Such splitting and measure of association are then applied recursively to each of the respective groups of observations based on another of the explanatory variables. This process is repeated a number of times, selecting different variables to split the data. The cutoff points and predictive success may provide recommended thresholds for identifying animals that need attention from the stockperson.

Results and discussion

Effect of shading on the thermal environment of calves

Table 3 displays the average temperatures measured in the morning (8:00), at noon (12:00), in the afternoon (16:00) and in the evening (20:00) in the outdoor area of the shaded and unshade groups.

The measuring site (hutch or outdoor area) did not have a significant effect on ambient temperature ($p=0.77$). As expected, average temperatures differed with the time of day ($p<0.001$), being higher at noon and in the afternoon. Shading had a significant effect ($p<0.05$) on the ambient temperature at 12:00 and 16:00.

Table 3 Temperatures measured in the outdoor area and inside the hutch at different times of day in shaded and unshaded groups (°C). Means with different letters in superscript indicate significant differences ($p<0.05$) within a column. Means with different numbers in superscript indicate significant differences ($p<0.05$) between unshaded and shaded groups at a given site.

	Outdoor area				Hutch			
	Unshaded		Shaded		Unshaded		Shaded	
	mean	sd	mean	sd	mean	sd	mean	sd
8:00	26.5 ^a	2.8	25.9 ^a	3.2	26.2 ^a	3.0	26.1 ^a	2.9
12:00	36.2 ^{b1}	3.3	35.3 ^{b2}	3.2	38.8 ^{b1}	4.2	34.7 ^{b2}	3.5
16:00	40.2 ^b	4.3	37.5 ^b	2.9	37.8 ^{b1}	2.2	35.9 ^{b2}	2.3
20:00	28.6 ^c	1.1	29.0 ^c	1.3	29.3 ^a	1.2	29.2 ^a	1.0

The average ambient temperatures in the outdoor area, as expected, differed significantly by time of day but – except for noon measurements – did not differ with the presence or absence of shading. The latter can be explained by the fact that dry bulb thermometers must be shaded, therefore, they do not measure the heat transferred by solar radiation. However, on one occasion, the thermometers in the unshaded group measured an exceedingly high value above 45°C (**Figure 2**), which is presumably a consequence of direct sunlight that could have hit the sensor at that time point, which biased the result upwards. Also, skin temperatures did not show a drastic increase that would be expected to follow such a steep increase in ambient temperature (see **Figure 2**). To account for the outlier nature of this particular value, we have performed comparisons with and without this value. Yet, the significance of differences was similar in both scenarios. Shading reduced the heat accumulation in the outdoor area and the hutch material, therefore provided significantly lower ambient temperatures measured inside the hutch in the hottest period of the days. The physiological relevance of the average 2-3°C difference is, however, questionable, as average temperatures in both the hutch were well above the accepted upper critical temperature of dairy calves (26°C). Despite the not significant / not relevant effects, we have controlled for shading during the rest of the analysis.

Rectal and skin temperatures in the unshaded and shaded groups

The animal-based temperature measurements and the ambient temperature at the time of measurements in the sunny and shaded groups are displayed in **Figure 2** and **Figure 2**.

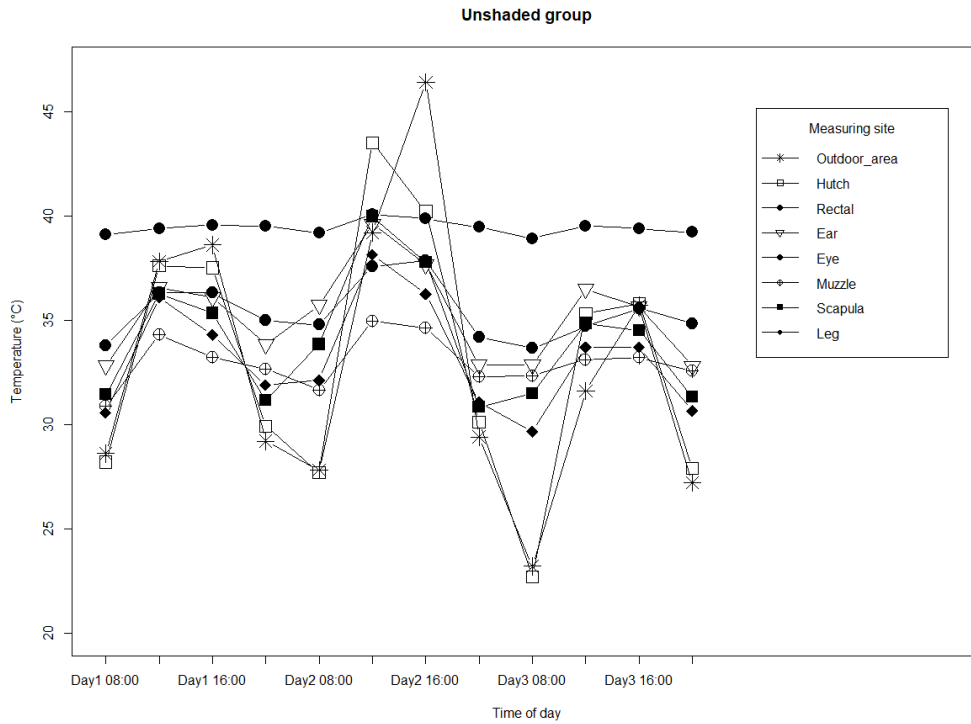


Figure 2 Dry bulb temperature in the outdoor area and body temperatures measured at different body regions during the daytime hours of the three hottest days of the study in the unshaded group.

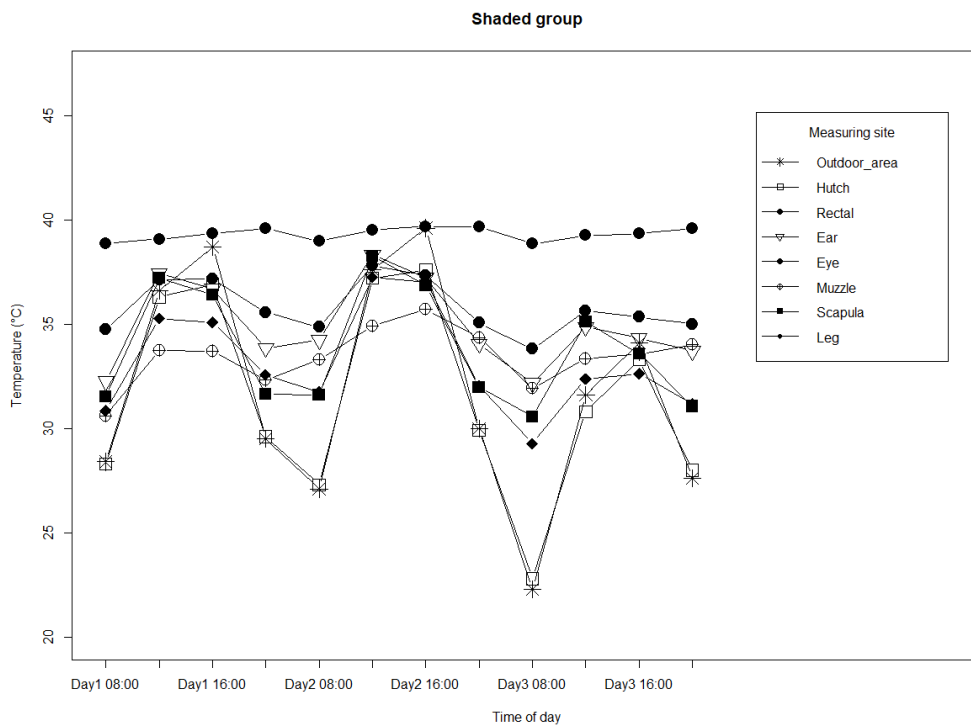


Figure 3 Dry bulb temperature in the outdoor area and body temperatures measured at different body regions during the daytime hours of the three hottest days of the study in the shaded group.

Table 4 Average ambient and body temperatures in the groups. Means with different superscripts indicate a significant difference in the same column (p<0.05)

Temperature	Unshaded group	Shaded group
Rectal	39.4 ^a +- 0.45	39.3 ^a +- 0.39
Ear	35.2 ^b +- 2.49	34.9 ^b +- 2.32
Eye	35.3 ^b +- 1.56	35.7 ^b +- 1.51
Leg	33.2 ^c +- 2.95	33.1 ^c +- 2.76
Muzzle	32.9 ^c +- 1.88	33.4 ^c +- 1.86
Scapula	34.1 ^d +- 3.15	33.8 ^d +- 2.97

Though the time points involve only the daytime hours, it still shows the diurnal rhythm of rectal temperature with higher values in the hottest parts of the day and lower values in the morning and evening hours. As compared to the study of Piccione et al. (2003), where the core body temperature of preweaning dairy calves showed an average of 38.3 °C with an amplitude of 1.4 °C, in our study, the temperature values had a similar variation, but oscillating around a mean temperature of 39.3-39.4 °C, which is 1°C higher than in the study of Piccione et al. (2003) that was conducted at lower environmental temperatures (22-28°C). It suggests that the temperature conditions both in shaded and unshaded groups imposed a severe heat load on the calves.

The temperature of the body shell, as represented by skin temperatures, show a much more significant variation, similar to ambient temperature. Understandably, as the body surface is the scene of constant heat transfer between the core and the environment, skin temperatures depend on the temperature gradient between the core and the environment. **Figure 2** and **Figure 3** display that when the difference between ambient temperature and core body temperature is higher (8:00 and 20:00), skin temperature values measured at different sites tend to scatter more widely, however, when the temperature gradient is smaller (12:00 and 16:00) they tend to show less variability. As expected, areas that are closer to the core of the body (ear and eye) show less difference from rectal temperature and show a narrower range (lower variance) as more distal regions (leg, scapula) which have a wider range (show greater variance; **Table 4**). On most measuring occasions, skin temperatures were above 35°C, limiting the efficiency of heat-flux from the core to the shell and thereby causes a disturbance in maintaining a constant body temperature (Berman, 2005). **Figure 2** and **Figure 3** shows that in the hottest hours of the day, most of the body regions had a skin temperature above 35°C, which could explain the average elevated core body temperature.

Association between temperature measures

The correlation of repeated measures between the different temperatures in unshaded and shaded environments are summed up in **Table 5**. Classification of strength based on correlation coefficients is based on Taylor (1990).

Table 5 Repeated measures correlation between temperature measures in unshaded (no fill) and shaded (grey fill) conditions. All correlations were significant ($p < 0.0001$). Correlations considered to be strong are written in bold.

Temperature	Ambient	Rectal	Ear	Muzzle	Scapula	Leg
Ambient	-	0.61	0.68	0.54	0.76	0.77
Rectal	0.36	-	0.58	0.55	0.53	0.55
Ear	0.76	0.31	-	0.52	0.82	0.81
Muzzle	0.48	0.43	0.57	-	0.52	0.66
Scapula	0.83	0.18	0.74	0.40	-	0.83
Leg	0.84	0.36	0.76	0.57	0.82	-

Surface temperatures show a stronger association to ambient temperature than to core body temperature. The explanation is that in the measured ambient temperature range, most of the animals could maintain their body temperature close to normothermia, therefore variability did not exceed that originating from the normal diurnal rhythm (Piccione et al., 2003). Indeed, a high correlation between surface temperatures and the rectal temperature could only be detected in artificially induced febrile states (George et al., 2014; Hovinen et al., 2008) or inflammation when body temperatures strongly deviate from physiological (Poikalainen et al., 2012). The correlation between surface and rectal temperatures is influenced by the surrounding environmental conditions, as it is seen from the table. Skin temperatures measured with infrared thermometry are usually significantly lower than rectal temperature (Hoffmann, 2013; George et al., 2014), and the magnitude of the difference is influenced by the thermal environment (Pusta et al., 2012).

The association between ambient temperature and the gradient between core and surface temperatures gradient between the body core and surface temperatures are displayed in **Table 6**.

Table 6 Relationship between temperature gradient between core and surface temperatures and ambient temperature in unshaded and shaded groups. Different superscripts indicate significant differences between slopes in shaded and unshaded groups. Models were fit with and without the extreme ambient temperature value of 46.4 °C.

Difference between rectal and body surface temperature	Unshaded	Shaded
Ear mean \pm SE	4.2 \pm 0.2 °C	4.4 \pm 0.3 °C
range	0.2 – 8.4 °C	0.9 – 8.2 °C
$R^2 = 0.457$		
Model fit without outlier $R^2 = 0.457$		
Muzzle mean \pm SE	6.5 \pm 0.2 °C	5.8 \pm 0.2 °C
range	3.9 – 9.0 °C	3.2 – 8.9 °C
$R^2 = 0.278$		
Model fit without outlier $R^2 = 0.266$		

Difference between rectal and body surface temperature	Unshaded	Shaded
Scapula mean \pm SE	5.4 ± 0.3 °C	5.5 ± 0.3 °C
range	-0.2 – 9.2 °C	0.6 – 9.7 °C
$R^2 = 0.569$		
Model fit without outlier $R^2 = 0.561$		
Leg mean \pm SE	6.3 ± 0.3 °C	6.2 ± 0.3 °C
range	1.4 – 10.3 °C	1.8 – 9.8 °C
$R^2 = 0.605$		
Model fit without outlier $R^2 = 0.622$		

The association between the gradient between rectal and surface temperatures during the daytime and the ambient temperature was significant ($p < 0.001$) in all measuring sites. The temperature gradient, and therefore the heat dissipation capacity through conductive heat transfer, is maximal in thermoneutral conditions and decreases with increasing ambient temperature. The average rate of decrease is reflected in the slope of the regression equations, which is an estimate of the average decrease in the temperature gradient between core and surface per 1°C increase in ambient temperature. The R^2 value stands for how much of the variance in the temperature gradients can be explained by taking the effect of ambient temperature, shading and the individual effect of calves into account. We assumed that regions with a broader range of gradient with higher sensitivity to the changes of ambient temperature and higher explanatory power are the major scenes of heat transfer. Based on the slopes of regressions and the coefficients of determination, ear, leg and scapula temperatures were considered more informative of the actual heat dissipation capacity of the animal than muzzle temperature. Unlike the other regions, the muzzle is hairless and usually wet, which promotes evaporative heat loss, which supports lower surface temperature and higher temperature gradient between its surface and the core (George et al., 2014). Heat dissipation through the muzzle is even at higher temperatures, however, the surface area of the muzzle is very small compared to the flank or limbs.

