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MORPHO-PHYSIOLOGICAL RESPONSES OF *Bos indicus*, *Bos taurus* AND CROSSBRED WEANED HEIFERS TO SEASONAL VARIATIONS

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ABSTRACT - Beef cattle kept in extensive systems are constantly exposed to the effects of the climate. In tropical and intertropical regions, the reproductive management of *Bos taurus* animals with *B. indicus* animals is an alternative for zootechnical improvement. The objective was to evaluate morphophysiological characteristics of heat adaptation of beef heifers on pasture. For this, fifty-four animals from four distinct genetic groups: (1) Nellore, (2) Senepol, (3) Angus x Nellore and (4) ¼ Brahman x ¼ Nellore x ½ Senepol (Tri-cross), with six months-old, were evaluated during the summer and autumn seasons of 2016. The morphological characteristics of the coat (length, diameter, thickness, density and number of hairs and coat color) and physiological (skin, hair and rectal temperature; sweat rate; respiratory and heart rate) were evaluated. The environment was evaluated using the temperature and humidity index, black globe temperature, humidity index and radiation thermal load. We observed that the production environment presented thermal discomfort for the animals. Thus, the animals' rectal temperature differed between seasons. All groups studied showed some adaptive characteristic to the tropical environment. Angus x Nellore heifers had the highest values for the morphological measures in the months evaluated, coinciding with thermal comfort indexes above those considered comfortable for cattle and the lowest value of respiratory rate when compared between the crossing. In conclusion, all genetic groups have at least three adaptive characteristics to the tropical environment when analyzing the physiological responses.
Keywords: adaptability, animal welfare, crossbreeding, hair coat, respiratory rate.

RESPOSTAS MORFO-FISIOLÓGICAS DE Bos indicus, Bos taurus E NOVILHAS DESGASTADAS À VARIAÇÕES SAZONAIS

RESUMO - Bovinos de corte mantidos em sistemas extensivos estão constantemente expostos aos efeitos do clima. Em regiões tropicais e intertropicais, o manejo reprodutivo de animais Bos taurus com animais B. indicus é uma alternativa para o melhoramento zootécnico. Objetivou-se avaliar as características morfofisiológicas da adaptação ao calor de novilhas de corte a pasto. Para isso, cinquenta e quatro animais de quatro grupos genéticos distintos: (1) Nelore, (2) Senepol, (3) Angus x Nelore e (4) ¼ Brahman x ¼ Nelore x ½ Senepol (Tri-cross), com seis meses de idade, foram avaliados durante as estações de verão e outono de 2016. As características morfológicas da pelagem (comprimento, diâmetro, espessura, densidade e número de pelos e cor da pelagem) e fisiológicos (pele, cabelo e temperatura retal; taxa de sudação; frequência respiratória e cardíaca). O ambiente foi avaliado através do Índice de Temperatura e Umidade, Índice de Temperatura, Umidade de Globo Negro e Carga Térmica de Radiação. Observamos que o ambiente de produção apresentou desconforto térmico para os animais. Assim, a temperatura retal dos animais diferiu entre as estações. Todos os grupos estudados apresentaram algumas características adaptativas ao ambiente tropical. Novilhas Angus x Nelore apresentaram os maiores valores para as medidas morfológicas nos meses avaliados, coincidindo com índices de conforto térmico acima dos considerados confortáveis para bovinos e o menor valor de frequência respiratória quando comparados entre os cruzamentos. Em conclusão, todos os grupos genéticos apresentam pelo menos três características adaptativas ao ambiente tropical ao analisar as respostas fisiológicas e o período tem forte influência sobre as respostas avaliadas.
Palavras-chave: adaptabilidade, bem-estar animal, cruzamento, frequência respiratória, pelagem.

