



Can *Bos indicus* cattle breeds be discriminated by differences in the changes of their sweat gland traits across summer and winter seasons?

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ABSTRACT

This study addresses the hypothesis that *Bos indicus* cattle breeds can be discriminated by the changes that occur in their sweat gland traits between summer and winter seasons in tropical conditions. Samples of the skin were taken from six *Bos indicus* cattle breeds (eight subjects per breed), including Nellore, Cangaian, Gyr, Guzerat, Punganur, and Sindhi in winter and summer. The sweat gland epithelium (μm), glandular portion length (μm), sweat gland duct length (μm), gland depth (μm), and sweat gland density (cm^2) were determined. Principal component analyses were performed to address the overall structure of breed's group, together with confirmatory analyses by the least squares procedures. Exploratory analysis showed that cattle breeds presented patterns of dissimilarity in the changes in their skin and sweat glands traits between winter and summer seasons. Breeds were separated into three groups under the two principal components, which represented 77.26% of the total variance. The first group was composed of Sindhi and Guzerat cattle, which did not present modifications in the parameters assessed between seasons. The most visible alterations were observed in Gyr cattle (third group). In fact, confirmatory analyses showed that glandular portion length, sweat gland duct length, gland depth, and sweat gland density of the Gyr cattle increased ($P < 0.05$) during the summer season. In conclusion, the results of this investigation demonstrated that morphological traits of the skin and sweat glands associated with seasonal changes in tropical conditions were able to discriminate among *Bos indicus* cattle breeds.

1. Introduction

Adaptation is a broad term used to describe the ability of animals to adjust to environmental conditions or to infer genetic modifications that make animals more suitable for existence under specific ecological conditions (Turner, 1980). Tropical zones have high levels of solar radiation and high air temperature, which seem to be the main factors that influence livestock adaptation in this environment (daSilva and Maia, 2013). *Bos indicus* (Zebu) cattle breeds arose in the tropics, with most of these animals derived from India before being translocated to Africa a thousand years ago or to the Americas and Australia in the last hundred years (Mariane and Egito, 2002; Barendse, 2017). The *Bos indicus* cattle are widely recognized by their thermal adaptive attributes to tropical and subtropical environments, including a larger proportion of skin

surface to body mass ratio, darker skin pigmentation, shorter hair, and light colored coats, larger and more numerous sweat glands, plus the capacity to substantially increase the blood supply to skin when compared with *Bos taurus* breeds (Landaeta-Hernandez et al., 2011; Fonsêca et al., 2016; DeMelo Costa et al., 2018). These traits may imply that capability for cutaneous evaporative heat loss could be related to skin morphology and explain the superior heat tolerance of the Zebu in comparison to European cattle (Hansen, 2004).

Studies comparing skin traits of *Bos taurus* and *Bos indicus* breeds are abundant in the literature (Nay and Hayman, 1956; Turner, 1980; Carvalho et al., 1995; Hansen, 2004). For instance, using multivariate techniques, McManus et al. (2011) were able to discriminate naturalized Brazilian *Bos indicus* cattle (Curraleira, Pantaneira, Junqueira, Mocho Nacional, and Crioula Lageana) and imported *Bos taurus* breeds

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(Holstein) based on the cutaneous surface traits. Differences between *Bos indicus* and *Bos taurus* breeds regarding seasonal changes in sweat gland morphology also were described by Hayman and Nay (1957). Nevertheless, to date, we are unaware of any findings that have described distinct patterns of heat tolerance in *Bos indicus* cattle breeds based on skin and sweat gland morphology. Therefore, this research aims to answer the following questions: (I) Can *Bos indicus* cattle breeds be discriminated based on the skin and sweat gland morphology? (II) Are there apparent changes in such traits throughout the year under tropical conditions? We hypothesized that tropical *Bos indicus* cattle breeds, even housed under tropical conditions, can be discriminated by the modifications that occur in the sweat gland parameters throughout the year. Research dealing with this aspect will help us to understand how *Bos indicus* cattle breeds can cope with the thermal challenges in tropical conditions, giving the need for selection of breed most likely to thrive under the predicted climate changes in the region of interest.

2. Material and methods

2.1. Animals and experimental design

The study was carried out in the Veterinary Science Faculty of Uberlândia Federal University (18° 54' S, 48° 15' W, 843 m altitude), Brazil. At the beginning of March and August 2014, samples of the skin were taken from six cattle breeds (eight animals per breed), including Nellore, Cangaian, Gyr, Guzarat, Punganur, and Sindhi. All subjects had between 50 and 60 months of age and were non-lactating adult cows managed on a pasture-based system with access to water and shade provided by trees. Meteorological variables, including air temperature (T_{Air} , °C), relative humidity (R_H , %) and solar radiation (R_s , $W\ m^{-2}$), were recorded from a weather station of the Institute of Meteorology (INMET) during the summer (January, February and March 2014) and winter (June, July and August 2014) seasons. The Temperature and Humidity Index (THI) was calculated as described by Thom, 1959. All procedures described in this study were approved by the Ethics, Bioethics, and Animal Welfare Committee at the Federal University of Uberlândia (Protocol number: 162/13).

