Effects of heat stress on milk yield of primiparous Holstein cows at regional scale using large data bases

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Abstract: With the prospects of global warming, heat stress, the depressive (summer) heat effect on milk yield, has become a high priority research problem in temperate zones. The effect of summer present day heat and lag heat effects on milk yield of first lactation grazing Holstein cows was assessed through the temperature and humidity index (THI). Additionally, THI thresholds were calculated. Daily air temperature and humidity data from three locations for six summer seasons (December-March in years 2001 – 2006) were used. Data of 35500 monthly test days from 8875 cows in 54 farms within the influence zones of the respective meteorological stations were analyzed. Mixed linear models were adjusted, considering the animal as random effect and location, farm, days in milking, age at calving, year of calving and THI as fixed effects. Four measures per animal were taken into account and modelled as repeated measures. A significant depressing heat effect on milk yield was found for the present day (THI) and also for one-day and two-days before (THI1 and THI2). Significant interactions between THI and days in milk, farm and year were found. The lag heat effects explained more variability on milk yield than the heat effect for the present day. Threshold THI-values were different depending on the considered day: 75, 75 and 72 were estimated for THI, THI1 and THI2, respectively. Heat stress caused a decrease in milk yield of 1.3%, 1.9%, and 0.9% of average daily production (per THI unit increase above threshold), depending on the THI measure used.

Key words: Dairy cow, Mixed models, Summer heat, THI

Efectos del estrés por calor en la producción de leche de vacas Holstein primíparas a escala regional utilizando grandes bases de datos

Resumen: Con las perspectivas de calentamiento global, el estrés por calor, el efecto depresivo (verano) sobre la producción de leche, se ha convertido en un problema de investigación de alta prioridad en zonas templadas. El efecto del calor actual del verano y los efectos del retraso en el rendimiento de la leche de las vacas Holstein que pasaron la primera lactación se evaluó a través del índice de temperatura y humedad (THI). Además, se calcularon los umbrales de THI. Se utilizaron datos diarios de temperatura y humedad del aire de tres ubicaciones durante seis temporadas de verano (diciembre-marzo en los años 2001 - 2006). Se analizaron datos de 35500 días de prueba mensuales de 8875 vacas en 54 granjas dentro de las zonas de influencia de las estaciones meteorológicas respectivas. Se ajustaron modelos lineales mixtos, considerando al animal como efecto aleatorio y ubicación, granja, días de ordeño, edad al parto, año de parto y THI como efectos fijos. Se tomaron en cuenta cuatro medidas por animal y se modelaron como medidas repetidas. Se encontró un efecto de calor deprimente significativo en el rendimiento de la leche para el día presente (THI) y también para un día y dos días antes (THI1 y THI2). Se encontraron interacciones significativas entre THI y días en leche, granja y año. Los efectos del calor rezagado explicaron más variabilidad en el rendimiento de la leche que el efecto del calor para el día de hoy. Los valores de umbral de THI fueron diferentes según el día considerado: 75, 75 y 72 se estimaron para THI, THI1 y THI2, respectivamente. El estrés por calor provocó una disminución en la producción de leche de 1.3%, 1.9% y 0.9% de la producción diaria promedio (por unidad de THI aumenta por encima del umbral), dependiendo de la medida de THI utilizada.

Palabras clave: vaca lechera, modelos mixtos, calor de verano, THI

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Introduction

In past decades, milk yield in dairy cows has increased as a consequence of breeding and management. Consequently, dairy cattle have become more heat sensitive: as individual milk yield increases, so does feed intake and heat production (Collier, 1982; Turner et al., 1989; Johnson, 1994; Kadzere *et al.*, 2002). Development of strategies to deal with heat stress in the frame of global warming is mentioned as the first objective in some studies (e.g. Hahn, 2001; Collier and Zimbelman, 2007; Hill and Wall, 2015). The magnitude of the heat stress effect upon milk yield depends on the extreme values of temperature and humidity, length of the event, acclimatization, breed and management (Turner *et al.*, 1989; Kadzere *et al.*, 2002).

Dairy production in temperate regions accounts for more than a third of dairy production worldwide (Silanikove and Koluman, 2015). With increasing warmer climates heat stress could seriously affect the dairy production in many countries, especially during summer. For instance, in South America, studies of air temperature trends for 1931-2000 with data from several locations of Uruguay, Argentina and SW of Brazil, showed a significant rise of daily minimum temperature (Travasso *et al.*, 2007). This is important because during nights is when animals can recover its energetic balance, dissipating the excess of heat from the diurnal phase of the day.