INTRODUCTION

Beef cattle kept in extensive production systems are constantly exposed to the effects of climatic environment, especially high radiant thermal load (SILVA and MAIA, 2013). This particular aspect favours the thermal discomfort and impairs animal welfare, in addition to decreasing productive and reproductive performances, mainly in animals with low adaptability to a tropical environment (SHIOTA et

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al., 2013; KARVATTE Jr. et al., 2016). Distinct breeds present different tolerance to the tropical environment, which is related to their efficiency in activating thermoregulatory mechanisms, these intrinsically correlated to morphological characteristics that provide adaptation (RENAUDEAU et al., 2012; MAIBAM et al., 2017). Skin and hair coat are anatomical structures that explain the differences in heat tolerance between species and among breeds (PRAYAGA, 2003), in which more pigmented skin and clear hair favour the animals.

Over the centuries, evolutionary processes lead *Bos indicus* animals to become more heat tolerant when compared to *B. taurus* animals because of their thermoregulatory ability (LEMOS et al., 2018). Because of utilization of crossbreeding among breeds that are adapted and poorly adapted to the tropical environment, there is a need for studies with those genetic groups, in which breeds with a great or small degree of adaptability present morphological characteristics of their forming breeds, and wide thermal comfort zone, ranging from 5ºC to 31ºC (SILANIKOVE, 2000). Such amplitude can become a complicating factor in extensive range production systems located in high-temperature regions, as in the Brazilian Midwest. Thus, the use of more heat-tolerant breeds in crossbreeding systems aimed at enhancing the quality of the final product, that fits the market requirements, becomes an efficient management tool and consistent with the Brazilian productive conditions.

Considering the aforementioned, the objective was to evaluate morphophysiological characteristics of heat adaptation of beef heifers kept on pasture.

MATERIALS AND METHODS

This study was performed in a commercial farm (São Carlos's Farm) located in Três Lagoas, Mato Grosso do Sul State, Brazil (20°45'04" S and 51°40'42" W). The weather is classed as *AW* (tropical hot and humid), with rainy season during the summer and dry winter (MARCUZZO et al., 2012).

The average annual temperature is 26ºC and annual rain precipitation ranging from 900 to 1.400 mm (ALVARES, 2014). The experimental period started in late summer and in autumn 2016. The research procedures were previously approved by the Committee of Ethics on the Use of Animals of the Federal University of Goiás (protocol number 072/15).

Fifty-four recently weaned heifers, from four distinct genetic groups (14 Nellore; 12 ½ Angus x ½ Nellore; 14 Senepol and 14 ¼ Brahman x ¼ Nellore x ½ Senepol), with initial average BW of 197, 235, 169, and 233 kg, respectively, and six months-old, were evaluated. All heifers were from the same farm and subjected to the same feed and sanitary managements. During the cow-calf phase, the animals were kept in *Brachiaria brizantha* cv. Xaraés pastures with free-choice access to water and provided with supplementation in a creep feeding through.

The microclimate was evaluated from 07:00 to 17:00 (GMT = -04:00), at 1-h intervals, at 1.5 m from the soil surface, in two points: one point under the sun and the other under the shade in the cow pens. Dry bulb temperature, wet bulb temperature and relative humidity, were measured using a digital thermo-hygrometer (model HT-500; Instrutherm Instrumentos de Medição Ltda, São Paulo, Brazil), inserted in a perforated PVC shield (TRUMBO et al., 2012). Black globe temperature was measured by using an adapted thermometer inserted in plastic spheres. Wind speed was measured by using a portable digital anemometer (model HMM 489; Homis Controle e Instrumentação Ltda, São Paulo, Brazil), in 3-min. intervals, obtaining minimum and maximum speed. To characterize the thermal environment, the Temperature and Humidity Index (THI) was calculated according Thom (1958), Black Globe Temperature and Humidity Index (BGHI), according Buffington et al. (1981) and radiant thermal load (RTL) according Esmay (1978), for each season and evaluation hour.

