## ORIGINAL PAPER

# Genotype effects on body temperature in dairy cows under grazing conditions in a hot climate including evidence for heterosis

S. Dikmen · L. Martins · E. Pontes · P. J. Hansen

Received: 18 December 2008 / Revised: 11 February 2009 / Accepted: 11 February 2009 / Published online: 5 March 2009 © ISB 2009

Abstract We compared diurnal patterns of vaginal temperature in lactating cows under grazing conditions to evaluate genotype effects on body temperature regulation. Genotypes evaluated were Holstein, Jersey, Jersey × Holstein and Swedish Red × Holstein. The comparison of Holstein and Jersey versus Jersey × Holstein provided a test of whether heterosis effects body temperature regulation. Cows were fitted with intravaginal temperature recording devices that measured vaginal temperature every 15 min for 7 days. Vaginal temperature was affected by time of day (P < 0.0001) and genotype  $\times$  time (P < 0.0001) regardless of whether days in milk and milk yield were used as covariates. Additional analyses indicated that the Swedish Red × Holstein had a different pattern of vaginal temperatures than the other three genotypes (Swedish Red × Holstein vs others  $\times$  time; P < 0.0001) and that Holstein and Jersey had a different pattern than Jersey × Holstein [(Holstein + Jersey vs Jersey  $\times$  Holstein)  $\times$  time, P< 0.0001]. However, Holstein had a similar pattern to Jersey [(Holstein vs Jersey)  $\times$  time, P > 0.10]. These genotype  $\times$ time interactions reflect two effects. First, Swedish Red × Holstein had higher vaginal temperatures than the other genotypes in the late morning and afternoon but not after the evening milking. Secondly, Jersey × Holstein had lower vaginal temperatures than other genotypes in the late morning and afternoon and again in the late night and early morning. Results point out that there are effects of specific genotypes and evidence for heterosis on regulation of body temperature of lactating cows maintained under grazing conditions and suggest that genetic improvement for thermotolerance through breed choice or genetic selection is possible.

 $\textbf{Keywords} \ \ \text{Holstein} \cdot \text{Jersey} \cdot \text{Swedish Red} \cdot \text{Crossbreeding} \cdot \\ \text{Heat stress} \cdot \text{Milk yield}$ 

## Introduction

Heat stress in dairy cattle leads to milk yield depression (West 1999) and reduced fertility (Hansen 2007). One potential strategy for reducing effects of heat stress is to change the cow genetically to improve thermoregulation. A specific gene controlling thermotolerance in cattle has been identified (the slick hair gene that controls hair length; Olson et al. 2003; Dikmen et al. 2008) and there are undoubtedly others. Selection for thermotolerance is possible (Ravagnolo and Misztal 2000, 2002). There are also prospects for improving thermotolerance through breed selection or crossbreeding. Holsteins have been reported to be less thermotolerant than Jerseys or Brown Swiss (Ruvuna et al. 1983; Correa-Calderón et al. 2004; Garcia-Peniche et al. 2005). Heterosis achieved by crossbreeding can affect milk yield, fat and protein percentage of the milk, incidence of diseases, and reproductive traits (Sørensen et al. 2008), but no experiments have been conducted to determine whether there are effects of heterosis on body temperature regulation during heat stress. Here, we compared diurnal

S. Dikmen

Department of Animal Science, Faculty of Veterinary Medicine, University of Uludag, Bursa 16059, Turkey

L. Martins · E. Pontes · P. J. Hansen (⋈)
Department of Animal Sciences, University of Florida,
Gainesville, FL 32611-0910, USA
e-mail: hansen@animal.ufl.edu

L. Martins · E. Pontes Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil



patterns of vaginal temperature in lactating cows under grazing conditions to evaluate genotype effects on body temperature regulation. Genotypes evaluated were Holstein, Jersey, Jersey × Holstein and Swedish Red × Holstein. The comparison of Holstein and Jersey versus Jersey × Holstein provided a test of whether heterosis effects body temperature regulation. The Swedish Red × Holstein was included because this mating combination has been considered a favorable cross for improved reproduction and longevity under California conditions (Heins et al. 2006).

