



RESEARCH ARTICLE

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## PHENOTYPIC PLASTICITY AND BEHAVIORAL RESPONSES OF GIROLANDO HEIFERS ON CONTINUOUS GRAZING IN THE SEMIARID OF PERNAMBUCO

**<sup>1</sup>Ingrid do Nascimento Bezerra, <sup>4</sup>Gledson Luiz Pontes de Almeida, <sup>4</sup>Cristiane Guiselini, <sup>4</sup>Héilton Pandorfi, <sup>3</sup>Pedro Henrique Dias Batista, <sup>\*3</sup>Marcos Vinícius da Silva, <sup>3</sup>Rodes Angelo Batista da Silva, <sup>1</sup>Maria Eduarda Gonçalves de Oliveira, <sup>2</sup>Wesley Amaro da Silva and <sup>2</sup>Adriel Sales Coutinho**

<sup>1</sup>University Federal Rural of Pernambuco, Undergraduate Student in Agricultural Engineering, Rua Manuel de Medeiros, s/n, 52171-900, Dois Irmãos, Recife, PE, Brasil;

<sup>2</sup>University Federal Rural of Pernambuco, Master's student in Agricultural Engineering, Rua Manuel de Medeiros, s/n, 52171-900, Dois Irmãos, Recife, PE, Brasil;

<sup>3</sup>University Federal Rural of Pernambuco, PhD student in Agricultural Engineering, Rua Manuel de Medeiros, s/n, 52171-900, Dois Irmãos, Recife, PE, Brasil;

<sup>4</sup>University Federal Rural of Pernambuco, Professor of the Agricultural Engineering Department, Rua Manuel de Medeiros, s/n, 52171-900, Dois Irmãos, Recife, PE, Brasil

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**\*Corresponding author: Marcos Vinícius da Silva,**

### ABSTRACT

The indicators of thermal stress for ruminants are described according to the magnitude of the meteorological elements, physiological, behavioral adjustments, and productive response. This research was conducted to evaluate the ingestive behavior and phenotypic plasticity of Girolando heifers subjected to continuous grazing in the semiarid of Pernambuco. Three heifers of 7/8 Dutch-Gir genetic composition were evaluated, submitted to a paddock with 40 x 40 m brachiaria, for 21 days. The meteorological variables recorded in the production environment were the dry bulb temperature (°C) and the relative humidity of the air (%), which allowed the thermal characterization through the temperature and humidity index (THI). The behavior of heifers was quantified through observation by instantaneous scanning of activities, walking, drinking, eating, ruminating, lying down, leisure and standing. The physiological responses recorded were rectal temperature (°C) and respiratory rate (movement min<sup>-1</sup>). The experimental design was completely randomized in a 2 x 3 factorial arrangement (shifts x evaluation period). Behavioral variables were subjected to the chi-square and probability test. The physiological parameters were subjected to analysis of variance and application of the Tukey test at the level of 5% significance. The THI did not exceed 89 units, which characterizes the critical limit of moderate stress for the animals. The heifers proved to be adapted to the climate of the region, as, during the study period, they showed sufficient physiological and behavioral alteration capacity to maintain homeothermic.

