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Adaptive integumentary features of beef cattle raised on afforested or non-shaded tropical pastures

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We aimed to analyze the seasonal acclimatization process of Nelore and Canchim cattle raised on two production systems (non-shaded, NS, and integrated crop-livestock-forest, ICLF), based on the dynamics of the morphological and functional attributes of the hair coat and skin during winter and summer. The study was conducted in Brazil, in a low-altitude tropical climate region. A completely randomized 2 × 2 factorial design was adopted as follows: two production systems (NS and ICLF), two breeds (Nelore and Canchim) in a longitudinal structure, with measurements repeated over time through two stations (winter and summer). The experimental animals consisted of 32 Nelore (*Bos indicus*) and 32 Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*) bulls. The animals were equally distributed between two intensive rotational grazing systems. In both breeds, the hair coat was significantly thicker in winter but longer in summer, which increased epidermal protection. The Nelore bulls had shorter, wider, and thicker hairs, which are attributes that promote heat loss via conduction. The Canchim bulls showed significantly lower hair density and higher epithelium distance to sweat glands, which resulted in higher core temperature and respiratory rate. In turn, Nelore bulls had higher serum concentrations of triiodothyronine and lower serum concentrations of cortisol. However, Canchim bulls more frequently and intensely activated their thermoregulatory system and markedly adjusted their hair coat and hair features to reduce heat gain, especially in summer. Therefore, the anatomical plasticity and functional integumentary characteristics of Nelore and Canchim bulls reflect their acclimatization to tropical conditions.

Most pastures grazed on by ruminants are located in tropical and subtropical regions, accounting for more than 50% of the meat and 60% of milk produced worldwide[1]. The geographic distribution of cattle herds follows land-use dynamics. More than half of the world's total cattle population, estimated to be approximately 940 million animals, is located in tropical environments[2]. However, tropical climates are characterized by high solar radiation levels and high heat loads, which may limit optimal animal productivity[3], especially when raising cattle on pastures. This scenario requires adopting strategies capable of improving the efficiency of production systems through positive interventions to their biotic or abiotic components.

Regarding environmental management, integrated crop-livestock-forest (ICLF) systems are the most environmentally friendly and have proved to be an interesting alternative to monoculture production systems from an ecophysiological standpoint. ICLF systems are associated with environmental benefits, including low greenhouse gas emissions, increases in carbon storage compared to monoculture systems, and amelioration of microclimate conditions[4]. The ICLF system also benefits soils, pastures, and animals[5], which can take advantage of the natural shading provided by the forest for greater thermal comfort[6,7]. However, different production practices may yield

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different results for a given component of the system, such as the genotype of the animals. Cattle bred under temperate conditions may be more sensitive and, therefore, have a lower heat stress threshold[8,9]. Large-scale studies show that the commonly used thresholds to define heat stress risk classes should be differentiated for different cattle types, considering their productivity level, housing type, and heat protection strategies[10].

Zebuine cattle (*Bos taurus indicus*) have developed specific attributes and functions that favor heat exchange to adapt to hot environments, such as a low metabolic rate and large body surface area[11,12]. In contrast, taurine cattle (*Bos taurus taurus*) have a more compact body, higher metabolic and growth rates, and better carcass quality[13]. Nevertheless, the thermoregulatory capacity of taurine breeds decreases with increasing air temperature[14]. Zebu cattle's superior ability to regulate body temperature during heat stress also results from their morphology. Differences in the tegument between breeds may contribute to higher thermotolerance as Zebu cattle are characterized by shorter and thinner hair than taurines[15,16]. Thus, exploiting heterosis by crossbreeding taurine and zebuine cattle is one strategy for generating more productive progeny and simultaneously making them more resilient to heat stress[17]. The Canchim (CAN) is one of the best-known crossbreeds developed and raised in Brazil, resulting from a cross between the Charolais breed and Zebu animals[18], including the Nellore breed (NEL). The development of Canchim aimed to combine the adaptable characteristics of the Zebu with the higher reproductive performance and meat quality of the Charolais[19].

Heat stress is one of the main factors involved in reducing animal performance and productivity[20,21]. Under heat stress conditions, animals attempt to dissipate heat by activating integumentary, cardiorespiratory, and endocrine mechanisms, which are essential for adaptability[22]. Therefore, some morphological features of cattle are crucial for thermal adaptation, directly affecting the heat exchange mechanisms, such as convection, radiation, and evaporation, between the animal and the surrounding environment[23]. Evaporative cooling by sweating is the primary mechanism of heat dissipation in hot climates[24]. Skin plays an important role in heat loss[25], and the physical characteristics of the hair coat, such as thickness and density, in addition to hair length and diameter, affect thermoregulation efficiency[26]. Furthermore, the intrinsic characteristics of the skin, including epidermal thickness and the number and morphology of sweat glands and hair follicles, play critical roles in thermal insulation[25].

In this context, more accurately identifying these characteristics to improve heat tolerance may help increase phenotyping and the selection of animals with more efficient thermoregulatory responses when raised under adverse climatic conditions[27,28]. Consequently, actions that prioritize the identification of heat-tolerant animals may have a direct positive impact by enhancing animal welfare in areas subjected to different frequencies of heat loads. However, there is a lack of scientific knowledge on relevant integumentary features for the adaptation of different cattle breeds, whether Zebu or composite, raised in a tropical climate. The hypothesis is that the Nelore and Canchim pasture-raised animals present similar integumentary characteristics related to thermoregulation, whose functional attributes are dynamic and sensitive to climatic variations. Therefore, this study aimed to investigate the seasonal acclimatization of Nelore (*Bos indicus*) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*) cattle raised on pasture, based on the dynamics of the morphological and functional attributes of the hair coat and skin in summer and winter.

Results

All anatomical regions analyzed, except for the wither region, showed a significant breed*season interaction effect on hair coat thickness ($p < 0.01$). Canchim bulls had thicker hair coats than Nelore bulls in winter and summer ($p < 0.0001$). The hair coat was thicker in both breeds in winter ($p < 0.0001$; Table 1).

The breed*season interaction significantly affected hair length in the shoulder, wither, and rump regions ($p < 0.05$). The hair was always significantly longer in Canchim than in Nelore bulls ($p < 0.0001$), regardless of the season (Table 2). The longest hair length in both breeds was recorded in summer ($p < 0.0001$).

No significant two-way interactions were observed when the largest hair and medulla diameters were analyzed. These characteristics were significantly higher in Nelore than in Canchim bulls ($p < 0.0001$; Table 3). The breed*season interaction affected the smallest hair and medulla diameters. In Canchim bulls, the smallest hair and medulla diameters were significantly higher in summer than in winter ($p < 0.0001$). However, this effect was not observed in Nelore bulls.

