Influence of dietary fiber content and horn status on thermoregulatory responses of Brown Swiss dairy cows under thermoneutral and short-term heat stress conditions

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ABSTRACT

In the present experiment, 10 horned and 10 disbudded mid-lactating Brown Swiss cows were included in a crossover feeding trial with a hay or hay and concentrate diet. The effects of dietary neutral detergent fiber (NDF) content and horn status on thermoregulatory responses under thermoneutral and short-term heat stress conditions were studied, as both are considered to ease the cow's thermoregulation under an environmental heat load. Cows received either ad libitum hay and alfalfa pellets (85:15, C−, NDF content: 41.0%) or restricted amounts of hay and concentrate (70:30, C+, NDF content: 34.5%). The level of restriction applied with the C+ diet was determined from pre-experimental ad libitum intakes, ensuring that both diets provided the same intake of net energy for lactation (NE_L). For data collection, cows were housed in respiration chambers for 5 d. The climatic conditions were 10°C and 60% relative humidity (RH), considered thermoneutral (TN) conditions (temperature–humidity index (THI): 52) for d 1 and 2, and 25°C and 70% RH, considered heat stress (HS) conditions (THI: 74), for d 4 and 5. On d 3, the temperature and RH were increased gradually. Compared with TN, HS conditions increased the water intake, skin temperature, respiration and heart rates, and endogenous heat production. They did not affect body temperature, feed intake, or milk production. Lowering dietary fiber content via concentrate supplementation lowered methane and increased carbon dioxide production. It did not mitigate physiological responses to HS. Although the responses of horned and disbudded cows were generally similar, the slower respiration rates of horned cows under HS conditions indicate a possible, albeit minor, role of the horn in thermoregulation. In conclusion, future investigations on nutritional strategies must be conducted to mitigate mild heat stress.

Key words: cattle, temperature–humidity index, hay, horns, respiration chamber, methane

INTRODUCTION

Due to the high metabolic load related to milk synthesis and the associated endogenous heat production, dairy cows are sensitive to environmental heat load. Heat stress (**HS**) has been given various definitions; it may refer to any response to external heat load that helps the animal avoid physiological dysfunction (Kadzere et al., 2002), or to the increase of the animal's optimum core body temperature when external and internal heat loads exceed the animal's heat dissipation capacity (Dikmen and Hansen, 2009; Bernabucci and Mele, 2014). Heat stress may have negative effects on animal welfare (Polsky and von Keyserlingk, 2017) and performance (e.g., reduced feed intake, milk yield and composition or fertility disorders). The threshold at which HS begins has been reported differently by different authors. While the threshold for HS was once thought to be a temperature–humidity index (**THI**) of 72 (Armstrong, 1994), later, a threshold of a THI >68 was proposed, as this was shown to increase body temperature and decrease milk production in dairy cows (Bouraoui et al., 2002; Zimbelman et al., 2009). More recently, a THI of 47 was determined as the threshold for certain heat-stress-related behavioral changes in moderate climate zones (Heinicke et al., 2018). Studies investigating not only the effect of the heat load intensity but also of the heat load duration on HS are relatively new (Foroushani and Amon, 2022). The duration of heat exposure may be relatively short (i.e., several hours to one day) to cause behavioral changes

(Heinicke et al., 2018), of one day to decrease milk components (Ouellet et al., 2019), and more than 2 d can decrease feed intake and milk yield (Spiers et al., 2004) and induce changes in the milk fatty acid profile (Liu et al., 2017). Obviously, these time spans depend on environmental conditions, such as temperature, RH, and solar radiation, as well as animal-related factors, such as breed, production level, lactation stage, and genotypes (reviewed by Kadzere et al., 2002). To date, little is known about the effects of sporadic short-term exposure to heat load. As short periods of heat load are more frequent than longer ones (Rodriguez-Venegas et al., 2022), the effects of short-term heat loads require investigation to detect HS in its early stages and thus improve animal welfare.

Forage-based production systems aim to optimize the valorization of human-inedible resources by ruminants and thereby minimize feed–food competition. Digestible fiber, the major type of nutrient in forage-based diets, is considered to cause a greater heat increment than other nutrients—that is, starch, proteins, or fat—due to the greater heat production of acetate metabolism compared with propionate metabolism (West, 1999). Consequently, metabolic heat production increases with greater dietary fiber content (Kadzere et al., 2002), and therefore, dairy cows in forage-based production systems are particularly exposed to HS. The risk of HS may be further aggravated by grazing, because on pasture, cows may be directly exposed to a great heat load, including high ambient temperature, humidity, and solar radiation. It has been shown that decreasing the content of NDF of total mixed rations containing 60% to 70% of concentrate decreases heat-stress-related physiological responses and negative effects on animal performance in high-yielding dairy cows (West, 1999; Miron et al., 2008). However, it has not been determined whether the reduction in dietary NDF content also has a mitigating effect in forage-based diets.

The role of horns in the thermoregulation of cattle has frequently been discussed. Picard et al. (1994, 1999) concluded from ex vivo studies that the horns of cattle may dissipate heat. A more recent study (Baars et al., 2019), in which differences in single milk fatty acids of horned and disbudded cows housed under cold ambient temperatures were observed, revived this hypothesis by suggesting that the differences might be caused by heat loss via the horns and a thereby altered metabolism. In cassowaries, under field conditions, it was found that their casque, a horned integument appendage, plays a role in heat dissipation (Eastick et al., 2019). Increased casque surface temperatures, recorded using thermography images, identified the casque as the body part dissipating most heat under high ambient temperatures. Despite these indications, to date, the role of horns in thermoregulation under HS conditions has not yet been investigated. In case horns had a distinct role, this would be of importance as most dairy cows are disbudded as calves. Due to earlier described influences of horn status on stress physiology (Reiche et al., 2019, 2020) and milk fatty acids (Baars et al., 2019), the horn status was taken into account in the present study to elucidate possible interferences with responses to HS and dietary NDF content.

The aim of the present study was to investigate the influence of the NDF content of hay-based diets and horn status on the responses, namely feed and water intake, milk production and composition, indicators of HS and heat production, of mid-lactating Brown Swiss dairy cows to short-term HS conditions. We hypothesized that reducing dietary NDF content decreases endogenous heat production and therefore mitigates HS. We further hypothesized that the horn, despite its small size and surface area compared with the surface area of the whole body, has an influence on the heat dissipation of dairy cows.

MATERIALS AND METHODS

Animals

This experiment was conducted in accordance with the Swiss laws of animal protection and was approved by the cantonal veterinary office of Zurich, Switzerland (ZH 127/2020).