On the evaluation day, heifers were conducted to the cow pens, where they were kept in a waiting corral with no cover until the moment of data collection. Evaluations of physiological parameters were conducted in the cow pens, realized randomly according to the entrance of the animals in the squeezing chute that was covered with a roof. Time of entrance in the squeezing chute was recorded to be utilised in the statistical analysis. Rectal temperature was measured utilising a digital clinic thermometer. Superficial skin and superficial hair coat temperatures were measured on the back of the animal (20 cm below the spine), using a portable infrared pyrometer (model TI-890, Instrutherm Instrumentos de Medição Ltda., São Paulo, Brazil).

Heart (HR) rate was determined by auscultation of the heart beats, using a veterinary clinical stethoscope positioned on the left side of the animals. Respiratory (RR) rate was obtained by direct observing the movements of the flank during 1 min. Sweating rate was determined on the back of the animal (corresponding to the point of higher solar radiation) and was based on the time needed for dehydrated filter paper discs (0.5 cm in diameter), impregnated with 10% cobalt chloride, to change colour from violet to bright rose (SCHLEGER and TURNER, 1965). The time taken for the discs to change colour was measured with a digital chronometer and realized by the same technician.

Skin and hair colours were determined on the animal's back by visual observation comparing with a graduated colour chart (SILVA and MAIA, 2013) in which 0% corresponds to white and 100% to black or red. Hair thickness was measured on the same moment when heifers were restrained in the squeezing chute, using an electronic digital pachymeter (model EC799A-6/150, Starrett Indústria e Comécio Ltda., Itu, SP, Brazil), according to Bertipaglia (2007). Afterwards, a 1-cm² hair sample was collected from the back of each animal (20 cm below the spine) and placed in envelopes previously weighed and identified (SILVA and MAIA, 2013). Number of hairs was estimated through visual counting of all hair of the sample. Hair length was determined considering the average of the 10 longest hairs collected, measured with an electronic digital pachymeter; then, the average hair diameter was determined by measuring in an optic microscope with a graduated lamina. The average density was determined by counting the number of hairs in a 1 cm^2 area (SILVA and MAIA, 2013).

The experimental design was the completely randomized with four treatments (genetic group) and replicates corresponding to the number of animals of each genetic group. Physiological and morphological variables were analysed by adjusting to a mixed model with time-repeated (summer and autumn) measures considered as the fixed effects. The physiological variables contained as fixed genetic group of the animal, sampling date, BGHI measured under the shade at the sampling hour (linear covariable) and sampling hour (linear covariable). Animal within genetic group and standard error were considered as random effects. The mathematical model was represented by Equation 1:

$$PVijk = \mu + GGj + SDj + BGHIsj + SHj + ANj + eijk$$ (Equation 1)

Which:

PV_{ijk} = physiological variables of the ith animal in the jth repetition (time), at the kth time,

μ = inherent constant of all observations,

GG_i = genetic group of the ith animal,

SD_j = sampling date, in the jth replicate,

$BGHI_{sj}$ = black globe temperature and humidity index in the shade at jth sampling hour,

SH_j = sampling hour of jth replicate,

AN_k = animal within kth genetic group and

e_{ijk} = random error associated with each observation.

For the morphological variables, the fixed effects were genetic group of the animal and sampling date, and the random effects were animal within genetic group, and error, according to the mathematical model, represented by Equation 2:

$$MVijk = \mu + GGi + SDj + ANk + eijk$$ (Equation 2)

Which:

MV = morphological variables of the ith animal in the jth repetition (time),

μ = inherent constant of all observations,

GG_i = genetic group of ith animals,

SD_j = jth sampling date,

AN_k = animal within kth genetic group and

e_{ijk} = random error associated with each observation.

It was considered a composite symmetric variance structure, in which the correlation between the measurements of the same animal is considered equal, and the variance is constant at the different times, for both variables. Analyses were performed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA; version 9.4). Least square means were compared by t test, at 5% of significance (SAS Inst., Inc., Cary, NC, USA; version 9.4).

