Thermoregulatory, behavioral, and production traits of ½ and ¾ Holstein/Gir dairy cows

Características termorreguladoras, comportamentais e produtivas de vacas leiteiras ½ e ¾ Holandês/Gir

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Abstract

The aim of this study was to evaluate thermoregulatory, behavioral, and production traits of Holstein/ Gir dairy cows of different genetic compositions. The experiment was carried out in the municipality of Turvânia - GO, Brazil, from July to December 2017. Twenty lactating cows of two genetic groups (½ and ¾ Holstein/Gir [HG]) were used in a completely randomized design where they were subjected to a rotational-grazing system in 24 irrigated Mombasa grass pastures with one day of occupation and 24 days of rest, and fed a 22% CP supplemental concentrate diet. During the experimental period, thermoregulatory, behavioral, and production data were collected on 12 occasions, with 15-day intervals. Environmental data such as ambient temperature, air relative humidity, and temperaturehumidity index were recorded. Number of hairs, hair length and thickness, body surface temperature, rectal temperature, and respiratory frequency were analyzed as thermoregulatory traits. The behavioral assessment was undertaken by observing the feeding behavior, rumination, rest, and other activities. To determine production traits, milk yield and composition were analyzed. A higher respiratory frequency was observed in \(^3\)4 HG cows compared with \(^1\)2 HG cows (p < 0.05). Cows of the \(^1\)2 HG group produced milk with higher protein contents (p < 0.05). Rectal temperature, body temperature, number of hairs, hair length and thickness, feeding behavior, rumination, rest, and other activities were similar (p > 0.05) between the two genetic groups, and the same was true for milk yield, fat and lactose contents, and somatic cell count.

Key words: Dairy cattle. Ethology. Genetic groups. Milk production. Physiological responses.

Resumo

Objetivou-se avaliar as características termorreguladoras, comportamentais e produtivas de vacas leiteiras Holandês/Gir de diferentes composições genéticas. O experimento foi realizado no município de Turvânia, GO, entre os meses de julho a dezembro de 2017. Foram utilizadas 20 vacas em lactação de dois grupos genéticos ½ e ¾ Holandês/Gir, distribuídas em delineamento inteiramente ao acaso, submetidas a pastejo rotacionado irrigados com 24 piquetes de Mombaça, com um dia de ocupação e 24 dias de

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descanso, com uma dieta suplementar concentrada com 22% de PB. Durante o período experimental, foram coletados os dados das características termorreguladoras, comportamentais e produtivas com um intervalo de 15 dias, totalizando 12 coletas. Foram registrados dados ambientais como temperatura ambiente, umidade relativa do ar e índice de temperatura e umidade. O número de pelos, comprimento e espessura do pelo, temperatura de superfície corporal, temperatura retal e frequência respiratória foram analisados como características termorreguladoras. A avaliação comportamental foi realizada observando o comportamento alimentar, ruminação, descanso e outras atividades. Para determinação das características produtivas foi verificado a produção e composição química do leite. As vacas ¾ HG apresentaram frequência respiratória superior aos animais ½ HG (p < 0,05). Os animais ½ HG apresentaram maior teor de proteína no leite (p < 0,05). A temperatura retal, temperatura de superfície corporal, número de pelo, comprimento e espessura de pelo, comportamento alimentar, ruminação, descanso e outras atividades foram semelhantes (p > 0,05) entre os dois grupos genéticos, assim como a produção de leite e os teores de gordura, lactose e contagem de células somáticas.

Palavras-chave: Bovino leiteiro. Etologia. Grupos genéticos. Produção de leite. Respostas fisiológicas.

Introduction

Milk production is greatly influenced by climate conditions such as climatic elements and factors. Some of the main climatic elements are temperature, humidity, and solar radiation. The combination of climatic elements such as high temperature and elevated humidity is one of the main causes of thermal stress in dairy cattle.

In a stress situation, these animals make use of body thermoregulatory (REZENDE et al., 2015) and behavioral alterations to reduce the stress caused by heat or cold, adopting sensible and/or latent mechanisms for the dissipation or gain of heat in an attempt to maintain their body temperature within the thermoneutral zone.