It was carried out between March and July 2021 at the metabolic research center of AgroVet-Strickhof, Lindau, canton of Zurich, Switzerland. We used 20 lactating Brown Swiss cows. The Brown Swiss breed was chosen, because it is the second most prevalent dairy breed in Switzerland, that has an important percentage of horned cows compared with other breeds, for example Holstein. Therefore, studying the presence of horns and its potential consequences is relevant to dairy farming. The cows were selected from the entire Brown Swiss population based on herdbook data and breeding values of Braunvieh Schweiz (https://homepage.braunvieh.ch/) and farm visits, according to the following criteria: they had similar genetic indices (mean milk index: 109, ranging from 99 to 121), mean fitness index: 100 (ranging from 90 to 110; Braunvieh Schweiz, 2020), and body conformation (Table 1). At the start of the experiment, the animals were pregnant and in mid-lactation (mean DIM: 161 d) of their second or third lactation. Half of the cows were horned, and half were disbudded as calves. During farm visits, one to 2 mo before the start of the experiment, the cows' body dimensions—wither height, sternum height, hip height, hip width, heart girth, and body length—were

Table 1. Characteristics related to milk production and body dimensions of experimental cows

| | | Horn status[1] | | | |
| | | H$^+$ (n = 9) | | H− (n = 10) | |
Item	All	Mean	SD	Mean	SD
Lactation number	2.3	2.3	0.5	2.2	0.4
DIM	152	150	6.4	153	12.5
Milk yield previous lactation (kg)	5,933	5,998	958	5,869	656
BW (kg)	636	632	44	640	39
Body dimensions (cm)					
Withers height	147	148	4	145	4
Hook height	147	149	3	145	3
Sternum height	69	70	4	68	6
Hip width	57	58	3	56	2
Heart girth	203	202	5	204	7
Body length	170	172	5	168	5
Backfat thickness (mm)	0.81	0.81	0.3	0.81	0.2

[1]H$^+$ = horned; H− = disbudded.

measured using a measuring stick (custom-made by Braunvieh Schweiz, Zug, Switzerland), as described in Raschka et al. (2016). This selection process resulted in obtaining 20 cows from 17 different Swiss dairy farms located in the Uri, Schwyz, Appenzell Innerrhoden, Sankt Gallen, and Graubünden cantons. For the experiment, the cows were transported to the metabolic research center farm and returned to their home farms afterward. Before the arrival on the experimental farm, the cows were kept indoors where they were fed forage-based diets. One cow was not pregnant upon arriving at the experimental farm but was successfully inseminated on the sixth day after arrival. Each animal underwent a medical check before transport and was clinically healthy upon arrival at the experimental farm. For each cow, the experiment lasted 35 d.

Experimental Design

The experiment was a crossover study using 2 experimental diets: (i) a diet composed of hay and alfalfa pellets (85:15, **C−**) offered with ad libitum access, allowing for 10% hay orts; and (ii) a diet containing a restricted amount of hay and concentrate (70:30, **C+**; Table 2). All cows received a mineral supplement (150 g/animal/d MINEX 974, UFA, Herzogenbuchsee, Switzerland, and 60 g/animal/d livestock salt). The amounts of alfalfa pellets in the C− diet were calculated to meet a crude protein content of 13% of the entire diet. Aiming for a similar daily energy intake, the restricted amounts of the C+ diet were calculated based on the cows' individual daily NE_L intake (Agroscope, 2021) when receiving the C− diet during the first 5 d after arrival on the experimental farm (preparation period). On d 6 after arrival, cows were equally allocated to one of the 2 experimental diets for the next 15 d (first pe-

riod). On d 21, the diet was changed from C− to C+ or from C+ to C− for the next 15 d (second period). Each period contained a 10-d adaptation and a subsequent 5-d sampling period. For technical reasons, 4 cows went through the experiment at a time, resulting in 5 blocks, each containing horned and disbudded cows.

During the 5-d preparation and 10-d adaptation periods, the cows were housed in a tie-stall on rubber mats, interspersed with straw. The animals had free access to fresh water and could physically interact with their neighbors. The cows were milked at 0600 and at 1700 h,

Table 2. Ingredients and means of chemical composition of experimental diets

| | Diet[1] | |
Item	C−	C+
Ingredients (% of total diet)		
Hay	85	70
Alfalfa pellets	15	—
Concentrate	—	30
Wheat	—	12.6
Corn	—	8.9
Oat	—	3.0
Barley	—	2.9
Soybean meal	—	1.5
Corn gluten meal	—	1.1
Analyzed composition		
DM (% of original substance)	96.3	93.8
CP (% of DM)	12.8	13.1
NDF (% of DM)	41.6	33.7
Calculated energy and protein supply[2]		
NE_L (MJ/kg DM)	5.7	6.5
APDE (% of DM)	10.2	11.0
APDN (% of DM)	9.1	9.8

[1]C− = ad libitum hay and alfalfa pellets; C+ = restricted amounts of hay and concentrate.

[2]Calculated according to Agroscope (2021). APDE/APDN = absorbable protein at the duodenum consisting of rumen-bypass protein and microbial protein from fermentable energy/rumen-degradable protein.

and feed was provided immediately after milking. Hay was offered using weighing plates (model PFA575, floor scale, Mettler-Toledo, Greifensee, Switzerland), and alfalfa pellets and concentrates in a manger mounted in each individual stall. The ambient temperatures in the tie-stall barn ranged from 9 to 17°C over the course of the experiment. Every other day, the cows had access to an outdoor pen for approximately 30 min.

During the 5-d sampling periods, cows were housed individually in respiration chambers (**RC**) with a volume of 38 m^3 (No Pollution, Industrial Systems Ltd., Edinburgh, UK), described in detail by Zhang et al. (2022) and Keller et al. (2022), to measure their gaseous exchange. The gas measurements were continuous, with a measurement every 10 min for each RC. Zero and standard gas calibrations of the RC were conducted daily (between 0700 and 0900 h). In the RC, the light circle was set from 0600 to 2000 h. Before and during the experiment, 3 recovery tests (total system test) were performed (beginning of March, end of April, and mid-June 2021). For the recovery tests, gas (CO_2 and CH_4) was injected individually into each RC using the same RC settings as for the animal measurements. The average recovery rates of all chambers were about 88% for CO_2 and 99% for CH_4. Before the data analysis, the values of produced CH_4 and CO_2 and consumed O_2 were corrected with the recovery rates of the corresponding RC closest in time.

