**Thermoregulatory responses related to coat traits of Brazilian native ewes: an adaptive approach**

Jacinara Hody Gurgel Morais Leite, Débora Andréa Evangelista Façanha, Wirton Peixoto Costa, Dowglish Ferreira Chaves, Magda Maria Guilhermino, Wallace Sostene Tavares Silva and Luis Alberto Bermejo

+Department of Animal Science, Universidade Federal Rural do Semiárido, Mossoró, RN, Brazil; +Laboratory of Physiology and Control of Reproduction, Veterinary School, State University of Ceará, Fortaleza, Ceará, Brazil; +Academic Unit of Agricultural Science, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil; +Department of Science Universidad de La Laguna, Santa Cruz de Tenerife, Canary Islands, Spain

**ABSTRACT**

Semi-arid conditions can adversely affect livestock productivity and change certain physiological parameters. The relationship between hair coat and thyroxine levels in Morada Nova ewes was evaluated through environmental factors, such as air temperature, relative air humidity, radiant and Black Globe Humidity Index to gain a better understanding of thermoregulation mechanisms in these animals. Measurements were taken from July to January in 383 Morada Nova ewes. The variables studied included rectal temperature (RT), respiratory rate (RR), coat thickness (CT), hair length (HL), hair diameter (HD), hair density (D) and thyroid hormones. The data underwent multivariate statistical analyses and a significant inverse relationship was found between coat traits and Thyroxine ($T_4$). The animals that exhibited greater HL, coat density and CT showed lower $T_4$ concentrations. Coat traits showed a strong interaction with physiological mechanisms and can be considered relevant in maintaining homeostasis. Hair traits play an important role in this process, since $T_4$ reduction was stronger in animals that showed difficulties in eliminating heat, which were the ones that had greater HL, hair density and HD. By contrast, animals with a hair coat more favourable to heat losses had higher levels of thyroid hormones.

**1. Introduction**

There is a strong relationship between environmental conditions and animal response. In environments with high levels of thermal radiation, livestock show important reductions in productivity because of health disturbances (Marai et al. 2007). Therefore, livestock farming has to be based on breeds that can adapt to stressful conditions, since they can maintain productive and reproductive efficiency through their higher stress tolerance. Animals tend to develop some adaptive features in order to guarantee surviving under heat stress conditions.

Several features are used to assess animal adaptation to high air temperature and solar radiation. Among them, we can highlight hair characteristics that are directly related to heat loss or gain from the environment. Hair structure has two important roles, first it can protect the skin against direct solar radiation, and, second, it can promote the convection and heat loss by evaporation (Silva 2008). The efficiency of these functions depends on the hair structure. The physical structure of the hair coat is the first layer of defence protecting animals from direct sunlight. This protection differs according to many factors such as coat thickness (CT) and hair characteristics (Silva 2008; Maia et al. 2009). Substantial research has been conducted to analyse the relationship between coat characteristics and livestock productivity and other traits that are not directly related to thermoregulation (Bertipaglia et al. 2007, 2008). McManus et al. (2009), analysing five sheep groups, found that animals that showed lower respiratory rate (RR), rectal temperature (RT) and heart rate are characterized by better coat traits to heat adaptation. Paim et al. (2013) found that hair density, coat height and hair length (HL) were the most important traits for heat tolerance characterization.

When animals are not able to lose surplus heat, they can suffer certain physiological disturbances such as reduction in hypothalamic activity, which, in turn, can reduce thyroid hormone concentrations (Pugh 2002; Marai et al. 2007). These hormones have thermogenesis capacity and play an important role in certain physiological functions related to growth, reproductive efficiency and dairy production (West 2003; Al-Haidary 2004). The animals exposed to thermal stress conditions can reduce these hormones when the air temperature increases sharply (Todini et al. 2007; Helal et al. 2010; Lourenço et al. 2010; Maurya et al. 2010).

