

2018

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Arias, Rodrigo A.; Herrera, Camila; Larraín, Rafael; González, Fernando; Mader, Terry L.; and Velásquez, Alejandro, "Physiological and behavioural response of two dairy cows' genotypes during summertime in the central region of Chile" (2018). *Faculty Papers and Publications in Animal Science*. 998.
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Physiological and behavioural response of two dairy cows' genotypes during summertime in the central region of Chile

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ABSTRACT. Heat stress has been recognised as a serious problem in dairy farms. The study goal was to assess the effects of climatic conditions on physiological and behavioural responses of dairy cows in Central Chile. Data of tympanic temperature (TT), panting score, respiration rate (RR), and shade utilization of cows from two genotypes, Holstein (H) and Holstein x Montbeliarde (HM), were collected twice per day (AM/PM) during three periods of the summer season in Central Chile. Moreover, three thermal comfort indices: Comprehensive climate index (CCI), temperature humidity-index (THI), and adjusted THI were estimated using meteorological data. The hour of each day was classified as "Normal" or "Stressful" based on CCI threshold of 25 °C. Statistical analysis included ANOVA, repeated measures analysis and Chi square test ($\alpha=0.05$). There was an interaction of genotype x CCI condition x period ($P=0.0026$) with the highest TT of both genotypes under a stressful condition within each period. In addition, interactions of genotype x hour ($P<0.0001$) and genotype x CCI condition ($P<0.0002$) were also observed. The HM cows showed greater TT than H cows in both CCI conditions. The RR was higher during the afternoon and a greater proportion of cows used shade at "Mild" and "Moderate" CCI categories ($P<0.001$). Both genotypes showed some degree of heat stress, but cool nights and shade seem to be enough to allow to cows' cope with the challenging diurnal conditions observed in the summer season. A study of these effects on milk production is necessary to confirm or discard the previous.

Key words: tympanic temperature, thermal comfort, respiration rate, heat stress.

INTRODUCTION

Heat stress has been recognised as a serious problem around the world, but especially on dairy farms using breeds with a high potential of milk production (Arias *et al* 2008, Silanikove 2000). Usually, these animals fail to maintain an adequate thermal balance causing negative consequences for milk production and animal welfare (Collier *et al* 2006, Kadzere *et al* 2002). The poor performance and efficiency in milk yield have been associated mainly with a decrease in dry matter intake (West 2003), resulting in significant economic losses (St-Pierre *et al* 2003), estimated to be \$897 million only for the dairy industry at the USA. A summary of the major behavioural responses includes changes on feeding, defecating and urinating frequency, water intake, lying time, standing time, shade seeking behaviour (Ratnakaran *et al* 2017). In addition, body temperature as well behaviour has been affected by heat stress (Jara *et al* 2016, Tucker *et al* 2008).

In Chile, there is limited information regarding the effect of heat stress on dairy cattle. However, there is a risk of heat stress for the central region of Chile, but with a moderate to

low risk in the southern regions (Arias and Mader 2010). The aforementioned is relevant considering that dairy farms located in the central region of Chile have a greater degree of intensification (total mixed rations and high production genotypes) and higher milk yield per cow than those located in the southern regions, where the systems are based on pasture. On the other hand, this subject is acquiring major attention and concern among producers due the changes in animal welfare requirements as well as changes observed on climate, with an increasing proportion of heat waves and extended periods of drought. We hypothesised that cows of the central region of Chile experience physiological and behavioural changes as consequence of the summer weather conditions. Therefore, the objectives of the study were to assess in two dairy genotypes changes on patterns of tympanic temperature, panting scores, respiration rates and utilisation of artificial shade during summer conditions in the central region of Chile.

