Relationship among the thermal environment, body characteristics, production, and milk constituents of dairy Gyr cows raised on pasture

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Short title: **Features of dairy Gyr cows' lactation raised on pasture**

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SUPPLEMENTARY FILE

Material and Methods

Experimental area and climate pattern

This study was carried out at the Brazilian Association of Dairy Gyr Breeders (acronym in Portuguese: ABCGIL), Uberaba, Minas Gerais state, Southeast of Brazil (19°44′54″ S, 47°55′55″W). The climate of the region is characterized as subtropical (CWa) with warm and rainy summers and relatively dry winters according to the Köppen's classification (Alvares *et al*. 2013). The study was performed from August 2013 to July 2015, covering the dry (April to October) and rainy (November to March) seasons from the Southern hemisphere. Local microclimatic variable (air temperature, relative humidity, dew point and precipitation) by hour was obtained from the The National Aeronautics and Space Administration Prediction of Worldwide Energy Resources (NASA POWER). The NASA POWER database demonstrated to supply weather data satisfactorily for Brazilian territory (Monteiro *et al.* 2018), also, had been validated to determine thermal comfort indicator for dairy cows (Manica *et al.* 2022; Rockett *et al.* 2023).

The details of the thermal environment variables are described in Table 1. In general, during the dry season, the average air temperature was 22.9°C, ranging from 19.5°C to 27.8°C while the average air temperature during rainy season was 25.3°C, ranging from 23.8°C to 27.8°C.

Table S1. Mean values of the month and standard deviation (SD) of air temperature (AT, °C), relative humidity (RH, %) and accumulated precipitation (PP, mm) during August 2013 to July 2015 in Uberaba, Minas Gerais state, Southeast of Brazil.

Year	Month	Season	AT (°C)		RH (%)		PP (mm)
			Mean	SD	Mean	SD	
2013	May		21.5	4.1	74.5	15.6	89.65
2013	June		21.3	3.7	77.8	14.6	21.09
2013	July	Dry	19.5	5.3	67.6	18.1	10.55
2013	August		21.6	5.7	52.8	17.9	0.0
2013	September		24.8	5.5	52.6	19.1	68.55
2013	October		24.7	4.6	65.6	18.4	110.74
2013	November		25.3	4.2	69.8	18.3	137.11
2013	December		24.9	3.1	78.5	13.8	200.39
2014	January	Rainy	25.8	3.8	69.1	17.5	89.65
2014	February		26.3	4.5	63.4	20.3	110.74
2014	March		25.1	3.4	74.8	15.8	105.47
2014	April		24.3	4.1	73.2	16.9	84.38
2014	May		22.2	4.9	61.2	18.4	0.0
2014	June		22.3	4.9	55.6	16.7	0.0
2014	July	Dry	21.4	5.1	55.9	20.4	47.46
2014	August		23.5	5.6	46.8	16.8	0.0
2014	September		26.8	5.3	43.5	17.9	21.09
2014	October		27.8	5.9	44.1	22.2	52.73
2014	November		25.3	3.7	72.3	18.7	216.21
2014	December		24.5	3.5	78.5	14.9	195.12
2015	January	Rainy	26.9	4.5	64.8	20.3	73.83
2015	February		24.7	3.3	77.9	15.1	195.12
2015	March		23.8	3.1	82.1	12.7	179.3
2015	April		23.7	3.3	80.2	13.6	79.1
2015	May	Dry	21.1	3.9	77.2	15.2	73.83
2015	June		20.9	4.3	69.5	16.8	10.55
2015	July		22.1	4.6	62.8	18.1	5.27

With the microclimatic data, we determined the Temperature Humidity Index (THI) by equation (1) developed by (Thom 1959). The obtained values were classified according to Reis et al. (2021) in normal (£74), alert (75-83), emergency (³84).

$$THI = DBT + 0.36*DPT + 41.2 \quad (1)$$

Where:

DBT is the dry-bulb temperature (°C), and

DPT is the dew point temperature (°C).

Animals and management

A total of forty-five nulliparous dairy Gyr cows participated in this study from prepartum (average age of 35.4 ± 5.3 months, range: 27 – 48 months) to 10 months of lactation. The cows participated in two pasture-based milk production performance tests, in which the objective was to identify cows with potential for milk production in pasture-based systems since these systems are used for milk production with zebu breeds in Brazil.