For the first 48 h of the sampling periods, the THI conditions (**THI-C**) of the RC were set to thermoneutrality (**TN**; ambient temperature: 10°C, relative humidity (**RH**): 60%, and THI: 52 (calculated following Dikmen and Hansen, 2009). Afterward, the ambient temperature and RH were gradually increased over 24 h to reach, at the end of the 24-h period, heat stress conditions (**HS**; ambient temperature: 25°C, RH: 70%, THI: 74). The HS conditions were maintained for 48 h. Afterward, the cows were released from the RC and returned to the tie-stall. With a THI of 74, a comparably mild but distinct heat load level was chosen, which has been shown to decrease the milk yield of Holstein dairy cows in Italy (when the THI ranged from 71 to 76 (Bernabucci and Mele, 2014). On the first day of each sampling period, the cows entered the RC after the morning milking at around 0800 h. The gas measurements started at midday to ensure that the gases in the RC had reached approximately stable values. For habituation to the RC, each cow spent once 4 to 8 h in the RC during the first adaptation period.

Sampling and Sample Preparation

Each cow's body weight was measured in the first, third, and last experimental week using a livestock scale (Ammann Waagen, Ermatingen, Switzerland). The subcutaneous backfat thickness was measured during the second adaptation period using an ultrasound device with a linear probe (LOGIQ e R8, GE HealthCare, Illinois, USA) by the same person following Raschka et al. (2016). Throughout the experiment, the cows' milk yields and feed intakes were recorded daily. Both the tie-stall and RC were equipped with bucket milking systems. The amounts collected in the bucket were weighed using a scale (preparation and adaptation periods: PFA575, floor scale model, Mettler-Toledo, Greifensee, Switzerland; sampling period: KC300s, Mettler Instrumente AG, Greifensee, Switzerland). During the adaptation and sampling periods, milk samples were collected from the milk buckets directly after each morning and evening milking using a measuring cup. The sampled milk was transferred into 50 mL tubes containing a bronopol-based preservative and chilled until analyzed for fat, protein, lactose, and urea content. For the analysis of milk fatty acids during sampling periods, an additional 50 mL of milk was taken at the last morning milking under TN and HS conditions. These samples were immediately frozen at −20°C until analysis.

The amounts of distributed feed and leftovers were weighed using the same scales as for milk weighing (KC300s, Mettler Instrumente AG). Feed intake was calculated by subtracting the amount of leftovers of each of the 3 feed components from the amount of distributed feed. For feed analysis, a pooled sample for each of the 3 feed components—hay, alfalfa pellets, and concentrate—was taken during each sampling period. Feed samples were stored until analysis in a dry and dark place.

During the 5-d sampling periods, the cows were equipped with Polar devices (Polar Team Pro, Finland) to record their heart rates (**HR**) continuously. The records lasted for a maximum of 8 h, and the devices were replaced after each milking to ensure a recording of 16 h per day. Forty minutes before milking (i.e., at 0520 and 1620 h), the respiratory rate (**RR**) of each cow was recorded using visual observation via video cameras (SNV-L5083RN/P, Samsung, South Korea) installed in the RC. The RR was counted for 15 s and multiplied by 4 to obtain the breaths per minute. Ten minutes before milking, rectal temperature was measured using a digital thermometer with an accuracy of 2 decimal digits and a flexible tip (Domotherm Rapid, UEBE Medical GmbH, Eilheim, Germany). To measure the surface temperature, thermographic images were collected during the last hour under both TN and HS conditions (i.e., between 1100 h and midday). Three body locations were recorded using an infrared camera (Fluke Ti25, Fluke Corporation, Everett, WA, USA),

including the neck, the back of the ears, and the back and front of the horns. All thermographic images were taken by the same person and from predefined positions in the RC to ensure similar distances between the recorded body parts of the cows and the camera. The thermographic images were analyzed using the software SmartView (Fluke) to obtain the minimum, mean, and maximum surface temperatures of the recorded body locations.

Laboratory Analyses

The analyses for milk fat, protein, lactose, and urea were carried out on the bronopol-preserved, 50-mL milk samples by Fourier transformed infrared spectrometry using a MilkoScan FT6000 (Foss, Hillerød, Denmark) at SuisseLab (Zollikofen, Switzerland). The milk fatty acid (**FA**) composition was analyzed using gas chromatography with flame ionization detection (Bär et al., 2020). In brief, after transesterification, the milk lipids were extracted with hexane, containing the internal standard C9 and esterified with Methanol/KOH solution in methyl ester according to ISO 15884:2002. Separated on a capillary column (CP-SIL 88, 100m, 250 μm i.d., 0.20 μm film thickness, Agilent J and W Part No CP958570), the fatty acids were determined using a gas chromatograph (Agilent 6890 Plus, Agilent Technologies, Basel, Switzerland) and manually integrated. Feed samples were analyzed for DM, ADF, NDF, and N. The contents of DM and OM were determined gravimetrically by oven drying at 105°C for 3 h and ashing at 550°C for 4 h. The NDF and ADF contents were determined using Fibertherm (Gerhardt, Königswinter, Germany; methods ISO 16472:2006 for NDF and ISO 13906:2008 for ADF). The total N content was analyzed using the Kjeldahl method (AOAC International, 1995; method 988.05) and multiplied by 6.25 to calculate the CP content. The dietary contents of NEL and absorbable protein in the duodenum were calculated as described by Agroscope (2021).

Calculations and Statistical Analysis

The daily heat production (**HP**) per unit of metabolic BW (**mBW**) was calculated according to Brouwer (1965), using the volumes of produced CH_4 (V_{CH4}) and CO_2 (V_{CO2}) production and consumed O_2 (V_{O2}):

$$\frac{HP}{mBW}\left(\frac{kJ}{kg^{0.75}}\right) = \frac{16.18 \times V_{O2}(L) + 5.02 \times V_{CO2}(L) - 2.17 \times V_{CH4}(L) - 5.99 \times N_u(g)}{mBW\left(kg^{0.75}\right)}.$$

Urinary N excretion (N_u) was estimated according to Weiss et al. (2009):

$$N_u = -75 + (0.412 \times N_i)$$

with N_i being the daily ingested nitrogen amount (g). Conductance was calculated according to Klaus et al. (2005), using the HP, rectal temperature (RT), ambient temperature (AT), and metabolic BW: $C[kJ/(K \times kg^{0.75})] = HP(kJ)/\{[RT(°C) - AT(°C)] \times mBW\}$.

Before the experiment, a power analysis was performed on the variable HP using the software G*Power (Faul et al., 2007). Setting the type-I error to $\alpha = 0.01$ and the power to 0.80, n = 20 was necessary to detect effects of an effect size of 0.4 in a repeated measure model.