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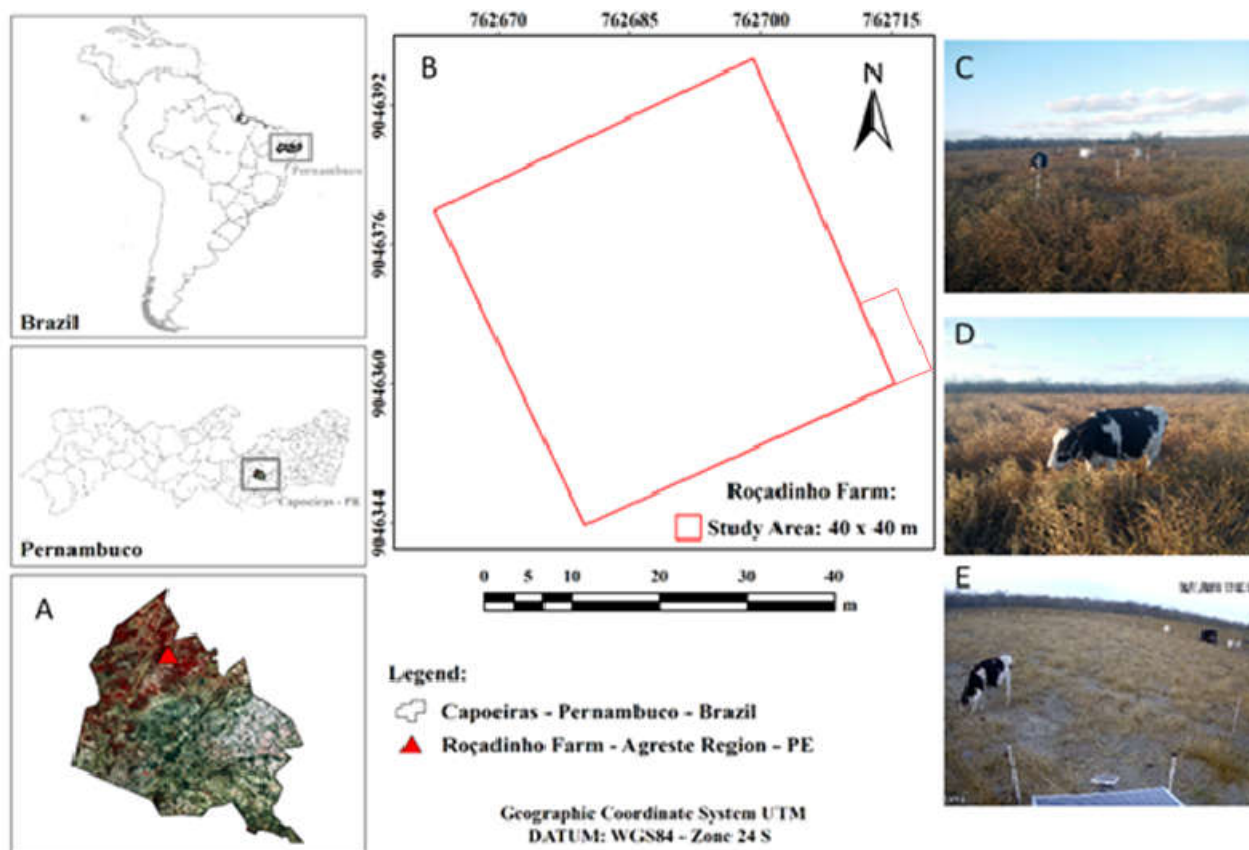
### INTRODUCTION

The action of meteorological elements can interfere in the behavior of animals, in physiological responses and production, attributed to the need to adapt and shift energy from production to maintenance, impacting zoo technical indices and economic losses, especially in the semi-arid region, where high temperatures in this region imply the thermal imbalance of animals (Batista *et al.*, 2019). The ideal climatic conditions for

the exploration of dairy cattle is conditioned by the meteorological elements, temperature and relative humidity of the air, which vary from 10 to 27 °C and 60 and 70%, respectively (Baêta& Souza, 2010). In this context, the temperature and humidity index (THI) is used for the thermal characterization of the environment and thermal comfort for dairy cattle (Ji *et al.*, 2019). According to Armstrong (1994), THI below 72 characterizes an environment without heat stress by heat, between 72 and 78 it is classified as mild stress, from

79 to 88 moderate stress and from 89 to 98 severe stress. One of the ways to evaluate the animals' responses to the thermal environment is through phenotypic plasticity, through physiological variables, respiratory frequency, and rectal temperature, which consists of the animal's ability to change these variables when influenced by intrinsic factors (age, race, physiological condition) and extrinsic factors (time of day, ambient temperature, wind speed, season, food and water intake) (Almeida *et al.*, 2014).

Brachiariadecumbens pasture, during a 21 days monitoring period, in a 40 × 40 m paddock, with an 8 × 10 m resting space, with a drinking fountain and shading mesh (80%), which provided an area of 15 m<sup>2</sup> of artificial shade (Figure 1B). Three Girolando heifers, with an average weight of 300 kg, were used in a continuous grazing system. The height of the forage canopy (pasture) was determined at the beginning (week 1), intermediate (week 2) and end of the grazing period (week 3), using a ruler graduated in centimeters.