### Materials and methods

### Farm

Data were collected from a rotational grazing dairy located in Trenton, Florida (29°38′N, 82°49′W). The dairy milked approximately 1,200 cows/day using twice-daily milking. Bovine somatotropin was not used. Cows were maintained in rye grass pastures and were moved to a new paddock daily. In addition, all cows had free access to a mixed ration after the morning and evening milking.

#### Vaginal temperatures

The experiment was conducted between 23 July and 26 September 2007 using a total of 19 Holstein, 24 Jersey, 32 Jersey × Holstein and 14 Swedish Red × Holstein cows. Cows were in their first 150 days of lactation and were first or second parity.

Cows were part of a 300-cow fresh group located on a pasture divided into 7 triangular paddocks of about 30,000 m<sup>2</sup> each. Cows were rotated into a new paddock each day. Milking was twice per day. The milking parlor was at a distance of ~500 m from the farthest paddock. Cows initiated travel to the parlor at ~0530 and ~1730 hours and returned to the field between ~0900 and 1000 and ~2100 and 2200 hours.

The field contained one centrally-located irrigation pivot which covered 0.81 km<sup>2</sup>. The pivot was turned on each day from 1000 to 1800 hours and moved at a rate of 15 m/h. While in the parlor, cows were cooled with sprinklers that turned on for 5 min every 15 min.

Devices to record vaginal temperatures were HOBO water temperature Pro V2 data loggers (Onset, Bourne, MA, USA) attached to a blank (i.e. without progesterone) controlled internal release device (CIDR; Pfizer Animal Health, New York, NY) inserted into the vagina. Each week, 12–15 cows not analysed previously received devices. The devices were inserted into the vagina after the morning milking and remained in place for 7 days. After removal, data were downloaded from the data loggers to a laptop computer.

Milk yield data were obtained from farm records. Milk yield was measured monthly with Waikato milk meters (Waikato Milking Systems, Hamilton, NZ) and the milk yield record at the closest test day to the start of the experiment was used to estimate milk yield.

#### Statistical analysis

The data on vaginal temperatures were analysed with the MIXED procedure of SAS (v. 9.1.3; SAS Institute, Cary, NC) with genotype, time of day, cow nested within genotype and all interactions in the model. Cow was considered random and other main effects considered fixed. Initial analyses with parity and breed × parity indicated these effects were not significant and these terms were not used in the final model. The analysis was performed with and without milk yield and days in milk as covariates. Data were analysed as a complete dataset and then re-analysed after recoding breeds to determine genotype × time of day interactions for the following three orthogonal comparisons: Swedish Red × Holstein versus other breeds, Jersey × Holstein versus Holstein and Jersey, and Holstein versus Jersey.

Data on milk yield, parity and days in milk were subjected to analysis of variance using the Proc GLM procedure of SAS with genotype and parity in the model.

Simple regression analysis was used to determine the relationship between milk yield and average vaginal temperature.

## Results

There were no differences in days in milk or parity between genotypes (Table 1). There was a tendency (P=0.07) for genotype to affect milk yield. The numerically greatest milk yields were for Swedish Red × Holstein. Use of orthogonal contrasts indicated a tendency (P=0.07) for milk yield of Holstein and Jersey to be greater than milk yield for Jersey × Holstein and milk yield for Holstein to be greater than milk yield for Jersey.

Daily variation in vaginal temperature is shown in Fig. 1. Data are shown for models in which no covariates were used (Fig. 1, top panel) or where days in milk and milk yield were included as covariates (Fig. 1, bottom panel). Results were very similar for both models. Throughout most of the day, including at night, vaginal temperatures were above those considered characteristic of homeothermy (i.e., 38.3–38.6°C). Coincident with the morning milking, there was a precipitous drop in vaginal temperatures for all groups. Thereafter, vaginal temperature rose during the day to reach a peak at 1115–1130 hours. After a subsequent, slow decline, vaginal temperatures rose again beginning at ~1530 hours until a peak just before the afternoon milking,