Body weight was averaged by cow. Backfat thickness was measured on the frozen ultrasound images, as described by Raschka et al. (2016), using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Data on milk yield were obtained by summing up morning and evening milk yields for each experimental day. Morning and evening values of the milk composition were averaged, weighted by milk yield, for every experimental day. The ECM (kg/d) was calculated as milk (kg/d) × [0.38 × fat (%) + 0.24 × protein (%) + 0.17 × lactose (%)]/3.14 (Agroscope, 2021). Due to influencing outliers in the data of milk yield and composition, only observations within the interval mean ± 1.96 × SD, namely 93%, 96%, 99%, 99%, and 96% of the data for milk yield, ECM, lactose, protein and fat, respectively, were used for statistical analysis. Due to the limit of quantification, only FAs with a proportion of >0.1 g/ 100 g fat are presented.

For the analysis of HR, only 8-h measurements with a relative standard error <5% were used (Gygax et al., 2008). The measurements were averaged by cow and an 8-h measurement period. Thermographic images and ultrasound images of insufficient quality (i.e., images that were not sharp) were removed from analysis.

For statistical analysis, the R environment was used (Core, 2018), specifically the package lme4 (Bates et al., 2014). Only data collected during the sampling periods were used for analysis. During the experiment, one horned cow developed endometritis and miscarried, and was therefore removed from the data analysis. To investigate the effects of diet, horn status, THI condition, and their interactions, linear mixed models with repeated measures were computed with diet, horn status, THI-C, diet × THI-C, and horn status × THI-C, as fixed effects. The interaction of diet × horn status was not significant and was therefore excluded from the model. For respiration rate, heart rate, and body temperature, the time of day (**TOD**; a.m. vs. p.m.) was introduced as an additional fixed effect. Animal, block, and period were used as random effects. Effects

are presented if they were present in at least 4 blocks, if not otherwise indicated.

Principal component analyses (PCA) were conducted using the R package factoMineR (Lê et al., 2008). Only variables with a cos2 > 0.3 and >0.8 were retained and presented in the plots, respectively.

RESULTS

Feed and Water Intake

The THI-C did not affect feed intake ($P = 0.70$). As intended, the NEL intake did not differ between diet treatments ($P = 0.80$; Table 3). Consequently, cows receiving the C− diet had a greater DMI ($P < 0.001$), NDF intake ($P < 0.001$), and CP intake ($P < 0.001$) than C+ cows. Water intake was greater ($P < 0.001$) when cows received the C− diet and under HS than when receiving the C+ diet and under TN. Horn status did not influence feed and water intakes (all $P \geq 0.47$). No interactions were present ($P \geq 0.32$).

Milk Yield and Composition

The THI-C did not affect milk yield and composition ($P \geq 0.42$), except for milk lactose content, which was greater under TN than under HS conditions ($P = 0.020$). Cows receiving the C− diet had lower daily milk and ECM yields ($P < 0.001$ and $P = 0.023$, respectively) and milk lactose content ($P = 0.043$) and greater ($P = 0.045$) protein content than C+ cows (Table 3). Milk fat content was not affected by diet ($P = 0.15$). There was no effect of horn status on milk yield or composition. The MUN content did not differ by to THI, diet, or horn status (Table 3). No interactions were present for milk yield and composition ($P \geq 0.25$).

The THI-C did not alter the FA profile ($P \geq 0.15$). Milk of the C− cows had greater proportions of SFA C15:0, C16:0, C17:0 and therefore total SFA (all $P < 0.001$), of most branched-chain FA (C15:0 iso and aiso, C16:0 iso, and C17:0 iso), MUFA C14:1 *cis*9 (n-5), C16:1 u1, C16:1 *cis*9 (n-7), and C17:1 *cis*10 (n-7), and PUFA C18:2 u2, C18:2 *trans*9,*cis*12 (n-6), and C18:3 *cis*9,*cis*12,*cis*15 (n-3) than milk of C+ cows (all $P \leq 0.026$; Table 4). In contrast, the C− cows had lower proportions of short-chain FA C 8:0 and C10:0 and therefore total SCFA, SFA C18:0, most C18:1 isomers, and certain PUFA (C18:2 u2, C18:2 u3, C18:2 *cis*9,*cis*12 [n-6], and C22:5 *cis*7,*cis*10,*cis*13,*cis*16,*cis*19 [n-3]) than C+ cows (all $P \leq 0.016$). Overall, the milk of C− cows contained greater proportions of total medium-chain and total n-3 FA and lower proportions of the long-chain FA, oleic (C18:1), linoleic (C18:2), MUFA, PUFA, total UFA and n-6 FA (all $P < 0.001$).

Table 3. Feed and water intake, milk production, and composition as affected by THI condition, diet, and horn status[1]

Item	n	THI condition (THI-C)		Diet		Horn status		SEM	P-value				
		TN	HS	C-	C+	H+	H-		THI-C	Diet	Horns	THI-C × diet	THI-C × horns
Intake													
Total DM (kg/d)	152	18.3	18.3	19.4	17.1	18.2	18.4	0.17	0.70	<0.001	0.97	0.96	0.70
Hay	152	14.8	14.6	16.8	12.3	14.8	14.7	0.23	0.88	<0.001	0.54	0.32	0.89
Concentrate	152	2.4	2.5	—	4.8	2.4	2.6	0.21	0.95	<0.001	0.47	0.55	0.88
Alfalfa pellets	152	1.1	1.1	2.6	—	1.0	1.2	0.09	0.92	<0.001	0.74	0.41	0.79
NE$_L$ (MJ/d)	152	110	110	112	109	109	111	0.95	0.70	0.36	0.80	0.73	0.65
NDF (g/d)	152	6,986	6,921	7,887	5,901	6,922	6,981	102.7	0.80	<0.001	0.80	0.61	0.86
CP (g/d)	152	2,369	2,374	2,494	2,233	2,339	2,399	22.6	0.64	<0.001	0.80	0.56	0.64
Water (L/d)	152	78.4	88.2	89.8	77.2	82.9	83.9	0.82	<0.001	<0.001	0.66	0.93	0.46
Milk production[2]													
Milk yield (kg/d)	142	19.1	19.8	19.0	19.9	19.2	19.7	0.21	0.86	<0.001	0.46	0.56	0.042*
ECM (kg/d)	146	20.1	20.6	19.9	20.8	20.3	20.4	0.25	0.78	0.023	0.49	0.67	0.093*
Fat (%)	146	4.20	4.20	4.22	4.18	4.25	4.15	0.038	0.67	0.15	0.87	0.70	0.80
Protein (%)	151	3.79	3.74	3.79	3.74	3.80	3.74	0.024	0.48	0.045	0.45	0.82	0.25
Lactose (%)	150	4.78	4.75	4.76	4.77	4.77	4.76	0.023	0.020	0.043	0.83	0.82	0.49
MUN (mg/dL)	145	5.22	5.31	5.08	5.45	4.94	5.64	0.218	0.42	0.066*	0.095*	0.070*	0.95

[1]C−, = ad libitum hay and alfalfa pellets; C+ = restricted amounts of hay and concentrate; H+, horned; H−, disbudded; HS, heat stress; THI, temperature–humidity index; TN, thermoneutrality.