**Figure 1. Location of Fazenda Roçadinho in Capoeiras, Pernambuco, Brazil (A); Delimitation of the area where the experiment was carried out (B); Images of animal registration in the experiment area (C, D, E)**

The study of animal behavior is one of the most important indicators for adequate agricultural exploitation, in the search for management techniques, feeding, and facilities that allow the increase of productive responses. Thus, it is necessary to monitor the environment in which the animals are inserted, so that the cattle can maintain their homeotherm, and thus, minimize the deviation of maintenance energy and expand their production capacity (Borchers *et al.*, 2016; Wang *et al.*, 2018). This research was conducted to evaluate the ingestive behavior and phenotypic plasticity of Girolando heifers subjected to continuous grazing in the semiarid of Pernambuco.

## MATERIAL AND METHODS

The experiment was carried out at Farm Roçadinho, commercial ownership of dairy cattle, located in the municipality of Capoeiras, Mesoregion Agreste in the state of Pernambuco, latitude 8° 36' S, longitude 36° 37' W and altitude of 700 m (Figure 1). The region's climate is characterized as semi-arid (Bsh), according to the Köppen climate classification. The region's average annual rainfall is 588 mm, with an average annual temperature of 22.1 °C (Alvares *et al.*, 2013). The study was carried out in an area under

Meteorological variables, dry bulb temperature (TBS, °C) and relative air humidity (RH, %) were recorded every ten minutes, in the animals' resting area and the meteorological shelter, using a Hobo U12 – data logger. 12 (Onset Computer Corporation Bourne, MA, USA). The sensors were fixed inside the meteorological shelter installed at 1.50 m from the ground, and under the shading mesh, in the rest area. The thermal characterization of the environment was performed using the temperature and humidity index (THI) (Eq. 1), proposed by Thom (1959):

$$\text{THI} = \text{TDB} + 0,36 \times \text{TDP} + 41,5 \quad \text{Eq. 1}$$

on what,

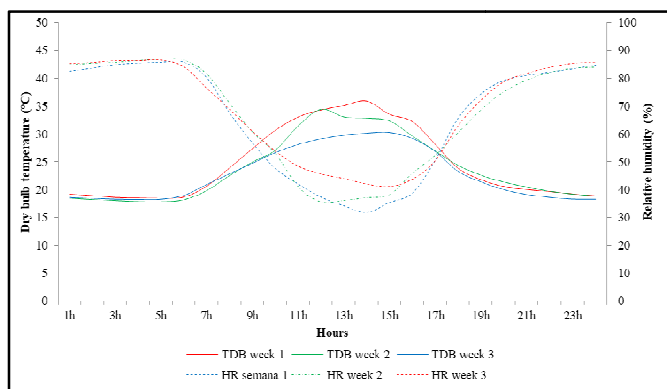
TDB – dry bulb temperature, °C; and;  
TDP – dew point temperature, °C.

The animals' recorded physiological parameters were: rectal temperature (RT, °C), and respiratory rate (RR, mov min<sup>-1</sup>) from 6:00 am to 6:00 pm, in the interval of 3:00 hours, for two days a week. To record the animals' behavior, a video camera, equipped with infrared, was used to assist in nighttime monitoring, positioned so that the visual field included the

entire grazing area, triggered once a week on days not coincident with the recording of physiological variables. The description of behaviors was organized according to each reality. Behavioral quantification (walking, drinking, eating, ruminating, leisure, standing and lying down) was based on the model developed by Almeida *et al.* (2013), adapted from the Altman instant scan method (1974), in which the video was paused every 10 min, with a record of the behavior observed at that moment, being performed once every week during the 24 h. The experimental design was completely randomized in a 2 × 3 factorial arrangement. For behavior analysis was divided into 2 (two) daily shifts (day – 6:00 am to 6:00 pm; night – 6:00 pm to 6:00 am) and 3 (three) periods of behavioral assessment (1st, 2nd and 3rd week of grazing). Behavioral variables were subjected to distribution and percentage analysis, using the chi-square and probability test. The physiological parameters were subjected to analysis of variance and application of the Tukey test at the level of 5% significance for multiple comparisons of means when there are differences between the hourly intervals considered.