The breed*season interaction significantly affected the cortex thickness ($p = 0.008$) and area ($p = 0.02$). Cortex thickness and area differed between breeds in winter ($p < 0.05$) and were more significant in Nelore than in Canchim bulls. The medulla area significantly differed between breeds in winter and summer ($p < 0.05$) and was also higher in Nelore bulls (Table 4). The cortex perimeter was significantly greater in Nelore than in Canchim bulls ($p < 0.0001$). In addition, this characteristic was significantly greater in summer than in winter ($p < 0.0001$). Breed ($p < 0.0001$) and season ($p < 0.0001$) significantly affected the medulla perimeter.

No two-way interaction effect was observed in the histomorphometric analysis. Epithelium thickness did not differ significantly ($p > 0.05$) between breeds, regardless of the anatomical region (Fig. 1). The epithelium distance to sweat glands differed between breeds, reaching significantly higher values in Canchim than in Nelore bulls ($p < 0.0001$). Nelore bulls had significantly higher mean values of the area of sweat glands ($p < 0.05$), total area of sweat glands ($p < 0.006$), and proportion of the glandular area ($p < 0.01$), especially in the wither region. In line with the breed effect on hair follicle density, Nelore bulls had higher values than Canchim bulls (NEL: 23.8 ± 0.75 vs. CAN: 17.0 ± 0.78 hairs/mm^2; $p < 0.05$). Histomorphometric differences resulting from the individual effects of the production systems ($p < 0.05$) and season ($p < 0.05$) are shown in Supplementary Fig. S1.

No significant two-way interactions were found in the analyses of rectal temperature, respiratory frequency, and serum concentrations of hormones. Rectal temperature significantly differed between breeds (Fig. 2), reaching higher values in Canchim than in Nelore bulls (CAN: 39.47 ± 0.05 vs. NEL: 39.28 ± 0.05 °C; $p < 0.01$). Significant differences were also observed between production systems, with higher values in animals maintained

Season	Nelore	Canchim	Overall average
	Shoulder (mm)		
Winter	1.70±0.06[Ba]	2.11±0.06[Aa]	1.91±0.04
Summer	1.06±0.04[Bb]	1.19±0.04[Ab]	1.13±0.03
Overall average	1.38±0.04	1.65±0.04	
	Wither (mm)		
Winter	1.81±0.07	2.07±0.07	1.94±0.05[a]
Summer	1.06±0.0	1.11±0.04	1.08±0.03[b]
Overall average	1.43±0.04[B]	1.59±0.04[A]	
	Back (mm)		
Winter	1.79±0.06[Ba]	2.19±0.06[Aa]	1.99±0.05
Summer	1.14±0.04[Ab]	1.20±0.04[Ab]	1.17±0.03
Overall average	1.46±0.04	1.69±0.04	
	Rump (mm)		
Winter	1.74±0.06[Ba]	2.09±0.06[Aa]	1.91±0.04
Summer	1.07±0.04[Ab]	1.14±0.04[Ab]	1.11±0.03
Overall average	1.41±0.03	1.61±0.03	

Table 1. Mean (± standard error) hair coat thickness measured in different anatomical regions of Nelore (*Bos indicus*) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*) bulls raised on tropical pastures[Ω]. [A,B]Different uppercase letters indicate significant differences between columns ($p < 0.05$). [a,b]Different lowercase letters indicate significant differences between rows ($p < 0.05$). [Ω]n = 480 evaluations per breed per anatomic region.

Season	Nelore	Canchim	Overall average
	Shoulder (mm)		
Winter	6.51±0.51[Bb]	11.79±0.51[Ab]	9.15±0.36
Summer	9.38±0.51[Ba]	16.82±0.51[Aa]	13.10±0.36
Overall average	7.94±0.36	14.31±0.36	
	Wither (mm)		
Winter	6.77±0.41[Bb]	11.90±0.41[Ab]	9.34±0.29
Summer	9.10±0.53[Ba]	16.48±0.53[Aa]	12.79±0.37
Overall average	7.94±0.38	14.19±0.38	
	Back (mm)		
Winter	8.78±0.39	12.76±0.39	10.77±0.27[b]
Summer	10.73±0.46	16.00±0.46	13.36±0.33[a]
Overall average	9.76±0.33[B]	14.38±0.33[A]	
	Rump (mm)		
Winter	8.40±0.36[Bb]	12.04±0.36[Ab]	10.22±0.26
Summer	9.53±0.36[Ba]	14.52±0.36[Aa]	12.02±0.26
Overall average	8.96±0.26	13.28±0.26	

Table 2. Mean (± standard error) hair length measured in different anatomical regions of Nelore (*Bos indicus*) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*) bulls raised on tropical pastures[Ω]. [A,B]Different uppercase letters indicate significant differences between columns ($p < 0.05$). [a,b]Different lowercase letters indicate significant differences between rows ($p < 0.05$). [Ω]n = 4800 hair units evaluated per breed per anatomic region.

in the non-shaded (NS) system than in animals in the ICLF system (NS: 39.45 ± 0.05 vs. ICLF: 39.29 ± 0.05 °C; $p < 0.05$). B breed also affected respiratory frequency, reaching higher values in Canchim than in Nelore bulls (CAN: 35.97 ± 0.83 vs. NEL: 26.03 ± 0.82 breaths/min; $p < 0.0001$). Finally, respiratory frequency varied with the season, showing lower values in winter than in summer (winter: 24.55 ± 0.83 vs. summer: 37.45 ± 0.82 breaths/min; $p < 0.0001$).

Nelore bulls had higher serum T3 (NEL: 1.21 ± 0.02 vs. CAN: 1.10 ± 0.02 ng/mL; $p < 0.01$) and lower cortisol (NEL: 5.3 ± 1.11 vs. CAN: 8.5 ± 1.07 ng/mL; $p < 0.05$) concentrations than Canchim bulls. The serum concentrations of T3 were higher in summer than in winter, regardless of breed (summer: 1.27 ± 0.02 vs. winter: 1.04 ± 0.02 ng/mL; $p < 0.0001$), whereas the serum concentration of cortisol was higher in winter than in summer (winter: 9.36 ± 1.03 vs. summer: 4.62 ± 1.15 ng/mL; $p < 0.01$).