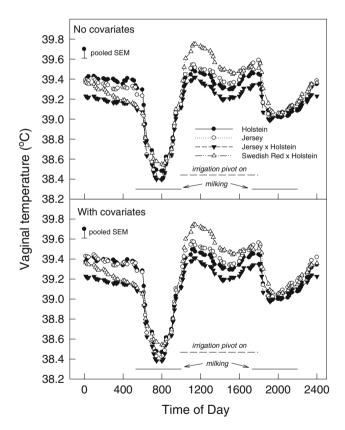
Table 1 Days in milk, parity and milk yield as affected by genotype (least-squares means ±SE)

	Holstein	Jersey	Jersey × Holstein	Swedish Red × Holstein
Days in milk	110±13	98±13	109±13	79±27
Parity	$1.4 \pm 0.1$	$1.2 \pm 0.01$	$1.1 \pm 0.01$	$1.1 \pm 0.1$
Milk yield (kg/day) <sup>a</sup>	$19.4 \pm 1.0$	$16.3 \pm 1.1$	15.8±1.2	$20.4 \pm 2.3$

<sup>&</sup>lt;sup>a</sup> Genotype effect P=0.07, Holstein and Jersey versus Jersey × Holstein P=0.07, Holstein versus Jersey P=0.07

when vaginal temperatures declined again. The decrease in vaginal temperature coincident with the afternoon milking was less than the decline associated with the morning milking.

Vaginal temperature was affected by time of day (P< 0.0001) and genotype × time (P<0.0001) regardless of whether covariates were used in the analysis or not. Additional analyses indicated that the Swedish Red × Holstein had a different pattern of vaginal temperatures than the other three genotypes [(Swedish Red × Holstein vs



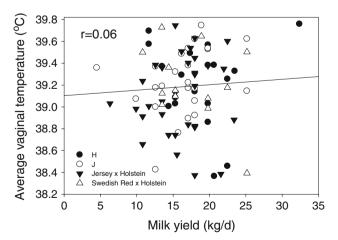
**Fig. 1** Genotype effects on diurnal changes in vaginal temperatures of lactating dairy cows maintained on pasture as affected by genotype: Holstein (*filled circle*), Jersey (*open circle*), Jersey × Holstein (*filled triangle*), Swedish Red × Holstein (*open triangle*). The *top panel* represents an analysis without milk yield and days in milk covariates and the *bottom panel* represents results of analysis with milk yield as a covariate. The times when the group of cows were being milked and exposed to sprinklers is shown by the *horizontal solid lines* at the bottom of each graph and the period when cows were exposed to an irrigation pivot is shown by the *dashed line* 

others)  $\times$  time; P < 0.0001] and that Holstein and Jersey had a different pattern than Jersey  $\times$  Holstein [(Holstein + Jersey vs Jersey  $\times$  Holstein)  $\times$  time, P < 0.0001]. However, Holstein had a similar pattern to Jersey (Holstein vs Jersey  $\times$  time, P > 0.10). These genotype  $\times$  time interactions reflect two effects of genotype. First, Swedish Red  $\times$  Holstein had higher vaginal temperatures than the other genotypes in the late morning and afternoon but not after the evening milking. Secondly, Jersey  $\times$  Holstein had lower vaginal temperatures than other genotypes in the late morning and afternoon and again in the late night and early morning.

Two analyses were used to determine the relationship between milk yield and vaginal temperature. First, milk yield was included as a covariate in the analysis of variance of vaginal temperature. There was no significant effect of milk yield (P=0.69). Secondly, simple regression analysis was performed to relate milk yield with average vaginal temperature. The correlation between milk yield and average vaginal temperature was 0.06 (P=0.58) (Fig. 2).