MATERIAL AND METHODS

The study was conducted during the summer of 2011 at the dairy research farm of the Pontificia Universidad Católica de Chile, located in the foothills of the Andes mountains of the central region of Chile (33°40'22.4"S 70°35'33.8"W, 654 meters above sea level). The study consisted of three periods of data collection: January 19 to 25th; February 5 to 8th; and March 20 to 24th. In each period, 6 Holstein (H) and 6 Holstein x Montbeliarde (HM) cows were selected to receive a data logger device to collect tympanic temperatures (TT) and to study its behaviour. Thus, a total of 36 mature multiparous and healthy cows (67 ± 6 d on milk), body condition score = 2.75 to 3.00 (1 to

Accepted: 09.08.2017.

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5 scale) at the beginning of the experiment, were randomly selected from the commercial herd. All the cows were fed with the same diet three times a day (08:00 h, 11:30 h, and 18:00 h), which included (all expressed as DM/cow/day) corn silage (4.0 kg), alfalfa soiling (4.9 kg), barley brewers (2.9 kg), and supplements (0.25 kg).

Animals were kept in open pens (80 m x 80 m) with *ad libitum* access to water and shade, and milked three times per day (03:00, 12:00 and 18:00 h). However, milk yield was not considered in this analysis. The shading structure consisted of treated wood poles of approximately 4.0 m high, with a black raschel mesh (80% shade, 2.1 m wide) located over the wood poles, providing 2.8 m² of shade by cow.

TYMPANIC TEMPERATURE AND ANIMAL BEHAVIOR

Each cow within each period received an iButton data logger device (Maxim Integrated Products Inc., CA, USA) located manually in its tympanic canal. The devices were programmed to collect TT at 10 minute intervals and subsequently compiled into hourly readings. The same cows used to collect TT were observed within each period to collect behavioural data. One trained observer recorded the proportion of cows using shade on the pen and also collected data of respiration rates (RR; breaths per minute; bpm). The RR were estimated by timing and counting 10 flank movements in each animal. Measurements of the panting score (PS) were also collected following the description of Mader and Davis (2002). First, the observer identified cows with the ibutton device within the herd, recording its location (shaded or unshaded), then recorded the count of flank movement and PS score independent of cow's location in the pen.

ENVIRONMENTAL DATA COLLECTION AND THERMAL COMFORT INDICES

Ambient temperature (AT, °C), wind speed (WS, m/s), relative humidity (RH, %), solar radiation (SR, W/m²), and precipitation (P, mm/d) were collected continuously at 10 minute intervals by using a weather station (U30 Hobo Onset, MA, USA) located at the dairy farm. Later, these data were compiled into hourly values to match TT dataset. Likewise, these climatic data were used to estimate three thermal comfort indices: temperature-humidity index (THI), adjusted THI (THI_{adj}), and comprehensive climate index (CCI) by using the following equations:

$$THI = 0.8 * AT + ((RH/100) * (AT-14.4)) + 46.4$$

(equation 1; Hahn *et al* (2009))

$$THI_{adj} = THI + 4.51 - (1.992 * WS) + (0.068 * SR)$$

(equation 2; Mader *et al* (2006))

$$CCI = AT + F_{RH} + F_{WS} + F_{SR}$$

(equation 3; Mader *et al* (2010))

Where:

F_{RH} corresponds to the correction factor for AT due to relative humidity;

$$e^{(0.00182 * RH + 1.8*10^{-5} * AT * RH)} * (0.000054 * AT^2 + 0.00192 * AT - 0.0246) * (RH - 30)$$

F_{WS} corresponds to the correction factor for AT due to wind speed;

$$\left[\frac{-6.56}{e^{\left\{ \frac{1}{2.26*WS+0.23^{0.45}} * 2.9+1.14*10^{-6}*WS^{2.5} - \log_{0.3}(2.26*WS+0.33)^{-2} \right\}}} \right] -0.00566 * WS^2 + 3.33$$

and F_{SR} corresponds to the correction factor for AT due to solar radiation.

$$0.0076 * SR - 0.00002 * SR * AT + 0.00005 * AT^2 * \sqrt{SR} + 0.1 * AT - 2$$

The risk of thermal stress is given by the following categories of CCI (Mader *et al* 2010): No stress (CCI ≤ 25); Mild (> 25 and ≤ 30); Moderate (> 30 and ≤ 35); Severe (> 35 and ≤ 40); Extreme (> 40 and ≤ 45); and Extreme danger (CCI > 45).