Predictive value of surface temperatures in assessing the risk of hyperthermia

In the classification algorithm, the ear, the scapula and the leg temperatures were applied as explanatory variables to classify individuals being at risk or at no risk of hyperthermia at a given time point. Referring to the study of Piccione et al. (2003) and the average rectal temperatures of the two groups, we have defined a high risk of hyperthermia as having a rectal temperature above 39.5°C. **Figure 4** displays the cutoff points in skin temperatures that best separated between animals being or not being at risk of hyperthermia. (n stands for the number of observations in the given group).

The figure shows that an ear temperature above or below 38°C and a leg temperature above or below 30.8 separates the cases with creating the greatest homogeneity in terms of the outcome variable. The bar plots depict the frequency distribution of the outcome within that node. Predictions were made by classifying the observations to each of the „nodes” on the basis of the skin temperatures, and the outcome with the higher probability in the given node was assigned to that observation. The risk of hyperthermia that was predicted this way was

compared to the risk that was assessed on the basis of the rectal temperature. The agreement with this approach was 72%. The figure shows that despite achieving fairly good homogeneity in animals with an ear temperature above 38°C or low risk of hyperthermia in animals with a leg temperature below 30.8°C, most observations (n=124) were classified in a group that was heterogenous in terms of the outcome.

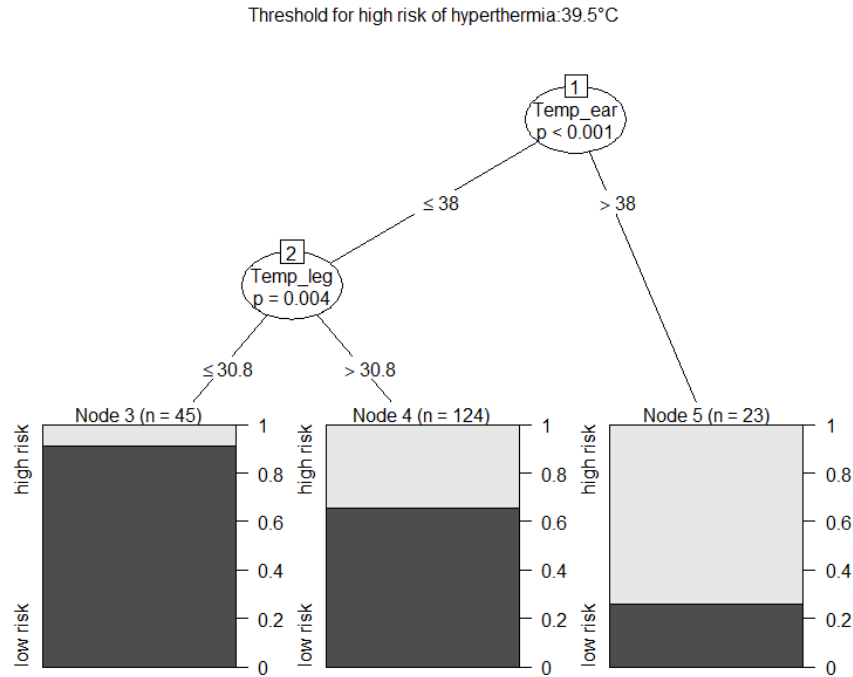


Figure 4 The output of a decision tree on classifying cases as being or not being at risk of hyperthermia (defined as a rectal temperature above 39.5 °C). Nodes 1 and 2 indicate cutoffs, Nodes 3-5 indicate grouped observations and the probability of the outcome in the given node

With a similar approach, other thresholds of the risk of hyperthermia were also defined and tested for prediction accuracy. The results are summed up in **Table 7**.

Table 7 Thresholds for defining an animal being at risk of hyperthermia at a given time point and the accuracy of predicting the risk of hyperthermia on the basis of the skin temperature cutoff obtained from a decision tree algorithm.

Rectal temperature threshold for the definition of high/low risk of hyperthermia	Skin temperature cutoff (°C) in predicting high/low risk of hyperthermia	Predictive ability of the model
39.0	Leg skin temperature > / < 31.9.	71.3 %
39.1	Leg skin temperature > / < 31.9.	69 %
39.2	Leg skin temperature > / < 31.9.	67 %
39.3	Ear skin temperature > / < 32.6 Leg skin temperature > / < 35	63%
39.4	Leg skin temperature > / < 32	59%
39.5	Ear skin temperature > / < 38.0 Leg skin temperature > / < 30.8	72%

Table 7 shows that predictive ability and skin temperature cutoffs are very sensitive to the thresholds defining hyperthermia. Accuracy of the device, measuring distance, hair depth or colour can also greatly influence the predictive ability of this approach. Another explanation of the low interpretability is that surface, and core temperatures show less agreement in clinically sound animals (Hoffmann et al., 2013). Despite the significant fluctuations seen in surface temperatures and the consequent changes in heat dissipation capacity, the duration of the exposure to high temperatures during the day did not induce large variability in the rectal temperature.

4.3. The effect of hutch orientation on primary heat stress responses in dairy calves in a continental region³

Objectives

In Chapter 3, it has been reviewed that the material of calf hutches may considerably impact the inner microclimate due to the absorbance of radiant heat. Conventional fibreglass hutches are still in use in many Hungarian dairies. We assumed that the compass direction in which the hutch entrance is facing could affect the hutch microclimate and primary heat stress responses of calves on sunny days. The compass direction of the hutch entrance also affects the availability of shaded resting areas both inside and outside the hutch. The amount of radiant heat that can accumulate in the material is the resultant of the solar incidence angle and the duration of exposure, and both can vary between hutches oriented toward different compass directions. The oriented alignment of hutches could serve as a no-cost measure for improving the thermal environment and the welfare of calves. The study aimed to monitor temperature conditions in differently oriented calf hutches and the primary behavioural and respiration response of dairy calves. We assumed that orientation has an influence on climatic conditions inside the hutches, and differences were expected to primarily occur between the east or north-facing hutches as compared to those facing south or west.

Materials and methods

Animals and measurements

For the site of the measurements, a commercial dairy farm in Beled, Hungary (47°28'09.3"N 17°04'14.6"E) was chosen due to the circumstance that the study design fitted well into the regular management practice. The animal population of the farm is around 900 Holstein Friesian cows and their offspring. Calves are kept outdoors from birth till weaning (mean: 60 days, min/max: 56/70 days) in individual fibreglass-reinforced polyester hutches (Agrobox-1; Agroplast Ltd, Gyál, Hungary; **Picture 2**) with an adjacent fenced outdoor area (henceforth 'outdoor area'), placed on pebblestone and bedded with straw both inside the

³ Bakony et al.: Animal Welfare, 2021. accepted for publication

hutch and in the outdoor area. The hutches are in an open-air area and aligned in a manner that several hutches are facing with their openings to each of the four compass directions. At the time of the investigation, most of the hutches were inhabited by young calves. Measurements were carried out on a bright, mid-August day from 7:20-19:00. We have chosen altogether 20 of the inhabited hutches, 5 of them facing north, south, east, and west, respectively. We have chosen hutches so that no buildings or trees provided shade to any of them throughout the measurement period. All studied calves were female and between 7-17 days of age. Climatic parameters (DBT, BGT, and wind speed) were recorded in 20 min intervals from 7:20-19:00 inside four empty hutches, each of them facing with its opening to east, north, south, and west, respectively. The same parameters of the outdoor area were measured at one sunlit site outside the hutches. It was representative of the outdoor area of all hutches.



Picture 2 The calf hutches on the farm



Picture 3 Kestrel cattle heat stress tracker in use

Climatic measurements were performed with Kestrel 5400AG Cattle Heat Stress Tracker (Nielsen-Kellerman Co., Boothwyn, PA, USA, **Picture 3**) in hutches facing east and west and outside the hutches, and with Testo 480 (Testo SE & Co. KgaA, Lenzkirch, Germany) in the south and north-facing hutches. (Measurement accuracy of the two types of devices were similar that allowed direct comparisons.). Thermometers inside the hutches were placed at approximately the height where the head of a lying calf would be (approx. 30-40 cm above ground). The outside thermometer was placed 1.5 m above ground in an open area without shade. In parallel with temperature measurements, we have counted the respiration rates (RR) in 20-min intervals by counting flank movements (for 30 sec and multiplying by 2) from a distance of 3.5 – 4 m to avoid disturbing the calf. At the same time, we also recorded whether the calves were inside or outside the hutch, in a lying or a standing posture and exposed to mainly sun or shade. (Location preference, body posture and exposure to sun or shade shall be collectively termed 'behavioural measures' hereinafter). The periods before feeding, when calves were alert and excited, were not involved in data collection.

Statistical analysis

To describe the temperature conditions for hutches facing different compass directions, periods of the day were distinguished, namely morning (07:20 – 11:00), midday (11:20 – 15:00) and afternoon (15:20 – 19:00). The mean temperatures were compared between compass directions and periods of the day using variance analysis.

The daily average of RR values was compared by fitting a linear mixed model with compass direction and location as the independent variables and calf as a random term. In another model, the period of the day was also included as an explanatory variable to assess time-related differences between compass directions.

To study the measure of association between temperatures and RR, a general linear mixed model was fit with RR as the dependent variable and BGT / DBT, compass direction, period of the day and their interactions as independent variables, with calf id as a random term. Model selection was based on removing non-significant terms to achieve the lowest Akaike information criterion. Behavioural measures were dichotomized and compared using generalized linear mixed models. Compass direction, the period of the day and their interaction were included as explanatory variables and calf as a random term. Multiple comparisons were made using the Bonferroni correction method. The level of significance in all tests was set to $p < 0.05$. All statistical analyses were performed in the R statistical environment (R Core Team 2019).

Results and discussion

Climatic parameters

Average wind speed was 0.07 m/s (min: 0.02 m/s, max: 0.8 m/s) in hutch environments and 0.79 m/s (min: 0 m/s, max: 1.6 m/s) in the outdoor area. In the study of Dado-Senn et al. (2020) an air velocity of 2 m/s provided active cooling for calves; thus, we regarded the wind speed in our study as not being influential on the thermal comfort of the calves. BGT and DBT at each time point are displayed in **Figure 5**.

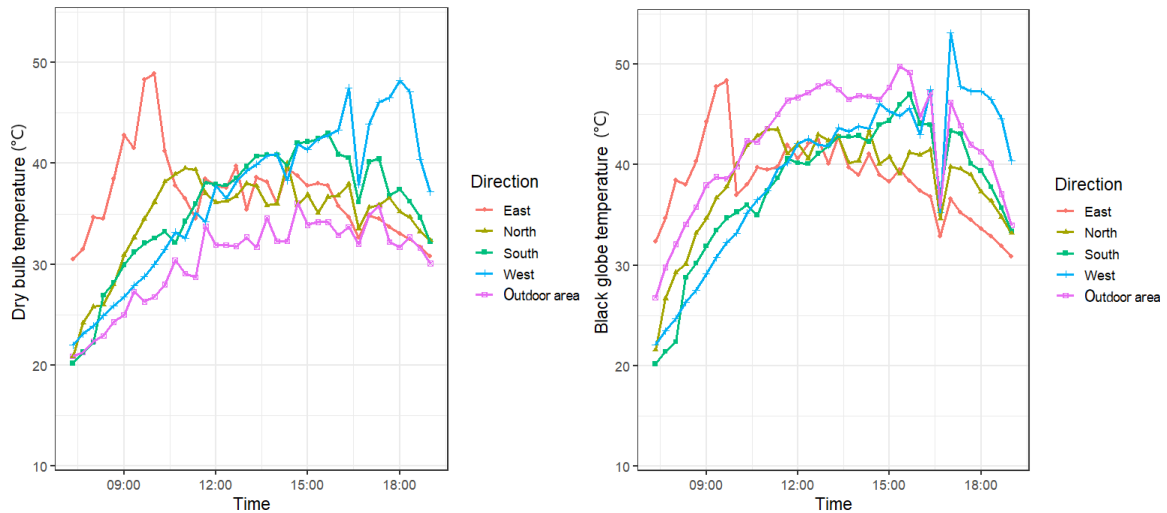


Figure 5 Temporal pattern of changes in the dry bulb and black globe temperatures (°C) measured at 20-min intervals between 7:00 and 19:00 inside hutches facing with their entrance to each of the four compass direction (East, North, South, West) and in a sunny area

In the early morning hours, both BGT and DBT moved in a similar range in all hutches. In the early afternoon hours, temperatures measured in the east and north facing hutches started to decrease. In contrast, those measured in the south- and west-facing hutches continued to increase. The separation of temperature curves suggests that the south- and west-facing hutches were exposed to greater solar radiation in the afternoon. BGT measured in the outdoor area were higher than BGT inside the hutches, except for east-facing hutches in the morning and west-facing hutches in the afternoon. Interestingly, DBT in the outdoor area were at almost all times lower than DBT measured inside the hutches.