Thyroid hormones can affect thermogenesis, and the variation in the secretion of these hormones can be considered a physiological index of environmental adaptation (Façanha et al. 2010). Generally, animals exposed to high temperatures need to reduce thyroid activity and, consequently, metabolic rate and endogenous heat production. However, this decrease in metabolic processes can affect the productive performance,
such as growth and fattening (West 2003). Undesirable hair coat traits can cause overheating in animals and can be related to variations in hormone concentrations (Bertipaglia et al. 2008). On the other hand, it has been established that low CT and less dense coats composed of short and thick hairs can promote the dissipation of excessive body heat and contribute to homeostasis and better performance (Cena & Monteith 1975; Finch et al. 1984).

In the Tropical region, the scenario of environmental conditions such as high air temperature and radiation through the year necessitates active thermoregulatory mechanisms of the animals in order to maintain homeostasis; however some animals could present more difficulty to adapt to these conditions. Locally adapted animals showed a good interaction with environment and thus, it is necessary to characterize the genetic resources and understand all the mechanisms related to the adaptability of these animals to the production system, especially the animals on grazing, directly exposed to the environmental conditions (Mariante et al. 2005). In this context, it should be noted that Morada Nova sheep, the only locally acclimated breed of the northeastern Brazil, is characterized by lack of wool, high prolificacy, rusticity and good maternal ability (Facó et al. 2008; Lôbo et al. 2011). These animals are considered as very adapted to the Semi-arid conditions, but some aspects of the thermoregulatory processes that ease their thermal balance with the environment need additional studies.

This paper hypothesizes that the coat characteristics developed over the years of natural selection could influence some physiological parameters in Morada Nova hair sheep, managed under natural conditions. Previous research has considered both controlled environment conditions and natural experiments (Clarke & Warwick 2001). In particular, the literature emphasizes the importance of experiments conducted under natural conditions because these show the interaction between animals and the environmental conditions, considering all the abiotic stressors that are not present in a laboratory environment. The focus of this paper is to evaluate how animals respond to natural conditions in adaptive terms, since these natural conditions are the most appropriate experimental design. The interaction between animals and natural conditions in the environment should always be considered, since the animals are constantly subjected to different environmental factors. Therefore, this paper aims to characterize the coat profile and its relations with physiological responses of local adapted Brazilian ewes. This means that animals could be selected with hair coats that favour heat loss under natural conditions, and would have less need to reduce the activation of thermoregulatory mechanisms and could result in a better productive performance.

2. Material and methods
2.1. Area and period description

The experiment (approved by the University Ethics committee on the use of animals, CEUA-UFERSA, number 23091003895/2014-71) was carried out in different farms registered with the Brazilian Association of Morada Nova Sheep Breeders (ABMOVA). These animals represent an important genetic resource in northeastern of Brazil. Data were collected from 383 adult ewes, once per animal, all at reproductive age, as estimated by dental chronology, and were classified according to Pugh (2002). Data were recorded in 23 commercial herds in the northeastern region of Brazil, always in the morning. The study was carried out from July to January, the most stressful season of the year in the region. The animals were clinically examined and checked for good health before sampling.

2.2. Environmental data

Environmental and physiological variables were recorded simultaneously in the same places the animals were. Air temperature (Air T; °C) and relative humidity (H; %) readings were recorded at the same time as each ewe’s morphological and physiological data using a digital psychrometer, so that for each animal, there were individual data for the corresponding meteorological variables. With these data, indexes of environmental comfort were estimated: the Black Globe Humidity Index (BGHI) and the Radiant Thermal Load (RTL), according to Silva et al. (2007).

2.3. Animal morphological variables

Examined traits included CT, determined in situ in the middle of the thorax of each animal about 20 cm below the dorsal line with a thin metal rule. Hair samples were taken from the same region where CT was measured and were removed using pliers (Silva 2008). The sample hair was removed, stored in plastic envelopes and the measurements of HL, hair diameter (HD) and hair density were recorded in the laboratory.