STATISTICAL ANALYSIS

During the study, eight data logger devices were lost (one per period for H genotype and 4 and 1 for HM genotype in periods 2 and 3, respectively). In addition, data from 2 animals were removed from data set due to incomplete records of TT (less than 24 h/day or less than 4 days/period), resulting in an unbalanced number of animals per period (total n = 26). Meteorological and TT data were analysed by using a complete randomised experimental design arrangement, with each animal as experimental and observational unit. Additionally, a dummy variable (CCI condition) was created based on threshold of CCI defined by Mader *et al* (2010). Thus, hour of each day with CCI ≤ 25 °C were considered as “Normal”, otherwise it was considered as “Stressful”. Thus, genotype, moment of day (AM vs. PM), and CCI condition (Normal vs. Stressful) were considered like study factors in a factorial arrangement. In addition, TT was modeled using a repeated measurements analysis by using the MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC) with TT as the dependent variable with genotype, hour and its interaction as independent variables in the model. The hour was the repeated measurement and the animal(genotype) statement was used as random effect. The period was not included in the model because different animals were used across

the periods. Finally, categorical data were analysed by using a Chi square test (Likelihood ratio test). The level of significance for all the statistical analyses was 0.05.

RESULTS

A summary of meteorological variables, thermal indices, and TT by period, type and moment of day are presented on table 1. Most of climatic variables showed a decrease on periods 2 and 3 regarding period 1, with the exception

of HR. In addition, even though AT reached values >30 °C during all days in periods 1 and 2, there was a considerable drop during night-time (mean 8.7 °C), representing over 20 points of fluctuation (Figure 1).

Figure 2 shows interaction period x CCI condition x genotype ($P=0.003$). Cows of both genotypes showed the highest TT during stressful hours within each period. There were also interactions for genotype x CCI condition ($P<0.001$), with HM cows showing greater TT than H cows in both CCI conditions; and for genotype x period

Table 1. Least square means ± SEM of climate variables, tympanic temperature and thermal comfort indices by period, type and time of day during the summer time in central Chile.

Period	TT, °C	AT, °C	RH, %	WS, m/s	SR, W/m ²	THI	THI _{adj}	CCI, °C
Period 1	37.83 ± 0.02 ^a	20.5 ± 0.2 ^a	63.8 ± 0.8 ^a	0.21 ± 0.08 ^a	353.3 ± 10.4 ^a	65.0 ± 0.3 ^a	71.5 ± 0.3 ^a	23.1 ± 0.3 ^a
Period 2	37.55 ± 0.02 ^c	19.7 ± 0.3 ^a	67.7 ± 0.9 ^a	0.12 ± 0.08 ^b	323.6 ± 14.9 ^a	64.2 ± 0.4 ^a	70.6 ± 0.4 ^a	21.7 ± 0.5 ^a
Period 3	37.71 ± 0.01 ^b	16.2 ± 0.2 ^b	69.9 ± 0.7 ^b	0.13 ± 0.08 ^b	246.9 ± 9.7 ^b	58.9 ± 0.3 ^b	64.8 ± 0.4 ^b	16.5 ± 0.4 ^b
Mean	37.74 ± 0.01	18.9 ± 0.1	66.6 ± 0.4	0.17 ± 0.01	312.5 ± 6.6	62.9 ± 0.2	69.2 ± 0.2	20.7 ± 0.2