Solar radiation, air temperature (T) and air relative humidity (RH) are the most relevant climatic variables that explain heat stress (Turner *et al.*, 1989). Because temperature and humidity are common variables recorded at any meteorological station, the temperature and humidity index (THI) is the most widely index utilized for associating heat stress and milk yield. Johnson *et al.* (1961) determined that the THI threshold value at which milk yield starts to decline was 72 for lactating Holstein cows in climatic chambers. Field conditions are the actual environment of animals and are often more complex to evaluate than climatic chambers. The combination of commercial dairy conditions and animal breeding can change the animal response to heat stress, including the threshold values (Collier and Zimbelman, 2007; Nardone *et al.*, 2010). Milk reduction because of heat in field conditions using large available meteorological and commercial data bases has been reported (Ravagnolo *et al.*, 2000; Barash *et al.*, 2001; Bohmanova *et al.*, 2007). The identification of adequate THI thresholds is an essential pre-requisite for identification of genetic components of heat stress (Ravagnolo and Misztal, 2000; Aguilar *et al.*, 2010). Additionally, some studies indicate the existence of a "lag heat effect" (West *et al.*, 2003; Bouaroui *et al.*, 2002; Lambertz *et a*l, 2014). Due to the time required to digest and metabolize nutrients, the effect of intake reduction on milk yield needs between one and two days to be fully expressed (West *et al.*, 2003). According to these authors, the lag heat effect on milk yield can be more important than the effect in the day of measure.

Therefore, in order to plan adaptation measures for climate change, it is increasingly relevant to quantify the heat stress effects on grazing dairy cows on field conditions, in temperate regions using THI.

The goals of this research were to: 1) assess the THI effect on milk yield, 2) evaluate the influence of lag heat effect, and 3) quantify the THI threshold values at which milk yield starts to decline on primiparous Holstein grazing cows.

Materials and Methods

Data

Uruguay is located in a temperate zone (30°30'S, 57°41'W to 34°30'S, 53°38'W) (Figure 1) and it has developed a milk industry based mainly on Holstein cows grazing on pastures. The present study was carried out for three locations of Uruguay: San José, Florida and Paysandú (Figure 1), which are the main Uruguayan dairy production zones.

Three meteorological analyses for associating heat stress and milk production in Uruguay were determined prior to this study. First, in order to achieve adequate temporal representativeness, it was estimated that a minimum of 6 years with daily THI information was required given the inter-annual variability of THI in Uruguay (R^2=0.75; Cruz and Urioste, 2009). Second, in order to achieve adequate spatial representativeness, the area represented by each meteorological station (in each location), was determined utilizing data from thirteen Uruguayan meteorological stations (R^2=0.85; Cruz and Urioste, 2009). Finally, quality of all series of meteorological data was conducted following the recommendations of the World Meteorological Organization (2004).

Subsequently, daily THI values from meteorological stations from San José, Florida and Paysandú were merged with daily milk test data from farms within each THI representative area (Table 1). All farms considered for each location were within the distances presented in Table 1, therefore, in all cases THI at any farm is associated with THI at the meteorological station with R^2 =0.85 (Cruz and Urioste, 2009).