In a comparison of breeds, the thermoneutral zone for adult European cattle is −10 °C for the lower critical temperature (LCT) to 27 °C for the upper critical temperature (UCT), with an optimal range between −1 and 16 °C, whereas adult Zebu cattle have a LCT around 0 °C and an UCT of 35 °C, with an optimal range between 10 and 27 °C (FERREIRA, 2005).

As the ambient temperature exceeds the critical temperature, cattle enter a state of thermal stress, which triggers thermoregulatory and behavioral changes that affect their milk production and its composition. In a stressful environment, milk yield can be increased through the use of higher-yielding

genotypes adapted to the local environmental conditions (FAÇANHA et al., 2013).

Part of the dairy herd in Brazil includes temperateclimate breeds (taurine breeds), e.g. Holstein, which have higher milk yields but do not tolerate the heat. In this scenario, a recommended viable alternative is the adoption of crosses involving zebu and taurine breeds, which can provide a rational use of the adaptation to the tropical climates of zebu breeds coupled with the yield potential of taurine breeds. This fact is only possible because of the maintenance of genetic variability present in the various genetic groups comprised by the breed, which grants productive traits and a greater thermoregulatory capacity to crossbred animals (CANAZA-CAYO et al., 2014).

The thermoregulation process in dairy cattle is related to physiological conditions, namely rectal temperature, respiratory frequency, and surface temperature (COSTA-E-SILVA et al., 2009). Structures of the hair coat are also essential for the animal to adapt to heat, and these comprise the hair length thickness and the number of hairs, which can determine the adaptability of an animal to thermal stress from solar radiation (MATA-E-SILVA et al., 2013).

In addition to affecting the physiological part associated with thermoregulation, heat stress can influence the normal behavior of animals. Examples of such influences are reduced feed intake, increased idle times, increased water intake, anomalous behavior, among others, which negatively affect the welfare, milk yield, and milk composition of the animal.

It is thus necessary to know the influence of the environment on thermoregulatory, behavioral, and production traits of Holstein/Gir cows of different genetic compositions, identifying which genetic composition is most affected by thermal stress. On this basis, the present study was developed to evaluate the thermoregulatory, behavioral, and production traits of Holstein/Gir dairy cows of different genetic compositions.

Material and Methods

The study was conducted between July and December 2017 on a dairy farm in the municipality of Turvânia - GO, Brazil (16° 36' 29" S latitude 50° 7' 25" W longitude, 204 m altitude). The climate in the region is a tropical Aw type, according to the Köppen classification. The experiment was executed after approval of the project by the State University of Goiás with code 2515.

Cows were subjected to a rotational-grazing system in 24 irrigated Mombasa grass pastures with one day of occupation and 24 days of rest, and fed a 22% CP supplemental concentrate diet. Mineral salt was available ad libitum all year long, and all animals had free access to drinking water and natural shading.

Twenty lactating multiparous crossbred cows at similar ages, with an average body weight of 560 \pm 27 kg and average daily milk yield of 15 \pm 2.80 kg, were divided into two genetic groups (Holstein [H] and Gir [G]; ten $\frac{1}{2}$ H + $\frac{1}{2}$ G and ten $\frac{3}{4}$ H + $\frac{1}{4}$ G). Cows were evaluated in a completely randomized design where each animal represented one replicate. The animals were identified by their morphological traits and numbered earrings.

Cows were milked twice daily using the bucket milking method, the first milking event occurred at 05h00, and the second at 16h00. During the experimental period, thermoregulatory, behavioral, and production traits were collected on 12 occasions, with 15-day intervals.

Ambient temperature and air relative humidity were recorded weekly by a thermo-hygrometer. These variables were measured on three occasions during a predefined day (08h00, 13h00, and 17h00).

Dry (DBT) and wet (WBT) bulb temperatures were recorded with a psychrometer to determine the temperature-humidity index (THI), using the following formula: THI = DBT + $0.36 \times WBT + 41.5$ (SANTOS et al., 2013).