Type/moment of day	TT, °C	AT, °C	RH, %	WS, m/s	SR, W/m ²	THI	THI _{adj}	CCI, °C
Normal	37.53 ± 0.01	13.4 ± 0.2	82.4 ± 0.3	0.00 ± 0.00	47.2 ± 2.52	55.7 ± 0.8	60.6 ± 0.2	10.5 ± 0.2
Stressful	38.03 ± 0.01	27.0 ± 0.3	43.4 ± 0.2	0.41 ± 0.01	702.7 ± 7.68	73.3 ± 0.1	81.8 ± 0.1	35.6 ± 0.1
AM	37.45 ± 0.01	13.9 ± 0.2	81.2 ± 0.4	0.04 ± 0.03	168.8 ± 6.61	56.2 ± 0.2	61.8 ± 0.3	12.5 ± 0.3
PM	38.02 ± 0.01	23.9 ± 0.2	52.0 ± 0.4	0.30 ± 0.08	456.1 ± 10.31	69.5 ± 0.2	76.5 ± 0.2	28.8 ± 0.3

TT = Tympanic temperature (genotypes pooled); AT= Ambient temperature; RH= Relative Humidity; WS= Wind speed; SR= Solar radiation; THI= Temperature humidity index; THI_{adj} = Adjusted temperature humidity index; CCI = Comprehensive Climate Index. Different letters within columns indicate significant differences between periods ($P<0.05$). In addition, all variables showed significant differences when compared Normal vs. Stressful and AM vs. PM ($P<0.001$). Period 1 = January 19 to 25; Period 2 = February 05 to 08; and Period 3 = March 20 to 24 of 2011.

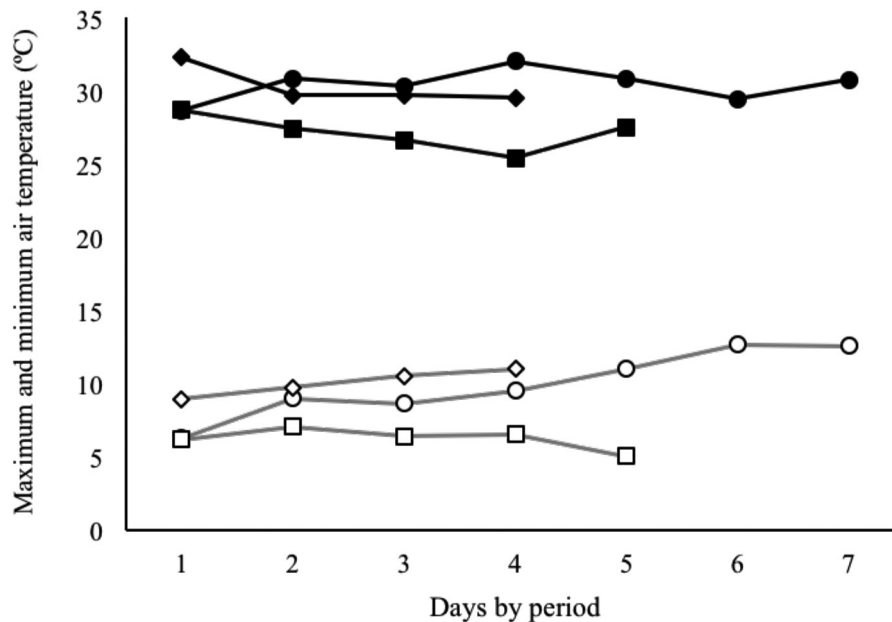


Figure 1. Daily maximum (dark line) and minimum (pale line) air temperatures for each day and period of collection data. Circles = Period 1(January 19 to 25); Diamonds = Period 2 (February 05 to 08); and Squares = Period 3 (March 20 to 24 of 2011).