#### Discussion

Here we show that the capacity for resistance to disruption of body temperature regulation during heat stress, as



**Fig. 2** Relationship between milk yield at the closest test date and average vaginal temperature collected every 15 min for 7 days. Holstein (*filled circle*), Jersey (*open circle*), Jersey × Holstein (*filled triangle*), Swedish Red × Holstein (*open triangle*)



measured by diurnal changes in vaginal temperature, was less for Swedish Red × Holstein than for other breeds. In addition, thermoregulation was superior for Jersey × Holstein than for other breeds, including cows of both parent breeds (Jersey, Holstein). The greater thermotolerance for Jersey × Holstein than either parent breed is evidence for heterosis. The genotype effects on thermotolerance seen here, where milk yield was low compared to cows fed total mixed rations in confinement, were not due to differences in milk yield because breed differences in milk yield were small and genotype effects on vaginal temperature persisted when days in milk and milk yield were used as covariates.

To our knowledge, this is the first report of body temperature regulation in Swedish Red crossbred cows exposed to heat stress. The Swedish Red × Holstein has exhibited superior reproduction and longevity traits under California conditions as compared to Holsteins (Heins et al. 2006). Given the fact that this breed was developed in Northern Europe and has not gone much selection under North American conditions, where heat stress is widespread (Oseni et al. 2003), it is not surprising that cows of this genotype were less able to regulate body temperature. Other genotypes examined were also developed in Northern Europe but cattle from these breeds have gone intensive selection under North American conditions.

Heterosis has been demonstrated for several traits in dairy cattle including milk yield, fat and protein percentage of the milk, incidence of diseases and reproductive traits (Sørensen et al. 2008). The observation that Jersey × Holstein cows had lower vaginal temperatures during much of the day than Holstein or Jerseys suggests that thermotolerance is another trait controlled by heterosis. Thus, one possible advantage of crossbreeding schemes in hot climates is the potential for increased milk yield and improved reproductive performance during periods of heat stress because of heterosis in thermotolerance. Indeed, experiments by Ruvuna et al. (1983) evaluating seasonal effects on genetic differences between Holstein, Brown Swiss and Jersey and their crosses in lactational and reproductive performance indicated greater heterosis in the warm season.

More research should be conducted into specific breed crosses that provide similar or greater degrees of heterosis for body temperature regulation as seen here. One crossbreeding scheme that is being practiced extensively is the use of *Bos indicus* to produce crossbred animals with superior thermotolerance. Milk yield can be higher for *B. indicus* × Holstein crosses than for cattle in which the proportion of Holstein breeding is higher, particularly when the level of management is poor (Madalena et al. 1990). However, crosses of *B. indicus* and *B. taurus* can have reduced lactation lengths (McDowell et al. 1996), and it is likely that crosses of specific breed combinations that do not involve *B. indicus* will be more profitable in many situations.

This experiment was performed under grazing conditions where the degree of environmental modification of the cows' surroundings was much less than can be accomplished for cows housed in barns or sheds. Despite the inclusion of a shade structure and an irrigation pivot that provided evaporative cooling, cows remained hyperthermic throughout most of the day. In fact, average vaginal temperatures reached temperatures characteristic of homeothermy (38.3-38.6°C) only at the morning milking. Given the prolonged period each day during which cows were unable to successfully regulate body temperature, genetic differences in thermoregulation are likely to be important for cow production and health. Differences between genotypes in vaginal temperature might be lower than reported here in situations where the degree of heat stress was less.

Differences in milk vield between genotypes were small. probably because the level of nutrition did not allow Holsteins to realize their genetic potential for milk yield. Perhaps genotype differences in thermoregulatory ability would be greater than seen here if there were greater differences in milk yield between genotypes. Regulation of body temperature during hyperthermia can be reduced as milk yield increases (Berman et al. 1985). Surprisingly, there was no significant relationship between milk yield and average vaginal temperature in the present study. The lack of a relationship may reflect the fact that milk yield was not measured coincidentally with vaginal temperature or that milk yields were low. However, no relationship between milk yield and rectal temperature was observed in another study in which cows with higher milk yields were examined (Dikmen and Hansen 2009). Some of the relationship between milk yield and vaginal temperature may have been obscured because cows that are better able to regulate body temperature may have higher milk yields as a result.