Hair density was obtained by counting the number of hairs removed in 0.1399 cm² using pliers according to Lee (1953) and then converted into 1 cm² (Maia et al. 2003). Hair length (mm) was taken as the average length of the 10 longest hairs in the sample, according to Udo’s method (1978). The number of hairs per unit area (hair/cm²) was obtained by direct counting of all hairs in the sample. A digital micrometer, Mitutoyo model, with scale 0–25 µm was used to measure HD (µm); the hairs were the same used for the measurement of HL. The average HD was also based on measurements of the 10 longest hairs in the sample.

2.4. Animal physiological variables

Rectal temperature (RT, °C) was measured with a clinical digital thermometer inserted into the animal’s rectum. The RR (breaths/min) was recorded by counting flank movements for over a minute. Blood was collected from the jugular vein (10 ml) into vacuum tubes. Plasma total triiodothyronine (T₃) and thyroxine (T₄) concentrations were determined as the mean of duplicate determinations using Human In Vitro® commercial kits and an automatic Elisa device (Elysium Uno®, Human®).
2.5. Statistical analysis

To determine the variables that affect the distribution of the sampled animals, a Principal Components Analysis (PCA) was carried out, including environmental, morphological and hormonal variables. Since PCA axis 1 showed environmental conditions, samples were grouped into five groups based on the percentile values of this axis (same number of samples in each group). These groups consisted of ewes that had been exposed to five different environmental situations from the most comfortable, characterized by lower air temperature, RTL, BGHI and higher relative humidity (group 1) to the most stressful with the highest values of air temperature, RTL, BGHI and lower relative humidity (group 5). Therefore, animals were sampled over a range of environmental conditions.

To analyse the relationship between hair traits and hormonal variables over the range of environmental conditions (groups 1–5), we performed regression models with hormone content, as the dependent variable, and one selected explanatory variable from the group of variables related to component 2, in each environmental condition class. Zuur et al. (2007) suggests that this is a valid approach in linear regression models because specific relationships among variables that emerge from PCA analysis allow us to explain the overall behaviour of one variable.

3. Results and discussion

The Principal Component Analysis showed that the first component is related to environmental variables such as air temperature (AirT), air humidity (RH) and solar radiation (Radiant Heat Load (RHL)), whereas the second component is related to coat and hormonal traits (Figure 1). An inverse relationship between AirT, RHL, BGHI and RH showed that the environment was hot and dry. Animals characterized by greater HL, hair density (number of hair (NH)) and hair diameter (D) tended to show lower levels of thyroxine hormone (T₄). These coat traits difficult the animal’s heat loss to the environment which may favour the reduction in the concentration of T₄ in order to reduce the metabolic heat production.

The animals were exposed to different environmental conditions, which were grouped into five classes (Table 1). The animals that were evaluated under the environmental conditions present in group 1 were exposed to lower Air temperature (AirT), radiation (RHL) and Black Globe Humidity Index (BGHI). However, the environmental conditions in group 5 were characterized by high AirT, radiation and thermal comfort index associated with low air humidity. From groups 3 to 5, air temperature were above of thermal comfort zone for sheep, between 20°C and 30°C according to Bianca and Kunz (1978), or the critical temperature for adult sheep 32°C (Hahn 1985). The BGHI higher than 85 is considered a severe conditions to animals according to National Weather Service – USA. The RHL is related to heat exchange by radiation between the animal and the environment, and it is an essential variable for determine environmental tolerance (Silva 2008); therefore, lower RHL values describe proper conditions for animals. From group 3, animals suffered high radiation levels according to values reported by Maia et al. (2015) in tropical environment (670 W m⁻²) was closed to our results. Therefore, it is likely that under these conditions the animals were exposed to the most stressful conditions. The natural environment to which the animals are exposed, along the year, can cause heat stress. According to Salama et al. (2014), in heat stress situations, ambient temperature is normally greater than skin temperature, which causes a high load, as animals tend to gain heat by convection and radiation. In these cases, water evaporation is the most efficient way to dissipate heat to maintain internal temperature within the normal range; this occurs by respiratory system and sweating.