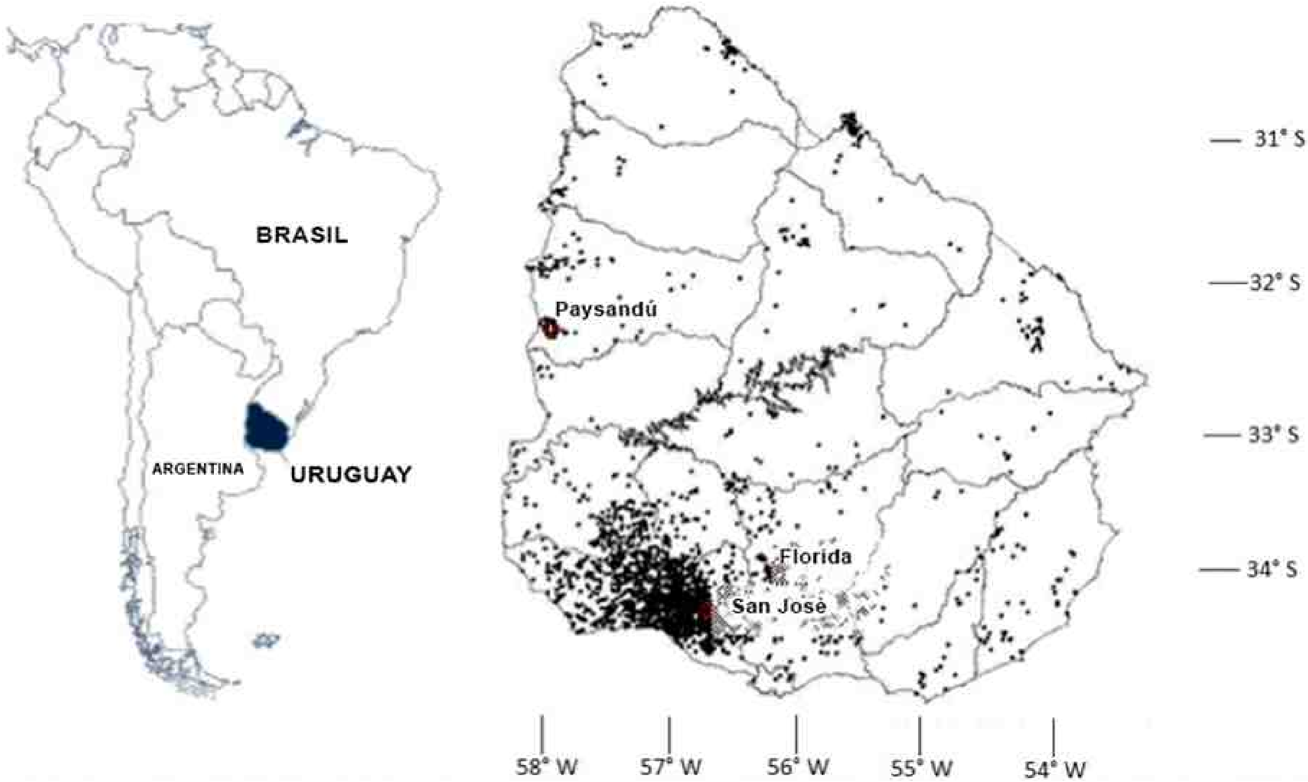

Figure 1. Geographical location of Uruguayan milk production zones and Meteorological Stations utilized in this study. Dots indicate milk farms

Table 1. Distances of spatial representativeness (R2=0.85) from Meteorological Station of each study location

	Distances (km)			
	North	South	East	West
San José	50	20	30	70
Florida	90	25	50	50
Paysandú	80	90	70	-

Meteorological information was provided by the Uruguayan Meteorological Institute. Daily air temperature (T) and relative humidity (RH) from three public weather stations placed in Uruguayan dairy areas were considered: San José (34º21'25"S, 56º42'05"W), Florida (34º4'0"S, 56º14'3"W) and Paysandú (32º20'57"S, 58º02'13"W) (Figure 1). T was calculated as the average of maximum and minimum daily temperature, since the availability of public meteorological information is at daily (not hourly) intervals. Observation of RH at 0900h was used as an estimator of the daily RH, following recommendations by Saravia *et al.* (2002), who found this indicator as the most representative of daily RH for Uruguayan climate. Additionally, the Uruguayan 0900h meteorological observation coincides with the 0600h coordinated universal time (UTC), which is mandatory by the World Meteorological Organization and all MS record this observation. Daily THI was calculated as reported by Valtorta and Gallardo (1996) and Bohmanova *et al.* (2007):

$THI = (1.8\ T + 32) - (0.55 - 0.55\ RH/100)\ (1.8\ T - 26)$; T (°C) and RH (%)

This formula is widely used in the region and allows the comparison of results from neighbor Latin-American countries. The study included 6 consecutive summer seasons (December, January, February and March, summers 2000-2001 to 2005-2006). The THI effect was defined in 21 one-degree classes between 60 and 80, and the few extreme data were included in two classes:

THI≤60 and THI≥80.

Milk yield data of first calving Holsteins were provided by the National Dairy Breeding Institute, consisting in monthly test-day (TD) records from farms located in the influence zone of the meteorological stations referred above. Animal records included TD-information, farm code, cow individual identification, milk yield in TD, cow's birth and calving date. Records from TD lower than 3 litres/day and DIM less than 7 days were rejected. Only animals with four Summer TD records were considered. The number of animals per herd averaged 160 cows. More than 75% of the herds provided information for at least 5 of the 6 considered years. Finally, a total of 35500 TD data from 8875 cows of 54 herds were used.

Statistical procedure

A general descriptive analysis was made as a first step to summarize productive and meteorological data, consisting in calculating averages, standard deviations, percentiles and probabilities of occurrence of THI values.

In preliminary analyses and always considering the effect of cow as repeated measure during lactation for each TD, different fixed and mixed linear models were evaluated, testing the different residual structure correlations and variances provided by the free statistical software Infostat (Infostat Statistical Package, 2009). After examining the graphic outputs of the models and the Akaike (AIC) and Bayesian (BIC) values, mixed models with cow as random effect with autoregressive correlation (AR1) and homogeneous variances were chosen. AIC and BIC are penalty likelihood criteria for model choice, indicating the best model to describe the data (Di Rienzo *et al.*, 2008).