Season	Nelore	Canchim	Overall average
	Largest hair diameter (µm)		
Winter	114.38 ± 1.90	90.79 ± 1.90	102.59 ± 1.35[b]
Summer	124.76 ± 3.20	110.23 ± 3.25	117.50 ± 2.28[a]
Overall average	119.57 ± 1.89[A]	100.51 ± 1.91[B]	
	Smallest hair diameter (µm)		
Winter	65.37 ± 1.45[Aa]	55.61 ± 1.45[Bb]	60.49 ± 1.03
Summer	66.46 ± 1.96[Aa]	66.69 ± 1.99[Aa]	66.58 ± 1.40
Overall average	65.92 ± 1.45	61.15 ± 1.46	
	Largest medulla diameter (µm)		
Winter	68.13 ± 2.04	48.61 ± 2.04	58.37 ± 1.44[b]
Summer	80.59 ± 3.21	63.25 ± 3.26	71.92 ± 2.28[a]
Overall average	74.36 ± 2.09[A]	55.93 ± 2.11[B]	
	Smallest medulla diameter (µm)		
Winter	27.96 ± 0.93[Aa]	22.35 ± 0.93[Bb]	25.15 ± 0.66
Summer	28.23 ± 1.28[Aa]	28.06 ± 1.29[Aa]	28.14 ± 0.91
Overall average	28.09 ± 0.96	25.20 ± 0.97	

Table 3. Mean (± standard error) values of the largest and smallest hair and medulla diameters of Nelore (*Bos indicus*) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*) bulls raised on tropical pastures[Ω]. [A,B]Different uppercase letters indicate significant differences between columns ($p < 0.05$). [a,b]Different lowercase letters indicate significant differences between rows ($p < 0.05$). [Ω]n = 320 images evaluated per breed.

Season	Nelore	Canchim	Overall average
	Cortex thickness (µm)		
Winter	21.04 ± 0.66[Aa]	19.01 ± 0.66[Bb]	20.03 ± 0.47
Summer	20.91 ± 0.51[Aa]	21.43 ± 0.52[Aa]	21.17 ± 0.36
Overall average	20.98 ± 0.49	20.22 ± 0.49	
	Cortex perimeter (µm)		
Winter	297.88 ± 5.37	233.21 ± 5.37	265.55 ± 3.80[b]
Summer	319.08 ± 9.84	274.10 ± 9.99	296.59 ± 7.01[a]
Overall average	308.48 ± 6.31[A]	253.65 ± 6.37[B]	
	Cortex area (× 10³ µm²)		
Winter	4.4 ± 0.16[Aa]	3.1 ± 0.16[Bb]	3.8 ± 0.11
Summer	4.5 ± 0.21[Aa]	4.5 ± 0.21[Aa]	4.7 ± 0.15
Overall average	4.6 ± 0.13	3.8 ± 0.13	
	Medulla perimeter (µm)		
Winter	165.35 ± 4.79	119.02 ± 4.79	142.19 ± 3.39[b]
Summer	190.15 ± 7.55	149.00 ± 7.67	169.58 ± 5.38[a]
Overall average	177.75 ± 5.07[A]	134.01 ± 511[B]	
	Medulla area (× 10³ µm²)		
Winter	1.58 ± 0.07	0.94 ± 0.07	1.26 ± 0.05[b]
Summer	1.93 ± 0.13	1.52 ± 0.13	1.73 ± 0.10[a]
Overall average	1.75 ± 0.09[A]	1.23 ± 0.09[B]	

Table 4. Mean (± standard error) ultrastructural parameters of the hair of Nelore (*Bos indicus*) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*) bulls raised on tropical pastures[Ω]. [A,B]Different uppercase letters indicate significant differences between columns ($p < 0.05$). [a,b]Different lowercase letters indicate significant differences between rows ($p < 0.05$). [Ω]n = 320 images evaluated per breed.

Discussion

Cattle kept in an environment that promotes thermal comfort are better at expressing their genetic potential, thus improving their productive performance[29]. The climatic variable records demonstrate that the average air temperature was lower in the ICLF system, mainly in summer, hence showing the favorable effect of trees in warm seasons, as previously reported[7,21]. In the ICLF system, the BGHI values remained below those observed in the NS system, particularly during the hottest season. The physical barrier provided by the tree canopy helped reduce the radiant heat load, which was lower in the ICLF than in the NS system both in winter (474 vs. 450 W/
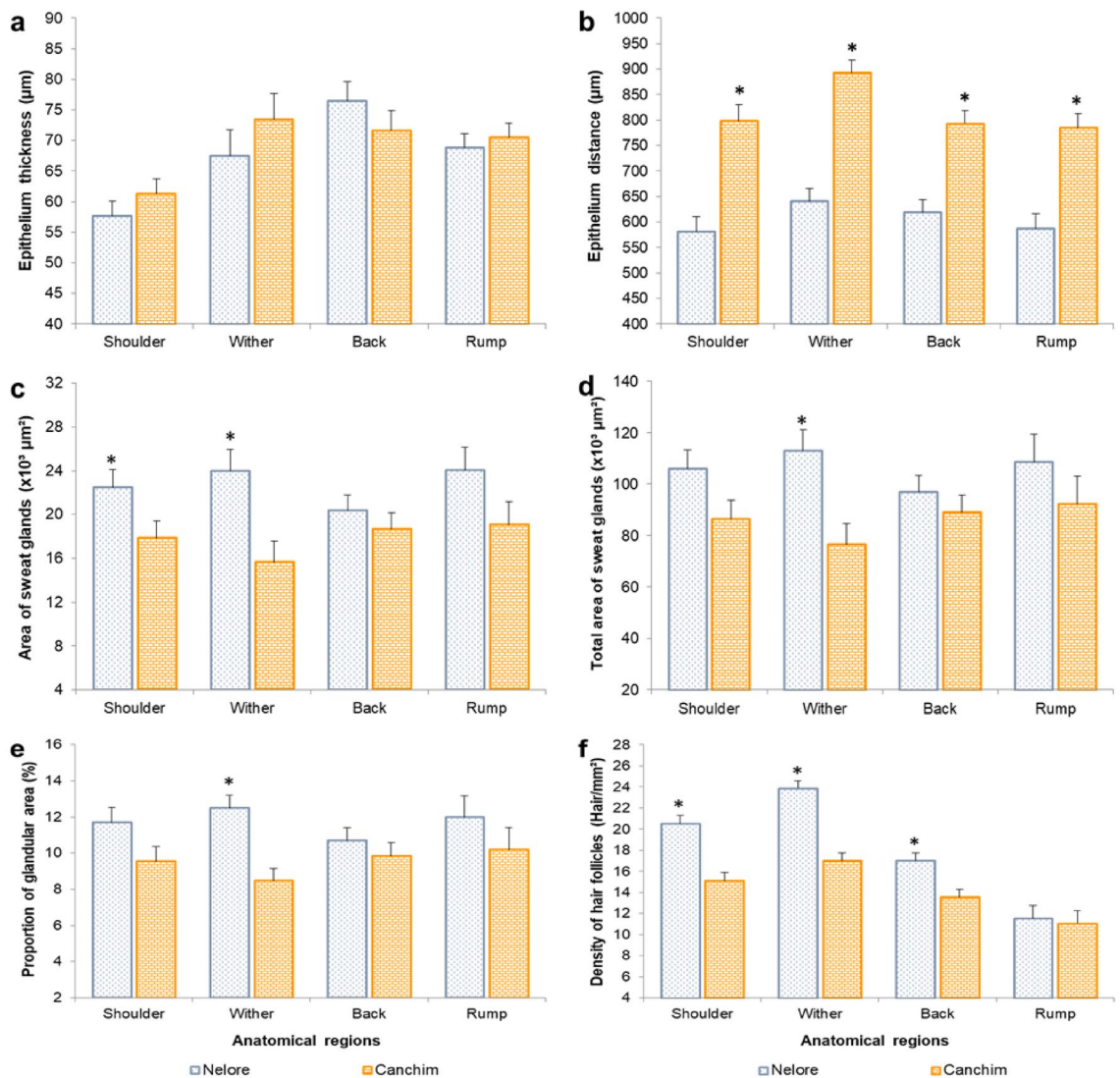
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Figure 1. Histomorphometric analysis of anatomical regions of the (**a**) epithelium thickness[α], (**b**) epithelium distance to sweat glands[α], (**c**) average area of sweat glands[β], (**d**) total area of sweat glands[β], (**e**) proportion of glandular area[β], and (**f**) hair follicle density of Nelore (*Bos indicus*, n = 10) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*, n = 10) bulls. Mean ± standard error by breed (The asterisks indicate $p < 0.05$). [α]n = 1200 measurements per anatomical region for each breed, [β]n = 2400 measurements per anatomical region for each breed.