RESULTS AND DISCUSSION

Increased air temperature values, solar radiation and humidity, promote an uncomfortable thermal environment, demanding compensation systems so that animals keep within their thermal equilibrium (MOORE et al., 2012). Although efficient, these mechanisms require a high-energy cost, based on heat exchanges of sensible heat (radiation, convection and conduction) and, if not sufficient, latent heat exchange (skin evaporation), resulting in losses in production (MADER et al., 2010; ALFONZO et al., 2016). In the summer, the maximum values of THI, BGHI and RTL, were obtained under the sun, at 10:00. Under the shade, values of these variables were lesser than the values obtained under the sun (Table 1).

During the autumn, the greatest values of THI, BGHI and RTL, were obtained at 12:00 under the sun (Table 2). Karvatte Jr. et al (2016) considered environments as comfortable that present THI ≤70 and BGHI ≤74, for the studied beef breeds. According to these authors, these values provide an environment with favourable thermal conditions to maximize the genetic potential expression of each species. However, many regions where intensive cattle production is present extrapolate such indices. Souza et al. (2010), for example, reported values above those considered comfortable (THI = 81) in other region of the same state where the present study was performed, even in autumn. In the present study, the same conditions happened, and the study site was considered as having an alert and caution situation for production animals.

All genetic groups studied showed have some adaptive characteristic that favors their exploration in the tropical environment. The use of *Bos indicus* animals in all crosses and breeds may explain this fact. For the physiological variables, sweat rate, skin, and hair surface temperature of heifers belonging to four groups only the respiratory rate showed a difference between then. In thermally stressing environments, cattle utilize physiological mechanisms for heat loss to avoid hyperthermia.

Rectal temperature (physiological range from 38.5 to 39.7°C) and respiratory rate (physiological range from 24 to 36 movements per minute), are considered the best parameters used to estimate heat tolerance by animals (ALFONZO et al., 2016; VIEIRA, 2016). This second, is fundamental means of characterizing thermal discomfort in cattle. Its increase is the first visible sing that the animal is under thermal stress. Heart rate can also be used as indicative of thermal stress in cattle. Reference values of 60 to 80 bpm are considered normal for cattle (FEITOSA et al., 2008). Although different groups were used, heterosis between races and mainly with the Nellore race may have provided the others an adaptation to the environment. In both seasons, the values obtained were within the normal range, although differences were observed between seasons.

TABLE 1 - Thermal comfort indices under the shade and sun during the end of the summer season.

Hour	Shade			Sun		
	THI	BGHI	RTL	THI	BGHI	RTL
07:00	71	71	314.6	71	71	330.6
08:00	71	71	314.6	71	71	330.6
09:00	74	75	318.1	76	79	370.7
10:00	77	79	320.6	80	87	490.5
11:00	77	79	316.4	78	82	347.1
12:00	75	77	315.4	75	76	248.5
13:00	76	78	320.2	76	80	385.8
14:00	77	79	319.1	79	84	377.1
15:00	76	78	319.4	75	79	346.3
16:00	74	75	315.6	73	75	327.5
17:00	74	75	315.6	73	75	327.5

THI = temperature humidity index (dimensionless), BGHI = black-globe temperature and humidity index (dimensionless), RTL = radiant thermal load (W m^{-2}).

TABLE 2 - Thermal comfort indices under the shade and sun during the end of the autumn season.

Hour	Shade			Sun		
	THI	BGHI	RTL	THI	BGHI	RTL
07:00	71	76	315.7	71	75	315.7
08:00	73	78	415.1	75	80	338.4
09:00	78	83	399.2	78	84	359.2
10:00	81	87	315.7	81	87	315.7
11:00	78	86	372.5	80	88	366.1
12:00	78	87	426.2	81	89	394.6
13:00	79	87	379.6	81	89	371.2
14:00	79	87	374.1	79	87	362.1
15:00	78	87	368.6	79	86	312.8
16:00	75	81	315.7	75	81	315.7
17:00	72	76	315.7	71	75	315.7

THI = temperature humidity index (dimensionless), BGHI = black-globe temperature and humidity index (dimensionless), RTL = radiant thermal load (W m^{-2}).