Heat exchange requires a temperature gradient and the amount of heat that flows depends on the gradient between the animal and the environment and the resistance to heat flow (the insulation). When air temperature is higher than the animal’s temperature, heat flows from the air to the animal are proportional to the insulation provided by coat traits. McManus et al. (2009) found significant differences in responses to increasing air temperature between wool and non-wool sheep breeds, in order to non-wool sheep showed a lower respiratory frequency than wool sheep, which suggests better adaptive capacity, probably due to more suitable hair traits to dissipate heat. Therefore, in the present study it is possible that the animals exposed to environmental conditions in groups 3, 4 and 5, gain heat and activate thermolysis mechanisms to maintain homeostasis.

Increasing RRs as a response by animals to heat stress has been studied widely (Panagakis 2011) and is considered a very important mechanism to cope with stressful situations, especially in sheep because it is a panting species. On the other hand, maintaining RRs high for a long time can cause some physiological disturbance, such as acid-basic disequilibrium (Srikanthakumar et al. 2003; Hamzaoui et al. 2013). However, we found that, considering the natural environmental conditions analysed, there were no changes in stress response parameters, such as RR and RT, as shown in Figure 1. Roger (2008) proposed 17–22 breaths/min as a normal value for sheep, under thermo-neutral conditions, when normally the animals do not require thermoregulatory mechanisms. In this paper, it was verified that the animals trigger respiratory thermolysis, once the means of RRs oscillated between 40.02 and 46.82 breaths/min. A very interesting finding was that the ewes were able to maintain RR in low-stressful situations over different environmental conditions, according to Silanikove (2000), which classified low-stressful values of RR range from 40 to 60 breaths/min. Thus, the RR of Morada Nova ewes under natural environmental conditions indicates a high adaptive capacity. However, as conditions become more stressful, other physiological mechanisms begin to play a more relevant role as way to ensure homeostasis. When the animals are exposed to solar radiation, skin temperature elevates and can increase the sweat secretion to stimulate output from the hypothalamus to the sweat glands, resulting in a higher production of the sweat gland rather than panting (Dmi’el & Robertshaw 1983). Starling et al. (2002) assessed RR and cutaneous evaporation with increasing air temperature from 30°C to 40°C and did not find RR variation, rather a significant increase in cutaneous evaporation as a way of losing heat. According to Silva and Starling (2003), cutaneous evaporation accounts for 63% of all
evaporation in sheep, although respiratory evaporation can be a suitable mechanism for rapid and short-term response to heat stress. Srikandakumar et al. (2003) found similar results in which two different genetic groups presented important increases in respiratory thermolysis, and observed higher magnitudes in RRs in Australian Merino sheep (128/min) than in locally adapted sheep (68/min) exposed to the same heat stress conditions; the lower magnitude of increase in RR suggests that the latter breed was less stressed by high air temperature.

Rectal temperature is generally considered as a good index of deep body temperature; the native hair sheep evaluated did not change RTs over different natural environmental conditions, maintaining it within the normal range reported by Roger (2008) between 38.8°C and 39.7°C. The maintenance of body temperature within physiological limits is necessary for animals to remain healthy, survive and maintain their productivity (Marai et al. 2007). Therefore, these animals were able to maintain homeothermy regardless of environmental conditions and ensure normal conditions for physiological processes, even under the most stressful environmental conditions in this experiment (Table 1). Certainly the thermolysis mechanisms, especially latent heat loss, were triggered to maintain the animals’ normal temperatures, confirming their homeothermic capacity. Hamzaoui et al. (2013) reported that animals kept under heat stress condition into climatic chambers, presented the highest values of RT and RR during the first week, but after this period were observed slight decreasing of these variables, which indicates a partial adaptation to the heat stress conditions. These results could explain the low variability of RT and RR of animals among environmental conditions (from

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**Figure 1.** Principal Component Analysis (PCA) for environmental variables and physiological and hormonal traits. Measurements were taken for: H: humidity, DPT: Dew Point Temperature, AirT: Air Temperature, GlobT: Globe Temperature, BGHI: Black Globe Humidity Index, RTL: Radiant Thermal Load, T4: Thyroxine content, T3: Triiodothyronine, HL: Hair Length, NH: Number of hairs, HD: Hair diameter. N = 383.