[2]Due to influential outliers, only data within the interval mean ± 1.96 SD are included.

*Effect present in only ≤3 blocks.

The diet-related differences in the C10:0 and C18:2 *cis*9 and *cis*12 (n-6) proportions were more pronounced under HS than under TN (interaction effects $P = 0.027$ and $P = 0.004$, respectively; Supplemental Table S1). The milk of H^+ cows had greater proportions of SFA C10:0 and C18:0 and branched-chain FA C17:0 iso (all $P \leq 0.034$), and had greater proportions of C18:1 *cis*12 (n-6; $P = 0.05$) and C18:3 *cis*9,*cis*12,*cis*15 (n-3; $P = 0.087$) than the milk of H− cows. Further, the milk of H^+ cows had lower proportions of C16:0 ($P = 0.039$), C16:1 *cis*9 (n-7; $P < 0.001$), C18:2 *cis*9,*trans*11 (n-7; $P = 0.092$) than the milk of H− cows. Overall, H^+ cows had greater proportions of SCFA, and lower proportions of medium-chain FA, total CLA, and n-6 FA (all $P \leq 0.10$). No interactions of THI-C with horn status were found (all $P \geq 0.11$).

The natural clustering of milk samples of C− and C+ cows based on fatty acid composition is graphically illustrated in a PCA plot (Figure 1). The first axis is strongly related to total n-6 FA, total UFA, total C18:2 FA, and total C18:1 FA, whereas the second axis is strongly related to total CLA.

Physiological Indicators of HS

In comparison to TN, HS increased the cows' respiration rates ($P < 0.001$), HR ($P = 0.014$), and surface temperatures ($P < 0.001$; **Table 5**), but had no effect on body temperature. Cows receiving the C− diet had lower HR than C+ cows ($P = 0.013$; **Table 5**). Diet had no influence on respiration rate or body and surface temperatures. Horn status had no overall effect on the physiological indicators. Under TN conditions, horn surface temperatures were greater in horned C− cows than horned C+ cows (interaction effect: $P < 0.001$; Supplemental Table S1). Under HS conditions, H^+ cows showed lower respiration rates (30.5 ± 1.0 bpm) than H− cows (36.6 ± 1.3 bpm; interaction effect: $P = 0.015$; Supplemental Table S1). No other interactions of horn status and THI-C on physiological indicators were found, and no interactions were found for heart rate or body temperature (all $P \geq 0.10$). Heart rates were slower ($P < 0.001$) and body temperatures were lower ($P < 0.001$) for a.m. than for p.m. measurements.

Gas Exchange and Heat Production

Under TN conditions, cows consumed less oxygen ($P < 0.001$) and produced less heat ($P < 0.001$) than under HS conditions. Cows receiving the C− diet produced more carbon dioxide ($P = 0.002$) and methane ($P < 0.001$) per day than C+ cows (Table 5). The HP was not influenced by diet ($P = 0.72$). Horn status did not influence the gas exchange or HP of the cows. No interactions were found ($P \geq 0.10$).

DISCUSSION

We investigated the responses of horned and disbudded dairy cows to HS conditions over 2 d while being fed 2 diets that differed in their NDF content. Individually, the 3 experimental factors—THI conditions, diet, and horn status—influenced the cows' physiology and milk production in different ways. However, the mostly lacking interactions illustrate that neither variations in dietary NDF content nor horn status altered the cows' responses to the imposed HS conditions.

In response to the imposed HS conditions, the cows increased their respiration and HR, skin surface temperature, water intake, and HP. These adaptive physiological responses are in line with many previous studies and are part of the thermoregulatory mechanisms that become activated under conditions of increased environmental heat load (Kadzere et al., 2002, Sejian et al., 2018). Thermoregulatory responses aim at maintaining homeostasis (i.e., a steady body temperature), which, in the case of an increased heat load, includes the dissipation of heat from the body. An immediate heat dissipation mechanism is peripheral vasodilatation, which aims to transport heat via blood to the skin, where it is lost through radiation, convection, and conduction, or evaporatively by sweating. In response to increased skin blood flow, the heart rate increases to maintain blood pressure (Kadzere et al., 2002; Sejian et al., 2018; Hoffmann et al., 2020; Henderson et al., 2021). An increased respiration rate aims to increase evaporative heat loss via exhaled air and to increase the O_2 supply in response to greater cellular O_2 demand for cardiac activity and accelerated metabolic rate (Ames et al., 1971; Henderson et al., 2021). In turn, the increased respiration rates also increase the O_2 demand (for breathing muscles; Henderson et al. (2021). Finally, to compensate for the water losses due to evaporation through respiration and sweating, the animal increases its water intake. The thermoregulatory physiological changes generate heat, and the heat-stress-related greater O_2 consumption and CO_2 production lead to greater HP, as found in the present study.

In this study, short-term HS conditions did not increase the cows' body temperatures, suggesting that thermoregulatory responses were effective in maintaining a steady body temperature. In a previous study (Garner et al., 2017) with late-lactating Holstein dairy cattle of a similar daily milk yield (~18 kg) housed in climate chambers under similar THI conditions for 4 d, the cows' rectal temperatures increased at a THI of 74 to 84 (daytime: 80–84, nighttime: 74) compared

Table 4. Proportions of milk fatty acids (g/100 g of total fatty acids) as affected by THI condition, diet, and horn status[1,2]