Similarly, Cardoso et al. (2015), evaluating tropical-adapted breeds' females (Nellore, Indo-Brazilian, Gyr-Holstein, Red Sindhi, and Gyr) in the Brazilian savanna, did not observe changes on rectal temperature, and heart rates. This fact could indicate that the evolution heritage regarding heat tolerance is present in these genetic groups, similarly to the present study. An effect (*P*-0.001) was found between seasons for rectal temperature, respiratory and heart rate and sweating rate, with the greatest averages obtained in the summer (Table 3).

TABLE 3 - Average of physiological variables, sweating rate, and surface skin and hair temperatures of heifers under thermal stress in the summer and autumn.

Item	Summer	Autumn	*P*-value
Rectal temperature (°C)	40.1a*	39.6b	0.001
Respiratory rate (movements per minute)	43.0a	33.0b	0.001
Heart rate (beats per minute)	72.0a	49.0b	0.001
Sweating rate (g m^{-2} per hour)	301.0a	121.0b	0.001
Superficial skin temperature (°C)	34.2	35.0	0.156
Superficial hair temperature (°C)	34.3	35.0	0.282

*Means within a row with different lowercase letters differ ($P<0.05$).

However, in the present study, despite the high thermal discomfort indexes, no changes in rectal temperature were observed among animals of the different genetic groups, that is, other thermoregulatory mechanisms were efficient to maintain homeothermy (Table 4). The observed averages were 39.9°C for rectal temperature, 38 movements per minute for respiratory rate; 61 beats per minute (bpm) for heart rate, 211 g m^{-2} per hour for sweating rate, 34.6°C for superficial temperature of the skin, and 34.6°C for superficial temperature of the hair coat. Similarly, no effects of season and genetic group (Tables 3 and 4).

Hair coat temperature is a measure related with environment temperature. According to Martello et al. (2004), values between 31.6 to 34.7°C are considered normal. When that temperature is below 35°C, as observed in all genetic groups, the gradient between rectal and body surface temperature is effective in heat exchange with the environment, not requiring other physiological mechanisms such as evaporation, which requires increased energy expenditure to maintain homeothermy (SILVA and MAIA, 2013).

TABLE 4 - Average of physiological variables, sweating rate, and surface skin and hair temperatures of heifers pertaining to four distinct genetic groups under thermal stress.

Item	Genetic group				
	Angus x Nellore	Nellore	Senepol	Tri-cross	*P*-value
Rectal temperature (°C)	39.9	39.7	39.9	39.9	0.630
Respiratory rate (movements per minute)	33.0c*	37.0bc	42.0a	39.0ab	0.008
Heart rate (beats per minute)	50.0	65.0	67.0	60.0	0.064
Sweating rate (g m^{-2} per hour)	182.0	209.7	235.1	217.1	0.405
Superficial skin temperature (°C)	35.2	34.3	34.8	34.0	0.215
Superficial hair temperature (°C)	34.7	34.5	34.4	34.6	0.962

*Means within a row with different lowercase letters differ ($P<0.05$).

An effect (*P*-0.001) of genetic group was observed for all morphologic hair coat characteristics (Table 5). As a comparison, Navarin et al. (2009) reported similar results from the present study. These authors evaluated the thermal comfort of grazing Nellore cattle under different shading conditions and reported 35.2°C for superficial skin and peltry temperatures, even for no shading condition, corroborating that hair coat acts as a physical barrier to the heat (SILVA and MAIA, 2013).

TABLE 5 - Average of morphological characteristics of heifers pertaining to four distinct genetic groups under thermal stress.

Item	Genetic group				
	Angus x Nellore	Nellore	Senepol	Tri-cross	*P*-value
Average hair length (cm)	19.831a*	10.902b	12.628b	10.325b	0.001
Average hair diameter (µm)	0.067b	0.086a	0.079a	0.087a	0.001
Number of hairs (hair cm^{-2})	1674a	852b	1052b	967b	0.001
Average density (g cm^{-3})	0.009	0.003	0.007	0.008	0.237
Hair coat thickness (mm)	4.100a	2.400b	2.700bc	2.300bd	0.001
Skin colour (%)	90a	88b	70c	66c	0.001
Hair coat colour (%)	91a	38b	51cd	52d	0.001

*Means within a row with different lowercase letters differ ($P<0.05$).