Cows that calved between May and October 2013 were included in the first milk test (n = 25), and cows that calved between August and October 2014 were included in the second milk test (n = 20). During the prepartum period, approximately 60 d before the estimated day to calving, the nulliparous cows were kept in a maternity paddock close to the milking parlor and received sorghum silage and concentrate (2 kg/cow/day) at the feeder.

After calving, the nulliparous cows were kept in an area of 7 ha, divided into 12 paddocks (0.6 ha) with *Brachiaria brizantha cv xaraes grass*. The pasture was managed by the rotational stocking method to provide three days of occupation and approximately 33 days of rest. Usually, the cows entered in the paddock when the pasture achieves 35 cm of height and left with 15-20 of height. Due to forage scarcity of dry season (August to September), the diet of cows was complemented with corn silage. Also, the cows received 6 kg of concentrate per day until the 35th day of lactation. After this period, the concentrate was supplied at a proportion of 1 kg per each 3 L of milk produced above 10 L, during the milking. The nulliparous cows were milked twice a day using a mechanical milking machine (EuroLatte 330/450 l, 50 kPa) in the presence of their calves. Until 90 days old, the calves were allowed to suckle in one teat of their mothers before milking to stimulate milk ejection, and after milking to suckle the residual milk (standard management for Zebu dairy cows). After 90 days old, the calves only drank the residual milk from their mothers.

Measurement

Body weight, body condition score (BCS), subcutaneous fat thickness, milk production, milk composition, and milk concentration of progesterone were all monthly recorded, from prepartum, approximately 60 d before the estimated day to calving (except milk recorded traits), to 10 months of lactation.

Body measurement

For body measurement, the nulliparous cows were conducted once a month to the management center, always in the morning milking (~7h). To evaluate body weight, cows were weighted using an automatic balance. Body condition was evaluated by assigning a score of 1 (debilitated), 2 (verythin), 3 (thin), 4 (borderline), 5 (moderate), 6 (good), 7 (verygood), 8 (fat) or 9 (obese), according to Ferreira *et al*. (2005).

Subcutaneous fat thickness

To assess subcutaneous fat thickness five ultrasound images (Figure S1) were taken using a Pie Medical Equipment B.V. (1996) and processed by the Echo Image Viewer 1.0 program. In this study, we only used the data of subcutaneous fat thickness from pelvic region (EGS2), as this region has high correlation with BSC before calving and during lactation (Miranda et al., 2022).

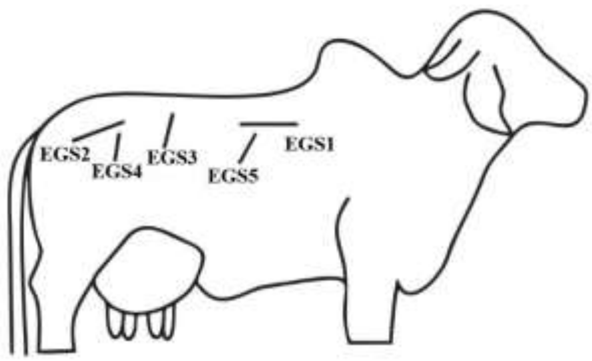

Figure S1. Schematic representation of the five ultrasound images taken to assess subcutaneous fat thickness. EGS1 - longitudinal image across the 11th−13th rib (BIF 2010); EGS 2 - pelvic region midway between the tuber coxae (hook) and tuber ischii (pin) (Schwager-Suter et al. 2000); EGS3 - transverse plane of the flank (Schwager-Suter et al. 2000); EGS4 - lumbar region midway between the last rib and tuber coxae (hook) (Schwager-Suter et al. 2000); EGS5 – image between the 12th and 13th rib (BIF 2010).