For the comparison of heat load in total and within different periods of the day, mean, minimum and maximum of the BGT and DBT are summed up in **Table 8**. Daily mean BGT measured inside the hutches were not different from the outdoor area, except for south-facing hutches, where inside temperatures were lower. It suggests that the hutch material has a minimal mitigating effect against solar radiation. It also indicates that the direction in which the hutch entrance is facing does not influence the calves' overall daily heat load. However, when comparing temperatures at different periods of the day, significant differences were found. It suggests that though overall heat load did not differ between compass direction, its temporal distribution can vary.

Table 8 Mean \pm sd of black globe temperature (BGT, °C) and ambient temperature (DBT, °C) measured inside hutches oriented to East, North, South and West and outside in a sunlit site (Outside) and respiratory rate (RR, breaths/min) of calves (n=5 / compass direction)

Time of observation	Measure	East	North	South	West	Outside
daily	BGT	38.5 ^{ab} \pm 4.1	38.2 ^{ab} \pm 5.1	37.7 ^a \pm 6.7	39.3 ^{ab} \pm 7.9	42.1 ^b \pm 5.9
	DBT	36.9 ^a \pm 4.2	34.5 ^a \pm 4.5	35.5 ^a \pm 6.1	36.6 ^a \pm 7.5	30.3 ^b \pm 4.2
	RR - ins	97.9 \pm 22.2	96.9 \pm 29.3	107.5 \pm 30.1	108.3 \pm 28.7	
	RR - outs	77.9 \pm 19.9	80.9 \pm 21.2	91.9 \pm 25.1	85.2 \pm 26.1	
morning	BGT	39.9 ^a \pm 4.8	34.9 ^{ab} \pm 6.9	30.6 ^b \pm 6.0	29.9 ^b \pm 5.1	36.8 ^a \pm 5.2
	DBT	38.9 ^a \pm 5.9	31.3 ^b \pm 6.3	28.7 ^{bc} \pm 4.9	27.6 ^{bc} \pm 3.7	25.4 ^c \pm 3.1
	RR - ins	86.0 ^a \pm 25.9	60.7 ^b \pm 19.4	81.9 ^{ab} \pm 23.9	66.0 ^{ab} \pm 14.6	
	RR - outs	81.3 \pm 21.5	75.2 \pm 21.5	76.0 \pm 24.1	70.1 \pm 14.6	
midday	BGT	40.6 ^a \pm 1.5	41.7 ^{ab} \pm 1.3	41.8 ^{ab} \pm 1.7	42.9 ^b \pm 1.8	46.9 ^c \pm 0.8
	DBT	37.7 ^{ab} \pm 1.6	37.2 ^a \pm 1.4	39.5 ^b \pm 1.8	38.7 ^{ab} \pm 2.5	32.6 ^c \pm 1.8
	RR - ins	104.3 \pm 16.7	109.4 \pm 22.1	120.2 \pm 23.5	111.6 \pm 20.7	
	RR - outs	90.7 \pm 10.1	93.0 \pm 11.5	110.7 \pm 34.5	98.0 \pm 26.2	
afternoon	BGT	35.1 ^a \pm 2.7	38.1 ^{ab} \pm 2.8	40.9 ^{bc} \pm 4.3	45.2 ^c \pm 4.4	42.6 ^{bc} \pm 5.1
	DBT	34.2 ^a \pm 2.2	35.3 ^a \pm 1.6	38.4 ^b \pm 3.3	43.6 ^c \pm 3.7	33 ^a \pm 1.6
	RR - ins	96.6 ^a \pm 23.8	106.3 ^a \pm 22.9	117.5 ^{ab} \pm 27.1	128.2 ^{bc} \pm 19.2	
	RR - outs	74.3 \pm 19.1	74.4 \pm 25.7	99.2 \pm 20.3	94.5 \pm 28.0	

^{a,b,c} Means with different superscripts indicate significant differences within a row

In the morning period, BGT in the east-facing hutches was on average 9.5°C higher ($p < 0.0001$), and the temperature in the outdoor area was on average 6.5 °C higher ($p < 0.05$) than both in the south and west-facing hutches.

In the midday period, BGT was on average 4-6 °C higher outside than inside hutches facing all four compass directions ($p < 0.0001$). A 2.2 °C average difference was also found between east and west-facing hutches ($p < 0.01$).

In the afternoon period, the lowest BGT were measured in east-facing hutches. It was, on average, 7.5°C lower than outside ($p < 0.001$) and 5°C and 10°C lower than in the south and west-facing hutches, respectively ($p < 0.01$). Black globe temperature inside north-facing hutches was also lower than inside west-facing hutches, with an average of 7.1°C ($p < 0.001$). Temperature conditions did not differ between south-facing and west-facing hutch interiors and outside. The underlying reason for differences between the periods of the day is the daily solar incidence angle pattern. In the morning hours, the BGT sensor in the east-facing hutch was exposed to full sun. Thereby it measured the heat irradiated by the hutch material and the heat conveyed by solar radiation. Before sunset, the same is true for the sensor in the west-facing hutch in the afternoon hours. The thermometer sensors were positioned in the

head height of calves, which entails that calves in east-facing hutches have no access to shade in the morning hours. In contrast, calves in the west-facing hutches have no access to shade in the afternoon hours. On both occasions, inside temperatures exceeded the BGT measured outside in the same period.

Daily mean DBT were similar between hutches facing different compass directions. Hutch inside averages were 4.2-6.4 °C higher than the outdoor area temperature ($p < 0.01$).

In the morning, DBT in east-facing hutches was on average 7.5 °C higher than in north-facing hutches ($p < 0.01$) and on average 10-13.5°C higher than in the south- and west-facing hutches and the outdoor area, respectively ($p < 0.0001$). The 6°C difference between north-facing hutches and outdoor area temperature was also significant ($p < 0.05$).

In the midday period, temperatures between hutch interiors were not significantly different. Still, they were in all compass directions 4.5-6.8 °C higher than outside temperatures ($p < 0.0001$).

In the afternoon, temperatures in the east- and north-facing hutches and outside were not different, but lower than temperatures in south-facing hutches (3-5 °C, $p < 0.05$) and west-facing hutches (8-10 °C, $p < 0.001$), respectively. The highest temperatures were measured in east-facing hutches in the morning hours and in the west-facing hutches in the afternoon hours. The dry bulb thermometers were positioned in the calves' head height and were given no extra shielding. It means that based on the solar incidence angle, the thermometer sensor was either shielded by the hutch roof or exposed to full sun. Due to the low solar incidence angle, the dry bulb thermometer was presumably exposed to full sun in the morning hours. For the same reason, in west-facing hutches, it was exposed to full sun in the afternoon hours, which increased the DBT values. Spain and Spiers (1996) observed a similar phenomenon and excluded it from the analysis of the air temperature (DBT) values measured in sunny conditions. In case the thermometer sensor is not shielded from solar radiation, measurements of dry bulb thermometers are considerably biased (Anderson and Baumgartner, 1998). In the south- and north-facing hutches, the solar incidence angle was never as low that the hutch roof would not block the thermometer from the sun. This way, shielding was provided throughout the whole measurement period. The DBT results suggest that the east-facing and west-facing hutches provide no shade for the indwelling calf in certain hours of the day, either standing or lying. The same conclusion was drawn with respect to BGT.

In contrast to BGT, the DBT was several degrees higher inside the hutches than outside, at nearly all periods and in all compass directions. We assume that the heat irradiated by the hutch material warms up the air inside the hutch; this way, the DBT increases.

Spain and Spiers (1996) also found air temperature to be higher inside the hutch, than outside in sunny conditions. They explained it by the increased heating of the hutch material by solar radiation. However, the difference was only around 0.5 °C. In their study, the hutch material was a different type than in our study (presumable polyethylene). Also, temperatures were measured not just in the hottest part of the day but also in the early morning, which could have decreased the average difference. There are only a low number of studies that assessed hutch and outdoor area thermal environment separately. Manriquez et al. (2018) studied the effect of aluminized film cover on the microclimate of hutches and found that the DBT within the hutch was a few degrees higher than in the outside area. Both studies were performed outdoors, and their findings are in accordance with our results. It is interesting that the two environmental measures (BGT and DBT) lead to divergent results. The hutch material's conductive properties and methodological issues, like positioning and shielding of thermometers, might also contribute to contradicting findings. However, it is tempting to speculate that in outdoor conditions, the use of only DBT can be misleading. It can make the researcher think that the thermal environment is similar or even better in the sunlit outdoor area than under shade inside the hutch. Calves generally seek shade inside the hutch in hot, sunny weather, which can be hardly explained by looking at the DBT values. DBT performs well in a barn environment but is less informative in outdoor conditions (Hahn et al., 2009). Based on temperature measurements, we concluded that since BGT and DBT measurements give contradicting results, it is advised to use BGTs in outdoor studies. The excellent review of Herbut et al. (2018) depicts the development of heat-stress indices used in heat stress assessment in dairy and beef cattle. Incorporating solar radiation into the indices either directly (adjusted THI and the Comprehensive Climate Index [Mader et al. 2006 and 2010]) or in the form of the black globe temperature (the Black Globe Humidity Index [Buffington et al., 1981], the Heat Load Index [Gaughan et al., 2003]), makes them more suitable for outdoor housing conditions. Such indices might also be adapted to studies on calves.

Our hypothesis on compass direction influencing the hutch inner microclimate was not fully confirmed. Given that the average daily BGT was not different between inside and outside the hutches, we concluded that the overall daily heat load of hutches was similar between

compass directions. However, periodic comparisons showed that the distribution of heat from solar radiation during the day is very different between compass directions, which should be considered when placing calf hutches. Though measurements were carried out for a single day, we concluded that conducting measurements for several other days would not lead to different conclusions. In an earlier, week-long study, we have concluded that the daily patterns of behavioural measures and RR were similar between the days (Kovács et al., 2018a,c). The chosen day well represented a typical hot summer day in a continental region when heat stress abatement measures would be necessary.

Respiratory rate

We expected that since radiant heat accumulates over time in the material of the hutch, the period of the day is influential in the thermal environment and, consequently, the respiratory heat stress response of calves. The location of the calf was also included in the model as a controlling variable. The number of observations of calves located outside was relatively low in number (which we expected to hinder establishing statistical significance), and on such occasions, calves were mainly in the shade. Since outdoor area temperatures were only measured in sunny conditions, we do not wish to compare the difference between inside and outside RR.

As increased respiration is among the primary heat dissipation mechanisms, we expected changes in RR and temperatures to occur in parallel. Hence, we primarily focused on the differences in RR of hutch-located calves and compared them to the differences found in inside temperature conditions.

Mean RR measured throughout the observation period are shown in **Figure 6**.

RR averaged for the periods of the day and location of calves are displayed in **Table 8**.

Location was found not to modify the effect of direction on RR. However, the period of the day was found to alter the differences in RR between compass directions or locations.

The daily average of RR was elevated above the physiological range of 50-70 breaths/min (Piccione et al., 2003) in all compass directions. A higher RR shows that calves in all hutches were experiencing some level of heat stress. In most of the daytime hours, DBT was above the calves' upper critical temperature of 26 °C (Spain and Spiers, 1996; Collier et al., 2019), explaining the increased RR.

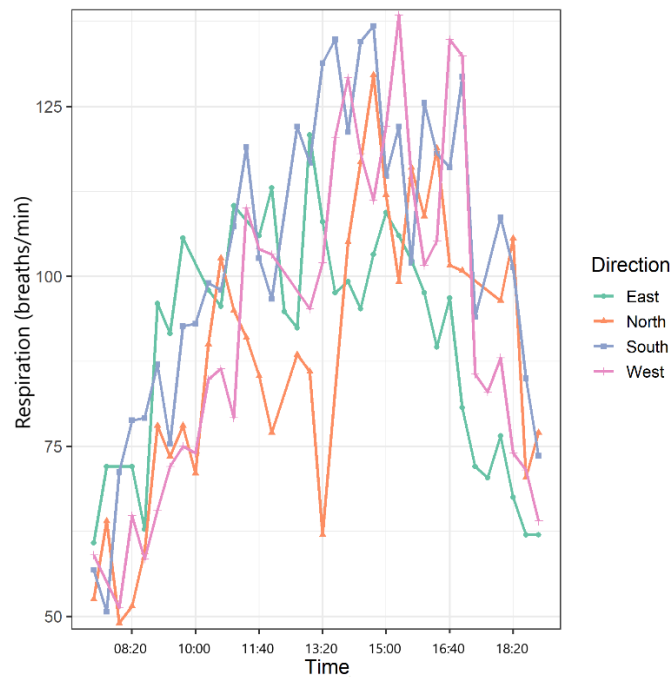


Figure 6 Mean respiratory rates of calves (n=5/direction) at different time points , located either inside or outside the hutch with respect to different compass directions.

Daily average RR did not differ significantly between calves housed in hutches facing different compass directions, located either inside the hutch or in the outdoor area. This finding is in parallel with the observations inside BGT and DBT. We assumed that the differences in RR and temperatures would reflect a similar trend and concluded that the overall heat load inside the differently oriented hutches was not different. However, the distribution of heat load throughout the day varied with the compass direction.