m²) and summer (537 vs. 484 W/m²). Throughout the seasons, the incidence of solar radiation strongly affected the air and black globe temperatures, which mainly increased in the environment without shade.

The hair coat is a characteristic related to adaptation that affects functional responses to the environment[30]. In the present study, Canchim bulls showed a thicker hair coat than Nelore bulls (Table 1). This is likely because of genetic inheritance, as the taurine subspecies has a thicker hair coat than that of the zebuine cattle[31]. Hair coat thickness can be affected by the angle of hair inclination relative to the epidermis. This is an essential trait for protection against thermal radiation and efficient heat loss because an increased angle of hair inclination reduces heat storage between hair fibers and increases heat dissipation[32]. When necessary, animals involuntarily reduce the angle of hair disposition to the epidermis, which increases the insulative capacity of the hair coat. Moreover, animals develop a thicker hair coat in winter, expressed by the vertical distance between the skin and the limit of the outer surface of the hair coat, as a self-protective characteristic at lower temperatures[33]. In contrast, the hair coat thins in the summer to increase heat loss efficiency. Angus (3.04 vs. 1.86 mm) and Simental (1.86 vs. 0.59 mm) breeds[33] showed a similar variation in hair coat thickness between cold and warm seasons. Therefore, the differences in hair coat thickness may be due to the adaptation of bulls to tropical climates[32,34].

Hair length is also regarded as a relevant feature in the dynamics of body heat dissipation[35] via both convection-conduction and evaporation. On average, Canchim bulls had hairs that were 5.4 mm longer than those of Nelore bulls (14.1 and 8.7 mm, respectively; Table 2), corroborating a previous report[36]. In cattle, the estimated heritability is 0.67 for short hair and 0.42 for long hair[15]. The Canchim is a composite breed with a higher Charolais proportion and a lower Zebu proportion[37]. Therefore, this genotypic property should have affected hair length because the higher the Zebu percentage in the racial composition is, the shorter the hair shafts will be[15].
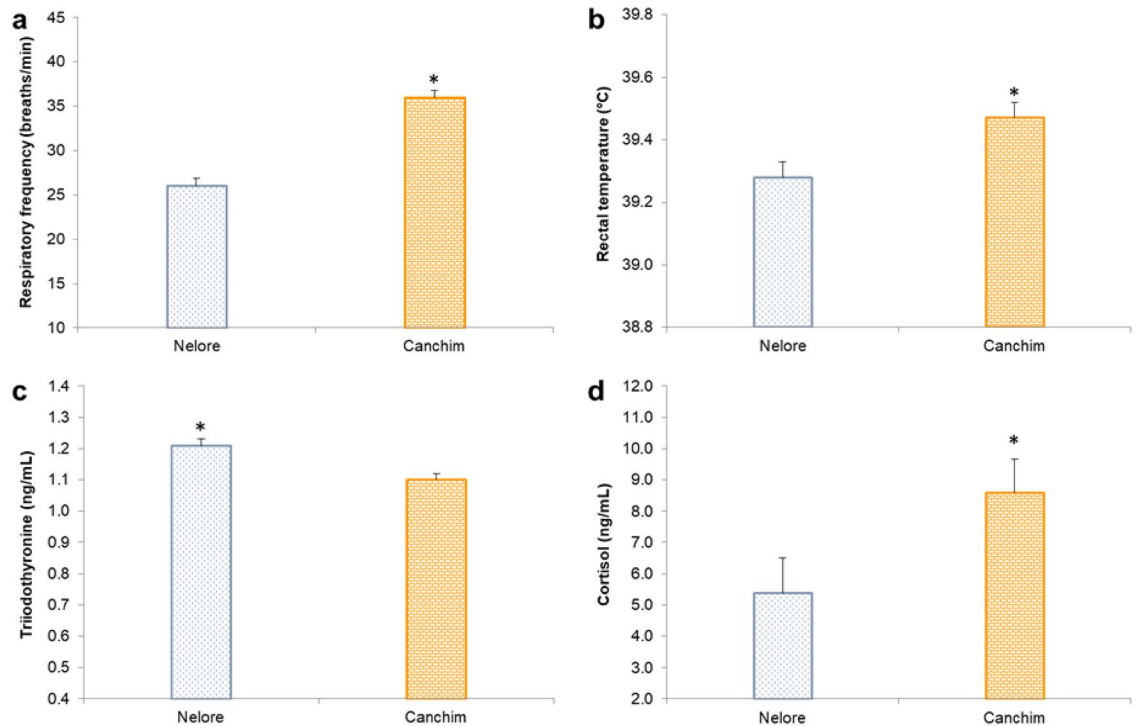
Figure 2. Mean (± standard error) of the physiological variables$^{\Omega}$: (**a**) respiratory frequency, (**b**) rectal temperature, and serum concentrations of (**c**) triiodothyronine and (**d**) cortisol of Nelore (*Bos indicus*) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*) bulls (n = 64). (The asterisks indicate $p < 0.05$. $^{\Omega}$n = 160 measurements per breed.

A related attribute that has recently attracted interest in genetic selection programs is slick hair. The slick-hair phenotype is derived from a mutation in the prolactin receptor gene[31] and determines the expression of a hair coat with very short, smooth, and shiny hairs[38], which increases heat tolerance[39]. A comparative study in Criollo Limonero cattle identified significant differences in hair length between animals with and without the slick-hair gene[40]. Also, the first slick mutation described (SLICK1) does not change the abundance of sweat glands in the skin but increases the functional properties of sweat glands[41]. Given the high heritability of the slick-hair gene and the expected genetic variability between individuals within the same breed, identifying this gene in Nelore and Canchim cattle may be advantageous. It should be addressed in future studies to gain further insight into its association with heat tolerance.