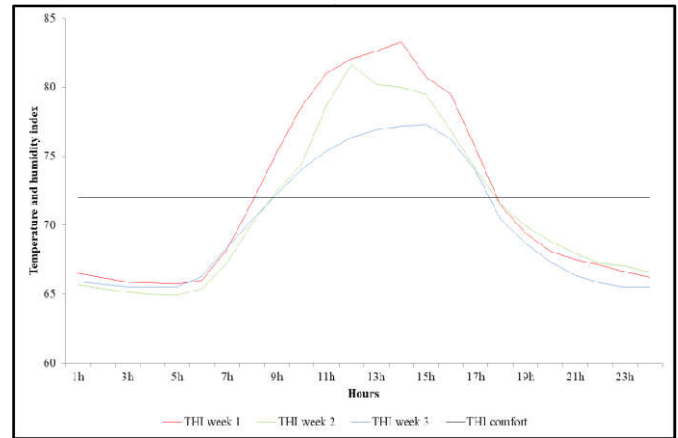
## RESULTS AND DISCUSSION

During week 1, the highest values of dry bulb temperature (TDB, °C) were recorded being that the relative humidity of the air (RH, %) showed a variation inversely proportional to TDB during all the weeks of study (Figure 2).



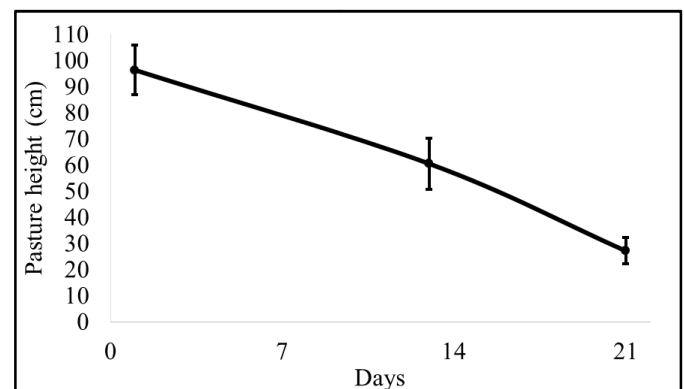
**Figure 2. Average weekly hourly variation of dry bulb temperature (TDB, °C) and the relative humidity of the air (HR, %) in the picket**

The conjunctures suggested by Armstrong (1994) of Temperature and Humidity Index (THI) less than or equal to 72, were overcome in the study environment, in week 1 in the range from 8 am to 6 pm, in week 2 in the range from 9 am to 5 pm and week 3 from 9 am to 6 pm. Therefore, at these times the animals were exposed to greater heat stress (Figure 3). In that, the THI of the first week reached the greatest amplitude, classified as moderate, therefore, in the second week it was found moderate THI from 13 to 14h, however, week 3 in the peak periods, the THI did not exceed the mild. In the present study, the animals were never subjected to severe thermal stress in any period, that is, when the THI is above 89 (Figure 3). However, Veissier *et al.* (2018), studying the behavior of cows subjected to moderate stress, showed important adaptive responses to eliminate excessive heat from the body, thus demonstrating the animals' adaptability to the temperate climate region.



**Figure 3. Average weekly variation hourly of the temperature and humidity index (THI) in the pasture**

It can be seen in Figure 4 reduction ( $p > 0.05$ ) of the height of the pasture during the study (21 days). Therefore, in week 3, due to the height and the quality of the pasture, which decreased over time, the animals increase the grazing time, to compensate for the low quality of the forage. Similar results were found by Trindade *et al.* (2016).