Due to limitations of computer speed and software availability (some functions were not available in the free version), models numbered 1 to 4 were selected to analyze the information.

Five classes for days in milk (DIM, less than 61; 61 to 90; 91 to120; 121 to180; and 181 or more days) and 4 classes for calving age (CA, less than 24; 24 to 35; 36 to 47; and 48 months or more) were defined.

Model 1was used for testing the effect of THI on milk yield, grouping by herd for each location (San José, Florida or Paysandú):

(1) $Y^{ijklmn} = \mu + Herd^{i} + DIM^{j} + CA^{k} + Year^{l} + THI^{m} + c^{n} + \varepsilon^{ijklmn}$

Where: Y: represents each TD value from a given cow from a specific herd, year, DIM class, CA class and THI level.

μ: is the general mean of Y

Herd: is the ith fixed effect of Herd

DIM: is the jth fixed effect of days in milk (5 classes)

CA: is the kth fixed effect of calving age (4)

Year: is the lth fixed effect of year (2001, 2002, 2003, 2004, 2005 and 2006)

THI: is the mth fixed effect of the ITH in the test-day (less of 60, ..., more than 80)

c: is the random effect of the cow n repeated during lactation for each TD

ε: is the residual effect

The lag heat effect on milk yield was tested using model (1) running the three locations together using THI, and after the THI effect was replaced by THI from the previous day (THI1) or from two days before (THI2). The effect of THIs was tested separately in each model run. Classes of THI1 and THI2 were the same as those previously defined for the THI classes.

To assess the interaction effects between each factor and THI, models (2) and (3) were used, running the three locations together.

(2) $Y^{ijklmn} = \mu + Herd^{i} + DIM^{j} + CA^{k} + Year^{l} + \beta THI + [Factor \times \beta THI]^{m} + c^{n} + \varepsilon^{ijklmn}$

(3) $Y^{ijklmn} = \mu + Location^{i} + DIM^{j} + CA^{k} + Year^{l} + \beta THI + [Location \times \beta THI]^{m} + c^{n} + \varepsilon^{ijklmn}$

Here, THI was considered as a linear covariable, where β represents the regression slope value, indicating the amount of decrease in milk yield per unit of increase in THI. The expression [Factor x βTHI] represents each of the following interactions: [Herd x βTHI], [DIM x βTHI], [CA x βTHI], and [Year x βTHI] using model (2), where each interaction expression was separately included for each model run. The [Location x βTHI] interaction was tested using model (3). The other factors were as in model (1), running the three locations together.

Model (4) was used for the identification of THI threshold values at which milk yield starts to decline, following the methodology reported by Bohmanova et al. (2007).

(4) $Y^{ijklm} = \mu + Herd^{i} + DIM^{j} + CA^{k} + Year^{l} + \beta\Delta THI + c^{m} + \varepsilon^{ijklm}$

Where ΔTHI is a dummy regression for the estimation of decline in milk production due to heat stress, with ΔTHI = 0 if THI ≤ threshold THI (normothermy), and ΔTHI = THI− threshold THI if THI > threshold THI (heat stress),

β = slope of the linear regression, representing the decrease in milk yield per unit of increase in THI above the threshold THI. Others factors are the same as in model (1).

Thresholds of THI, THI1 and THI2 between 70 and 77 were evaluated with model (4), using the AIC and BIC indexes for each output as comparison criteria; minimum AIC and BIC values were the criteria to choose the threshold value for each case.

A REML procedure, available at the free version of Infostat Statistical package (2009) was used in all cases.

Results and Discussion

Heat stress

The highest monthly average THI and probabilities of THI>74 were found in Paysandú (the Northern location) and January was the month with hottest and larger daily THI amplitude (Table 2). Also January was the month with lowest variation of THI between years, being March the month with highest variations. These results are consistent with a previous characterization of the thermal environment of Uruguayan summer for 1960 to 1990 (Cruz and Saravia, 2008).

Table 2. Monthly average THI (THI) and standard deviation (SD), daily THI amplitude (A); probability of THI>74 [P(THI>74) (%)]; average milk yield (MY) (l/cow/day) and standard deviation (SD) per month and location for years 2001-2006

	Paysandú				San José				Florida			
Month	THI±SD	A	P(THI>74)	MY±SD	THI±SD	A	P(THI>74)	MY±SD	THI±SD	A	P(THI>74)	MY±SD
December	70.6±4.0	18	18	15.7±4.3	68.6±4.5	19	11	16.9±4.0	67.7±4.5	21	9	14.8±4.2
January	73.7±4.2	19	49	13.7±3.8	70.4±4.5	20	35	14.7±3.7	71.2±4.6	21	28	13.1±3.9
February	70.5±4.6	18	37	13.4±4.0	70.1±4.5	18	28	15.0±4.0	70.3±4.6	19	26	13.0±3.9
March	69.4±5.5	17	27	14.3±5.4	68.9±5.1	18	20	15.8±4.0	67.4±5.2	20	9	13.9±4.1

The average milk yield ranged from 13.0 to 16.9 l/cow/day for the different months and locations for years 2001 - 2006. Milk yield decreases in Paysandú and Florida from December to February, and then increaseed in March. Changes in milk yield in San José were similar, but the lowest yield was in January (Table 2). This reduction in milk yield is consistent with the increase of THI. This is evident from December to January, where the reduction in milk yield and the increase in THI were the highest.