The average increase of hair length by 2.95 mm between winter and summer was unexpected because cattle undergo two physiological hair coat changes yearly during the transitional seasons. In fall, summer hairs in the telogen phase fall out and are replaced by anagen hairs that become long and thin. Winter hairs fall out in spring and are replaced by short, thick hairs[32]. Presumably, hair length increases in summer to form a sufficiently dense weave of α-keratin, a protein present in the cuticles of bovine hair[42], protecting the epidermis.

Hairs reduce the incidence of direct solar radiation on animal skin. Therefore, in light-coat animals, hairs further increase the surface reflectance, which was measured at 0.56 and 0.60 for Nelore and Canchim bulls, respectively, in the wavelength range from 300 to 850 nm[43]. In contrast, thicker coats reduce the transmittance and the absorbed solar radiation[43]. Consequently, the centripetal flow of thermal energy from the environment to the body core decreases, thereby protecting the animal from an increased radiant heat load during summer in a tropical climate.

Nelore bulls had greater hair and medulla diameters than Canchim bulls (Table 3), corroborating a previous study that reported hair and medulla diameters of 107.1 and 78.7 μm for zebuine bulls (Nelore breed) and of 101.4 and 61.4 μm for taurine bulls (Pantaneiro breed), respectively[44]. Thus, with great morphometric accuracy, our results demonstrate that Nelore bulls have thicker hair than Canchim bulls in different structural layers of the hair. Other studies, however, have reported a hair diameter smaller than those observed in the present study, for both Nelore (53.5 μm) and Canchim (47.0 μm) bulls, albeit without presenting methodological details of those measurements[36].

Considering that shorter and thicker hairs promote heat loss via conduction[45], the larger hair diameter observed in Nelore bulls may be a key trait that provides Zebu cattle with greater adaptation to tropical climates. Furthermore, given its physical characteristics, bovine hair conducts thermal energy more easily and quickly than air. Thus, the greater the hair diameter and density, the greater the total thermal energy released by the hair coat will be[46], facilitating the centrifugal flow of heat from the body to the external environment. Nelore bulls had larger cortex and medulla perimeters, a thicker cortex, and larger cortex and medulla areas (Table 4), directly affecting convection-conduction heat fluxes. These results may also indicate traits of adaptation to the

tropical environment because, despite being linear or two-dimensional measurements, the perimeter and area are physical characteristics directly proportional to the three-dimensional structure of the hair.

The histomorphometric analysis of the skin showed a non-significant difference in epithelium thickness between Nelore and Canchim bulls (Fig. 1a). Nonetheless, the production system significantly affected this characteristic. The greater epithelium thickness of the NS bulls (Fig. S1a) may be attributed to more significant cell proliferation in the outer integumentary layer of animals exposed to ultraviolet radiation. In non-afforested pasture systems, animals experience a higher radiant heat load and air temperature than in silvopastoral systems[21]. The thicker epidermis helps block radiation and its deleterious effects, such as the formation of pyrimidine dimers. These dimers are responsible for structural distortions in the DNA double helix, interfering with cellular mechanisms, threatening the functional integrity of cells, and consequently contributing to mutagenic and tumorigenic processes[47]. A thicker epidermis also increases the protection of the animal against overheating by reducing the amount of thermal energy transferred to the body core[48].

Sweat glands produce and secrete sweat, and its evaporation from the skin surface leads to heat loss[49]. In the Nelore bulls, the sweat glands were closer to the skin's surface than in the Canchim bulls (Fig. 1b), likely because they are fuller[50]. Therefore, the increased production of sweat has a direct and positive effect on the rates of sweating, evaporation, and dissipation of endogenous heat. Accordingly, in both breeds, the epithelium distance to the sweat glands was shorter in summer, indicating more intense sweating on the hottest days of the year.

Bos indicus animals have more numerous and larger sweat glands and hence a greater capacity to produce a larger volume of sweat[25,51]. As a result, they can maintain thermal equilibrium by evaporation more easily than *Bos taurus* animals and their crosses[25]. However, few studies have objectively parameterized these traits. In the present study, Nelore bulls had sweat glands with a larger area and, presumably, a higher volume, showing a greater glandular area and a higher proportion of the glandular area per sample than in the skin of Canchim bulls (Fig. 1c–e). These results indicate that sweat glands occupy a more significant area of the skin tissue in Zebu cattle than in composite breeds.

Although sweat production and sweating rate were not directly measured in the present study, the high tissue availability of sweat glands and high glandular activity can be inferred as prerequisites for an increased capacity to dissipate heat through sweating and as likely traits of adaptation to tropical climates[30]. Accordingly, Canchim animals may be less effective in the functional response of their sweat glands[50], requiring the activation of their thermoregulatory system more often than Nelore animals when facing a thermal challenge of the same intensity.

Our results (Fig. 1f) corroborate the findings of previous studies, indicating that *Bos indicus* have a higher hair density than *Bos taurus* or crossbred animals[25]. The hair follicle density values of Nelore (11.5 to 23.8 hairs/mm^2) and Canchim (11.0 to 17.0 hairs/mm^2) cattle were in line with those reported by Silva et al.[43] for the same breeds (18.0 and 12.0 hairs/mm^2, respectively). The increased hair density and diameter of Nelore bulls decrease photon transmission from the radiation source and shortwave radiation penetration into the interior of the hair[52]. Conversely, the amount of radiation effectively transmitted from the hair coat to the external environment largely depends on the physical structure of the hairs and, mainly, on the number of hairs per unit area[53]. Therefore, animals with denser hair coats and wider hairs benefit from greater heat flow from the skin surface to the external environment.

The ability of an animal to withstand heat-challenging conditions has been physiologically assessed by measuring the rectal temperature and respiratory frequency[54]. Although approximately 70–80% of the maximum heat loss in cattle occurs through sweating, endogenous heat dissipation also depends on thermal polypnea, with thermal exchanges inherent to breathing[55]. The respiratory frequency measured in this study differed between breeds (NEL: 26.03 ± 0.82 vs. CAN: 35.97 ± 0.83 breaths/min; $p < 0.0001$) (Fig. 2a), with Canchim bulls showing values slightly above the normal range for adult cattle, from 24 to 36 breaths/min[56], and with a magnitude similar to that previously observed in this breed (33.2 ± 1.1 to 40.8 ± 1.2 breaths/min)[57]. Nevertheless, in both breeds, the bulls did not reach the panting stage, which is characterized by values above 60 breaths/min[58] and indicates an increased need for instant heat loss[33]. Panting is favored by increased ventilation of the respiratory dead space and water evaporation from the mucous membranes of the respiratory tract. Despite being within the acceptable range, the respiratory frequency increased in Canchim bulls to favor heat dissipation and reduce their core temperature[59]. As expected, the animals of both breeds had a higher number of respiratory cycles in summer because of the higher air temperature and relative humidity. During hot seasons, cattle resort to physiologically adaptive mechanisms, including increased respiratory frequency, to avoid hyperthermia[60].