**Figure 4. Pasture height before and over 21 days of grazing**

Thus, it is observed that at week 3 the animals grazed for a longer time compared to week 1 and 2 (Table 1), even so, it did not necessarily result in greater consumption of fresh matter, however, with the reduction of pasture (Figure 4) the cattle increased the grazing activity to supply the nutritional demand, for these reasons, also, in the last week the animals reduced the rumination and idle time. Therefore, the findings in the present study corroborate Werner *et al.* (2019), in which it was found that cattle submitted to a lower supply of forage tend to stay longer in the eating activity and reduce the rumination time. However, there were no significant differences between weeks for eating activity (Table 1). However, when the day and night periods were compared separately, they showed a significant difference ( $p > 0.05$ ). During the study, a higher frequency of drinking behavior was observed at weeks 1 and 3 (Table 1), therefore, the animals ingested a greater amount of water during the period when the ITH was higher (Figure 3), as well as at the time of greater grazing. According to Giro *et al.* (2019), water consumption is related to room temperature and food intake. Therefore, statistically, there was no significant difference between water consumption between the 3 weeks and the periods (day and night) of the study ( $p > 0.05$ ), so water consumption occurred in a normal way, meeting the physiological needs of animals. As for the ruminating activity, the animals performed it for a

longer time in the first and second week, preferably at night. As for rumination, the values compared between the weeks did not show any significant difference, however, when comparing the day with the night during the 3 weeks, there was a significant difference ( $p < 0.05$ ), findings that corroborate those found by (Poulopoulou et al., 2019).

behavior of crossbred cows, the authors observed a negative correlation of the animals' leisure with the environmental variables, which indicated a moderate action of the environment on this behavior. Heifers spent more time walking during the day ( $p < 0.05$ ) when compared to the night period (Table 1).

**Table 1. Percentage of time spent by animals in each activity during the three weeks of study**

	Week 1	Week 2	Week 3	P Value	Average
Eating (minute)					
Day	360,00	366,70	410,00	0,4382	378,90
Night	123,30	103,30	120,00	0,7011	115,53
P Value	<0,0001	<0,0001	<0,0001	-	-
Total	483,30	470,00	530,00	-	-
Drinking (minute)					
Day	26,60	13,30	16,60	0,4560	18,83
Night	13,25	10,00	16,60	0,2670	13,28
P Value	0,2416	0,1772	0,9119	-	-
Total	39,85	23,30	33,20	-	-
Ruminating (minute)					
Day	120,00	116,60	53,40	0,0114	96,67
Night	166,70	206,60	196,60	0,2609	190,00
P Value	<0,0379	<0,0006	<0,0001	-	-
Total	286,70	323,20	250,00	-	-
Walking (minute)					
Day	26,70	20,10	30,00	0,4123	25,60
Night	3,45	3,10	10,10	0,2211	5,52
P Value	<0,0001	<0,0001	<0,0001	-	-
Total	30,15	23,20	40,10	-	-
Idleness (minute)					
Day	186,70	203,30	210,00	0,5205	200,00
Night	413,30	397,00	376,70	0,5598	395,67
P Value	<0,0001	<0,0001	<0,0001	-	-
Total	600,00	600,30	586,70	-	-
Standing (minute)					
Day	596,70	606,70	556,60	0,1447	586,70
Night	250,00	146,70	203,30	0,0038	200,00
P Value	<0,0001	<0,0001	<0,0001	-	-
Total	846,70	753,40	759,90	-	-
Lyingdown (minute)					
Day	123,30	113,30	163,40	0,1447	133,30
Night	470,00	573,30	516,70	0,0038	520,00
P Value	<0,0001	<0,0001	<0,0001	-	-
Total	593,30	686,60	680,10	-	-

**Table 2. Variation of physiological responses to respiratory rate (RR) and rectal temperature (RT) during the study period**

