

Supplementary material

Effects of natural tree shade on blood and physiological traits, milk yield and composition of lactating dairy cows during the summer in the subtropics

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Material and Method

Composition of the diets

Supplementary tables

Table S1 *Composition (DM basis) of corn silage, concentrate and mineral mix supplied to animals*

Feed (Trial 1)	Corn Silage	Concentrate	Pasture
Supply (Kg of DM/cow per day)	4.5	6.0	-
Dry Matter (g/kg)	300	880	280
Crude Protein (g/kg DM)	80	200	120
Neutral Digestible Fiber (g/kg DM)	490	20	650
Total Digestible Nutrients (g/kg DM)	680	750	600
Mineral Mix Composition			
Calcium (minimum – g/kg)	190.0		
Phosphorus (minimum – g/kg)	60.0		
Sulfur (minimum – g/kg)	20.0		
Magnesium (minimum – g/kg)	20.0		
Potassium (minimum – g/kg)	35.0		
Sodium (minimum – g/kg)	70.0		
Cobalt (minimum – mg/kg)	15.0		
Cooper (minimum – mg/kg)	700.0		
Chromium (minimum – mg/kg)	10.0		
Iron (minimum – mg/kg)	700.0		
Iodine (minimum – mg/kg)	40.0		
Manganese (minimum – mg/kg)	1 600.0		
Selenium (minimum – mg/kg)	19.0		
Zinc (minimum – mg/kg)	2 500.0		
Vitamin A (IU/kg)	2 10 ⁵		
Vitamin D ₃ (IU/kg)	5 10 ⁴		
Vitamin E (IU/kg)	1 500.0		
Fluorine Maximum (mg/kg)	600.0		
Sodium Bicarbonate (g/kg)	20.0		

Management of animals

Two groups of cows (with eight cows per group) were placed into two rectangular-shaped paddocks (40 x 700 m each) located side by side, with Tifton 85 (*Cynodon dactylon*) grass. Cows within groups grazed a new strip of pasture of approximately 0.8 ha every day. The shaded areas with approximately 200m² were located at end of the paddocks (shorter side of the rectangle).

Natural shade was provided by *Eucalyptus* trees, with more than 10 m in height. The amount of available shade was calculated dividing the area under the trees at 1200 h by the number of cows. Although the shaded area varied during the day, it was at least about 10m² per animal.

During the first 14 days (Pre-stress period) all cows had free access to the shaded areas, at least 10m² per animal. On days 15 to 24 (Stress period), one group of cows was deprived of accessing the shaded area by the use of a fence line behind the projection of the tree's shadow, so during this period no shade was available in this group's paddock; the other group of cows continued with free access to shade during Stress period. On days 25 to 38 (Post-stress period), fence line that impaired shade access from one group was removed and all 16 cows had free access to the shaded areas.

Cows were milked twice daily at 0600 h and 1700 h (GMT-0200 h) in a herringbone parlour with four milking machines. After morning and evening milkings, cows were individually fed in a barn for 60 minutes. Concentrate (3 kg as fed) and corn silage (2.3 kg as fed) were supplied for each cow per meal (see diet composition in Table S1).

Milk production and composition

Milk yield (kg) was recorded on days 1, 9, 14 (variables measured during the Pre-stress period were used as covariates, *Supplementary material*), 16, 17, 18, 22, 24 (Stress period), 28, 31, 35 and 38 (Post-stress period), at the morning and evening milking using Westfalia milk meter. On days (1, 9, 14, 18, 22, 24, 28, 31, 38) proportional volumes of milk from morning and evening milkings from each cow were mixed and composed 200-ml samples. Fat, total protein and lactose in milk were determined by an infrared analyzer (Bentley 2000® equipment); sodium in milk was measured by flame atomic absorption spectrophotometry. Acidity was determined by titration with 0.1 N NaOH solution ($\% \text{ of lactic acid} \times 100 = \text{°D}$); density was determined using thermo lactometer and results were adjusted to a standard temperature of 15°C.