In the morning period (7:20-11:00), average RR was higher in east-facing hutches than in north-facing hutches (with on average 25.3 ± 8.5 breaths/min, $p < 0.01$). In the same period, BGT did not show a difference in the given relation; however, a difference of 7.5 ± 1.6 °C was observed regarding the DBT ($p < 0.01$). Presumably, due to the relatively low sample size of 5 calves per group, the numerical difference in RR between calves in east-facing hutches as compared to the south- or west-facing ones, statistical significance could not be detected.

In the midday period (11:20-15:00), the RR of calves was not different between hutches facing different compass directions. Assuming that the RR is in correlation with the level of heat load, observing no difference between compass directions is following the result of temperature comparisons. Although an average 2.3°C degree difference was found between the highest and lowest values of inside BGT and DBT, respectively, it did not induce a difference in RR.

In the afternoon period (15:20-19:00), RR was on average 39.3 ± 9.7 breaths/min and 40.4 ± 10.2 breaths/min higher inside the hutches facing west than in the north and east-facing ones, respectively. In parallel, BGT temperatures were on average $10.2 \pm 4.4^\circ\text{C}$ and $7.1 \pm 1.4^\circ\text{C}$ higher in west-facing hutches than in east-and north-facing hutches, respectively ($p < 0.01$, $p < 0.001$). DBTs were $9.4 \pm 4.6^\circ\text{C}$ and 8.3 ± 3.5 higher in west-facing hutches than in the east and north-facing hutches, respectively ($p < 0.001$).

We concluded that the difference observed in RR could be explained by the differences in the animals' thermal environment. Even the lowest measured averages were well above the RR in thermoneutrality; thus, all calves experienced some level of heat stress.

Compass direction and period of the day had no significant influence on the association between temperature and respiration. We observed that in the measured temperature range, a 10°C increase in BGT was associated with an average 23.3 ± 0.22 increase in RR (95% CI: 1.89; 2.77, $p < 0.0001$). A rise of 10°C in DBT was associated with an average 25.3 breaths/min increase in RR (95%CI: 2.09; 2.96, $p < 0.0001$). This finding is in accordance with the differences observed in RRs between compass directions in different periods of the day.

So far, studies on heat stress alleviation methods in dairy calves have not listed the targeted compass direction of hutches amongst the applied strategies. We can thus compare our results with the effect of shading as an outdoor heat abatement measure. In the study of Kovács et al. (2018c) net shading was associated with a 40/min reduction in the average RR in the hottest hours of the day. Spain and Spiers (1996) observed only a 10/min difference in the RR in the afternoon (47 vs 57 in shaded and unshaded groups, respectively) due to net shading installed above the hutch and exercise area (2.1 m above ground, 80% shading rate). However, maximal air temperatures did not exceed 38.2°C in the latter study, and temperature and RR were measured only twice a day. The magnitude of difference between mean RR of calves inside the hutches facing different compass directions did not approach the observations of the mentioned studies. We concluded that the heat stress alleviating effect of orienting hutches is overall negligible compared to that of shading, and advantages can only be achieved in the morning and the afternoon hours. However, since newborn calves are more prone to heat strokes due to immature thermoregulation and an increased risk of dehydration, even the slightest reduction in heat load can be crucial in the first days of life. In case no other heat alleviation methods are applicable, the openings of hutches should be positioned to face east or north in the summer.

Behavioural measures

The relative frequency of behavioural measures was compared between compass directions in each period of the day. Correlation within subjects was taken into account during statistical analysis. Significant differences are concisely listed in the following, coupled with the biological meaning and welfare implications.

The relative frequency of observing a calf being in the sun vs shade is displayed in **Figure 7a**. In the morning period, the probability of a calf being in shade at the time of observation (henceforth 'exposure to shade') was higher in south (odds ratio (OR): 11.1; 95% CI: 1.88; 59; $p = 0.03$) and west-facing (OR: 8.22; 95% CI: 1.42; 47.61; $p = 0.01$) hutches than in east-facing. In the midday period, exposure to shade was higher inside the hutches facing east than those facing west (OR: 8.48; 95% CI: 1.41; 51; $p = 0.01$). In the afternoon period, exposure to shade was higher in east-facing hutches than in south- and west-facing hutches (OR: 5.8; 95% CI: 1.07; 31.46; $p < 0.05$ and OR: 16.9; 95% CI: 3.39; 85.11; $p < 0.001$, respectively). Also, exposure to shade was higher in north-facing hutches than in south- and west-facing hutches (OR: 33.25; 95% CI: 1.75; 629.54; $p = 0.03$, and OR: 97.2, 95% CI: 5.36; 1763, $p < 0.001$, respectively).

Above the critical upper temperature, a shaded resting area is usually preferred over one exposed to the sun if given access (Tucker et al., 2008). Consequently, we associated greater access to shade with better welfare (Spain and Spiers 1996; Kovács et al. 2018c). In the morning hours, the shade was not available in east-facing hutches; however, the DBT did not rise above the upper critical temperature of 26 °C until around 10:00. East-facing and north-facing hutches provide more access to shade in the hotter periods of the day than south- or west-facing hutches. Given the daily changes in the solar incidence angle, it seems obvious. However, it is rarely considered when placing the calf hutches. We found no available publications that have studied compass direction-induced differences in hutch microclimate. It is a limitation of our study that shade preference or availability was not measured continuously. The availability of shade – e.g. in terms of the shaded proportion of the calf's living space – would be more informative.

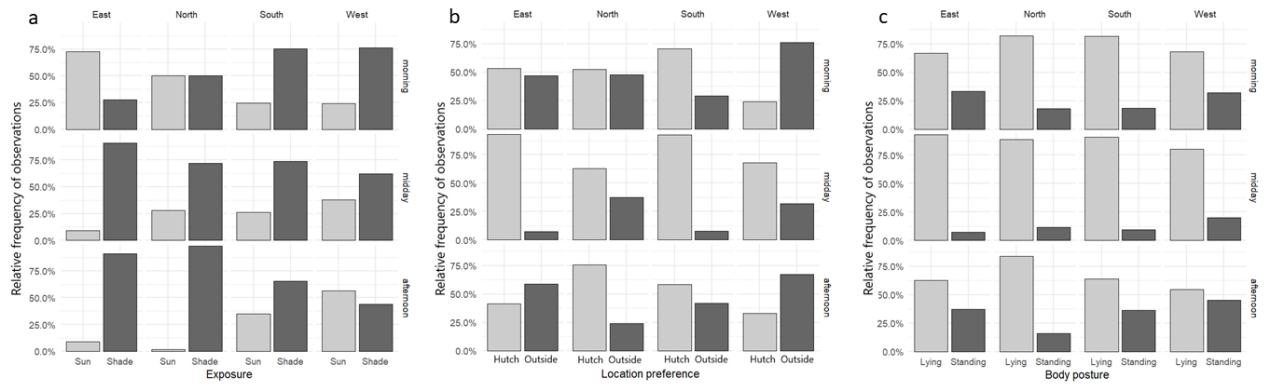


Figure 7 Relative frequency of a) being in the sun vs shade; b) location preference (hutch vs outdoor area); c) body posture (lying vs standing) at the time of observation among calves housed in hutches facing different compass directions. [The animals were observed every 20 min in the morning (7:20 – 11:00), midday (11:20 – 15:00) and afternoon (15:20 – 19:00) periods.]

The relative frequency of observing a calf being inside vs outside the hutch is displayed in **Figure 7b**. Hutch preference in the morning period was higher in east-facing hutches than west-facing hutches (OR: 14; 95% CI: 1.24; 159.1, $p < 0.05$). In the midday period, it was higher in east-facing hutches than in north-facing (OR: 23; 95% CI: 1.34; 394.6, $p < 0.05$) and west-facing ones (OR: 23.6; 95% CI: 1.46; 381.4, $p < 0.05$), respectively. We found no difference in the relative frequency of hutch preference between different compass directions in the afternoon period.

We assumed that access to shade is the priority in the hutch or outdoor area preference of calves. In the midday period, both shade access and hutch preference were higher in east-facing than west-facing hutches, which partially confirms our hypothesis. However, access to shade is not the only decisive factor in choosing a place to rest. In case shade is not available, or both the hutch and the outdoor area are shaded, other factors may also play a role. Calves tend to seek a microenvironment within or outside the hutch that best suits their comfort and well-being. Their selection depends on outdoor temperature and time of day (Brunsvold et al., 1985). Hutch or outdoor preference could not be linked directly to a single one (or two) of the climatic parameters. It is influenced by the resultant of all the factors that affect heat transfer. We concluded that the 'operative temperature', that is, the temperature as perceived by the animal, could be the appropriate measure determining location preference. It integrates mean radiant temperature (incorporating the amount of solar radiation), wind speed, humidity and hair coat characteristics. Operative temperature is used mainly in human studies, e.g. for assessing thermal comfort in workplaces. However, when used correctly, it also reliably models the relationship between an animal's thermal environment and its physiology (Dzialowski 2005).

The relative frequency of observing a calf lying vs standing is displayed in **Figure 7c**. Lying prevalence did not differ between compass directions in the morning and midday periods. It was higher in north-facing than west-facing hutches in the afternoon period, respectively (OR: 4.38; 95% CI: 1.32; 14.58, $p < 0.01$).

Observing body posture at distinct time points – even as frequently as every 20 min – does not hold as much information as continuous monitoring (Kovács et al., 2018a). In the study of Kovács et al. (2018a), a 75-80% higher frequency of lying down was observed in shaded vs unshaded calves. We assumed that if the difference in comfort level between east or north and south or west-facing hutches had approached the difference between sunny and shaded conditions, it could have been detected with the obtained sampling frequency. The significant difference between north and west-facing hutches in the afternoon period suggests that directing hutch entrance to the north has some advantages. However, it also means that merely the different compass direction of the hutch entrance can not reduce the heat load to an extent to that of shading (Spain and Spiers, 1996; Kovács et al., 2018c).

4.4. Possibilities and the effects of shading on calves in Hungarian dairy farms⁴

Objectives

In the study described in Chapter 4.3. we have demonstrated that orienting the calf hutch entrance does not improve the inner microclimate of hutches.

In the present study we aimed to assess the extent to which calf hutches protect against intense solar radiation during the summer, and whether this is improved by some technique to reduce heat load, namely covering with heat-reflecting cover (Binion et al., 2014), shielding mesh (Kovács et al., 2018c, 2019) or built-in insulated roof.

Materials and methods

The study was performed in two Hungarian dairy farms (Farm 1.: Beled, 47°28'09.3"N 17°04'14.6"E; Farm 2.: Alattyán, 47°25'07.0"N 20°03'37.5"E). The study was performed from late June to late August 2019. On Farm 1, the calves were kept in a straw-littered calf hutch (Agrobox-1, Agroplast Kft, Gyál) made of fibreglass-reinforced plastic after birth until weaning at the age of 56–60 days. There is no shading above the calf cages on this farm (**Picture 4**). On Farm 2., calves are also placed in a straw-littered individual cage after birth (Calf-Tel Compact, Hampel Co., Germantown, WI, USA). Still, most of the calf cages are under a built-in sandwich panel-insulated roof (**Picture 5**). At the time of our study, calves were also kept in areas not covered by the roof due to the large number.

The calf rearing protocol on the farms was similar. On both farms, the calves receive the colostrum of their mother two or three times in the first days of life. In the following, a mixture of milk from the calving barn and milk replacer is fed to calves in an amount of 2x3 or 2x4 litres per day. Weaning takes place around 60 days of life. Water and calf starter is offered ad libitum from the first or second week of life, on Farm 1 and 2, respectively. We have found that any changes in the calf rearing protocol were independent of the respiratory rate that was recorded during the study. All calves were clinically healthy at the time of measurements.

4 Bakony & Jurkovich: Magyar Állatorvosok Lapja, 2021. 143: 3-10.

The study was designed to compare the effects of different types of heat stress abatement on the thermal protective properties of the calf hutch. For this purpose, 33 hutches on Farm 1 was selected. The hutches were oriented similarly, facing east with their entrance. Eleven of them remained uncovered, 11 of them were covered in heat-reflecting foil (Cool-Calf Covers, Oceanside, CA, USA; **Picture 7**), and a shading mesh was installed above 11 of them (**Picture 6**). Calves born on the farm at the time of the study were placed subsequently in one of the uncovered, foil-covered and mesh shaded hutches in order to obtain a similar distribution of age of calves housed in the studied hutches. 10-10-10 calves were housed in the experimental and control hutches, and one hutch in each group was left empty for temperature measurements. During the course of the study, the experimental hutches remained the same (heat stress abatement techniques remained installed throughout the time of the study, and all hutches were inhabited by the same calf from the time of birth, so measurements were performed on the same hutches and animals, this way the age of the animals were gradually higher with the date of measurement. This farm was visited three times during the summer. Due to unforeseen bad weather, the shading mesh was torn down by the time of the third measurement. On Farm 2, the hutches placed under the shade and in the sun were already inhabited by calves. This way we have made measurement on all calves on the farm to obtain a similar distribution of age of the studied calves on both farms. This farm was visited once. The number of calves involved in the study is summed up in **Table 9**.