The physiological internal temperature for beef cattle ranges from 36.7 to 39.1 °C[61]. The difference in rectal temperature between breeds demonstrated that Canchim bulls were less effective than Nelore bulls at maintaining stable homeothermy (Fig. 2b). Composite animals may accumulate more heat than zebuines in pastures, as has also been observed during thermal challenges in climatic chambers[62]. Several factors may have contributed to the lower heat dissipation during the most thermally challenging times of the day. Among them, the longer coat and its relationship with the morphological and functional characteristics of the sweat glands may be responsible for the lower efficiency of evaporative losses[33], accumulating thermal energy, as shown by the higher rectal temperatures recorded in Canchim than in Nelore bulls. Generally, in the ICLF system, animals benefit from higher environmental thermal comfort with lower radiant heat loads and BGHI values. Therefore, the bulls in the ICLF system had an average rectal temperature of 0.16 °C lower than that of the bulls in the NS system, confirming that the afforested system provides a better thermal environment[7]. This result corroborates the findings of a study on the homeothermy of Canchim heifers, which showed that the animals had lower core temperatures in a silvopastoral system than in non-forested pastures (39.2 ± 0.07 vs. 39.4 ± 0.07 °C)[21].

Triiodothyronine is a hormone essential for maintaining the body's metabolic rate, heart and digestive functions, muscle control, brain development, and bone activity[63,64]. The Nelore bulls had higher T3 concentrations (Fig. 2c), which is unexpected because Zebu animals have a lower metabolic rate than taurine animals[65]. Additionally, greater T3 release induces greater endogenous heat production as a byproduct of increased metabolism.

Even though the animals experienced slight distress due to thermal loads, none presented classic signs of heat stress, such as panting and sialorrhea. Therefore, the effect of T3 release as a body growth factor was not detrimental, even if the activation of metabolism increases endogenous heat production, particularly in young animals during the growth phase.

The lower serum cortisol concentration observed in Nelore than in Canchim bulls indicates that they are less stressed due to being better adapted to hotter climates[66]. This adaptive capacity is related to their body size and metabolic differences, as well as to the set of phenotypic characteristics described in this study, such as shorter, thicker hair with constituent layers that are structurally more conducive to heat transfer, in addition to larger and more active sweat glands. These traits are considered essential for high heat tolerance[15]. In cattle, the physiological serum concentration of cortisol ranges from 5 to 10 ng/mL[67]. Thus, the animals of both breeds were within the normal range (Fig. 2d). However, in Canchim bulls, the cortisol concentration was closer to the upper limit of the reference values, which indicates that they are more sensitive to the thermal environment[68] than are Nelore bulls, which are one of the cattle breeds considered to be well adapted to heat[23].

The seasonal variation in the studied hormones was the opposite of that shown in some studies, with higher T3 and lower cortisol levels during summer than winter, regardless of the production system and breed. This result indicates that the thermal challenge was not severe enough to trigger thyroid endocrine responses and that the physiological stress level in the animals (chronic signs) was low. Low cortisol concentrations help decrease metabolic heat production and facilitate acclimatization in animals[69]. Because cortisol is a thermogenic hormone, its decrease in summer enables animals to maintain high levels of T3 secretion to preserve anabolic processes and provide metabolic energy for muscle growth and development. Similarly, serum cortisol concentrations above physiological limits impair nutrient availability, stimulate tissue catabolism, and mobilize nutrients for body maintenance that could otherwise be used for growth[70].

Conclusion

Nelore bulls show integumentary traits that promote adaptation to a hot climate, such as short hairs, with large hair and medulla diameters. In addition, their sweat glands are closer to the epidermis, which makes it easier to maintain homeothermy during summer in pasture production systems. Canchim bulls are also quite tolerant to heat, but they are more sensitive to environmental variables and activate their thermoregulatory system more frequently and intensely than the Nelore bulls do, especially during the hottest season. Additionally, Canchim bulls can adjust their hair coat and hair characteristics to increase protection against direct radiation and reduce heat gain. In general, the morphological traits of the Nelore and Canchim breeds related to integumentary hair and sweat glands, as well as their anatomical plasticity and functional characteristics, reflect their acclimatization to the tropical environment. Efforts to increase knowledge of the traits that cattle use to cope with heat stress are essential to improve their productive efficiency in environments with tropical or subtropical climates and in regions that have recently been exposed to heat loads. Therefore, the functionality of integumentary traits can be considered when selecting animals that are more adaptable to hot climates to promote the occurrence of more thermotolerant phenotypes in beef cattle herds.

Materials and methods
Study area and climatic characterization

The study was conducted at Embrapa Pecuária Sudeste, in São Carlos, Brazil (21°57′42″ S, 47°50′28″ W, 860 m), from March 2018 to January 2019 (totaling 297 days), in winter (June–August) and summer (December–January). The region has a low-altitude tropical climate type (Cwa) according to the Köppen-Geiger classification[71]. The summer is characterized by high monthly average air temperatures (22.7 °C), relative humidity (81.6%), and rainfall (183.8 mm). In contrast, winter is the season with the lowest monthly average air temperatures (19.7 °C), relative humidity (63.3%), and rainfall (46.6 mm)[72]. During the experimental period, the air temperature varied between 18.9 and 26.3 °C and the relative humidity between 52.8 and 84.3% during the day shift. During the night shift, the air temperature varied between 17.6 and 21.2 °C, and the relative humidity was between 68.9 and 84.1%.

Production systems

Two production systems were used, each with 12 ha of pasture [*Urochloa brizantha* (*syn. Brachiaria brizantha*) (Hochst. ex A. Rich.) Stapf cv. BRS Piatã], in intensive rotational grazing. The two systems were: (a) non-shaded (NS) system composed of pastures with minimal natural shading due to the presence of dispersed native trees, which determined a useful shading area of 1.02%, and (b) integrated crop-livestock-forest (ICLF) system, with eucalyptus (*Eucalyptus urograndis*) trees planted in single, east–west-oriented rows 15 m apart, with 4 m between trees (165 trees/ha), determining a useful shading area of 30.06%. During the study period, the trees had an average height of 29.7 m, and the photosynthetically active radiation (PAR) was reduced by 55%. Using SQ-301 linear quantum sensors (Apogee, Logan, UT, USA), PAR was continuously measured at a height of 70 cm above ground level in the NS system and at four positions in the ICLF system, thereby assessing PAR reduction based on the PAR ICLF/PAR NS ratio[73].