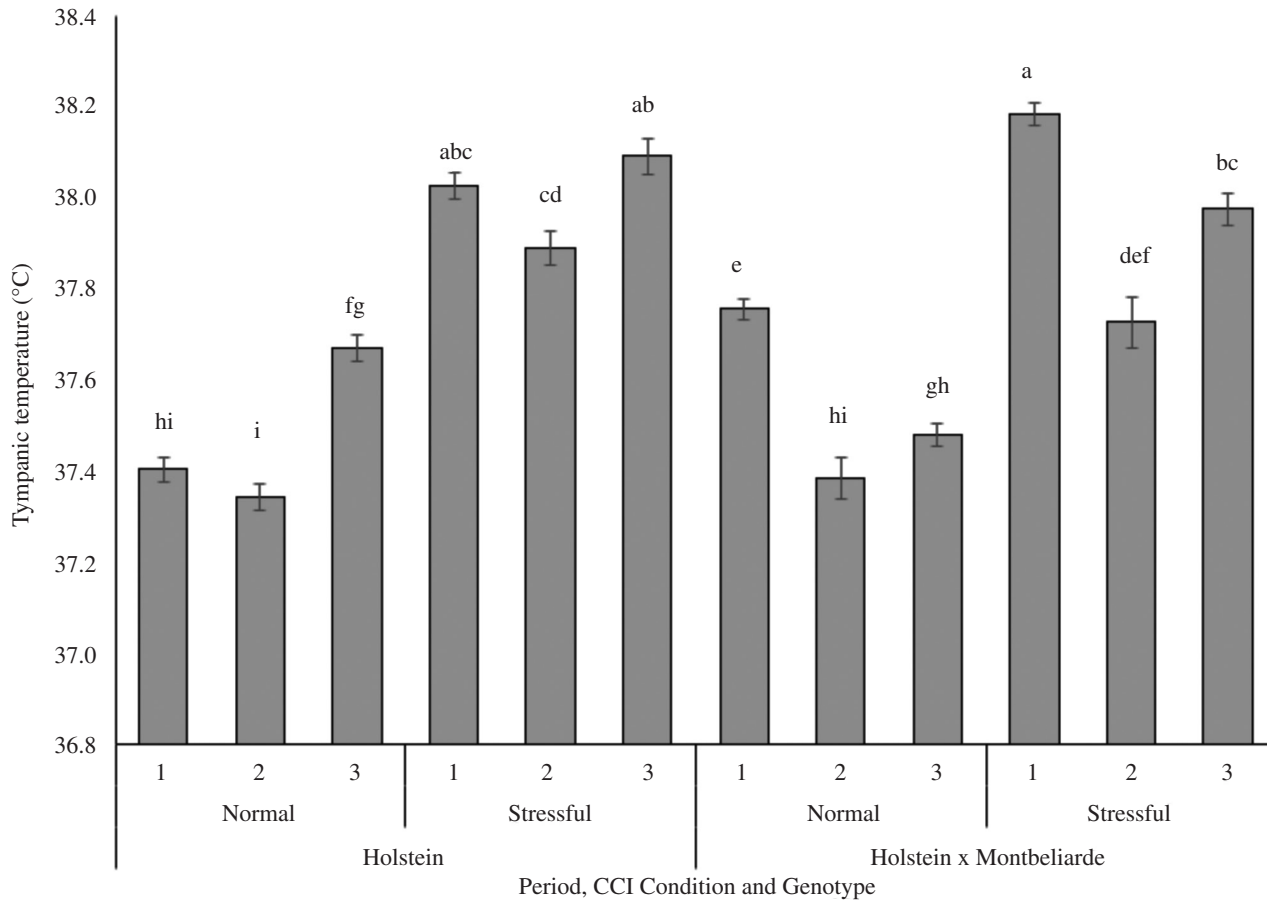


Figure 2. Least square means of tympanic temperatures of two dairy genotypes at each period and thermal index category (Comprehensive Climate Index = CCI) by period (1 = January 19 to 25th; 2 = February 5 to 8th; and 3 = March 20 to 24th) in the central region of Chile. Different letters within columns indicate significant differences between periods ($P=0.0026$).

($P<0.001$), with higher TT on period 1 in both genotypes. Finally, there was also an interaction of genotype x hour on TT ($P<0.001$; Figure 3), with H cows showing lower TT during great part of the day (from 21:00 to 11:00; $P<0.100$).