In conclusion, there are effects of specific genotypes and evidence for heterosis on regulation of body temperature of lactating cows maintained under grazing conditions. The existence of these genetic effects points out the existence of specific genes that control the physiologic and cellular processes underlying thermotolerance. These genes can be subject to selection, either by choosing breeds and breed crosses used to produce animals for dairy production or, perhaps, by identifying polymorphisms in these genes or gene markers located nearby to allow gene-specific selection for thermotolerance.

Acknowledgements Dr. Serdal Dikmen was supported by a grant from TUBITAK-BIDEB Ankara, Turkey. The authors thank to Eduardo Alava from University of Florida for his assistance in data collection; Ron St. John and the employees of Piedmont Dairy for access to their cows and active participation in the experiments, and Pfizer Animal Health for donating blank CIDR devices.



#### References

- Berman A, Folman Y, Kaim M, Mamen M, Herz Z, Wolfenson D, Arieli A, Graber Y (1985) Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical climate. J Dairy Sci 68:1488–1495
- Correa-Calderón A, Armstrong D, Ray D, DeNise S, Enns M, Howison C (2004) Thermoregulatory responses of Holstein and Brown Swiss heat stressed dairy cows to two different cooling systems. Int J Biometeorol 48:142–148. doi:10.1007/s00484-003-0194-y
- Dikmen S, Hansen PJ (2009) Is the Temperature-Humidity Index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J Dairy Sci (in press)
- Dikmen S, Alava E, Pontes E, Fear JM, Dikmen YK, Olson TA, Hansen PJ (2008) Differences in thermoregulatory ability between slickhaired and wild-type lactating Holstein cows in response to acute heat stress. J Dairy Sci 91:3395–3402. doi:10.3168/jds.2008-1072
- Garcia-Peniche TB, Cassell BG, Pearson RE, Misztal I (2005) Comparisons of Holsteins with Brown Swiss and Jersey Cows on the same farm for age at first calving and first calving interval. J Dairy Sci 88:790–796
- Hansen PJ (2007) Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. Theriogenology 68S:S242–S249. doi:10.1016/j.theriogenology.2007.04.008

- Heins BJ, Hansen LB, Seykora AJ (2006) Fertility and survival of pure Holsteins versus crossbreds of Holstein with Normande, Montbeliarde, and Scandinavian Red. J Dairy Sci 89:4944–4951
- Madalena FE, Lemos AM, Teodoro RL, Barbosa RT, Monteiro JBN (1990) Dairy production and reproduction in Holstein-Friesian and Guzera crosses. J Dairy Sci 73:1872–1886
- McDowell RE, Wilk JC, Talbott CW (1996) Economic viability of crosses of Bos taurus and Bos indicus for dairying in warm climates. J Dairy Sci 79:1292–1303
- Olson TA, Lucena C, Chase CC Jr, Hammond AC (2003) Evidence of a major gene influencing hair length and heat tolerance in *Bos* taurus cattle. J Anim Sci 81:80–90
- Oseni S, Misztal I, Tsuruta S, Rekaya R (2003) Seasonality of days open in US Holsteins. J Dairy Sci 86:3718–3725
- Ravagnolo O, Misztal I (2000) Genetic component of heat stress in dairy cattle, parameter estimation. J Dairy Sci 83:2126–2130
- Ravagnolo O, Misztal I (2002) Effect of heat stress on nonreturn rate in Holstein cows: genetic analyses. J Dairy Sci 85:3092–3100
- Ruvuna F, McDaniel BT, McDowell RE, Johnson JC Jr, Hollon BT, Brandt GW (1983) Crossbred and purebred dairy cattle in warm and cool seasons. J Dairy Sci 66:2408–2417
- Sørensen MK, Norberg E, Pedersen J, Christensen LG (2008) Invited review: crossbreeding in dairy cattle: a Danish perspective. J Dairy Sci 91:4116–4128. doi:10.3168/jds.2008-1273
- West JW (1999) Nutritional strategies for managing the heat-stressed dairy cow. J Anim Sci 77(Suppl 2):21–35