Table 9 The number of calves invoved the study

Date of measurement	Site of measurement	Type of heat stress abatement	Number of calves observed per type of heat stress abatement	Min-max temperature (11-17:00)
June	Farm1	No /Shading mesh /Foil coverage	10/10/10	25-33 °C
July	Farm1	No /Shading mesh /Foil coverage	10/10/10	29-43°C
August	Farm1	No / Foil coverage	10/10	31-36 °C
August	Farm2	No / Built roof	24/40	30-36°C

Climatic measurements were performed in the hottest hours of each day (11-17:00). On each visit on Farm 1, black globe and dry bulb temperatures were measured inside the empty hutches in each of the heat abatement groups (unprotected, foil-covered, mesh shaded) to represent the inner microclimate of hutches. BGT and DBT were also measured in the outdoor area of the unprotected sunny hutch. On Farm 2, black globe and dry bulb temperatures were measured inside and in the outdoor area of an empty hutch that was exposed to the sun, and inside an empty hutch that was shaded by the built roof structure. Temperatures were measured hourly with Kestrel 5400AG Cattle Heat Stress Tracker (Nielsen-Kellerman Co., Boothwyn, PA, USA) at head height (30–50 cm) of lying calves. The respiratory rate, body posture and location (inside the hutch or in the outdoor fenced area) of calves were recorded hourly on each visit. The experimental design on the farms are illustrated in **Figure 13** and **14** in the appendix.

We assumed that the effectiveness of a given heat reduction technique could be described by the temperature difference between the least favourable thermal condition that would occur if absolutely no heat reduction techniques were applied and the most favourable thermal condition that occurred as a result of the given heat reduction technique applied. The outdoor area of the unprotected hutch that was exposed to the sun served as control, representing the state of no heat abatement. We have compared the temperature difference between the outdoor area that was exposed to the sun and the inside temperature of the unprotected and cooled hutches, respectively. The magnitude of the difference reflects the heat-reducing ability of the given heat stress abatement technique. It is a limitation of the study that the temperature conditions in the outdoor fenced areas of hutches with different heat protection was not measured, which could have contributed to the comparison of the hutch inner microclimate and the outdoor area microclimate, respectively. We assumed that the heat reduction technique that has the greatest effect on the hutch inside microclimate has the most benefits also on the outdoor area and consequently on the overall heat load of calves.



Picture 4 Individual calf hutches without shading



Picture 5 Individual calf hutches under thermally insulated roof



Picture 6 Individual calf hutches under mesh shading



Picture 7 Individual calf hutches covered with heat reflective foil covers

The means of the temperature differences between the outdoor area of an unprotected hutch and the interior of the hutches were compared by analysis of variance as a function of the heat reduction technique applied. The mean respiration rate per minute, as an indicator of the heat load of the animals, was compared between each treatment group using a general linear mixed model that takes into account the relationship between the values measured on the same animal. Statistical analyses were performed using statistical software R (R Core Team, 2020). The cut-off for statistical significance was set at $p < 0.05$. Descriptive statistics on behavioural observations were generated.

Results and discussion

Measurements of calves' body temperature were discarded because frequent body temperature measurements would have been a severe confounding factor in behavioural observations and would have increased the already high stress due to hot weather. In our previous studies, mesh shading did not cause a significant change in body temperature [Kovács et al., 2019]. In dairy cows, the relative humidity or the temperature-humidity index

are also often used to characterize the ambient heat load. Using THI was omitted in this study because it was demonstrated in a previous study that ambient temperature was sufficient to describe the heat load in calves kept outdoors (Kovács et al., 2018b).

Temperature conditions

Figure 8 and **Figure 9** show the changes in the mean temperatures measured in the absence or presence of different heat reduction techniques. Separate figures for each day of measurement are included as **Figure 15-22** in the appendix.

Figure 8 shows that the average *ambient temperature* inside the unprotected hutches was higher than in the outdoor area of the unprotected hutch and in covered, mesh shaded or roof protected hutches. This may be due to the heat absorption of the plastic, which heats up the air inside the hutch (Kovács et al., 2018c). Heat reduction techniques prevent the ambient temperature inside the hutch to rise above the ambient temperature of the outdoor area.

The differences in ambient temperature between the hutch inner environment and the outdoor area of unprotected hutches are summed up in **Table 10**, according to the heat reduction technique used. In the case of unprotected hutches, the difference was positive, while the other heat reduction techniques caused the inside temperature to be lower than the outside air temperature. The greatest differences were observed in the case of heat abatement by a built roof. The effect of mesh shading and foil coverage on the inside hutch temperature was not significantly different.

In outdoor studies, ambient temperature is most often used to characterize the thermal environment. Still, the difference observed contradicts our practical observation that calves tend to migrate to the calf hutch to avoid direct solar radiation. This points out that ambient temperature is not the optimal indicator for outdoor studies. According to our objective, the present study focused primarily on the thermal characteristics of the calf hutch. The direction of the differences experienced carries important methodological information. It is important to keep in mind that the sensory heat load in a site exposed to the solar or another type of radiation is primarily determined by the radiation (black globe) temperature (Curtis et al., 2017).

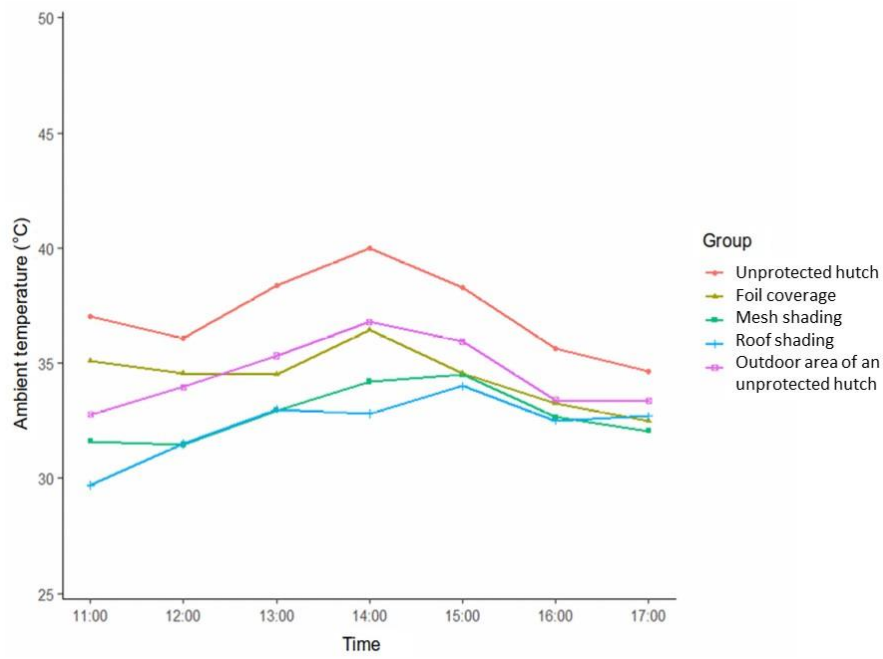


Figure 8 Changes in mean ambient temperature (°C) at the different measurement points

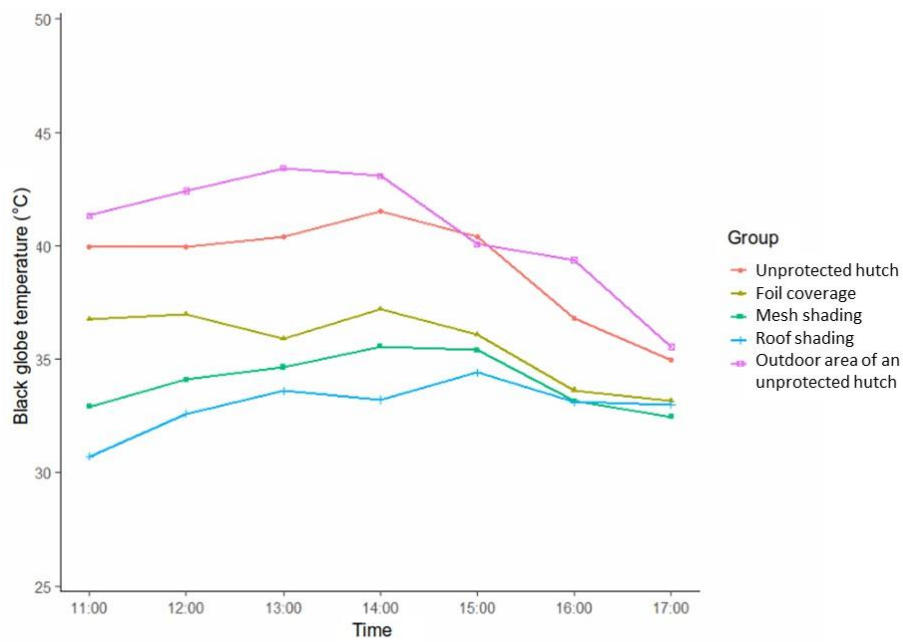


Figure 9 Changes in mean black globe temperature (°C) at the different measurement points

The diurnal changes in mean *radiant (black globe) temperature* are shown in **Figure 9**. Radiation temperatures inside the hutches were lower in all treatment groups than in the sunny outdoor area, suggesting that the calf house material provides some degree of protection against sunlight. This, in the case of an unshielded cage, contrasts with that found in dry air temperature and highlights the calves' preference when choosing a resting place. **Table 10** sums up the BGT difference between the outdoor area of unprotected hutches and the inside temperatures of the hutches equipped with different heat reduction techniques. It reflects the extent to which the given heat reduction technique reduces solar radiation. This result justifies using radiant temperature instead of ambient (air) temperature that better reflects the effect of solar radiation in outdoor studies to characterize the thermal environment (Curtis et al., 2017). It can be observed that the heat reduction techniques studied could reduce the heat absorption of the hutch material. The protective effect of shielding with foil and the use of mesh was also described by earlier other authors (Carter et al., 2014; Friend et al., 2014; Kovács et al., 2018c). The extent to which roof protection reduced the effect of solar radiation was significantly higher than the efficiency of mesh shading and foil coverage. We concluded that the built roof promotes the most favourable thermal environment.

Table 10 Differences in average ambient and black globe temperatures in the hutches compared to the open-air area of the control group

Treatment	Untreated	Foil	Mesh	Roof
The difference in ambient temperature between the outdoor area of the unprotected hutch and the inner space of heat-protected hutches (°C)	2.5 ± 2.06 ^a	-0.31±1.75 ^b	-1.87±1.94 ^{bc}	-2.17±1.10 ^c
The difference in radiant temperature between the outdoor area of the unprotected hutch and the inner space of heat-protected hutches (°C)area (°C)	-1.35±2.8 ^a	-4.86±3.52 ^b	-5.52±3.13 ^b	-9.38±4.50 ^c

^{a, b, c} Different superscripts show a significant difference within a row (p<0.05).

Respiratory rate

We compared the average respiration rate of calves in calf cages with different heat loads. The mean respiration rates measured at each time point are shown in **Figure 10**. We hypothesized that the calf would choose the most favourable thermal environment available

(Brunswold et al., 1985), which would be determined primarily by the thermal protection technique. Thus, the actual temperature values were not included in the model, instead, the average respiration rate was compared depending on the thermal protection technique used. The mean respiration rate estimated by the model taking into account the correlation between measurements on the same calf and the same day and the same farm was highest in calf houses without heat protection (113 ± 9.8 / min, mean \pm SE), and the respiratory rate of calves kept under heat-reflecting foil, mesh shading and roof was lower with an average of 13.5 ± 9.3 , 10.6 ± 8.2 , and 27 ± 3.7 resp./minute, respectively. Multiple comparisons showed that the respiration frequency was significantly lower in the case of roof protection as compared to the other groups ($p < 0.001$), but showed no significant difference between calves housed in unprotected, foil-covered, and mesh shaded hutches ($p < 0.05$). The upper limit of the respiration rate range of healthy calves is 70 (Piccione et al., 2003). Considering this, the order of magnitude of the averages shows that increased evaporative heat dissipation was observed in all groups, with extremely high maxima in unprotected calf hutches. Based on our observations, summer deaths may be due to heatstroke and other conditions associated with exhaustion (Morignat et al., 2014; Renaud et al., 2018). The magnitude of the differences was physiologically less significant when applying the heat protection foil and the mesh shielding. Extremely high values were rarely observed in the case of calves kept under the roof. The results are consistent with the numerical differences observed for the radiant temperature.

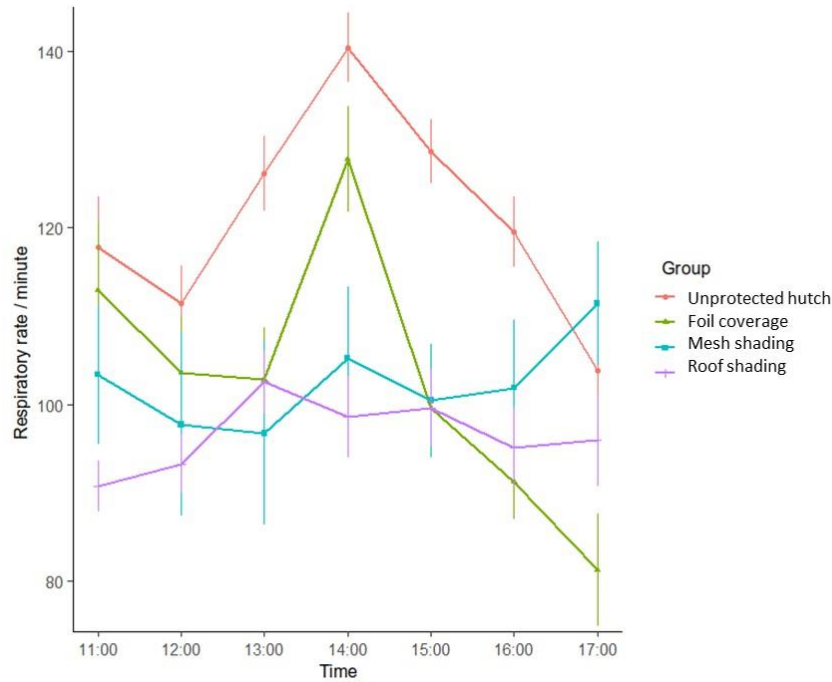


Figure 10 Changes in the respiratory rate of calves under different thermal protection

Behavioural thermoregulation

During the study, the animals were mostly in a lying position (**Figure 11**). We should note that the observations were made hourly and only during the hottest part of the day. However, our observations are consistent with the mention of increased heat inactivity as a concomitant of heat stress on calves (Holt, 2014; Roland et al., 2016). We found no differences in the frequency of lying between the groups.