Microclimate characterization

The microclimate of the production systems was characterized using two automatic weather stations, each consisting of a humidity and temperature probe (HC2-S3; Rotronic, Bassersdorf, Switzerland; accuracy: ± 0.8% RH, ± 0.1 °C AT), ultrasonic anemometer (WindSonic; Gill Instruments, Lymington, United Kingdom; accuracy: ± 2%), and copper-constantan thermocouple (Salcas, São Paulo, SP, Brazil; accuracy: ± 0.4%) inserted in a black globe. The sensors were installed 1.5 m above the ground and connected to a data logger (CR3000; Campbell Scientific, Logan, UT, USA). Data were recorded every 15 s, with outputs scheduled every 15 min, resulting in 38,011

records per device. The records between 8:00 a.m. and 4:00 p.m. were used according to the method adopted by Magalhães et al.[74], covering the period of the day with the highest air temperature and solar radiation[75]. The study variables were air temperature (°C), black globe temperature (°C), relative humidity (%), and wind speed (m/s). The black globe temperature and humidity index (BGHI)[76] was calculated according to the equation BGHI = BGT + 0.36 (DPT) + 41.5, in which: BGT is black globe temperature (°C) and DPT is dew point temperature (°C). The radiant heat load (RHL; W/m^2)[77] was calculated using the equation RHL = σ × (T$_m^2$), in which σ is the Stefan–Boltzman constant, 5.67×10^{-8} K^{-4} (W/m^2), and T$_m$ is the mean radiant temperature (W/m^2). The microclimatic conditions of both production systems during the study year are shown in Supplementary Fig. S2.

Animals and management

The experimental procedures were approved by the Animal Research Ethics Committee of Embrapa Pecuária Sudeste (Declaration CEUA-CPPSE 07/2017). The study sample consisted of 64 animals, Nelore (*Bos indicus,* n = 32) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus,* n = 32) bulls raised on the same plot in an extensive grazing system. For the purposes of this paper, animals had not previously been genotyped for the slick gene, so it was assumed that they did not inherit a SLICK1 allele (i.e., were wild-type). At the beginning of the experiment, the animals were 31.4 ± 1.6 months old and weighed 482.6 ± 47.7 kg. At the end of the experiment, the animals weighed 634.2 ± 51.5 kg, indicating an average weight gain of 0.758 kg/day. The average weights recorded during winter and summer are shown in Supplementary Fig. S3. The same animals were used in each production system during the experimental period. Thus, 16 Nelore and 16 Canchim animals were used in both NS (n = 32 bulls) and ICLF (n = 32 bulls). A rotational grazing strategy was used to manage the pastures. The animals had ad libitum access to water from automatic drinkers and mineral mix in troughs. The animals were introduced to the production systems 30 days before the start of the experimental period to allow them to adapt to the management practices.

Experimental design

A completely randomized 2 × 2 factorial design was adopted as follows: two production systems (NS and ICLF), two breeds (Nelore and Canchim), in a longitudinal structure, with measurements repeated over time through two stations (winter and summer). The experimental animals were equally distributed in the respective production systems such that each system had the same number of animals per breed, with a homogeneous average age and live weight. In winter and summer, biological samples were collected monthly to evaluate the response variables related to the hair matrix (hair coat thickness, hair length, hair and medulla diameter, cortex thickness, and cortex and medulla perimeter and area), blood (serum concentrations of triiodothyronine and cortisol), and instantaneous thermoregulation (respiratory frequency and rectal temperature). Animals were assessed on typical days of each climatic season[78], which allowed the selection of days within each experimental season that were representative of seasonal historical data[79].

On the assessment days, the animals were gently herded into a corral between 11:00 a.m. and 2:30 p.m., so that the evaluations could be performed during the warmest period of the day. The corral was close to the grazing areas, so the animals only had to walk 300 m to be assessed. Animals were individually evaluated in batches of eight animals, which were sequentially led to the corral in an alternating order that took into account the production system (e.g., NS-ICLF-NS-ICLF or ICLF-NS-ICLF-NS). Once in the corral, the animals remained in an uncovered pen for 10 min before being evaluated, in order to avoid the influence of the facilities on the variables analyzed[7]. This measurement criterion was adopted to (i) lead the animals directly from the production system to the site of evaluation and collection of biological samples and (ii) avoid sample bias determined by a fixed order of entry of the lots.

In winter (August) and summer (January), skin microbiopsies were taken from 20 randomly selected animals to evaluate the morphological features of the integumentary system histologically. Only a subset of the animals was used in this analysis in compliance with the bioethical principles of Reduction and Refinement, which support the adoption of adequate experimental strategies for statistical evaluation that help to gather comparable levels of data using fewer animals and applying techniques that do not cause or minimize pain and distress[78]. To avoid sampling bias, the animals subjected to skin sampling were blindly chosen from both groups according to their previously registered individual thermoregulatory responses. Ten animals were randomly selected from each production system and five from each breed for a balanced statistical evaluation. The experimental design is illustrated in Fig. 3.

Hair coat thickness and hair length

In winter and summer, the phenotypic characteristics of the hair and hair coat of all animals were evaluated monthly. Each animal was restrained in a chute to perform three consecutive coat thickness (mm) measurements using a digital metallic caliper with a millimetric scale (model 502.150; King Tools, Ontario, CA, USA). Coat thickness was measured by positioning the caliper perpendicular to the surface of the animal until it touched the skin, with the cursor positioned at the limit of the outer surface of the hair coat to measure the vertical distance in millimeters[26]. Four anatomical regions were assessed: shoulder, withers, back, and rump (Supplementary Fig. S4), for 960 measurements per anatomical region during the study period.

Subsequently, hair samples were collected from each animal to measure hair length (mm) in the same anatomical regions (shoulder, wither, back, and rump) used to measure hair coat thickness. All hairs within an area of 0.2 cm^2 were pulled out at once with adapted pliers[32,80]. Hair strands were epilated entirely in the direction of their growth to avoid mechanical artifacts[81]. The samples were individually placed in polypropylene envelopes labeled per animal, production system, breed, and anatomical region. In the laboratory, the hair units were separated and horizontally aligned on a smooth black ceramic surface under white lighting (10 W) for perfect
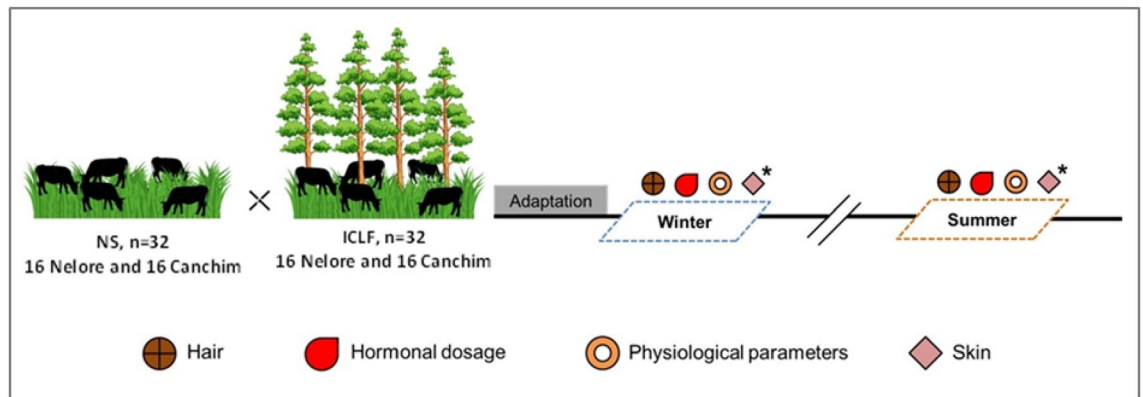
Figure 3. Schematic representation of the experimental design, indicating the production systems, number of animals, evaluation seasons and variables analyzed. The asterisks indicate that samples were collected from a subset of the experimental animals. *NS* non-shaded system, *ICLF* integrated crop-livestock-forest system.

individualization. For each sample, the 30 largest hair units from each anatomical region were measured, as recommended by Udo[82], using a digital caliper (model 502.150; King Tools, Ontario, CA, USA). The length of each hair unit was measured from the base of the bulb to the top of the hair shaft for 38,400 hair units, i.e., 4800 hair units from each anatomical region per breed.