Over 75% of cows were under shade during daytime. However, no differences were observed for morning vs. afternoon ($P=0.543$) for pooled data. Nevertheless, the proportion of cows using shade increased when the hourly CCI category moved from “Normal” to “Mild” or “Moderate” stress ($P=0.049$), but without differences between the last two categories. Similarly, this proportion also was higher ($P=0.007$) during stressful condition when compared with normal condition (89.0 vs. 75.3%). As expected, the mean value of CCI for a stressful condition (27.96 ± 0.21 °C) was greater than the normal condition (21.40 ± 0.28 °C; $P<0.001$). Cows showed a trend to reduce the use of shade during the third period of study ($P=0.094$), coinciding with the end of the summer season and a lower SR.

The proportion of cows under shade across the periods was slightly higher during the morning vs. afternoon (86% AM vs. 79% PM). By the contrary, cows had higher RR during the afternoon when compared with the morning

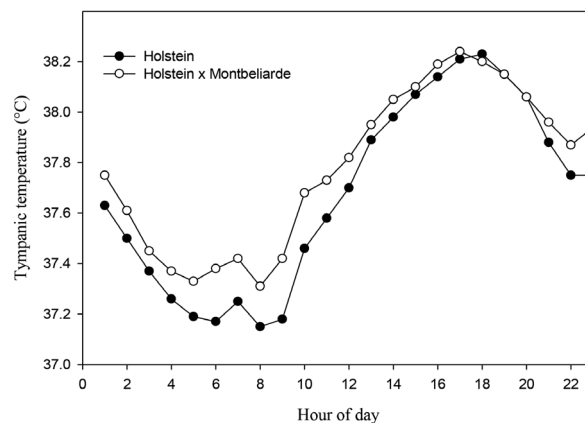


Figure 3. Least square means of tympanic temperature by genotype (All periods pooled: $P<0.10$ for hours = 0, 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 21, 22 and 23; $P<0.05$ for hour 5).

(67.51 ± 1.13 vs. 56.5 ± 0.99 bpm; $P < 0.001$). Likewise, RR increased ($P < 0.001$) from 59.98 ± 1.00 (Normal CCI condition) to 68.11 ± 1.35 bpm (Stressful CCI condition). When RR was compared among the hourly CCI categories showed an increase ($P < 0.001$) from 60.16 ± 0.95 bpm (Normal) to 69.13 ± 2.73 and 67.76 ± 1.58 for “Mild” and “Moderate” CCI categories, but without differences between them ($P > 0.100$). The PS changed across the day with a greater proportion of cows showing a PS=0 during the morning (57.4%), but decreasing during the afternoon (26.4%), whereas cows on PS=1 increased from 41.8% in the morning to 64.8% in the afternoon. The proportion of cows on PS=2 increased more than forty folds, from 0.2% to 8.8% when comparing morning vs. afternoon. A similar pattern was observed when PS of cows was compared across periods, but no effects of shade on PS were observed ($P = 0.765$).

DISCUSSION

Heat stress has tremendous effect on production, health, and reproduction in lactating dairy cows, and has been largely ignored from a management standpoint (Tao and Dahl 2013). However, in many countries there is a lack of information about the impacts of heat stress on animal production and wellbeing. The present study is one of the firsts in Chile addressing this topic.

A possible explanation to the difference observed on TT between the genotypes (Figure 3) could be the better adaptation of the H cows to the environment when compared with the HM cows, that represent a newer genotype in the area of study. The higher TT during nighttime of HM cows could be the resultant of a lower capacity to dissipate the heat accumulated during daytime, as demonstrated by Aharoni *et al* (2006) who compared H vs. HM dairy cows during the summer season in Jordan. These authors reported differences between H and HM in their diurnal patterns of heat production, suggesting that heat tolerance of HM was lower than H cows. In our study, we did not measure the heat production.