All the effects included in model (1) significantly affected milk yield. For our purposes, the main result was that the effect of THI on milk yield was significant ($p<0.01$) in the three locations. The autocorrelation coefficient between data of the same cow (repeated measure) for model (1) was 0.40 in Florida, 0.31 in San José and 0.44 in Paysandú. Results of model (1) allowed us to identify the existence of heat stress on dairy cows during Uruguayan summers.

Dairy cows in Uruguay graze during the whole year, and summer season is critical because of the water deficit due to a high evapotranspiration. In addition, forage availability and quality decline during summer. Considering these results, heat stress appears as another significance factor for explaining the reduction in milk production in Uruguayan summers. Further research is needed to develop strategies at farm level that mitigate heat stress effects. Current management recommendations for summer were mainly developed to mitigate the reduction in forage availability.

Lag heat effect

A significant depressing heat effect on milk yield was found, not only for the present day (THI) but also for one-day and two-days before (model (1)), where the THI effect was replaced by THI from the previous day (THI1) or from two days before (THI2). The lag heat effect explained more variability on milk yield (11% of total variability for THI1 and 10% for THI2) than the heat effect for the present day, which accounted for 8% of the variability.

Similarly to these results, West *et al.* (2003) and Bouraoui *et al.* (2002) reported that the depressing effect of 2-days earlier THI on milk yield was higher than the effect using the same day measure as the TD record. This lag is due to the time required to consume, digest, and metabolize nutrients. Intake is reduced in response to heat stress and there is a delay before the effect on milk yield is fully expressed. According to West *et a*l. (2003), these results have important implications for intake and milk yield prediction equations that typically rely on same day climatic measures to predict performance responses. For temperate climates, where hot environment "appears" for relatively short periods in the warm season and climate is not permanently hot, the delay in expressing the effect of heat on milk yield could be part of the acclimatization process, and finishes when the heat episode has ended. Ingraham et al. (1989) reported that animals under acute heat stress (short length) can return to its previous level of production, or acclimatize with lesser production, depending on the characteristics of the heat stress (heat load). If hot weather persists for many days, the animal adaptation capacity is overcome and milk yield is strongly affected. Also, chronic exposure to heat can eventually become in adaptive responses through the increase in the maximum critical temperature. In these cases, the depressing effect of heat stops after the adaptation.

Recent findings reveal the complexity of mechanism involves in heat stress situations. Wheelock *et* al. (2010) reported that in heat stress condition, the reduction of feed intake explains only 35% of the decrease in milk yield. A large portion of the effect of heat stress (no mediated by decreased feed intake) may be a consequence of energy intake-independent changes in nutrient partitioning. Moreover, the heat stressed animal initiates a variety of postabsorptive metabolic changes that are independent of reduced feed intake and whole animal energy balance. These changes in nutrient partitioning are adaptive mechanisms employed to prioritize the maintenance of normothermia (Baumgard and Rhoads, 2013).

Further research is needed to clarify the aspects mentioned above, having in mind that the magnitude of the heat stress effect on milk yield depends on several aspects, like the extreme values of temperature and humidity, length of the event, acclimatization, days in milk, breed, management and metabolism (Turner *et al.*, 1989; Kadzere *et al.*, 2002; Bohmanova *et al.*, 2007; Nardone *et al.*, 2010; Wheelock *et al.,* 2010).

Interactions

Models (2) and (3) were used to detect significance interactions with THI. Results showed significance interactions (p<0.01) between THI and herd, DIM and year (Table 3); location and calving age were not significant.

The THI by herd interaction (Table 3) suggest differences in management practices to deal with the heat effect, such as use of forestry areas for shadow, or good access to water. The fact that the effects of weather on milk yield depend on management had been demonstrated by Hill and Wall (2015) for Scotland conditions, with Holstein cows continuously housed indoors or grazed in summer. The understanding of animal responses to thermal stress and the ability to provide management options to prevent adverse consequences deserve an advanced planning of production management systems (Nienaber and Hahn, 2007). According to Nardone *et al.* (2010), adaptability is the key tool to improve sustainability of livestock production systems in the context of global warming. The significance of the THI by herd interaction found in this research give us a clue about effective management practices that are already applied at farm level that is necessary to identify.