Ethanol stability was determined by mixing 2 ml of milk and 2 ml of alcoholic solutions with ethanol concentration varying from 68 to 84 °GL in a Petri dish (Machado, Fischer, Stumpf & Stivanin, 2017); results were expressed as the minimal ethanol concentration in the alcoholic solution that induced milk coagulation. Heat stability was estimated using the coagulation time test (CTT). Briefly each milk sample was inserted in an individual glass capillary with 120 mm of length, 0.15 mm of external diameter and 0.08 mm of internal diameter. Both ends of the capillary were closed. After, each capillary was immersed in glycerin at 145 °C; the coagulation time (in seconds) was the time elapsed between the immersion and the visualization of clots inside the capillary (Negri et al., 2001). Somatic cell count (SCC) was determined by flow cytometry with Somacount 300®.

Environmental traits.

Air relative humidity (RH; %) and air temperature (Dry Bulb Temperature – Tdb, °C) were measured and registered hourly from 0800 h to 2100 h using meteorological stations (IncoTerm, USB – TFA 35.175) placed 1.5 m above the ground in the shaded area and in the non-shaded areas close to the paddocks.

The temperature and humidity index (THI) was used as an indicator of thermal comfort. It was calculated using the air temperature and relative humidity, using the formula proposed by Johnson, Ragsdale, Berry and Shanklin (1962): $THI = (1.8 \times Tdb + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26.8)]$, where Tdb = dry bulb temperature in °C and RH = relative humidity in % (RH, Tdb and THI values per day are shown in Figure 1). The THI was interpreted as normal if ≤ 74 , alert if $THI > 74$ and < 79 , danger if $THI \geq 79$ and < 84 and emergency if $THI \geq 84$ (Thom, 1959).

Results

Supplementary Table S1 LSmean values of physiological and blood traits, milk yield and composition of cows with or without access to shade at the pre-experimental period (used as covariates)

Trait	Treatment		s.m.e.
	No shade	Shade	
Respiratory rate (breaths/min).	75.4 a	81.6 a	4.2
Rectal temperature (°C)	40.1 a	40.0 a	0.26
Glucose (mg/dL)	61.3 a	61.8 a	1.9
Creatinine (mg/dL)	0.8 a	0.8 a	0.02
Blood Urea (mg/dL)	46.5 a	43.0 a	2.4
Blood Sodium (mmol/L)	137.7 a	137.0 a	0.8
Daily milk yield (L)	21.4 a	21.0 a	0.7

Ethanol Stability (°GL)	77.0 a	76.8 a	0.9
Coagulation time test (seconds)	213.3 a	244.6 a	22.4
Milk Fat (g/100g)	3.2 a	3.4 a	0.1
Crude protein (g/100g)	2.9 a	2.9 a	0.1
Milk Lactose (g/100g)	4.5 a	4.3 b	0.5
Titrateable Acidity (°D)	15.0 a	15.2 a	0.3
Log ₁₀ SCC	5.5 a	5.6 a	0.1
Milk Sodium (mg/L)	150.8 a	247.7 a	5.8
Milk Urea (mg/dL)	16.1 a	15.7 a	0.4

^{a-b} LSmeans within a row with different superscripts are statistically different (P<0.05).

NS = non significant (P>0.05)

During the Stress period, overall means (\pm SE) of AT (°C) were 25.9 ± 2.7 and 28.4 ± 3.1 ; RH (%) were 72.9 ± 8.3 and 60.0 ± 12.7 ; THI were 75.4 ± 3.7 and 77.4 ± 3.5 at shaded and unshaded areas of paddocks, respectively. During the Post-stress period, overall means (\pm SE) of AT (°C) were 25.1 ± 2.1 and 26.4 ± 2.1 ; RH (%) were 71.9 ± 10.0 and 701.8 ± 10 ; THI were 74.0 ± 2.5 and 76.0 ± 3.0 at shaded and unshaded areas of paddocks, respectively (Figure S1).