Thermoregulatory parameters such as rectal temperature (RT, °C), respiratory frequency (RF, mov min⁻¹), and surface temperature (ST, °C) were measured at 08h00 and 17h00, after the respective morning and afternoon milking events. Rectal temperature was measured using a digital clinical thermometer, in a covered restraint chute, immediately after milking.

After RT was evaluated, the animal hair was collected and its surface temperature was measured with an infrared thermometer in the regions below the eyes, chest, neck side, ribs, flank, rump, and udder, to calculate an average. At the end of the RT and ST measurements, the animals were released and their RF was counted as the number of flank movements in one minute.

The number of hairs, hair length and thickness, as well as the color of the epidermis were evaluated in the medium thoracic region, at 20 cm below the spine. Next, a sample of hairs was harvested from the same region as that used to measure the hair thickness, using long-nose pliers adapted to determine their quantification (NH, n cm⁻²); length and thickness of the largest ten hairs (HL and HT, mm), using a caliper; and color of the epidermis, by a direct visual assessment followed by a comparison with a printed sheet, in accordance with the methodology proposed by Silva (2000).

One day after the thermoregulatory traits were measured, feeding behavior, rumination, rest, and other activities (social, body-care, playful, locomotion, and anomalous behavior) were evaluated (ethogram; Table 1). This evaluation

was undertaken over a period of 12 h, at 15-min intervals, following the methodology proposed by Santana Junior et al. (2014), starting with 08h00 and ending at 20h00.

Table 1. Ethogram of behaviors observed.

Behavioral category	Description	
Feeding	Animals observed while feeding.	
Rumination	Process in which the swallowed feed returns to the mouth for a new breaking of particles through movements promoted by chewing.	
Other activities	Performing any activity other than those described.	
Rest and sleep	Animal lying, resting, or sleeping.	
Social	Playing with, rubbing against, dominating, or making contact with others.	
Body care	Self-cleaning, cleaning others, urination, defecation, and rubbing.	
Playful	Playful behavior.	
Locomotion	Animal moving.	
Anomalous	Related to stereotypies; anomalous behavior self-directed or directed towards the environment or another animal, such as sodomy, lignophagia, and geophagia.	

Behavior was analyzed visually by six trained observers who were divided in pairs positioned strategically so as not to disturb the animals. Digital stopwatches were used to count the time expended on each activity.

Milk control was performed fortnightly to evaluate the production capacity of each genetic group. Additionally, milk was harvested for laboratory analysis to determine its chemical composition.

The milk from each animal was weighed on a digital scale in the morning and afternoon milking events, to determine daily milk yield per cow. After weighing, a 40-mL sample of milk was harvested per cow (60% in the morning and 40% in the afternoon milking) for an individual analysis per cow of fat, protein, and lactose contents and SCC.

The bottles where samples were stored contained one tablet of the antimicrobial agent (preservative) bronopol at the concentration of 8 mg of the active ingredient for every 40 mL of sample. After

collection, bottlers were identified and immediately homogenized to dissolve the tablet.

Immediately after collection, the samples were placed in cool boxes containing reusable ice in sufficient quantity to maintain the internal temperature of the box at a maximum of 7 °C until the samples arrived at the laboratory accredited by the Ministry of Agriculture, Livestock and Supply.

The experiment was set up as a completely randomized design with ten replicates. Environmental variables and thermoregulatory, behavioral, and production traits of the animals were used in an analysis of variance, with means compared by Tukey's test at the 5% probability level. Statistical analyses were run using R statistical software version 2.15.2.

Results and Discussion

Elevated temperatures were recorded during the experimental period, averaging 30.77 °C. The

average relative humidity (RH) was 46.95%, which is considered low and as not having a great influence on heat dissipation by the animals.

The temperature-humidity index was 79.15, on average. This value is higher than the 72 recommended for these animals, and thus this variable can negatively affect their milk production. Azevedo et al. (2005) conducted an experiment using ½ and ¾ HG cows and estimated upper critical THI values of 79 and 77 for the respective genetic compositions.