The [Location x THI] interaction was not significance; indicating that at this scale the depressing effect of THI was independent of the location involved. This result could be explained because of the usual occurrence of heat waves in Uruguayan summers (Saravia and Cruz, 2006): the area affected by a heat wave exceeds the dimensions of the considered locations, showing no significance interaction between location and THI. Román *et* al., (2014), processing data from 47 summers in a location from the SW of Uruguay, reported an average of 4 heat waves per year with THI above 75. For locations and years studied in this work, the numbers of heat waves are similar and averaged 4.5 per year, also considering at least three consecutive days with THI>75.

Analysis including the occurrence of heat waves in autumn and spring deserve future works, since the occurrence of heat waves in Uruguay are not unusual at these seasons. For temperate climates, has been found significance THI (3 days lag measure) effect on milk yield for these intermediate seasons (Lambertz *et al.*, 2014).

The [DIM x THI] interaction was significance (p<0.05) with the β highest value at DIM class 2 (Table 3). That means the highest depressing effect on milk yield due to high THI happens at early lactation, as pointed out by Sharma *et al.* (1983) and Kadzere *et al.* (2002). Usually, early milking stages are critical, because high production is supported by mobilization of body resources (Sharma *et al.*, 1983). The whole energy balance is compromised in this situation: the environmental heat load effect is added to the negative energy balance typical at this stage, with difficulties to dissipate the excess heat (Kadzere *et al.*, 2002). According to Aguilera *et al.*, (2010), cows become more sensitive to heat stress with increasing parities. The significance of the [DIM x βTHI] interaction found in this research, even when

Table 3. Results of Model (2) to detect significant interactions between THI and Herd (a), DIM (b) and year(c)

a)

Fixed effect	β	SE	t	P
[Herd x βTHI]				
Intercept	20.29	1.64	12.38	***
THI:Herd-San José1	- 0.04	0.03	- 1.12	0.26
THI:Herd-San José2	0.12	0.04	3.45	***
THI:Herd-San José3	0.09	0.03	2.89	**
THI:Herd-San José4	0.03	0.04	0.76	0.45
THI:Herd-San José5	0.09	0.03	2.87	**
THI:Herd-San José6	0.14	00.4	3.76	***
THI:Herd-San José7	0.02	0.03	0.65	0.52
THI:Herd-San José8	0.16	0.03	5.25	***
THI:Herd-San José9	- 0.01	0.03	- 0.42	0.68
THI:Herd-San José10	0.12	0.03	4.15	***
THI:Herd-San José11	0.04	0.03	- 0.02	0.99
THI:Herd-San José12	0.01	0.03	0.40	0.69
THI:Herd-San José13	- 0.03	0.03	- 0.95	0.34
THI:Herd-San José14	- 0.12	0.03	- 4.10	***
THI:Herd-San José15	- 0.03	0.03	- 1.00	0.32
THI:Herd-San José16	0.19	0.03	5.66	***
THI:Herd-San José17	0.07	0.03	2.34	*
THI:Herd-San José18	- 0.05	0.03	- 1.64	0.10
THI:Herd-San José19	0.09	0.03	2.59	**
THI:Herd-Florida1	0.04	0.03	1.18	0.24
THI:Herd-Florida2	- 0.15	0.03	- 5.40	***
THI:Herd-Florida3	0.21	0.04	5.87	***
THI:Herd-Florida4	0.12	0.03	4.29	***
THI:Herd-Florida5	- 0.11	0.05	- 2.07	*
THI:Herd-Florida6	- 0.10	0.03	- 3.04	**
THI:Herd-Florida7	0.11	0.04	2.91	**
THI:Herd-Florida8	- 0.08	0.03	- 2.38	*
THI:Herd-Florida9	0.14	0.03	4.12	***
THI:Herd-Florida10	0.12	0.03	4.3	***
THI:Herd-Florida11	0.01	0.03	0.25	0.80
THI:Herd-Florida12	- 0.07	0.04	- 2.03	*
THI:Herd-Florida13	0.01	0.06	0.14	0.88
THI:Herd-Florida14	- 0.01	0.03	- 0.23	0.81
THI:Herd-Florida15	0.13	0.04	3.33	***
THI:Herd-Florida16	- 0.07	0.03	- 2.20	*