The thermal environment is probably the most critical factor in choosing to stay in the outdoor area or in the hutch (Brunswold et al., 1985). The location preference figure (**Figure 12**) clearly shows that the roof covering the runway also provides a favourable microclimate for the calves in the outdoor area (this cannot be supported by measurement data as the runway temperature under the roof was not measured).

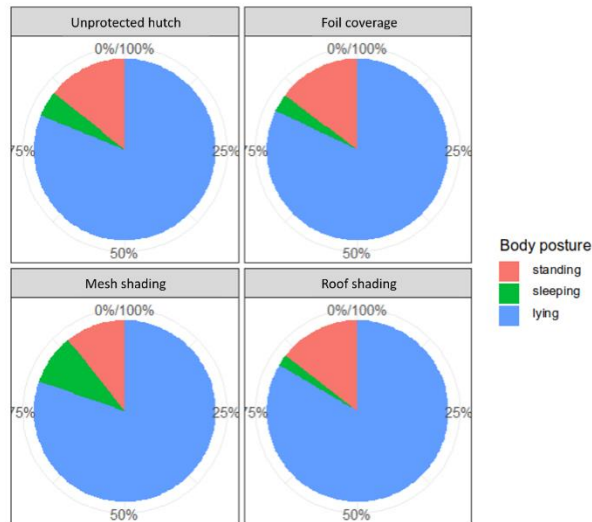


Figure 11 Activity of the calves in the different groups



Figure 12 Location preference of the calves in the different groups

Practical experiences

Although the study described here reports short-term observations, the thermal protection foil and shading mesh we installed have been on the calf cages for a more extended period. During this time, we made some observations that are also important for practice. The heat protection foil proved to be disposable, so its payback is doubtful. Because installation is cumbersome and the foil is not durable, in our opinion, it is not practical under farm conditions. When installing the shading mesh, the local weather conditions (mainly the

frequency of windy weather) must be taken into account when choosing the materials and installation method.

5. Comprehensive discussion

Our results provide evidence against the common belief that dairy calves cope well with heat. Increased calf mortality in the hottest month of the year highlights that heat stress abatement in preweaning calves is just as important as protection against cold. Heat stress reduction is advised in outdoor calf rearing when the average daily temperature reaches 22 °C, which is characteristic of summer weather in a continental region. In recent years, heatwaves are becoming more common, and the expected rise in average daily temperatures puts even more emphasis on heat stress in dairy calves. The exact causes of heat-related mortality are worth more fully investigated to improve the detection of animals at the highest risk of hyperthermia-related illnesses. The distribution of blood between the body shell and core in order to maximize heat dissipation capacity could be an underlying cause of the 'leaky gut' syndrome, a phenomenon that has already been described in heat-stressed lactating cows (Baumgard and Rhoads, 2013).

The body surface is the scene of constant heat exchange, and therefore, we investigated whether surface temperatures would prove informative in the heat dissipation capacity and thermal status of calves. The fact that ambient temperatures primarily influence surface temperatures in clinically sound animals limits the use of infrared thermometry in detecting heat stress-related hyperthermia. However, in the light of the recent heat dissipation limit theory, as proposed by Speakman and Krol (2014), the maximal heat dissipation capacity imposes a boundary of total energy expenditure of the animal body to avoid hyperthermia. A more precise estimation of changes in heat dissipation capacity in calves – which are influenced by surface temperatures – would contribute to a better understanding of coping mechanisms under hot weather conditions.

Besides the detection of animals needing more attention from the stockperson in times of hot weather, we have also investigated the heat load that calves are exposed to and the efficiency of different heat reduction techniques to be applied in practical conditions in outdoor calf rearing. Our experiments highlighted important methodological considerations. The proper description of the thermal environment of the calf is challenging. The inside of the hutch and the outdoor fenced area provides different thermal environments in terms of temperature and the availability of shade, and the animals' preference of location, which has to be taken into consideration when assessing the heat load of a given animal. These aspects make the proper description of the thermal environment of a calf challenging and require more clarification in further studies.

In view of how different microclimates can occur within a single calf hutch, the use of complex environmental indicators in outdoor studies was also confirmed during our investigations. In outdoor studies, indices incorporating the BGT picture the animal's thermal environment better than the DBT. The DBT masks how drastically high heat loads can calves experience during summer. Regarding welfare assessment, the results highlighted that it is crucial to use methods that reliably assess solar radiation when describing the thermal environment of livestock reared outdoors. Based on the environmental and animal-based parameters, we concluded that the positioning of the hutch entrance towards east or north in summer has some advantages in mitigating the drastic heat load. However, the differences in heat load between the most and least favourable microclimates are so low that hutch positioning may address only some acute heat stress effects. A second methodological issue that has arisen is that the other types of heat abatement techniques – heat-reflective covers, mesh shading or a built roof structure - affect either only the inside of the hutch (foil coverage) or both the inside and the outdoor fenced area (mesh shading or roof) which has to be taken into consideration when assessing efficiency. In recent years, we have experienced hotter, drier, and longer summers. It is gratifying that more and more dairy farms recognise the importance of protecting calves from the summer heat. Based on our present study, the shading mesh and the built, heat-insulated roof can be recommended among the available technologies. The shading mesh can bring relief during the hottest hours, and the local weather conditions must be considered so that it can be installed even for a long time at a relatively low cost. A built roof has the most significant benefits; however, it is the most expensive of the techniques listed. Both the shading mesh and the roof positively affect the workers (Coleman et al., 1996).

6. Conclusions

'...being conservative is a bigger problem than being too speculative.' (Brian McKnab; 2002)

Heat stress in dairy calves is still an overlooked area in dairy management. We need to focus scientifically and economically on this sensitive group of animals. It was shown that in terms of mortality, the summer heat could be just as detrimental to newborn calves as the winter cold. It follows that age is crucial in coping with heat stress and more attention needs to be paid to calves in the first two weeks of life. It is worth investigating whether the causes of death show a specific pattern in the summer months. Having monitored physiological indicators – e.g. rectal temperature, skin surface temperatures and respiration rate – we have realized little agreement in the literature on the reference values. It limits the interpretability of results and the clear definition of the thermoneutral zone of dairy calves. In terms of methodology, measurement of rectal temperatures with a digital thermometer still seems to be the only way to assess the thermal status of the calf accurately. It also became clear that heat abatement strategies are feasible on farms and can effectively improve animal welfare. However, they may require a significant investment. Interestingly, the work of the past years was most fruitful in gaining insight into what we don't know about heat stress in dairy calves. In the following, I collected a few thoughts on areas of further research.

Economic efficiency

Despite the growing body of evidence of adverse effects of heat stress on dairy calves from as early as the prenatal period, most dairy operations carry on without any cooling interventions for dry cows or preweaned calves. Translating the biological cost to financials could convince farm owners to invest in heat abatement. It could also speed up the much-needed change of thinking in dairy (calf) management that non-lactating animals require as much attention as lactating animals do.

Understanding the basics

Scarce literature on the upper end of the thermoneutral zone warns that there is room for improvement in understanding thermal requirements and heat dissipation capacities of dairy calves. The indices initially developed for indoor conditions, like dry bulb temperature or

the temperature-humidity index, can be misleading when assessing the thermal environment of outdoor reared calves. Upper critical thresholds should be formulated in a manner that suits the housing environment of calves. It necessitates a better understanding of how radiant heat, relative humidity and wind speed contribute to the thermal load of dairy calves.

Integrating the concept of in utero heat stress

Besides dry cow management, heat stress abatement in calf rearing is another overlooked area in dairy management. A longitudinal study involving a larger number of calves could shed light on whether adverse effects of pre- and postnatal heat stress are comparable and whether these effects add up when occurring. It would be interesting to study whether postnatal heat exposure without maternal heat stress results in the same adaptive metabolic and immune responses as that of the calf foetus. It is also worth investigating whether improved calf management and nutrition strategies could prove helpful in mitigating the effects of heat stress on growth and passive transfer of immunoglobulins.

Methodology

Real-time recording of environmental indices (radiant heat, humidity, wind speed) is feasible and could be easily integrated into precision livestock farming technologies. Automated monitoring of physiological parameters in outdoor kept calves is currently not widely available due to high costs or limited time of recording (10-14 days for indwelling thermometers). Respiratory rate can only be measured by labour-intensive visual observation. Adaptation of automated methods of measuring breathing rate – designed initially for cattle – would improve reliability and facilitate the determination of upper critical temperatures.

7. New scientific results

1. Hot weather in summer has similar effects on mortality of 0-14 day-old young calves than the cold stress in winter. This age group is more sensitive to the hot weather than the 15-60 day-old calves, where the winter mortality is much higher than that in summer.
2. The average mortality risk ratio in the 0-14 day age calf group in the risk periods (hot days with an average daily temperature of 22°C) was a least twice as high as in the reference periods (average daily temp. 5-18°C). With a daily mean temperature of 25°C or more (heatwaves), the risks were three times high as in the reference period.
3. Hutch reared dairy calves can experience a drastically high heat load in summer. It is advised to direct the entrance of fibreglass reinforced polyester hutches to the north in the summer period. This way, the dry bulb temperature inside the hutch can be reduced by up to 7.5°C in the morning hours (7:00-11:00) compared to the east-facing hutches, and by up to 10°C in the afternoon hours compared to the south- and west-facing hutches. Also, the black globe temperature in the north-facing hutches can be reduced by up to 7.1 °C compared to west-facing hutches in the afternoon hours. In parallel, the respiratory rate of calves in north-facing hutches was 25.3 breaths/min lower than that of calves in the east-facing hutches in the morning period and 39.3 breaths/min lower than that of calves in the west-facing hutches in the afternoon period.
4. The dry bulb temperatures inside the fibreglass reinforced polyester hutches was on average 2.5 °C higher than in the outdoor open-air area.
5. Black globe temperatures were lower inside the hutches than in the open-air area with an average of 1.4, 4.8, 5.5 and 9.4 °C in case of unprotected, reflective foil-covered, net shaded, and thermally insulated roof shaded hutches, respectively.

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9. Scientific publications

Scientific publications in peer-reviewed journals that form the basis of the thesis

1. Bakony, M., Jurkovich, V.: Heat stress in dairy calves from birth to weaning. *J. Dairy Res.*, 87(S1). 53–59, 2020.
2. Bakony, M., Kiss, G., Kovács, L., Jurkovich, V.: The effect of hutch compass direction on primary heat stress responses in dairy calves in a continental region. *Anim. Welfare*, accepted for publication
3. Bakony, M., Jurkovich, V.: Az árnyékolás lehetőségei és hatása a borjakban hazai tejelő tehenészetekben (Possibilities and the effects of shading on calves in Hungarian dairy farms). *Magy. Állatorvosok Lapja*, 143. 3–10, 2021. (in Hungarian)

Dissemination of the results at scientific conferences

1. Bakony, M., Könyves, L., Jurkovich, V.: Seasonal differences in the mortality rate of pre-weaned dairy calves. In: Szenci, O., Brydl, E. (eds) Proceedings of the 29th International Congress of the Hungarian Association for Buiatrics, 13-16 November 2019, Hévíz, Hungary, pp. 206–210. (in Hungarian)
2. Bakony, M., Kiss, G., Jurkovich, V.: The effect of hutch orientation on primary heat stress responses of dairy calves. In: Advancing Animal Welfare Science: How Do We Get There? – Who Is It Good For? Proceedings of UFAW International Animal Welfare Science Symposium, 3-4 July 2019, Bruges, Belgium, p. 52.
3. Bakony, M., Kovács, L., Kézér, F.L., Jurkovich, V.: Heat tolerance of dairy calves in sunny and shaded environments. In: Sebastian, Opaliński (ed) Proceedings of the XIXth International Congress of International Society for Animal Hygiene, 8-12 September 2019, Wrocław, Poland, pp. 31–33.

Other publications related to the topic of the thesis

1. Kovács, L., Kézér, F.L., Ruff, F., Szenci, O., Bakony, M., Jurkovich, V.: Effect of artificial shade on saliva cortisol concentrations of heat-stressed dairy calves. *Domest. Anim. Endocrinol.*, 66. 43–47, 2019.
2. Kovács, L., Kézér, F.L., Bakony, M., Jurkovich, V., Szenci, O.: Lying down frequency as a discomfort index in heat stressed Holstein bull calves. *Sci. Rep.*, 8. 15065, 2018.
3. Bakony, M., Könyves, L., Hejel, P., Kovács, L., Jurkovich, V.: Hőstressz tejelő teheneekben I. A tejtermelés-csökkenés háttérében álló élettani tényezők. Irodalmi összefoglaló. (Heat stress in dairy cows Part 1. – A review on physiological factors involved in milk yield loss) *Magy. Állatorvosok Lapja*, 141. 341–350, 2019. (in Hungarian)
4. Bakony, M., Könyves, L., Mézes, M., Kovács, L., Jurkovich, V.: Hőstressz tejelő teheneekben II. Az alkalmazkodást segítő takarmányozási megoldások. Irodalmi összefoglaló. (Heat stress in dairy cows 2. A review on nutritional strategies to alleviate losses) *Magy. Állatorvosok Lapja*, 141. 397–408, 2019. (in Hungarian)

Other publications not related to the thesis

1. Jurkovich, V., Bakony, M., Laky, E., Ruff, F., Kézér, F.L., Bende, A., Kovács, L.: Cardiac vagal tone, plasma cortisol and DHEA response to an ACTH challenge in lame and non-lame dairy cows. *Domest. Anim. Endocrinol.*, 71. 106388, 2020.
2. Jurkovich, V., Könyves, L., Bakony, M.: Association between feed sorting and the prevalence of metabolic disorders in Hungarian large-scale dairy herds. *J. Dairy Res.*, 86. 162–164, 2019.
3. Hejel, P., Jurkovich, V., Kovács, P., Bakony, M., Könyves, L.: A robotizált fejési rendszerek elterjedését és hatékony működtetését befolyásoló tényezők. Irodalmi összefoglaló (Automatic milking systems - factors involved in growing popularity and conditions of effective operation. Literature review). *Magy. Állatorvosok Lapja*, 140. 289–301, 2018. (in Hungarian)
4. Kézér, F.L., Tózsér, J., Bakony, M., Szenci, O., Jurkovich, V., Kovács, L.: Effect of physical activity on cardiac autonomic function of dairy cows on commercial dairy farms. *J. Dairy Res.*, 84. 395–400, 2017.