**Table 1.** Mean ± standard error of environmental and physiological traits in each group of environmental conditions from the most comfortable (group 1) to the most stressful environmental conditions (group 5).

| Groups of environmental conditions | 1 (N = 77) | 2 (N = 77) | 3 (N = 76) | 4 (N = 77) | 5 (N = 76) |
|-----------------------------------|-----------|-----------|-----------|-----------|-----------|
| RHL (W/m²)                        | 473.10³ ± 1.76 | 501.95⁴ ± 4.25 | 615.72³ ± 5.47 | 669.04³ ± 6.74 | 736.66³ ± 11.00 |
| BGHI                              | 75.57³ ± 0.17 | 77.92³ ± 0.34 | 85.71³ ± 0.34 | 89.14³ ± 0.31 | 94.16³ ± 0.50 |
| Air T(°C)                         | 28.30³ ± 0.15 | 29.89³ ± 0.25 | 32.22³ ± 0.29 | 33.20³ ± 0.36 | 34.97³ ± 0.49 |
| RH (%)                            | 68.55³ ± 0.90 | 54.31³ ± 1.13 | 40.43³ ± 1.52 | 37.84³ ± 1.10 | 35.53³ ± 1.47 |
| RT(°C)                            | 38.97³ ± 0.12 | 39.34³ ± 0.07 | 39.08³ ± 0.06 | 38.86³ ± 0.07 | 38.75³ ± 0.08 |
| RR (breaths/min)                  | 43.10³ ± 1.37 | 44.5³ ± 1.56 | 46.82³ ± 1.58 | 42.02³ ± 1.51 | 41.40³ ± 2.07 |
| T4 (µg/dl)                        | 5.97³ ± 0.28 | 4.27³ ± 0.21 | 3.82³ ± 0.29 | 3.32³ ± 0.25 | 3.69³ ± 0.28 |
| T3 (µg/dl)                        | 2.52³ ± 0.11 | 2.21³ ± 0.14 | 3.01³ ± 0.27 | 2.8³ ± 0.28 | 2.57³ ± 0.23 |

Notes: RHL: Radiant Heat Load, BGHI: Black Globe Humidity Index, AirT: Air Temperature, RR: Relative Humidity. Group 1 – The most comfortable conditions: lower Air temperature, radiant thermal load, Black globe Humidity Index and higher relative humidity. Group 5 – The most stressful conditions: highest air temperature, RTL, BGHI and lower relative humidity.

Mean within the same row with different letters were significantly different.
groups 1 to 5), possibly animals were acclimated to high radiation and air temperature in which they were exposed over the years. With regard to serum concentration of triiodothyronine ($T_3$) and thyroxine ($T_4$), the values for $T_4$ were higher in the most comfortable environmental conditions (group 1) and $T_3$ did not show differences among groups; similar results were reported by Costa et al. (2015) that evaluated $T_4$ and $T_3$ in this breed and found that thyroid hormone secretion in dry season was lower than in rainy season.