Item	THI condition (THI-C)		Diet		Horn status		SEM	P-value				
	TN	HS	C−	C+	H+	H−		THI-C	Diet	Horns	THI-C × diet	THI-C × horns
SFA												
C4:0	2.68	2.73	2.72	2.71	2.67	2.75	0.026	0.42	0.87	0.31	0.11	0.26
C6:0	1.92	1.96	1.91	1.97	1.96	1.92	0.015	0.097*	0.19	0.55	0.004*	0.65
C8:0	1.25	1.27	1.20	1.31	1.30	1.22	0.013	0.44	<0.001	0.012*	0.011*	0.94
C10:0	3.02	3.10	2.93	3.18	3.22	2.90	0.046	0.47	0.003	0.020*	0.027	0.96
C12:0	3.61	3.68	3.54	3.75	3.85	3.45	0.059	0.56	0.18	0.040*	0.097*	0.99
C14:0	11.2	11.4	11.3	11.4	11.5	11.1	0.099	0.45	0.98	0.29	0.074*	0.92
C15:0	1.27	1.22	1.43	1.10	1.31	1.22	0.033	0.62	<0.001	0.56	0.130	0.45
C16:0	34.4	33.8	35.8	32.7	33.0	35.4	0.355	0.93	<0.001	0.039	0.69	0.60
C17:0	0.49	0.48	0.55	0.43	0.50	0.48	0.009	0.61	<0.001	0.75	0.035*	0.27
C18:0	4.61	4.82	4.21	5.12	5.06	4.31	0.119	0.55	<0.001	0.034	0.73	0.96
Branched-chain fatty acids												
C15:0 iso	0.23	0.24	0.27	0.20	0.25	0.23	0.006	0.48	<0.001	0.16	0.035*	0.68
C15:0 aiso	0.49	0.50	0.54	0.44	0.49	0.49	0.010	0.96	<0.001	0.63	0.55	0.67
C16:0 iso	0.22	0.22	0.24	0.19	0.22	0.22	0.005	0.33	<0.001	0.45	0.58	0.82
C17:0 iso	0.33	0.33	0.35	0.32	0.35	0.32	0.005	0.76	0.026	0.011	0.28	0.99
MUFA												
C10:1 (n-1)	0.47	0.46	0.46	0.47	0.48	0.46	0.006	0.44	0.81	0.31	0.31	0.95
C14:1 *cis*9 (n-5)	1.21	1.21	1.22	1.19	1.16	1.24	0.015	0.78	0.019	0.18	0.092*	0.80
C16:1 u	0.12	0.12	0.12	0.12	0.12	0.12	0.002	0.32	0.011	0.65	0.55	0.46
C16:1 *cis*9 (n-7)	1.55	1.51	1.60	1.47	1.37	1.67	0.030	0.96	0.004	<0.001	0.31	0.95
C16:1 u	0.26	0.26	0.26	0.26	0.27	0.25	0.005	0.85	0.28	0.24	0.39	0.58
C17:1 *cis*10 (n-7)	0.21	0.20	0.23	0.18	0.20	0.21	0.004	0.49	<0.001	0.089*	0.004	0.34
C18:1 *cis*9 (n-9)	11.2	11.4	10.1	12.4	11.5	11.0	0.200	0.94	<0.001	0.57	0.58	0.37
C18:1 *cis*11 (n-7)	0.36	0.37	0.34	0.38	0.37	0.36	0.007	0.70	<0.001	0.93	0.88	0.043*
C18:1 *cis*12 (n-6)	0.12	0.13	0.09	0.16	0.14	0.11	0.005	0.52	<0.001	0.05	0.69	0.11
C18:1 *trans*6–9	0.24	0.24	0.19	0.28	0.23	0.24	0.008	1.00	<0.001	0.81	0.69	0.83
C18:1 *trans*10–11	1.21	1.35	1.15	1.33	1.14	1.33	0.069	0.69	0.648	0.51	0.45	0.68
C18:1 *trans*12 (n-6)	0.13	0.14	0.09	0.17	0.13	0.13	0.006	0.90	<0.001	0.80	0.47	0.48
C18:1 *trans*13,*cis*6–7	0.35	0.36	0.28	0.41	0.37	0.33	0.015	0.95	<0.001	0.54	0.90	0.61
C18:1 u	0.17	0.18	0.14	0.21	0.18	0.16	0.007	0.78	<0.001	0.41	0.66	0.66
C20:1 *cis*8–9	0.10	0.10	0.10	0.10	0.10	0.10	0.002	0.92	0.72	0.50	0.56	0.80
PUFA												
C18:2 u	0.14	0.14	0.12	0.16	0.14	0.14	0.005	0.68	<0.001	0.80	0.94	0.43
C18:2 *trans*9,*cis*12 (n-6)	0.18	0.19	0.22	0.15	0.18	0.19	0.008	0.81	<0.001	0.75	0.91	0.91
C18:2 *cis*9,*cis*12 (n-6)	1.08	1.16	0.75	1.46	1.19	1.02	0.050	0.62	<0.001	0.034	0.004	0.37
C18:2 *cis*9,*trans*11 (n-7)	0.61	0.64	0.62	0.61	0.54	0.69	0.027	0.92	0.279	0.092	0.54	0.856
C18:3 *cis*9,*cis*12,*cis*15 (n-3)	0.82	0.85	0.94	0.72	0.90	0.77	0.021	0.64	<0.001	0.087	0.21	0.073*
C22:5 *cis*7,*cis*10,*cis*13,*cis*16,*cis*19 (n-3)	0.10	0.10	0.09	0.11	0.11	0.09	0.004	0.85	<0.001	0.48	0.71	0.51
Sums of fatty acids												
Σ SCFA (C4:0 to C10:1)	9.41	9.59	9.29	9.70	9.70	9.31	0.070	0.15	0.016	0.058	0.007*	0.70
Σ medium-chain fatty acids	54.1	53.7	55.8	52.3	53.3	54.7	0.311	0.73	<0.001	0.058	0.92	0.74
Σ long-chain fatty acids	23.9	24.6	22.0	26.1	24.8	23.4	0.359	0.72	<0.001	0.23	0.97	0.56
Σ C18:1	13.9	14.3	12.5	15.4	14.2	13.8	0.232	0.83	<0.001	0.69	0.90	0.44
Σ C18:2	2.27	2.38	1.98	2.60	2.29	2.30	0.062	0.99	<0.001	0.95	0.18	0.92
Σ SFA	78.7	78.8	80.0	77.8	78.8	79.0	0.240	0.66	<0.001	0.70	1.00	0.62
Σ UFA	21.9	22.3	20.4	23.6	22.2	21.8	0.259	0.86	<0.001	0.93	0.74	0.30
Σ MUFA	18.3	18.6	17.0	19.7	18.5	18.3	0.217	0.81	<0.001	0.83	0.57	0.30
Σ PUFA	3.54	3.69	3.37	3.79	3.66	3.51	0.063	0.99	0.002	0.54	0.52	0.63
Σ CLA	0.71	0.74	0.72	0.69	0.62	0.78	0.029	0.88	0.22	0.10	0.60	0.81

Continued

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Table 4 (Continued). Proportions of milk fatty acids (g/100 g of total fatty acids) as affected by THI condition, diet, and horn status[1,2]

Item	THI condition (THI-C)		Diet		Horn status		SEM	P-value				
	TN	HS	C−	C+	H+	H−		THI-C	Diet	Horns	THI-C × diet	THI-C × horns
Σ trans fatty acids including trans CLA	2.91	3.12	2.76	3.13	2.78	3.10	0.115	0.81	<0.001	0.41	0.52	0.89
Σ n-3 fatty acids	1.19	1.24	1.31	1.11	1.29	1.14	0.025	0.72	<0.001	0.12	0.12	0.046*
Σ n-6 fatty acids	1.76	1.86	1.40	2.19	1.90	1.69	0.058	0.73	<0.001	0.058	0.027*	0.42