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7. Kovács, L., Kézér, F.L., Bakony, M., Hufnágel, L., Tózsér, J., Jurkovich, V.: Associations between heart rate variability parameters and housing- and individual-related variables in dairy cows using canonical correspondence analysis. *PLOS One*, 10. e0145313, 2015.
8. Kovács, L., Tózsér, J., Szenci, O., Póti, P., Kézér, L.F., Ruff, F., Gábríelné-Tózsér, Gy., Hoffmann, D., Bakony, M., Jurkovich, V.: Cardiac responses to palpation per rectum in lactating and non-lactating dairy cows. *J. Dairy Sci.*, 97. 6955–6963, 2014.
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10. Kovács, L., Bakony, M., Tózsér, J., Jurkovich, V.: Short communication: Changes in heart rate variability of dairy cows during conventional milking with non-voluntary exit. *J. Dairy Sci.*, 96. 7743–7747, 2013.

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Appendix

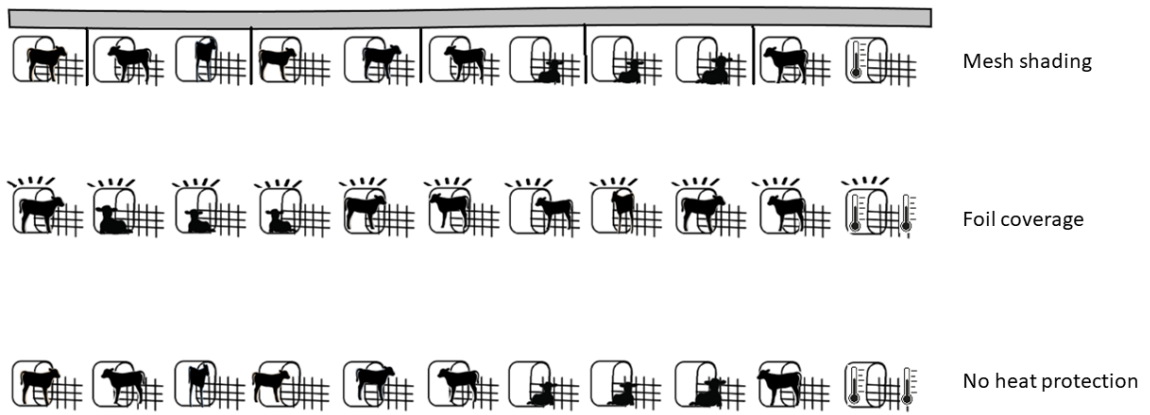


Figure 13 Schematic view of the experimental layout on investigating the effects of mesh shading and reflective foil coverage in comparison to unprotected hutches

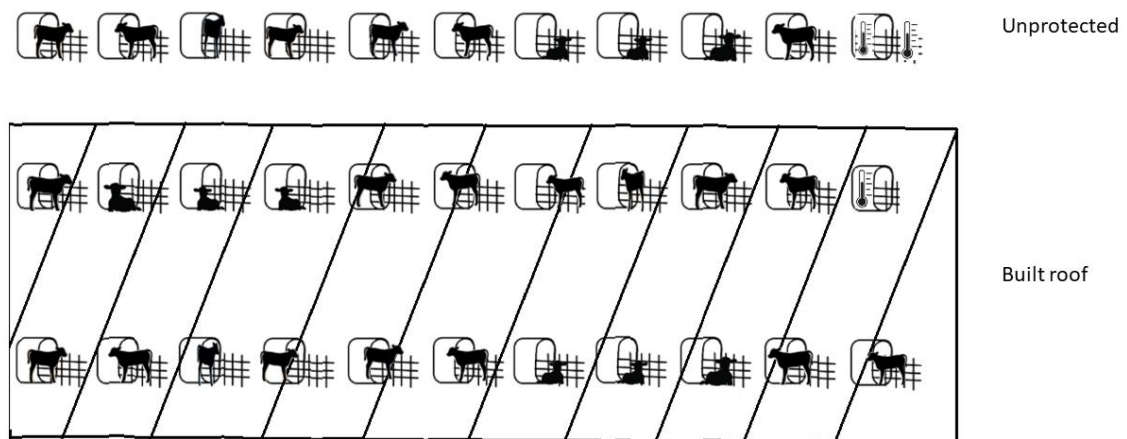


Figure 14 Schematic view of the experimental layout on investigating the effects of built roof in comparison to unprotected hutches

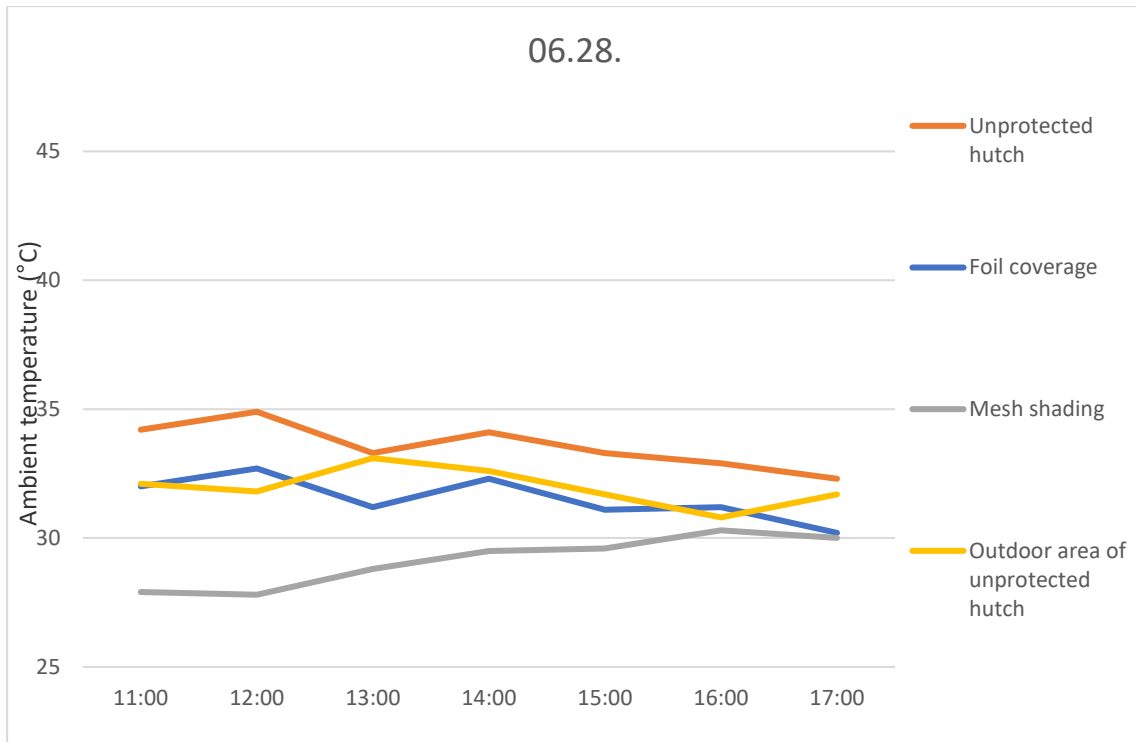


Figure 15 Hourly ambient temperatures in different measuring sites on Farm1 at the time of first measurement

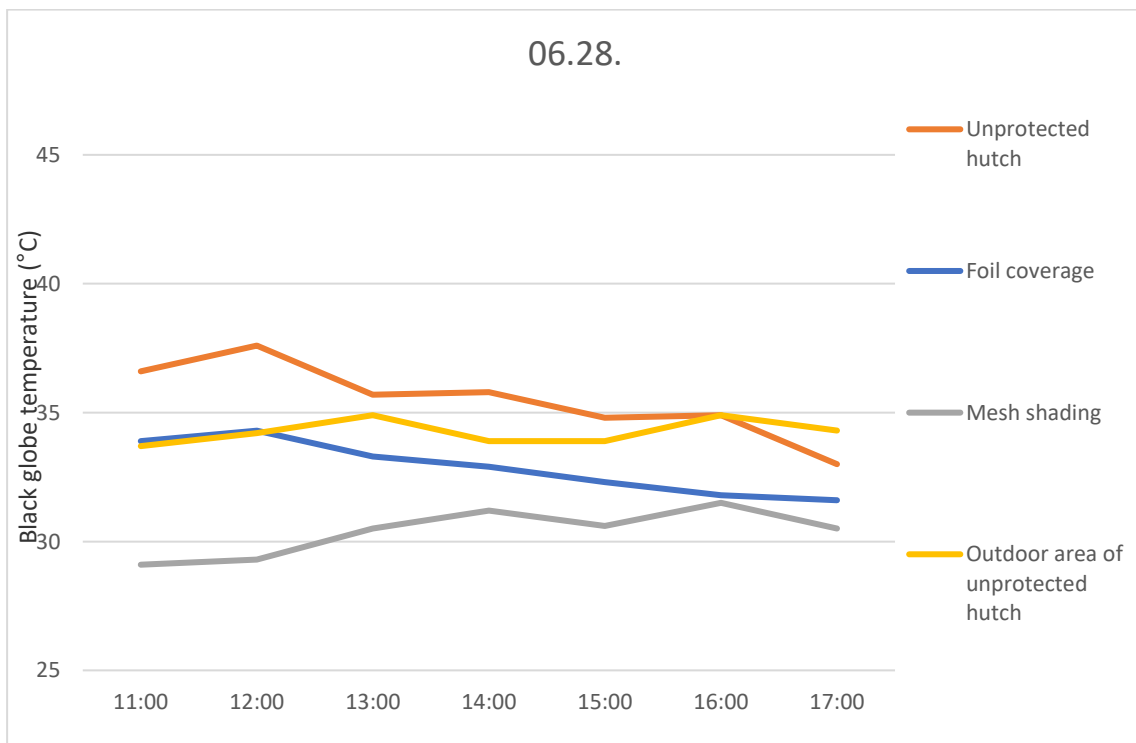


Figure 16 Hourly black globe temperatures in different measuring sites on Farm1 at the time of the first measurement

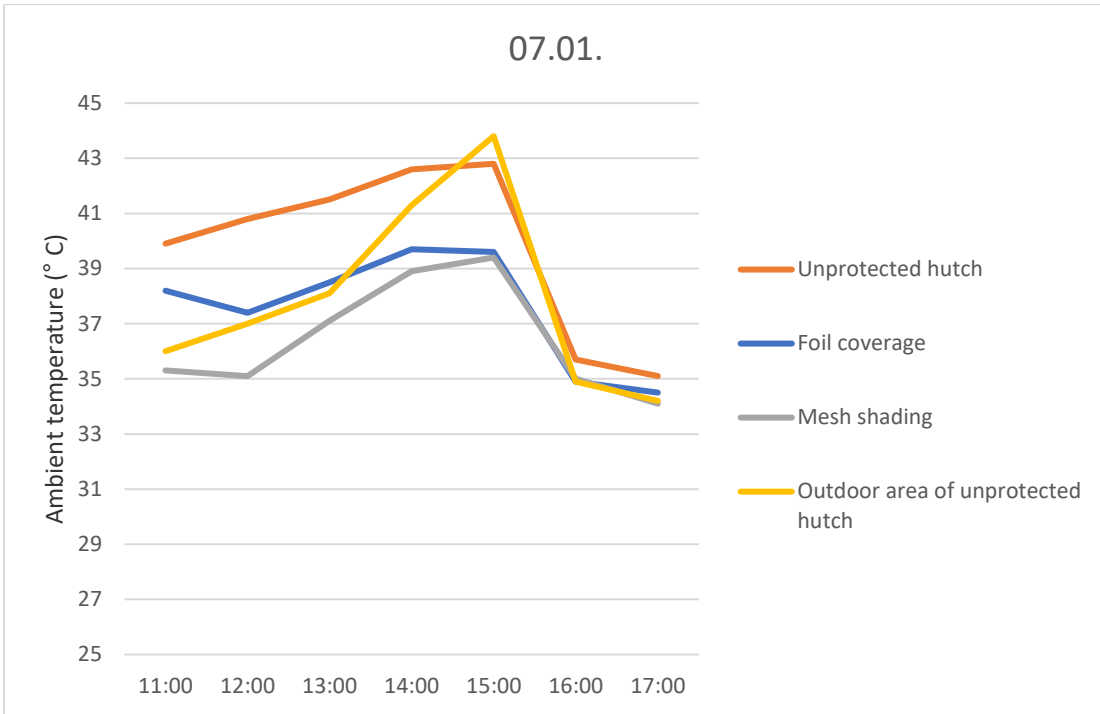


Figure 17 Hourly ambient temperatures in different measuring sites on Farm1 at the time of second measurement