The Angus x Nellore heifers presented the greatest averages for length, number, average density and thickness of hairs, and the colour of the skin and hair. In cattle, in addition to the skin, hair coat is also considered as an important factor to dissipate heat and, thus, in its tolerance since hair coat is considered the greatest tissue in contact with the external environment (SILVA and MAIA, 2013). The differences observed for all skin and hair coat-related traits (i.e., hair number, length, diameter, density, hair coat thickness and colour, and skin colour) between genetic groups, indicate the variability in the genotypes used and their differences acquired through natural selection to the environment of origin. Between seasons, during the summer an effect was observed for hair length. However, no effects were observed for the other morphological hair coat variables during the experimental period (Table 6).

The variation in hair length observed in both evaluation periods was also reported elsewhere with other breeds. Silva and Maia (2013), comparing different breeds, found hair coat thickness ranging from 2.7 to 3.7 mm, and hair length between 12.9 to 14.3 mm. The hair coat thickness results were similar to those obtained in the present study, even evaluating recently weaned heifers. For hair length, when the genetic group has increased *B. indicus* and tropical-adapted *B. taurus* influences, the results were similar. The difference observed between seasons for hair length, could be because of the process of shedding according to season due to the influence of the photoperiod, climatic conditions and seasonality (BERMAN, 2011). This process usually occurs in late spring, as a way of adjusting the skin to high temperatures (ALFONZO et al., 2016), which is related with heat transfer to the environment. Shorter hairs, such as those found in Tri-cross animals, aid heat dissipation by conduction and convection mechanisms, and are highly desirable for animals raised under tropical conditions (MAIA et al., 2009). In fact, number of hairs (hair coat density) is a variable commonly associated with physiological responses to thermal stress in cattle (ALFONZO et al., 2016).

TABLE 6 - Morphological characteristics of heifers under thermal stress in the summer and autumn.

Item	Summer	Autumn	*P*-value
Average hair length (cm)	11.800a*	15.100b	0.001
Average hair diameter (µm)	0.073a	0.086b	0.004
Number of hairs (hair/cm^2)	1168	1105	0.378
Average density (g/cm^3)	0.012a	0.002b	0.002
Hair coat thickness (mm)	2.930	2.850	0.577
Skin colour (%)	81	77	0.123
Hair coat colour (%)	55	61	0.186

*Means within a row with different lowercase letters differ (P<0.05).

Skin and hair coat colours were different between genetic groups because of the different phenotypes (white/cream for Nellore, black for Angus, cherry red for Senepol, and colour varied for Tri-cross, according to breeds used in the crossbreeding). The most advantageous type of hair coat for cattle in tropical regions is the one that presents white coloration, with well-settled hairs, on highly pigmented epidermis, to favour less absorption of amount of thermal energy (MAIA, 2009).

In animals with depigmented skin, the black hair, although presenting higher radiant balance, is preferable since melanin confers protection against ultraviolet rays. Turner and Schleger (1965), evaluating crossbred cattle in the summer and winter seasons, observed that the sample period influenced hair colour, in which darker hairs were found in the summer. However, because in the Mid-west region the thermal amplitude is not high, hair colour was not modified. The use of pure animals and not their crossings can reveal peculiarities of them regarding adaptation to heat.

CONCLUSION

All genetic groups have at least three adaptive characteristics to the tropical environment when analysing the physiological responses.

For morphological characteristics, the genetic groups that classify the best adaptive traits are those with the higher *Bos indicus* proportion and animals that were previously selected for tropical environments (i.e., Senepol).

Season interferes in physiological parameters, thermal gradients and hair coat characteristics of cattle.

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