[1]C−, = ad libitum hay and alfalfa pellets; C+ = restricted amounts of hay and concentrate; H+, horned; H−, disbudded; HS, heat stress; THI, temperature–humidity index; TN, thermoneutrality; u, unknown position of the double bond.
[2]76 milk samples were analyzed.
*Effect present in only ≤3 blocks.

with a THI of 55–61 on each of the 4 d of this experiment. It can be assumed that the greater daytime THI used in Garner et al.'s (2017) study—that is, a greater heat load—was responsible for this different observation. This assumption is supported by the markedly greater increase in RR (∼80 breaths per min) found in Garner et al.'s (2017) cows, which indicates a stronger thermoregulatory response under HS conditions than in the present study (∼30 breaths per min). Alternatively, the cows in the present study might have been less sensitive to the imposed heat load, as the Brown Swiss breed is considered to be more heat-tolerant than other dairy breeds (Mylostyvyi et al., 2021). The lack of an increase in body temperature could also be related to the experimental design, namely, the relatively short exposure (48 h) to HS conditions, which might correspond to a first (effective) phase of the onset of thermoregulatory responses preceding an increase in body temperature (D. Rico, personal communication). As previously stated, depending on the author, HS may be defined as any heat load-related response that helps the animal maintain physiological functioning (Kadzere et al., 2002). Therefore, and in view of the lack of increase in body temperature, we consider that the cows in the present work experienced mild or beginning HS while being exposed to a constant THI of 74 for 48 h. This statement is supported by the marked increase in heat production (+90%) under HS conditions, as the HP is considered to increase with increasing heat load and to subsequently decrease once the animal has acclimatized to a thermal environment (reviewed by Galán et al. (2018).

In the present study, the imposed HS conditions did not affect feed intake or milk yield. A reduction in feed intake is a thermoregulatory strategy to reduce HP related to ingestion, digestion, and metabolization (Galán et al., 2018). As the described physiological thermoregulatory responses were effective, as suggested by the stable RT, reducing feed intake was probably not (yet) necessary. The unchanged milk yield under HS conditions partly resulted from the unchanged feed intake and the experienced "only" mild or beginning heat stress, showing that despite the onset of energy-consuming thermoregulatory responses, manifested in almost twice as high metabolic energy losses via HP, the metabolizable energy for milk synthesis was still sufficient within 48 h of exposure to heat stress (Baumgard and Rhoads, 2013). However, the seemingly negligibly decreased milk lactose content under HS (−1%) may indicate that the uptake of glucose into the mammary gland was limited, probably due to its use in any thermoregulatory/acclimatization mechanisms or changes in the endocrine profile that regulate anabolic and catabolic pathways (Wheelock et al., 2010;

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Table 5. Physiological heat stress indicators, gas exchange, and heat production as affected by THI condition, diet, and horn status[1]

| Item | n | THI condition (THI-C) | | Diet | | Horn status | | Time of day (TOD) | | SEM | P-values | | | | | |
		TN	HS	C−	C+	H+	H−	a.m.	p.m.		THI-C	Diet	Horns	THI-C × diet	THI-C × horns	TOD
Physiological indicators																
Respiratory rate (bpm)	304	19.4	33.7	25.6	25.5	24.1	26.9	26.1	25.1	0.54	<0.001	0.31	0.99	0.33	0.015	0.59
Heart rate (bpm)	240	78.6	84.3	78.3	84.6	80.9	81.6	77.7	85.3	0.76	0.014	0.013	0.75	0.68	0.60	<0.001
Body temperature (°C)	218[2]	38.5	38.5	38.5	38.4	38.5	38.4	38.4	38.5	0.02	0.89	0.13	0.37	0.22	0.87	<0.001
Surface temperature (°C)																
Neck	131	27.6	32.8	30.2	30.2	30.1	30.3	—	—	0.46	<0.001	0.89	0.97	0.80	0.81	—
Ears	132	23.6	31.9	28.3	27.4	28.1	27.7	—	—	0.82	<0.001	0.50	0.32	0.13	0.10	—
Horns	67	22.1	29.8	26.2	26.3	26.2	—	—	—	0.98	<0.001	0.002	—	0.001	—	—
Gas exchange (L/d)																
O$_2$ consumption	152	6,085	12,732	9,487	9,287	9,408	9,363	—	—	365.7	<0.001	0.72	0.65	0.90	0.64	—
CO$_2$ production	152	6,519	6,445	6,660	6,307	6,500	6,463	—	—	45.6	0.71	0.002	0.94	0.082*	0.83	—
CH$_4$ production	152	544	514	596	461	537	519	—	—	7.6	0.29	<0.001	0.58	0.18	0.60	—
Heat production (kJ/kg BW$^{0.75}$)	152	979	1,829	1,420	1,383	1,403	1,400	—	—	46.9	<0.001	0.72	0.84	0.67	0.40	—
Conductance (kJ/K*kg BW$^{0.75}$)	112	16.7	120.0	68.7	67.8	70.8	65.0	—	—	4.86	<0.001	0.78	0.31	0.79	0.10	—

[1]bpm, beats per minute; C−, = ad libitum hay and alfalfa pellets; C+ = restricted amounts of hay and concentrate; H+, horned; H−, disbudded; HS, heat stress; THI, temperature–humidity index; TN, thermoneutrality.

[2]One value <37.25°C was removed.

*Effect present in only ≤3 blocks.

Baumgard and Rhoads, 2013). In a humid continental climate, milk lactose content was found to be negatively correlated to the THI of the second day before milk sampling but not to that of the day before or the sampling day (Ouellet et al., 2019). Further investigation is needed regarding the suitability of a decrease in milk lactose content as an early indicator of beginning HS. Milk protein and fat contents were not altered by THI conditions, which is consistent with some and in contrast with other earlier studies (reviewed by Tao et al., 2018).

As given by the experimental design, feeding restricted amounts of hay and concentrate, compared with feeding ad libitum hay and alfalfa pellets, decreased the DM, NDF, and CP intakes but not the calculated NE_L supply. The greater water intake of ad libitum hay-fed cows relates to their greater DM intake (Bannink et al., 1999; Kume et al., 2010, Khelil-Arfa et al., 2012). The differences in the fatty acid profile of C+ and C− fed cows are consistent with known dietary effects on fatty acid profiles, including the greater contents of unsaturated and n-3 FA in the milk of hay-fed cows (Dewhurst et al., 2006). For example, feeding hay and increased hay:concentrate ratios increased n-3 and branched-chain FA and reduced oleic acid contents (Loor et al., 2004; Riuzzi et al., 2021). Cows receiving concentrate supplementation showed faster HR than ad libitum hay-fed cows, which might reflect a hunger stress-related increased activity due to feed restriction (De Paula Vieira et al., 2008).