Statistical analysis

All analyses [influence, descriptive (average, standard deviation, coefficient of variation, minimum and maximum), and confirmatory] were performed through the statistical software R version 4.2.2 (R Core Team 2022). The database was built with 3,200 pieces of information composed of each measurement (body weight, body condition score, subcutaneous fat thickness, progesterone, milk production, and constituents). The data of body weight, body condition score, subcutaneous fat thickness, milk production, and constituents were categorized y month of lactation (1 to 10) and grouped by lactation stage (1st stage: 1d - 100d; 2nd stage: 101d - 200d; and 3rd: 201d - 305d). As this is an exploratory study, first we evaluate the influence of the lactation stage on the interest variables, and after we performed a deep analysis within the months of lactation (1 to 10) to obtain more information. The data were analyzed using a Generalized Linear Models (GLM) at a 95% confidence level. The models were adjusted through the maximum likelihood-Laplace approximation method in the statistical package lme4 (Bates *et al.*, 2015). The confidence intervals were estimated using Type II Wald chi-square tests and the fit of the model was given by a likelihood-test. The fitness of the models was tested by inspecting the residual in the graphs, a line of best fit. The normality of the random facts was given by quartile plot means with a confidence interval of 95%. The details of each statistical model performed for body measurement characteristics, progesterone, milk production, and milk constituents are described below.

Firstly, we evaluate the relationship between the lactation stage, milk production, and milk constituents. A mixed GLM model with Gamma distribution and logarithmic link function was built for each interest variable. The stage of lactation was used as a fixed effect, while cows were nested in milk tests (1 and 2) and used as a random effect. Secondly, we assess the influence of months of lactation on body weight, subcutaneous fat thickness, 305-day milk yield, and levels of progesterone. A generalized linear model with Gamma distribution was used and a confidence interval of 95%. The months of lactation (1 to 10) were used as a fixed factor, cows were nested in milk test (1 and 2) and used as a random effect, and the response variables were continuous values. The distribution of predicted values and standard error of the milk production and the percentage of constituents were plotted in graphics. In addition, the average temperature humidity index of two days before milk collection, milk production, and milk constituents were subjected to Pearson's correlation test with a 95% confidence level. We choose to work with an average THI of two days before milk collection because it is known that the negative impact of heat stress can occur with a delay (Bernabucci et al., 2014; Tao et al., 2020).

To determine whether weight loss influenced 305-day milk yield a GLM was performed with weight loss as a category variable. We determined the weight loss by subtracting the body weight for each postpartum month from the prepartum weights; and dividing it into three categories: Low: 0 to 27 kg; Medium: 28 to 50 27 kg; and High: greater than 51 kg. Furthermore, the 305-day milk yield of cows was submitted to confirmatory analysis by the Tukey test (95% of confidence level) to see if had a difference in the 305-day milk yield in relation to the three categories of weight loss.

Results

During the experimental period, there were hours of the day of thermal challenge for the nulliparous Gyr cows (Figure S2).