Hair ultrastructural analysis by scanning electron microscopy

The ultrastructure of hair samples from the back region of all bulls was analyzed using high-definition images generated by scanning electron microscopy. The samples were cleaned with pure acetone and absolute ethanol for 5 min to remove residue and grease[83]. Hair samples collected from each animal in winter and summer were mounted on aluminum stubs and cross-sectioned at their midpoints with a metal microblade for morphometric analysis of the internal structures. The samples were analyzed under a scanning electron microscope (Quanta 250; FEI Company, Eindhoven, the Netherlands), operated in low vacuum (40–50 Pa), at 2000× magnification. In total, 640 digital images were acquired and subsequently evaluated using ImageJ software (US National Institutes of Health, Bethesda, MD, USA). The largest and smallest hair and medulla diameters (μm) were determined by image analysis. The hair cortex was also assessed for thickness (cortex thickness, μm), perimeter (cortex perimeter, μm), and area (cortex area, μm^2), whereas the medullary region was assessed for perimeter (medulla perimeter, μm) and area (medulla area, μm^2) (Supplementary Fig. S5). Before analysis, the software was calibrated by adjusting the settings for the micrometric evaluation of each image[84].

Tissue preparation and skin histomorphometry

Skin tissue samples were collected via microbiopsy from the shoulder, wither, back, and rump areas, which are the same anatomical regions used for hair sampling. The microbiopsies were performed under local anesthesia (2% lidocaine hydrochloride without a vasoconstrictor) using an 8 mm punch. Two samples were collected per anatomical region to prepare serial histological sections, perpendicular (cross sections) and parallel (horizontal sections) to the skin surface. Samples were fixed in 10% neutral buffered formalin (pH 7.4) for 24 h[59]. After fixation, the samples were processed using routine histological techniques, embedded in paraffin blocks, and sectioned using a microtome (CM3050sIV; Leica Biosystems Nussloch GmbH, Nussloch, Germany). The histological sections (three 3–5 μm thick sections per sample) were fixed on a glass slide, stained using Masson's trichrome method[85], and observed under a bright field optical microscope (Zeiss Axioscope A.1; Carl Zeiss Microscopy GmbH, Jena, Germany) coupled to a high-resolution digital camera (Zeiss Axiocam 105 color, 5-megapixel CMOS sensor; Carl Zeiss Microscopy GmbH, Jena, Germany) for digital image acquisition.

The microphotographs were uploaded to a cloud image bank, and histomorphometric analysis was subsequently performed using ImageJ software version 1.52 (US National Institutes of Health, Bethesda, MD, USA). The epithelium thickness (μm) and distance to sweat glands (μm), the glandular area of the secretory coil of sweat glands (μm^2), and the proportion of glandular area (%) were measured in cross-section images. The number and density of hair follicles (hairs/mm^2) were determined from the horizontal-section images[84]. The analytical parameters adopted for each variable are outlined in the Supplementary Table S1. Supplementary Fig. S6 shows the approaches used to analyze the histomorphometric variables.

Physiological variables

Physiological variables related to thermoregulatory efficiency were assessed once every 30 days in winter and summer. During the warmest period of the day (11:00 a.m. to 2:30 p.m.), the bulls were restrained in a chute and sheltered from direct sunlight and rain. First, respiratory frequency (breaths/min) was assessed, followed by rectal temperature (°C) measurement. Respiratory frequency was recorded by counting the respiratory cycles per minute based on flank movements. Rectal temperature was assessed using a digital clinical thermometer (Termomed; Incoterm, Porto Alegre, RS, Brazil) with a measurement range from 32.0 to 42.0 °C, which is accurate to 0.1 °C with ± 0.2 °C maximum error[86].

Hormone concentrations

After assessing the physiological variables, blood samples were collected by jugular venipuncture into 10 mL siliconized vacuum tubes without anticoagulants to determine triiodothyronine (T3) and cortisol serum concentrations via radioimmunoassay. The samples were centrifuged at 3600 rpm for 15 min to separate the serum, aliquoted, and stored in polypropylene microtubes at − 20 °C. Triiodothyronine was quantified using T3 Antibody-Coated Tubes, T3 Tracer [125I], and T3 Standards Set (MP Biomedicals, LLC Diagnostics Division, Solon, OH, USA); the sensitivity and intra- and inter-assay coefficients of variability were 0.067 ng/mL, 9%, and 10%, respectively. Cortisol was quantified using the ImmuChem Cortisol Coated Tube RIA kit (MP Biomedicals, LLC Diagnostics Division, Solon, OH, USA). The sensitivity and intra-assay coefficient of variability were 170 ng/mL and 11%, respectively.

Statistical analysis

Data from the assessment of hair coat and hair parameters and from the histomorphometric analysis of the skin were obtained in a 2×2 factorial design with two production systems (NS and ICLF) and two breeds (Nelore and Canchim). The design was longitudinal, with measurements repeated over time through two stations (winter and summer). The data were subjected to an analysis of variance (ANOVA) using the SAS MIXED Procedure[87], and the best covariance matrix structure was selected based on Akaike's information criterion (AIC; smaller is better). The following statistical model was used: Y = mean + system + breed + system*breed + error1 + season + system*season + breed*season + system*breed*season + error2. Instantaneous thermoregulatory variables and hormone quantification were subjected to ANOVA, considering Y = mean + system + breed + season + system*breed + system*season + breed*season + system*breed*season + error. Significantly different means were compared with the post-hoc Tukey's test using the software NCSS 11.0 (NCSS LLC, Kaysville, UT, USA), with the significance level set at 5%. Because no significant triple interaction was observed, the results refer to double interactions and the main effects of interest.

Ethics approval

The experimental procedures were approved by the Animal Research Ethics Committee of Embrapa Pecuária Sudeste (Declaration CEUA-CPPSE 07/2017). The experiment was conducted in compliance with Brazilian laws and reported according to the precepts of the ARRIVE guidelines[78].

Data availability

All data generated or analyzed during this study are available from the institutional repository and accessible upon formal request.

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