As defined by Armstrong (1994), THI can be used to assess thermal stress in dairy cattle, with values lower than 72 characterizing a normal environment without the presence of stress. Higher or equal values, in turn, indicate the environment is already classified as stressful, ranging between mild (72 to 78), moderate (79 to 88), and severe (89 to 98).

In addition to ambient temperature, the mean values of the following variables were determined in both genetic compositions: number of hairs, hair length, hair thickness, surface temperature, respiratory frequency, and rectal temperature (Table 2).

Table 2. Thermoregulatory traits of ½ HG and ¾ HG cows.

Th	Genetic co	mposition ¹			
Thermoregulatory trait	½ H + ½ G	3/4 H + 1/4 G	p ²	CV%3	SD^4
Number of hairs (cm ²)	376.43a	469.14a	0.1554	15.65	66.18
Hair length (cm)	0.98a	1.07a	0.2738	25.36	0.26
Hair thickness (mm)	0.13a	0.20a	0.6506	1.82	0.003
Surface temperature (°C)	33.11a	32.97a	0.7939	7.72	2.55
RF (mov min ⁻¹)	38.02b	43.33a	< 0.05	25.05	10.19
Rectal temperature (°C)	38.43a	38.56a	0.2477	1.40	0.54

¹ Means followed by different letters in the same row differ at the 5% probability level by Tukey's test. ² Probability value of the F test in the analysis of variance; ³Coefficient of variation; ⁴Standard deviation. RF = respiratory frequency. HG = Holstein/Gir.

The evaluation of number of hairs between the genetic compositions revealed values lower than the 557.96 (½ HG) and 657.03 (¾ HG) reported by Aiura et al. (2014). Mean hair-length values, differed between genetic compositions as also reported by the above-mentioned authors (1.005 cm for ½ HG and 0.86 cm for ¾ HG), who stressed that lower values are acceptable, since shorter and denser hair is favorable for thermolysis. The authors went on to say that longer hairs mean greater thermal insulation, which may eventually compromise the animal's heat dissipation mechanism.

According to Azevedo et al. (2005) and Façanha et al. (2013), hair-coat differences such as number, thickness, and length of hairs are influenced by the genetic composition and climate to which the

animal is subjected. The number of hairs protects the epidermis against solar radiation. Thicker and longer hairs are a trait of animal with high Holstein genetics, since Zebu have more-lodged, short, and thinner hairs, which facilitate loss of thermal energy to the environment.

Body surface temperature was statistically equal between the genetic compositions. The present results were similar to the 30.69 °C found in ½ HG cows by Ferreira et al. (2009). Cruz et al. (2016) analyzed animals of different genetic compositions and also did not detect significant differences in the ST of ½ HG and ¾ HG animals (30.13 and 30.64, respectively) in similar experiment conditions.

A normal surface temperature (ST) for cattle should remain between 31.6 to 34.7 °C. This variable

is prone to variations, since it is associated with the environment, climatic changes, and physiological changes such as vascularization and perspiration (FERREIRA et al., 2006).

Façanha et al. (2010) submit that the hair-coat color influences the body temperature due to the greater heat absorption. In the present study, all animals had a black epidermis. In the opinion of Silva (2000), a black epidermis is fundamental to the protection of animals against ultraviolet rays.

The evaluation of RF revealed a significant difference between the two genetic compositions. However, no animals were identified as being under stress conditions. This demonstrates that animals with European traits suffer more in a tropical climate than Zebu animals, which elevates their the respiratory frequency for the dissipation heat and for thermolysis to occur. This difference was also observed by Cruz et al. (2016), who obtained RF values of 33.62 mov min⁻¹ in ½ HG and 38.11 mov min⁻¹ in ¾ HG cows.

Cows with a RF of 20 to 60 mov min⁻¹ are considered not under thermal stress; from 60 to 80 mov min⁻¹, under mild stress; 80 to 120 mov min⁻¹, moderate stress; and over 120 mov min⁻¹, under heat stress (PINHEIRO et al., 2015).