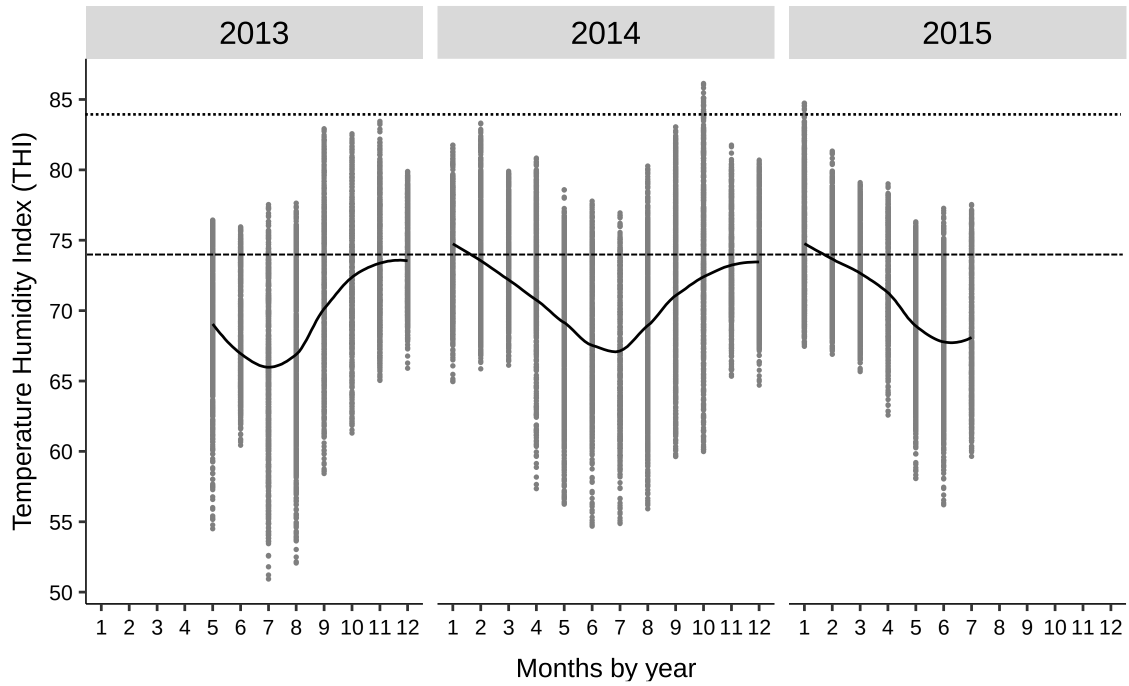

Figure S2. Distribution of hourly value of Temperature Humidity Index (THI) by month and year of experimental period. Values above the black dotted line (^{3}84) represents the category emergency for THI; values under the black dashed line (£74) represents the category comfort for THI and values between the lines (74 and 83) represents the category alert for THI.

As increased the months in lactation there was a decrease ($p<0.001$) in milk production, and milk lactose; in contrast, there was an increase ($p<0.05$) in milk fat, milk protein, and milk solid (Figure S3).