The difference of TT between the morning vs. afternoon is in agreement with those reported by Vickers *et al* (2010), who measured vaginal temperatures of dairy cows. But differs from those reported by Aharoni *et al* (2005) who found no differences for rectal temperatures and RR. In the ruminants, rectal temperatures are considerably affected by rumen metabolism and do not represent a reliable index of the regulated temperature (Berman 1971). In fact, Burfeind *et al* (2010) concluded that measures of rectal temperature could be influenced by the procedure itself, type of thermometer, and the penetration depth into the rectum. Thus, some care is required when rectal temperatures are used as estimator of body temperature.

Although values of TT on “stressful” conditions were higher than under the “normal” condition, these are still within the normal range (Hillman 2009). We speculate

that the cold nights observed during all the periods of data collection provide to the animals the opportunity to dissipate the heat accumulated during the daytime. In this context, some researchers have concluded that cattle that fail to or that do not cool down at night are prone to achieving greater body temperatures during hot days. Cattle that are prone to getting hot but can cool at night can keep peak body temperatures at or near those of cattle that tend to consistently maintain lower body temperatures (Mader and Johnson 2010, Mader *et al* 2010). The big drop in AT during night time, typical of the Andes foothills in that region (≥ 20 °C of fluctuation), apparently allowed the cows to maintain TT within a normal range and to cope in better way the impact of heat load.

In this study, THI and THI_{adj} ranged from 57.0 to 67.3 and 62.9 to 73.8 respectively. These values that are under the threshold at which milk production (du Preez *et al* 1990, Johnson 1985) and dry matter intake (Johnson 1985) begin to decrease (THI \geq 72). Nevertheless, Zimbelman *et al* (2009) proposed a new THI threshold of 68 for high producing dairy cows (≥ 35 L/day). The previous is in agreement with those reported by Markovich (2012) in New Zealand, where milk production began to decrease at THI of 65 for H and 75 for Jerseys. Bernabucci *et al* (2014) also reported a reduction in milk production at THI from 65 to 76 depending of the parity of cows. There are other reports showing a range of multiple values of THI thresholds ranging from 69 to 78.2 (Bohmanova *et al* 2007, Bouraoui *et al* 2002, Dikmen and Hansen 2009, Johnson *et al* 1962, Ravagnolo *et al* 2000). However, it is important to mention that most of those reports are associated with the same genetic (Holstein Friesian). Nevertheless, other breeds are less sensitive to thermal stress than Holstein Friesian (Sharma *et al* 1983). Thus, differences in response to heat stress between genotypes can be attributed to varying levels of adaptability to hot environments (Kadzere *et al* 2002).

There is strong evidence that lactating cows increase RR during heat stress events or even they are able to reduce RR when they are properly cooled (Hillman *et al* 2001, Smith *et al* 2006). In our study, cows had RR slightly higher than those reported by Kendall *et al* (2007) for the shade treatment in a study conducted in New Zealand, with similar weather conditions, but it was lower than the uncooled treatment. The differences of RR between stressful vs. normal conditions herein reported are in agreement with those reported by Muller *et al* (1994), who found differences in RR for cows in hot days (AT ≥ 25.1 °C), but not for cool days (AT ≤ 25 °C). In our study, RR were also influenced by moment of day (AM vs. PM). The differences in RR herein presented as well as those reported by Bouraoui *et al* (2002), and by Muller *et al* (1994) suggest that cows were primarily subject to heat stress during those hours of the day when AT and SR reach maximum values, resulting in significant increases of RR. Thus, SR and AT are two important drivers and triggers

of RR (Berman *et al* 1985, Harris *et al* 1960). The RR can be used as a valuable tool to assess the level of heat stress on cows. Finally, the high proportion of cows using shade, regardless of the moment of day, implies that cows use this mechanism to cope with heat load of daytime. In addition, when data were analysed by period, there was a trend to seek shade during the afternoons ($P=0.073$) in period 1 (January) which coincides with the highest values of SR. Based on the results of this study we can indicate that cows of both genotypes showed a slight degree of heat stress in Central region of Chile, particularly during daytime. However, they were able to cope with it because of the strong drop on AT during nighttime as well as by the using of shade.

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