There was observed an important relation between coat traits and thyroid hormones. Animals that showed lower $NH$ and $T_4$ may lead less difficulties for eliminating latent heat via cutaneous evaporation (Holmes 1981), and shortest coats (HL) also is considered as favourable for heat exchange. The coat traits are studied to analyse adaptation of animals (Maia et al. 2009; McManus et al. 2009; Helal et al. 2010). This declining in thyroid function during heat stress may be due to the effect of heat on hypothalamic pituitary axis to cause reduction in thyrotrropin-releasing hormone, which enable the animal to control the production of thyroid hormones. Animals that showed coat characteristics that promote heat dissipation, such as short hair and low hair density (Gebremedhin et al. 1983), were able to maintain the highest $T_4$ concentrations. The $T_4$ hormone plays an important role in livestock productivity (Todini et al. 2007). By contrast, when the animals had coat traits that makes heat loss difficult, such as longer hair and higher hair density, these animals tended to reduce $T_4$ hormone concentrations to reduce heat production (Table 2), probably due to the difficulty in dissipating heat through evaporative skin thermolysis (Maia et al. 2005, 2009). Bertipaglia et al. (2008) found a relationship between coat traits and reproductive indices, in which the animals that had short HL showed shorter calving intervals. However, this response does not increase linearly over the environmental conditions imposed on the groups. The strongest intensity of relationship among the traits was reached in animals of group 3, and then it began to decrease in groups 4 and 5 (Table 2). Therefore, in heat stress situations (groups 4 and 5), animals with coat traits that hinder thermolysis (higher HL, NH and D) showed a poor relationship with $T_4$ concentration, and from this point, regardless of coat traits the $T_4$ concentration diminished. In these environmental conditions (the highest solar radiation, air temperature and lowest air humidity) the animals were not able to reduce heat production and tend to reduce $T_4$ concentrations and endogenous heat production regardless of coat traits. Therefore, it is possible that, to maintain homoeothermic conditions, the animals activate other mechanisms to improve heat loss and reduce endogenous heat production, thus keeping internal temperature within the appropriate physiological range. Regardless of the hair coat traits, it is possible that these animals did not compensate the excessive heat and they needed to reduce $T_4$ concentration in order to maintain thermic equilibrium.

Therefore, whereas locally adapted breeds maintain physiological parameters under stressful tropical conditions through other mechanisms, breeds from temperate regions are not able to ensure homeostasis in these conditions. High phenotypic diversity of local breeds and inter-annual and seasonal variability of environmental conditions in tropical regions requires research into animal adaptive traits in field conditions through long-term and large-scale studies (Berkowitz et al. 1988; Franklin 1988). On the other hand, more stable regions do not need this type of study, since seasonal variability is lower than in arid and semi-arid environments (Ellis et al. 1993). In this context, research must not only focus on specific responses of animals but also on the range and intensity of change in responses because another important advantage of locally adapted breeds is probably related to their greater plasticity compared to non-local breeds. Therefore, acknowledging and understanding the effects of environmental variability should be one of the main avenues of livestock research in these regions.

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**Table 2. Regression between Thyroxine ($T_4$) and HD in each environmental group from the most comfortable environmental conditions (group 1) to the most stressful conditions (group 5).**

| Groups of environmental conditions | 1 | 2 | 3 | 4 | 5 |
|-----------------------------------|---|---|---|---|---|
| Pearson correlation               | 0.046 | -0.029 | -0.517 | -0.400 | -0.350 |
| $R^2$ adj                         | 0.002 | 0.001 | 0.267 | 0.160 | 0.122 |
| $F$                               | 0.158 | 0.063 | 26.946 | 14.272 | 10.319 |
| $P$ level                         | 0.692 | 0.803 | <0.001 | <0.001 | 0.002 |

Note: $R^2$ adj: Adjusted coefficient of determination.

Group 1 – The most comfortable conditions: lower air temperature, radiant thermal load, Black Globe Humidity Index and higher relative humidity. Group 5 – The most stressful conditions: highest air temperature, RTL, BGHI and lower relative humidity.

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4. Conclusions
Morada Nova hair sheep maintain homeostasis under heat stress conditions. The reduction of $T_a$ concentrations during heat stress situations depends on coat traits. The findings of this study suggest that selection of breeds with short hair, low thickness and density coats can improve heat exchange and promote homeostasis. Thus, coat traits can be considered good phenotypic markers to ensure the selection of heat-tolerant animals.

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ORCID
Luis Alberto Bermejo http://orcid.org/0000-0003-1661-3904

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