Supplementary Table S2 LSmeans values for physiological and blood traits, under alert THI conditions with or without access to shade at Stress and Post-stress periods

Trait	Period				s.e.m.
	Stress		Post-stress		
	No shade	Shade	No shade	Shade	

Respiratory rate (breaths/min)	114.5 a	87.2 b	77.8 a	73.9 a	4.6
Rectal temperature (°C)	40.6 a	39.7 b	39.8 a	40.0 a	0.2
Hematocrit (%)	26.6 a	26.4 a	26.8 a	26.2 a	1.5
Albumin (g/dL)	3.2 s	3.1 a	3.2 a	3.1 a	0.
Plasma creatinine (mg/dL)	0.9 a	0.7 b	0.8 a	0.7 a	0.04
Plasma glucose (mg/dL)	71.4 a	57.7 b	55.4 a	56.5 a	1.7
Plasma Sodium (mmol/L)	140.1 a	137.1 b	138.1 a	137.5 a	0.9

^{a-b} LSmeans within a row and with the experimental period with different superscripts are statistically different (P<0.05).

NS = non significant (P>0.05)

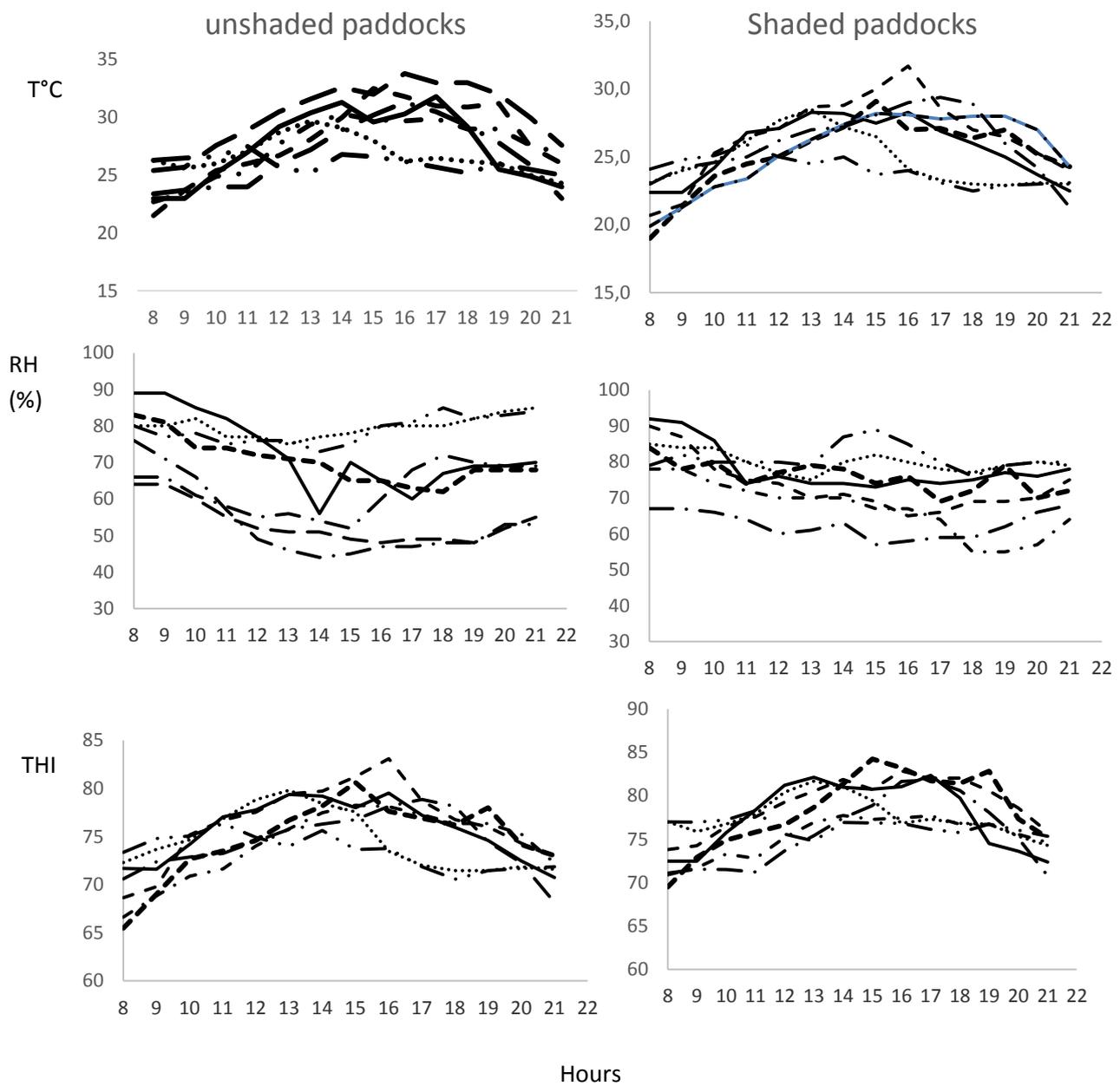


Figure S1 Values of air temperature, T°C (A), relative humidity, RH% (B), temperature-humidity index (THI) (C), and solar radiation (D) measured in the sun and in the shaded area of paddocks on days 9 (----), 14 (.....), 18 (___), 22 (-.-), 24 (___), 31 (___), (38 (___).)

Supplementary Figure 2

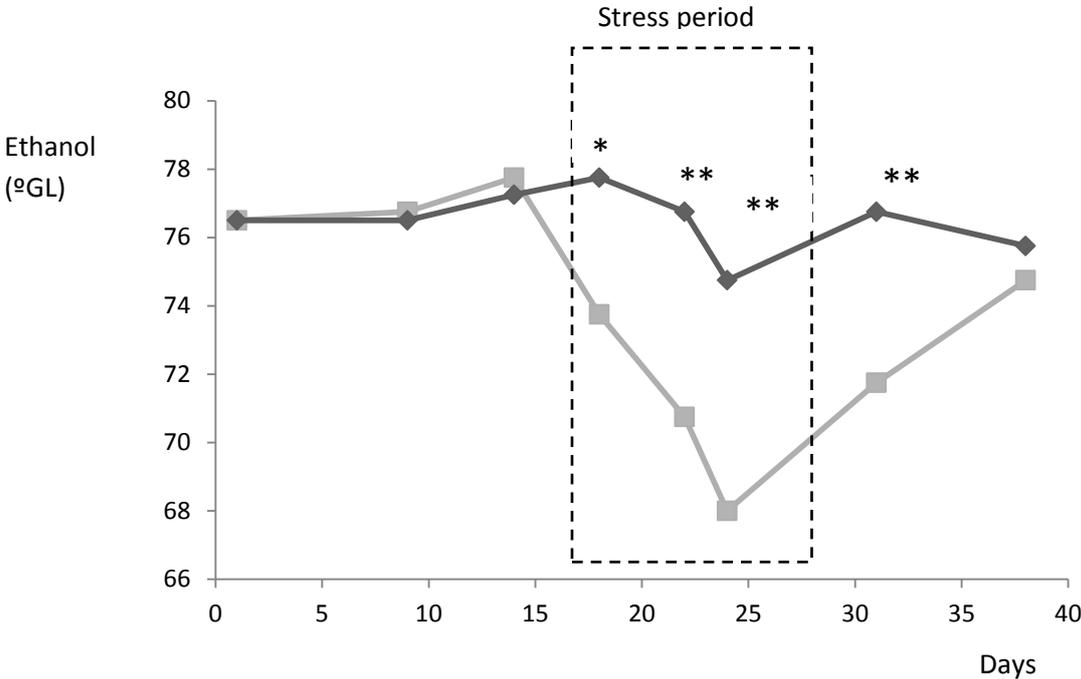


Figure S2 Values of milk stability at the alcohol test as the minimal concentration of ethanol (°GL) capable of inducing clots (shaded cows ◆ , unshaded cows ◻). Days 9, 14 (pre-stress); 18, 22 and 24 (stress); days 31 and 38 (post-stress period).