The concentrate supplementation slightly increased ECM and milk lactose content and decreased milk protein content. The around 1 kg greater milk and energy-corrected milk yield of C+ cows, despite a similar calculated energy intake, might be related to an overestimation of metabolizable energy of the hay. The lower milk protein percentages of concentrate supplemented cows are explained by their lower crude protein intake. Due to their greater fiber intake, cows receiving the C− diet produced more methane than C+ cows (reviewed by Beauchemin et al., 2020). The C− cows further produced more CO_2, probably due to the combination of greater DMI intake and lower milk production (Jentsch et al., 2009).

Greater NDF intakes did not engender a greater HP in C− fed cows in the present study. This is in contrast to findings in beef heifers, where a lower forage-to-concentrate ratio and consequently reduced dietary NDF content at a similar ME supply was found to reduce DMI and HP concomitantly (Reynolds et al., 1991). An explanation might be that the greater NDF intake in the present study did not increase digested NDF proportionally. Indeed, albeit with an equal estimated NE_L supply, the hay-fed cows produced less ECM than

the cows supplemented with concentrate, suggesting an unexpectedly lower fiber digestibility for the hay diet. As rumen fermentation (acetate metabolism) is considered to be the reason for the greater heat increment of fiber, a greater intake of raw NDF, but not digestible NDF, would not influence the cow's HP, as shown in the present study. The same phenomenon might have been present in an earlier study that did not find differences in HP when comparing Holstein dairy cows fed diets differing in their roughage NDF content (12% vs. 18%) under heat load conditions (Adin et al., 2008). The increased roughage NDF content did not engender a greater HP, which might have been partly related to the lower in vitro digestibility (59 vs. 69% for the 18%- and 12%-roughage NDF diets, respectively), apart from the lower DMI and milk production of the cows fed the diet with greater NDF contents. An unchanged HP has also been reported by other studies (Miron et al., 2008, Kanjanapruthipong et al., 2015) that lowered roughage NDF but not total NDF content in high-yielding dairy cows under moderate and severe HS conditions. However, in the 3 abovementioned studies, decreased NDF content reduced respiration rates and rectal temperatures. These studies used nonforage fiber sources, mainly soy hulls, to lower the roughage NDF content. The NDF fraction of soy hulls contains less lignin and more galactose, pectin, and glucan, and is therefore considered to be more digestible ("readily digestible NDF") and to have a faster passage rate than NDF fractions of forage (Ipharraguerre and Clark, 2003). If the heat-stress-mitigating potential of supplementing soybean hulls and thereby decreasing roughage NDF was related to a decrease in "work spent in eating," rumen activity, or the acetate/propionate ratio (due to different carbohydrate/sugar fractions), we could expect that supplementing concentrate would have similar consequences. However, this was not the case in the present study. Explanations may relate to the possible counteracting influences of DMI and milk production, lower digestibility, or too small difference in NDF proportions between diets (34.5% vs. 41%). Finally, none of these earlier and the present work investigated the true heat increment, but only the HP. This is why we cannot conclusively reject the general assumption that digestible fiber leads to a greater heat increment than other nutrients (West, 1999, Conte et al., 2018). To study the true heat increment, i.e., the increase in HP following the consumption of feed, the energy partitioning would need to be investigated in detail.

In the present study, horn status did not substantially affect heat stress indicators, except for respiration rates being lower, albeit not significantly, in horned than in disbudded cows under HS conditions. Although the horn is vascularized and is therefore—by force—able

to dissipate heat into the environment under conditions in which the horn surface temperature exceeds ambient temperatures, it has a very small surface area compared with the surface of the whole body of a cow. Peripheral vasodilatation, and consequently radiation and convection, are assumed to be of minor importance among heat dissipation mechanisms. Further, the greater the heat load, the more important evaporative heat loss becomes (Li et al., 2021), a mechanism that cannot take place on the horn surface (Picard et al., 1999). Therefore, the heat dissipation capacity of the horn is likely very limited, which was confirmed in the present study. Given the inherent important isolation properties of keratin (Lazarus et al., 2021), we would—also a priori—expect that the horn dissipates heat less easily than the skin and hair coat. Our findings are in contrast to the observations of Piccard et al. (1999), who estimated, based on ex vivo studies, considerable heat loss via horns. However, the horns' small contribution to heat dissipation in the present study might be related to the lower heat dissipation capacity of horns in temperate climate zones, where Brown Swiss cows originate, and from the relatively small horn surface area (horn length of the present study's cows: approximately 20–30 cm). Horns of greater surface area and thinner keratin sheaths might dissipate more heat, as has been described in cows from tropical climate zones (Piccard et al., 1999).

The FA profile of the milk differed according to the horn status of the cows, independently of the THI condition. Apart from nonsignificant differences, the milk of horned animals had greater proportions of total short-chain FA, C18:0 and C17:0 iso, and lower amounts of C16:0, of 2 C16-MUFA, and of 1 C18-PUFA. These results do not indicate a general trend. Rather, they indicate that only single fatty acids differed by horn status. This is in contrast with earlier studies, where under low ambient temperatures and in an on-farm study comparing pooled samples of milk of horned and disbudded cows, milk of horned cows had lower proportions of odd-chain SFA (C7:0, C9:0, C11:0, and C13:0) and greater proportions of 2 n-3 FA (C18:3n-3 and C18:4n-3), the branched-chain FA C17 iso, and *cis*-vaccenic acid (C18:1n-7 *cis*-11) than disbudded cows (Baars et al., 2019). The present study did not confirm these observations, except for C17:0 iso, which was of slightly greater proportions in the milk fat of horned cows than disbudded cows. C17:0 iso originates from the membrane lipids of rumen bacteria and may therefore reflect ruminal flora or the duodenal flow of microbial matter (Vlaeminck et al., 2005). In view of a lack of shift in the FA profile, the biological relevance of the slight differences in single FA between horned and disbudded cows is questionable.

CONCLUSIONS

In the present study, mild HS conditions engendered various physiological reactions in Brown Swiss dairy cows, but this did not alter the animals' body temperature or milk yield, suggesting that the cows were in mild or beginning HS. Further investigation is required with regard to the suitability of decreased milk lactose content as an early sign of beginning HS. In contrast to expectations, lowering dietary NDF content and the presence of horns mostly did not mitigate cows' responses to the imposed short-term HS conditions. This might be different to reducing dietary NDF content under severe or long-term HS. Future investigations of nutritional strategies must include information about digestibility and the source of NDF. Moreover, it needs to be clarified whether nutritional measures have an effect on mild HS of longer duration and whether feeding measures can reduce mild HS at all. Due to its low proportion of the body surface and its inability for evaporation, the horn is not likely to fulfill a major role in HS mitigation.

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