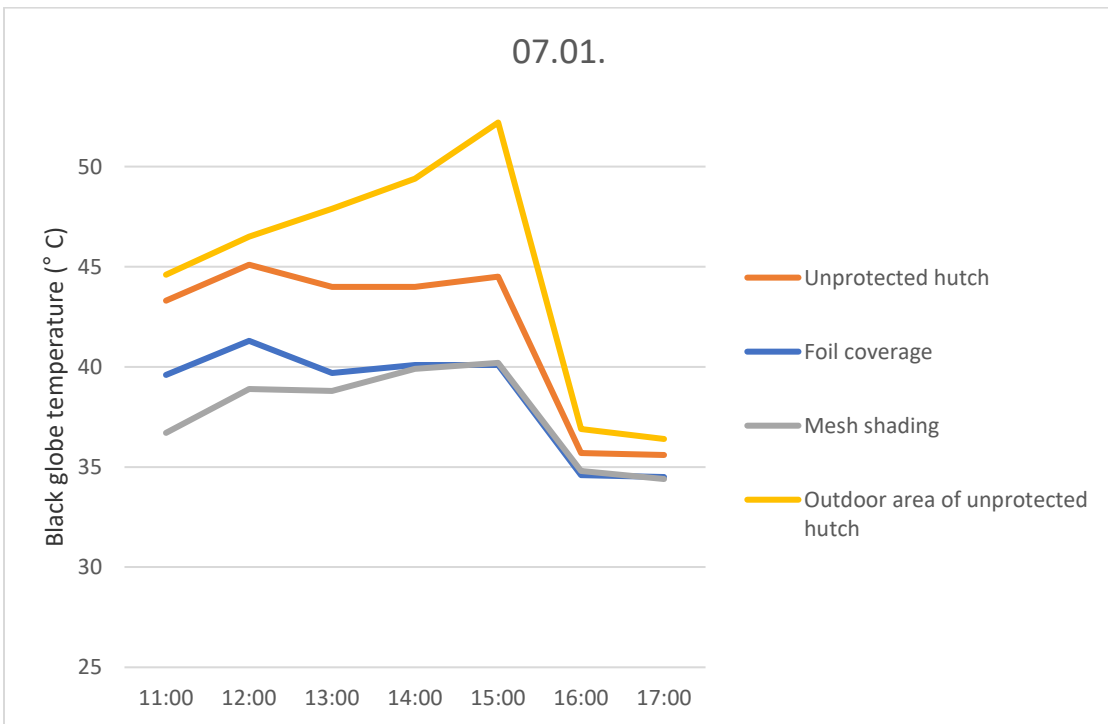


Figure 18 Hourly black globe temperatures in different measuring sites on Farm1 at the time of second measurement

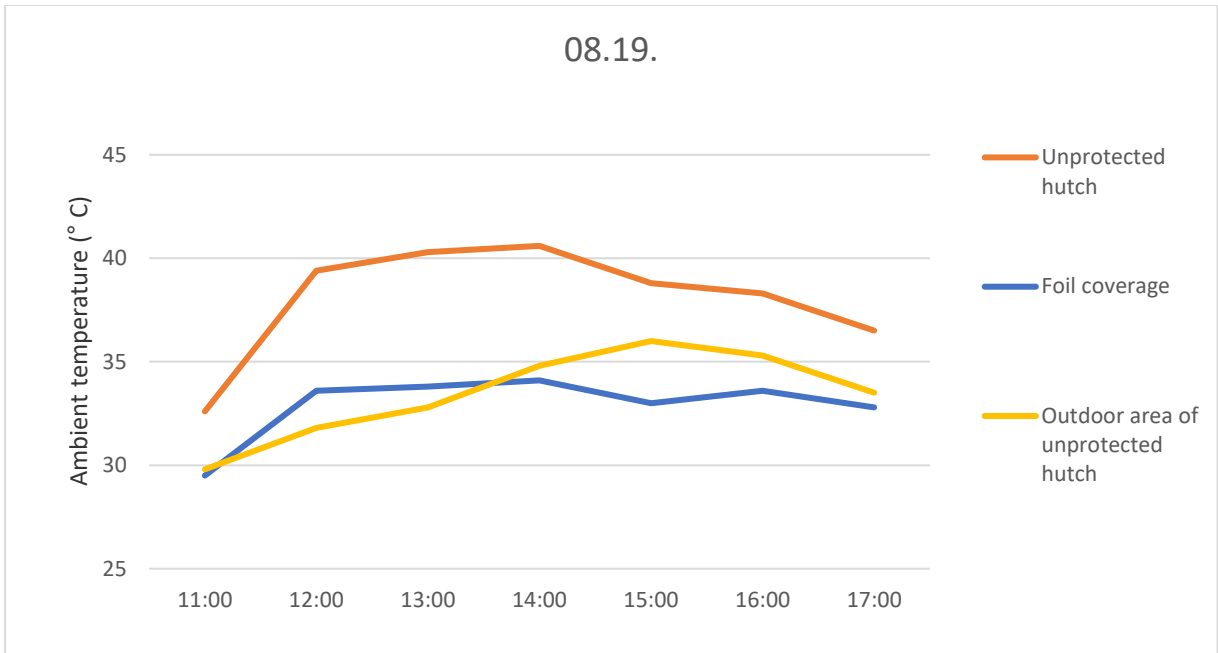


Figure 19 Hourly ambient temperatures in different measuring sites on Farm1 at the time of third measurement

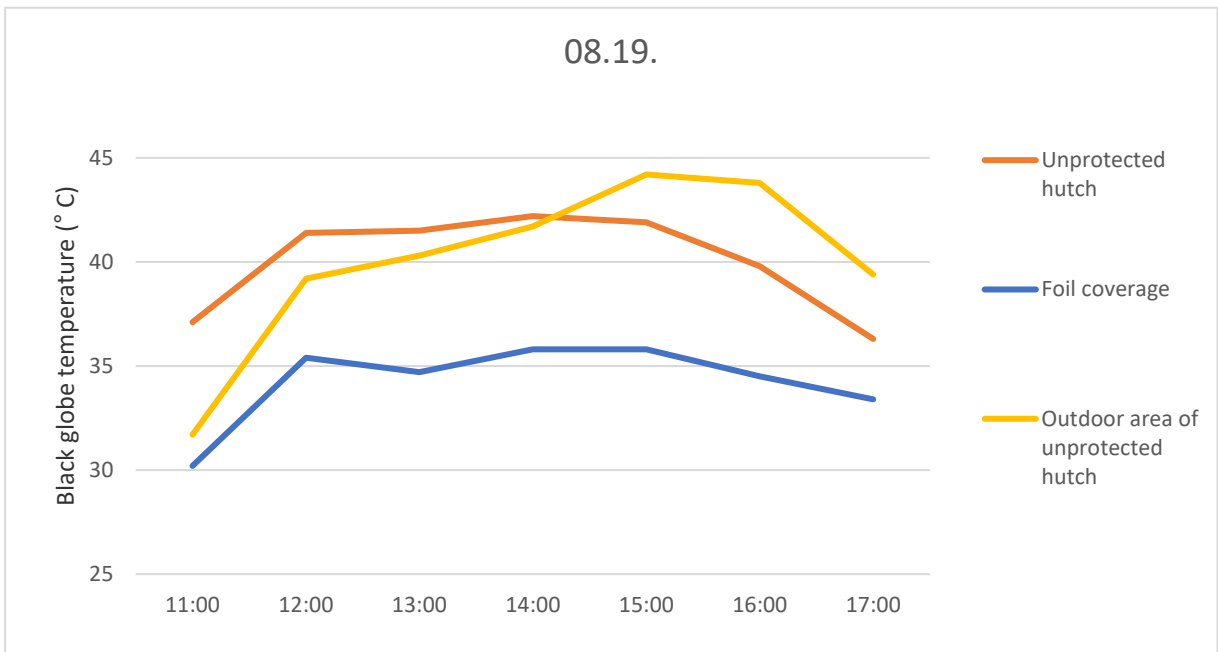


Figure 20 Hourly black globe temperatures in different measuring sites on Farm1 at the time of third measurement

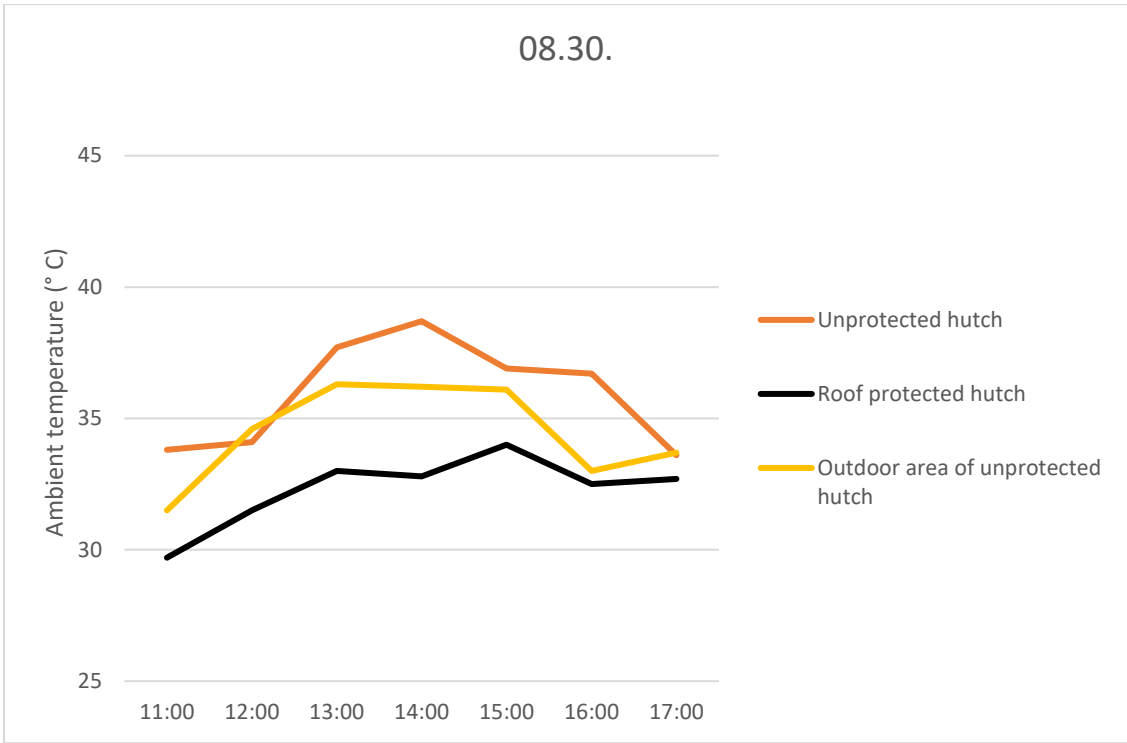


Figure 21 Hourly ambient temperatures in different measuring sites on Farm 2

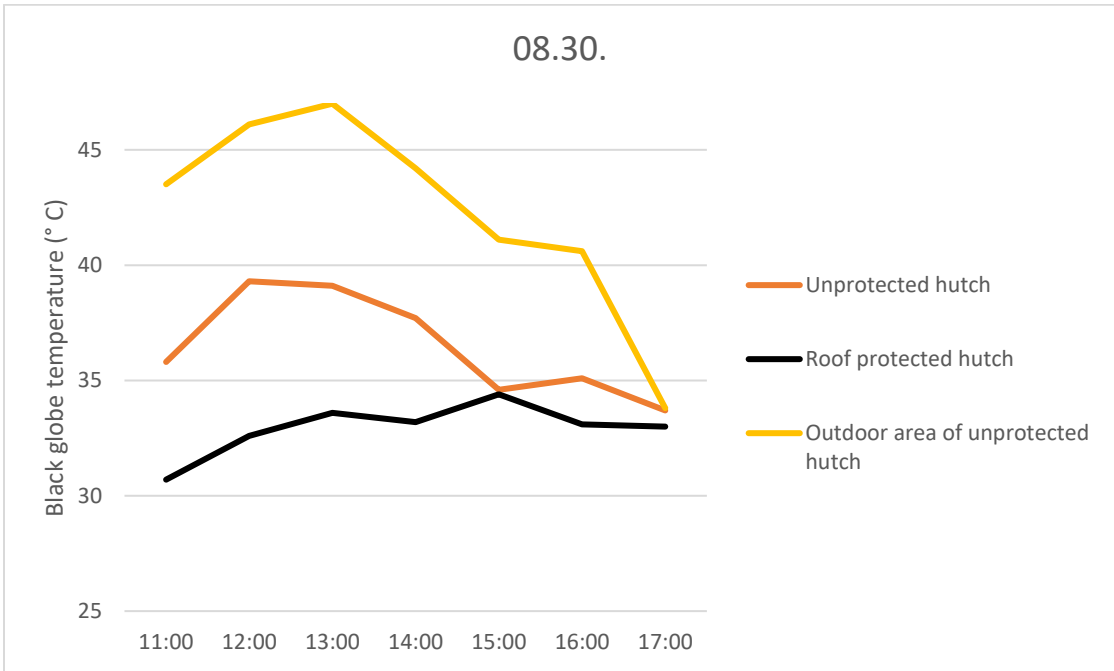


Figure 22 Hourly black globe temperatures in different measuring sites on Farm 2