Fixed effect	β	SE	t	P
[Herd x βTHI]				
Intercept	20.29	1.64	12.38	***
THI:Herd-Paysandu1	0.03	0.04	0.12	0.90
THI:Herd-Paysandu2	0.02	0.03	0.06	0.95
THI:Herd-Paysandu3	0.19	0.06	3.18	**
THI:Herd-Paysandu4	0.08	0.03	3.13	**
THI:Herd-Paysandu5	0.04	0.03	1.43	0.15
THI:Herd-Paysandu4	0.08	0.03	3.13	**
THI:Herd-Paysandu5	0.04	0.03	1.43	0.15
THI:Herd-Paysandu6	- 0.18	0.03	- 5.48	***
THI:Herd-Paysandu7	0.12	0.03	4.30	***
THI:Herd-Paysandu8	0.17	0.04	4.49	***
THI:Herd-Paysandu9	-0.01	0.03	- 0.27	0.79
THI:Herd-Paysandu10	-0.15	0.04	- 4.32	***
THI:Herd-Paysandu11	0.15	0.03	5.19	***
THI:Herd-Paysandu12	0.24	0.04	6.53	***
THI:Herd-Paysandu13	-0.21	0.04	- 5.79	***
THI:Herd-Paysandu14	0.18	0.04	4.19	***
THI:Herd-Paysandu15	-0.30	0.03	- 9.87	***
THI:Herd-Paysandu16	0.06	0.03	2.25	*
THI:Herd-Paysandu17	-0.05	0.04	- 1.39	0.16
THI:Herd-Paysandu18	0.10	0.03	3.12	**

b)

Fixed effect	β	SE	t	P
[DIM x βTHI]				
Intercept	18.9	0.95	19.2	***
THI:DIM Class 2	-0.04	0.02	-2.36	*
THI:DIM Class 3	-0.01	0.02	-0.79	0.42
THI:DIM Class 4	-0.003	0.01	-0.15	0.87
THI:DIM Class 5	-0.001	0.01	-0.10	0.91

c)

Fixed effect	β	SE	t	P
[Year x βTHI]				
Intercept	17.45	1.29	13.48	***
THI:Year 2002	0.02	0.02	1.00	0.31
THI:Year 2003	-0.03	0.02	-1.68	*
THI:Year 2004	-0.03	0.02	-1.45	0.14
THI:Year 2005	-0.01	0.02	-0.64	0.51
THI:Year 2006	-0.01	0.02	-0.64	0.52

increasing parities. The significance of the [DIM x βTHI] interaction found in this research, even when cows were first calvers and less sensitive to heat stress, is another evidence of the depressing effect of heat stress in Uruguayan dairy herds.

The [Year x βTHI] interaction was significance ($p<0.05$), with positive effect of THI on milk yield in year 2002 (using 2001 as reference) and negative for the rest of the considered period (Table 3). The most depressing THI effect corresponded to years 2003 and 2004. Although no significance was found for interaction in these years, tendency allows us to see the evolution between years.

2001 was the hottest year of the considered period. Taking Paysandú as a reference, the THI value at percentile 75 (P75) during 2001 to 2006 was 77.2, 75.0, 74.9, 74.3, 74.2 and 74.9, respectively. Although the maximum THI value at P75 occurred in 2001, milk yield was the highest in that year. In 2002, the interaction [Year x βTHI] was still positive and significance, and despite that THI values in 2003 and 2004 were decreasing, the most negative interaction with milk production values were found (Table 3).

A partial explanation of this trend is that year 2002 was critical to Uruguay: the country's economy collapsed, with strong effect on agriculture and livestock husbandry. Consecutive years were also critical for all activities in Uruguay and the neighbor countries. This results evidence that "year effect" is not synonymous of "inter-annual climate variability effect", which has been used in that sense for many agronomic researches in this region. Clearly, the year effect includes many aspects, and climate variability (inter annual variability of THI in our case), is only one in explaining variations in milk yield between years.

Thresholds of THI

Using model (4) and according with AIC and BIC criteria, the threshold THI values of 75, 75 and 72 were found for THI, THI1 and THI2, respectively (Table 4).

Table 4. AIC and BIC values from model (4) for testing thresholds of THI, THI1 and THI2, where thresholds between 70 and 77 were evaluated

Model (3)	THI		THI1		THI2	
THI Thresholds	AIC	BIC	AIC	BIC	AIC	BIC
70	177236	177829	177126	177719	177067	177661
71	177226	177819	177118	177711	177070	177663
72	177217	177810	177107	177700	177066	177659
73	177202	177795	177091	177685	177080	177673
74	177181	177774	177074	177668	177111	177704
75	177181	177773	176993	177586	177113	177706
76	177237	177830	176997	177591	177095	177688
77	177258	177851	177122	177715	177162	177756

Heat stress caused a decrease in milk yield of 0.19, 0.27 and 0.13 l/cow/day/THI unit above threshold, from an average daily production of 14 l/cow/day, which represents which represents a decrease of 1.3%, 1.9%, and 0.9%, depending on the THI measure used (THI, THI1 or THI2, respectively). In all cases the effect of THI on milk yield was highly significance ($p<0.01$) (Table 5).