There were no significant differences between the animals in the evaluation of rectal temperature. Cruz et al. (2016) also did not observe significant differences between the RT of ½ and ¾ HG animals, which averaged 38.55 and 38.77 °C, respectively. These values are considered normal and within the standard temperature range of 38.5 to 39.2 °C mentioned by Rocha et al. (2012). As stated by Silva et al. (2009), the normal rectal temperature of cows must be between 37.5 to 39 °C, and increases in °C reduce their production capacity.

Azevedo et al. (2005) analyzed the adaptability of crossbred animals by comparing ½, ¾, and ⅙ Holstein/Zebu (HZ) lactating cows for RT and RF values relative to THI. The authors observed that the ½, ¾, and ⅙ HZ animals reached a RT of 39.0 °C with THI of 80, 77, and 75, respectively. In establishing a THI of 85, RF was 86, 97, and 104 mov min⁻¹ for ½, ¾, and ⅙ HZ cows, respectively. This shows that ⅙ HZ animals, with greater participation of Holstein breed genes, are more sensitive to environmental variations.

There was no significant differences for feeding, rumination, rest, and other activities between ½ and ¾ HG cows (Table 3). The feeding behavior occurred mainly at the fresher times of the day. For the rumination and rest behaviors, the animals spent a good time lying in the shade, utilizing latent and sensible heat-dissipation mechanisms. As regards other activities, social, playful, anomalous, bodycare, and locomotion behavior was observed.

Table 3. Time (min) expended on feeding behavior, rumination, rest, and other activities performed by ½ HG and ¾ HG cows.

Behavior —	Genetic co	Genetic composition ¹		CV%3	SD^4
	¹⁄₂ H + ¹∕₂ G	3/4 H + 1/4 G	p^2	C V 7/0°	SD
Feeding	237.86a	221.20a	0.2117	24.19	55.52
Rumination	201.43a	224.28a	0.1068	21.32	45.39
Rest	430.00a	434.52a	0.8274	15.98	69.07
Other activities	52.14a	64.28a	0.2384	19.54	11.37

¹Means followed by different letters in the same row differ at the significance level of 5% by Tukey's test; ² Probability value of the F test in the analysis of variance; ³Coefficient of variation; ⁴Standard deviation. HG = Holstein/Gir.

The feeding-behavior values found for ½ and ¾ HG cows were 3.96 and 3.69 h. Nascimento et al. (2013) conducted a study evaluating the feeding behavior of Holstein × Zebu cows and found a feeding time of 1.68 h, due to the high temperature, and feeding peaks at the freshest times of the day: dawn and dusk.

Rumination periods for the ½ and ¾ HG categories were 3.36 and 3.74 h, which are close to the 3.70 h found by Zanine et al. (2008), but different from those reported by Nascimento et al. (2013), who observed longer rumination times (6.34 h).

The rest behavior of animals in this study lasted 7.17 and 7.24 h for the respective categories ½ and ¾ HG. Silva et al. (2005) found higher values for the 15-min interval: 11.5 h daily for ¾ HG crossbred animals. Zanine et al. (2008), working with Girolando, reported rest times of 4.16 and 7.26 h, with animals expending long periods lying in the shade, making use of latent and sensitive heat-dissipation mechanisms.

According to Silva (2016), animals may rest standing or lying; often near water sources or shades, in an effort to dissipate heat via convection and conduction.

Among the times spent on other activities for ½ HG animals, 5.48%, 41.10%, 0%, 1.37% and 52.05% were observed for social, body-care, playful, anomalous, and locomotion behaviors, respectively. Cows of the ¾ HG group, in turn, spent 11.11% of their time interacting socially, 40.00% performing body care, 0% playing, 1.11% performing anomalous acts, and 47.78% moving. The results showed no significant difference between the two genetic groups.

As stated by Broom and Fraser (2010), body care can be exemplified as self-cleaning, cleaning others, urination, defecation, and thermoregulation. It is a very broad and essential behavior for the life of animals, which explains its high percentage of occurrence.

Locomotion was the most frequent behavior among other activities, which may be explained by the rearing system to which the cows were subjected, as they were kept under rotational stocking. According to Silva (2016), the locomotion behavior is essential to the life of an animal, as it has a great influence on functional systems.