	Week 1	Week 2	Week 3	CV (%)	P value	Average			
RR (mov min <sup>-1</sup> )									
6h	28,7	a A	28,0	a A	29,0	13,1	0,891	28,5	
9h	36,0	a A	36,0	a BC	36,3	a A	20,1	0,985	36,1
12h	37,7	a A	40,0	a C	38,0	a A	14,9	0,754	38,6
15h	35,0	a A	35,0	a ABC	37,0	a A	17,6	0,819	35,7
18h	29,5	a A	30,0	a AB	33,0	a A	9,4	0,113	30,8
CV (%)	20,6		13,6		12,9		-	-	-
P value	0,114		0,001		0,011		-	-	-
Average	33,4		33,8		34,7		-	-	-
RT (°C)									
6h	37,7	a A	37,7	a A	37,6	a A	1,3	0,973	37,1
9h	37,9	a A	38,1	a AB	38,3	a B	1,5	0,374	38,1
12h	38,4	a A	38,5	a BC	38,4	a B	0,7	0,723	38,4
15h	38,6	a A	38,8	a C	38,7	a B	0,7	0,422	38,7
18h	38,3	a A	38,5	a BC	38,6	a B	1,9	0,849	38,5
CV (%)	1,9		1,0		0,7		-	-	-
P value	0,156		< 0,001		< 0,001		-	-	-
Average	38,2		38,3		38,3		-	-	-

Capital letters: Refers to the values compared between the hours; Lower case: Refers to the values compared during the weeks.

For leisure activity, the values between the weeks did not show the statistical change ( $p > 0.05$ ), however, when taking into account the day and night periods, there was a difference ( $p < 0.05$ ), with weeks 1 and 2 presented numerically higher values when compared to week 3, considering that in these periods higher values were recorded for the THI. Similar results were found by Pereira et al. (2018), when evaluating the effect of the climatic environment on the ingestive

This can be justified by the fact that the animals in that hour interval had presented more time in the activities standing and eating, having to move during the grazing. For standing activity, the animals spent more time in week 1, possibly due to the high THI values (Figure 2), since the cattle tend to remain standing when subjected to higher temperatures, to lose heat to the environment through sweating, corroborating (Matarazzo et al., 2007). For lying behavior, the animals spent

more time during week 2, which was the one with the highest rumination values. According to Batista et al. (2019), lying activity is directly linked to rumination activity, since cattle tend to ruminate in that position, thus favoring the positioning of the rumen. As for the physiological variables of the animals, in all analyzed hourly intervals, it was observed that there was no significant difference ( $p > 0.05$ ) between the weeks. When comparing the daily periods from 6 am to 6 pm, the only week 2 differed statistically ( $p < 0.05$ ) (Table 2). The animals showed a higher respiratory rate at week 3 (Table 2), although the environmental conditions were milder, they spent more time performing behavioral activities related to greater intensity of movements (eating, walking and standing) (Table 1). During both weeks, the time that showed the highest respiratory rate was at midnight, however, at no time did the animals exceed the limit of  $60 \text{ mov min}^{-1}$  considered normal by Hahn et al. (1997), that is, they were not under severe thermal discomfort.

The rectal temperature also did not show a statistical difference ( $p > 0.05$ ) when the three weeks of study were analyzed. Likewise, the second and third weeks showed no significant difference in the hours from 6 am to 6 pm (Table 2). However, about weekly hourly averages, there was little variation. The maximum rectal temperature did not exceed the value of  $39,1 \text{ }^\circ\text{C}$ , therefore, the animals were not subjected to thermal stress (Peng et al., 2019). Therefore, Araújo et al. (2016), in a survey carried out in the semi-arid region, observed that the animals remained within this ideal rectal temperature range during the period studied, proving that the cattle were not subjected to thermal stress. Therefore, it is emphasized that during grazing the animals in this study were not affected by environmental conditions, that is, the adaptability indicators associated with the transfer of energy by convection between animals and the environment attenuated the effects of stressors. This behavior is desirable and expected due to the rusticity and adaptability of the animals to the region and the management conditions in which they were inserted.

## CONCLUSION

The animals did not show significant differences for the behavioral parameters (eating, drinking, ruminating, walking and leisure) over the weeks analyzed, both for the daytime (6 am to 6 pm) and for the night time (6 pm to 6 am), except for standing and lying activity at night. The physiological variables did not show any significant difference between the weeks, but they did show a significant difference between the hours when compared in the same week. However, these statistical differences were not sufficient for the animals to be subjected to thermal stress. As a result, heifers proved to be adapted to the climate of the region, as, during the study period, they showed sufficient physiological and behavioral changes to maintain homeothermy.

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