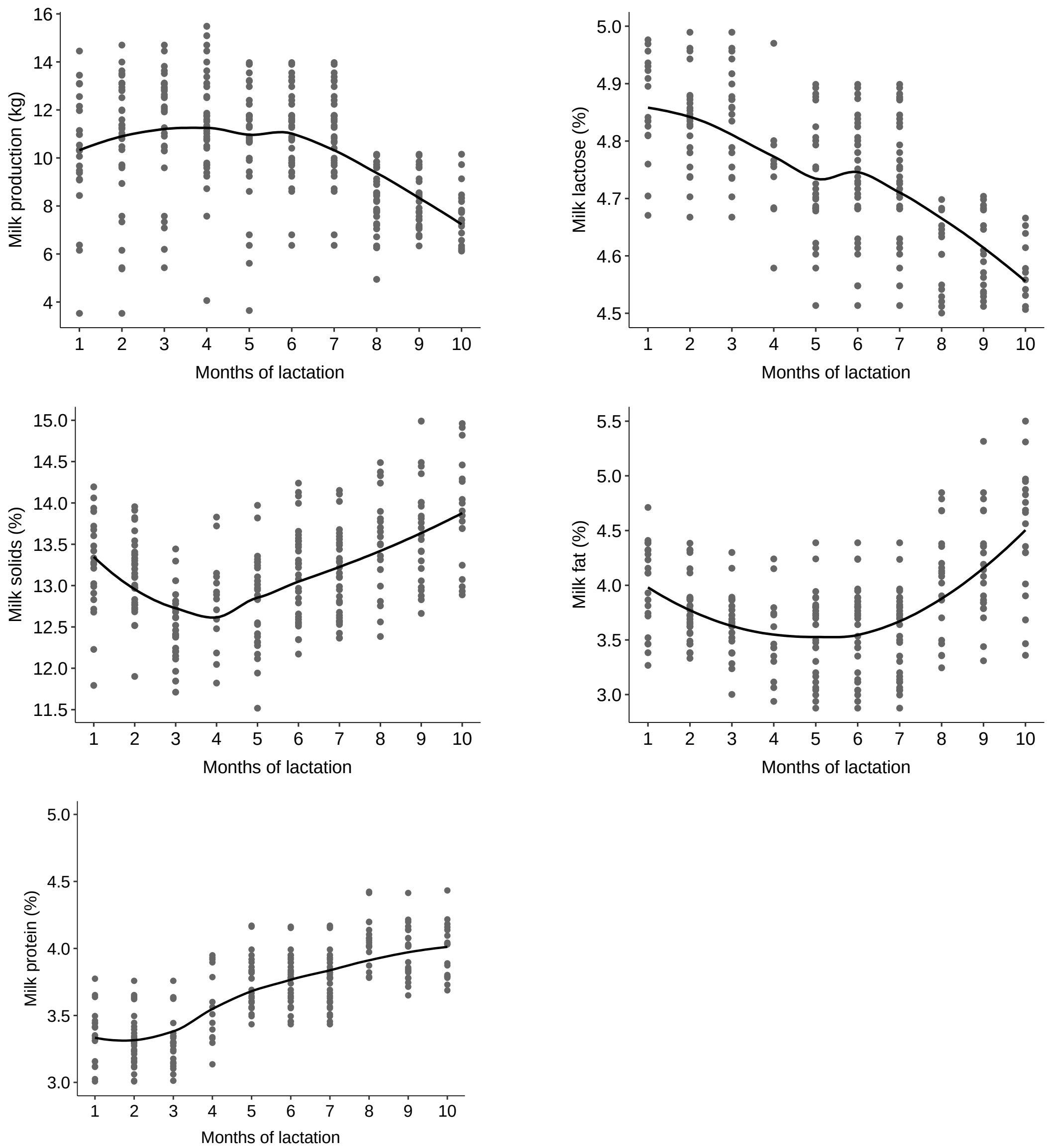

Figure S3. Distribution of values, line of tendency (black solid line) and standard error of milk production (kg) and percentage of constituents (lactose, solids, fat, and protein) for each cow throughout the ten months of lactation.

Most cows presented low values of P4 n/ mL (< 3 P4 n/ mL) until the fourth month of lactation (1^{st}: 82%; 2^{nd}: 52%; 3^{rd}: 48%; 4^{th}: 53%); while in the fifth month of lactation, most of cows (52%) presented values higher than 4 P4 n/ mL (Figure S4).

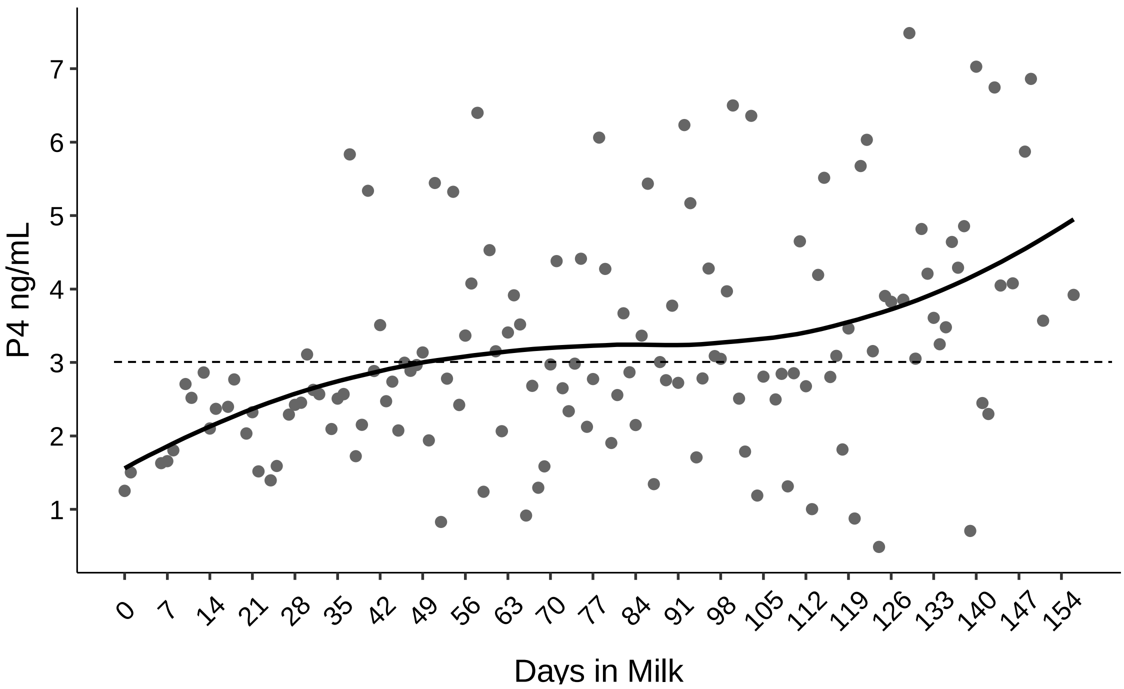

Figure S4. Distribution of values, line of tendency (black solid line), and standard error of milk progesterone (P4 ng/mL) from day 0 to 154 of lactation. The dashed line represents the threshold of 3 ng/mL, which corresponds to luteal activity (Bulman and Lamming, 1978).

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