Playful behavior is understood as play activities among the animals, with characteristics such as jumps, runs, among other demonstrations of joy. This type of behavior does not have an apparent purpose; it is more often observed in young animals, although it may occur in adult animals at a lower frequency (BROOM; FRASER, 2010), which explains why this behavior was not observed in the present experiment.

The anomalous behavior seen in ½ and ¾ HG animals included lignophagia, which, as explained by Navarrete et al. (2015), is an anomalous oral behavior characterized by wood consumption, and it is related to stress or nutritional deficiencies. Silva (2016) declared that the main types of anomalous behavior observed in cattle are lignophagia, licking, geophagia, and sodomy. The low incidence of anomalous acts in this experiment may be explained by their free-range rearing, on pasture, which allows for lower stress conditions on animals.

As can be seen in Table 4, there was no significant statistical difference for milk yield, fat, lactose, or SCC; only the protein content differed significantly. This finding can be explained by the fact that because the genetic correlation between milk yield and solids content is negative, higher-yielding cows are expected to have milk with a lower solids content. Porcianato et al. (2009) stated that reductions in protein concentrations are related to decreasing casein contents, which in turn results from a lower nutrient bioavailability caused by reduced feed intake. As a consequence, the amount of amino acids that would be used in the protein synthesis in the mammary gland decreases.

Table 4. Yield and composition of milk from ½ HG and ¾ HG cows.

Production trait	Genetic composition ¹		2	CV/0/3	CD4
	½ H + ½ G	3/4 H + 1/4 G	– p²	CV%³	SD^4
Milk yield (kg day-1)	19.58a	19.73a	0.8799	19.786	3.88
Fat (%)	3.40a	3.52a	0.7368	25.43	0.88
Protein (%)	3.55a	3.35b	< 0.05	7.33	0.25
Lactose (%)	4.67a	4.71a	0.5899	3.62	0.17
SCC (× 1000 SC mL ⁻¹)	114.78a	107.36a	0.1529	27.98	31.08

¹Means followed by different letters in the same row differ at the significance level of 5% by Tukey's test; ² Probability value of the F test in the analysis of variance; ³Coefficient of variation; ⁴Standard deviation. HG = Holstein/Gir.

Milk yield is related to ambient temperature and relative humidity, decreasing as these two variables rise. During the current experiment, the average relative humidity was 46.95%; temperature was 30.77 °C; and milk yield was 19.58 (½ HG) and 19.73 kg day⁻¹ (¾ HG) (Table 4).

During the experimental period, the average THI was 79.15. Azevedo et al. (2005) stated that THI values higher than 72 can negatively affect milk yield, especially when the cattle originate from temperate regions, as they are more sensitive to elevated temperatures when compared with those from tropical regions. In production terms, however, cattle from temperate climates have a greater potential. For this reason, these higher-yielding animals have been crossed with tropical-climate animals, which are better adapted to the climatic conditions, producing crossbred animals with satisfactory yield and adaptability.

In a situation of thermal comfort, higher milk yields are observed as the Holstein genetic composition is increased, considering that these are higher-yielding animals (BERTONCELLI et al., 2013).

According to Ferro et al. (2012), on farms with inadequate management for rearing specialized pure breeds, crossbred animals (½ HG) are recommended, as they are more rustic and better adapted to the tropical climate and thus able to express high genetic potential as compared with ¾ HG animals.

Dairy cows with a higher Holstein genetic composition have higher milk yields and higher metabolic rates, which corresponds to greater internal heat production; therefore, they are more sensitive to heat stress (SILVA et al., 2012). This greater sensitivity leads to reduced dry matter intake and consequently lower milk production (PERISSINOTTO et al., 2007).

Conclusions

Animals with a greater Holstein genetic composition suffer a greater deal of thermal stress, displaying higher rectal temperatures and respiratory rates than animals with less Holstein genetic composition. There were no differences for the hair traits or feeding, rumination, rest, and other types of behavior between the animals of different genetic compositions. Protein content was the only component in milk yield/composition that differed, with higher values for ½ HG animal.

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