The lower threshold at THI2 (Table 4) is consistent with the lag heat effect, which explained more variability on milk yield than the THI of the present day (10% of total variability for THI2 and 8% for THI). That implies not only the THI2 values should be considered because of the lag effect (West *et al*, 2003), but also the THI2 threshold is the one it should be considered instead of the estimated threshold for the present day.

Bouaroui *et a*l. (2002) reported decreases in daily milk yield per cow of 2.2% when THI was over 69. Comparing two locations of US, Bohmanova (2007) found decreases of 1.4% and 1% at THI thresholds of 72 and 74 respectively, using the same THI estimation than in the present work.

Table 5. Results of Model (4) for the best AIC and BIC values of THI, THI1 and THI2 thresholds.

Fixed effects	β	SE	t	P
Intercept	16.62	0.31	53.44	***
THI (75)	- 0.19	0.01	- 13.91	***
Intercept	16.63	0.31	53.62	***
THI1 (75)	- 0.27	0.01	- 19.58	***
Intercept	16.7	0.31	53.76	***

According to Collier *et al.* (2007), animals acclimatized to low THI present decreases in milk yield at low THI, which could explain the lower THI2 threshold we found. Knowing that meteorological data are always auto correlated, we calculated the autocorrelation of the THI time series for each location and autocorrelation was found for three days (results not showed); meaning that memory of atmosphere of THI values persists three days. In spite of this, in conditions of heat waves, could be more useful summarise THI and other meteorological data (such wind direction) at weekly (or more) as was suggested by Hill and Wall (2015), because is usual that duration of each heat wave exceeds three days in Uruguayan conditions (Román, 2014).

A significant depressing heat effect on milk yield of cows at first calving was found for the prevailing summer conditions of Uruguay. This effect was identified for the present day THI and also for one-day and two-days before (THI1 and THI2). Threshold at THI2 presented the best adjustment and the lowest value (72) comparing with THI and THI1. That implies the THI2 threshold should be considered instead of the others threshold estimation.

The differential effect of herd on milk losses suggests that management can mitigate this problem at farm level, which deserves further consideration in the context of increasing minimum temperatures for the whole region.

Knowledge of heat thresholds from these particular locations allows developing weather forecast to alert farmers for taking preventions. In grazing conditions, knowledge of heat lag and the associated THI threshold, suggests that some management practices to reduce the heat effect should be adopted before the hot air situation is installed.

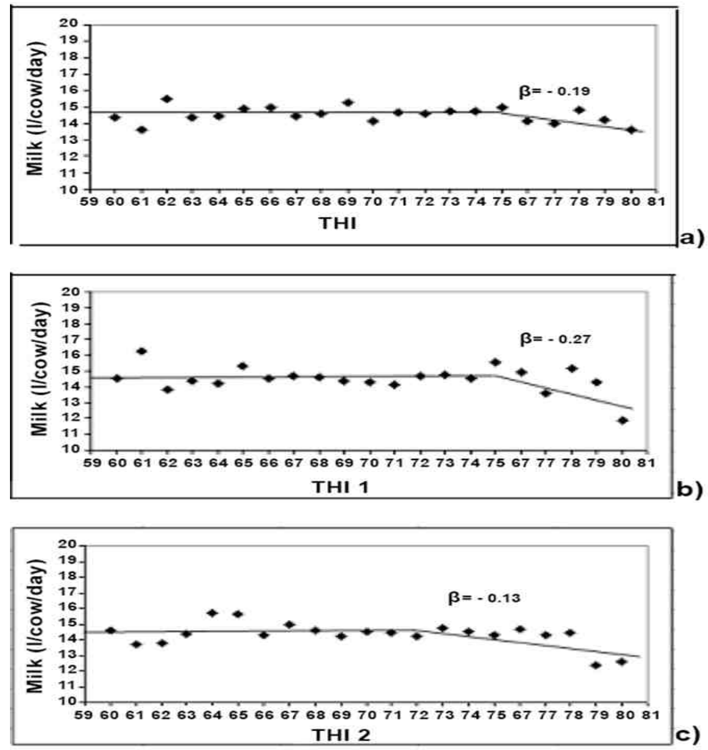

Figure 2. Milk yield (l/cow/day) and THI, according to model (4). Thresholds of THI = 75 (3.a), THI1 = 75 (3.b) and THI2 = 72 (3.c) and slopes β = -0.19, β = -0.27 y β = -0.13 respectively.

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