2.2. Sample preparation, and sweat gland traits

Skin samples ($n = 96$; one per animal during winter and summer season) were taken from the scapular region using the procedures described by Carter and Dowling (1954). Skin biopsies were obtained by first anesthetizing a section of the scapula region, using 5 ml of xylocaine (2%) per animal. The skin samples were removed using a skin biopsy punch (diameter = 1 cm) and were fixed in 10% formaldehyde for 48 h. All samples were taken between 15:00 to 18:00 h, and all subjects were in a resting condition and under the shade. Samples were dehydrated in an ethanol alcohol ladder (70, 90 and 100%) treated with xylol and infiltrated in liquid paraffin and stored until sectioned. The trimmed paraffin blocks were refrigerated for 10 min before sectioning to improve consistency and facilitate microtomy. A total of 30 samples per block were obtained for measurement of glandular parameters. The cuts were made from each block on a microtome (Model Leica RM 2025), with a thickness of 7 μm , and sections were placed on slides, then stained with Harris-Eosin Hematoxylin (Tolosa et al., 2003). All histological sections were analyzed by digitized images obtained on an Olympus Trimo-BX40 microscope coupled to the Oly-200 camera, attached to a computer by a Data Translation 3153 scanner; images were obtained with the microscope in 2X, 4X, 10X and 40 X, and analyzed using the HL Image 97 program (Mastrantonio et al., 2002). The sweat gland epithelium (H_{EP} , μm), glandular portion length (H_{GP} , μm), sweat gland duct length (H_D , μm), sweat gland depth (D_G , μm) and density (D_{EN} , sweat glands cm^{-2}) were determined. The gland depth was obtained by adding the glandular portion length and sweat gland duct. Ten animal samples from each breed were used to determine the sweat gland density

per millimeter, and multiplied by 100 to obtain the number of glands per cm^2 .

2.3. Statistical analysis

The principal component analyses of the sweat gland traits (sweat gland epithelium, glandular portion length, sweat gland duct length, gland depth and sweat gland density) were used to determine the structure of the samples of the six *Bos indicus* breeds in the study (Johnson, 1998; McManus et al., 2011). Before the exploratory analyses, variables were standardized to residuals normality attendance (i.e., mean = 0 and variance = 1). The confirmatory analyses were performed by least-squares method using the General Linear Models Procedure (Littell and Freund, 1991). The linear statistical model used to describe the traits was:

$$Y_{ijk} = \mu + B_i + S_j + I_{ij} + \epsilon_{ijk}$$

where Y_{ijk} is the k th observation of the sweat gland traits (glandular portion length, sweat gland duct length, gland depth and sweat gland density), B is the fixed effect of the i th breed (Nellore, Cangaian, Gyr, Guzarat, Punganur, Sindhi); S is the fixed effect of the j th season (summer and winter); I is the interaction between breed and season effects; ϵ_{ijk} is the residual term, and μ is the overall mean. The probability value that denotes statistical significance was at $P < 0.05$. All the statistical analyses were performed using the Statistical Analysis System, Version 8 (SAS Institute, 2002 NC, USA).

3. Results

Air temperature ranged between 19 and 36 °C in summer (relative humidity 60–73%) and between 16 and 22 °C in winter (relative humidity 35–65%). Overall, the mean (\pm S.D.) of air temperature, relative humidity, solar irradiance, and Temperature Humidity Index (THI) were 26.8 ± 3.5 and 20.6 ± 4.8 °C, $86 \pm 19\%$ and $55 \pm 11\%$, 877 ± 30 and 759 ± 40 $W\ m^{-2}$, 76 ± 3 and 67 ± 2 during the summer and winter seasons, respectively. The principal component analyses demonstrated that cattle breeds showed patterns of dissimilarity in the changes in skin and sweat gland traits that occurred between winter and summer (Fig. 1). Indeed, the least squares analyses revealed significant ($P < 0.05$) interaction between breed and season effects for all sweat gland parameters (Fig. 2). The principal components analyses revealed that breeds were separated into three groups under the two principal components ($Z_1 = 55.70\%$, and $Z_2 = 21.56\%$), which represented 77.26% of the total variance (Fig. 1). Notably, the most important variables (i.e., observing by the correlation coefficients between variables and the two principal components, Z_1 and Z_2) for this group separation were the glandular portion length ($r = 0.90$), sweat gland duct length ($r = 0.70$) and gland depth ($r = 0.90$) in the Z_1 , plus the sweat gland density ($r = 0.95$) in the Z_2 (Fig. 1). Both for Z_1 and Z_2 the sweat gland epithelium was poorly correlated (i.e., $r < 0.5$), and then removed from the confirmatory analyses. The first group (Group 1) was composed of Sindhi and Guzarat cattle, which did not present modifications in parameters assessed between the seasons. In fact, the least squares mean for all sweat gland traits showed no significant differences ($P > 0.05$) between seasons for these breeds (Fig. 2).

Changes for some sweat gland parameters were observed in a group formed by Nellore, Cangaian and Punganur cattle (Group 2). Nevertheless, for Punganur breed, higher values of least squares means for sweat gland density, sweat gland duct length, and gland depth observed during the summer were not significant ($P > 0.05$). Confirmatory analyses showed that Nellore cattle had greater ($P < 0.05$) glandular portion length during the summer, but there were no significant ($P > 0.05$) differences between seasons for sweat gland density, sweat gland duct length, and gland depth. In addition, the sweat gland duct length of Cangaian cattle was greater ($P < 0.05$) in the summer; however, the

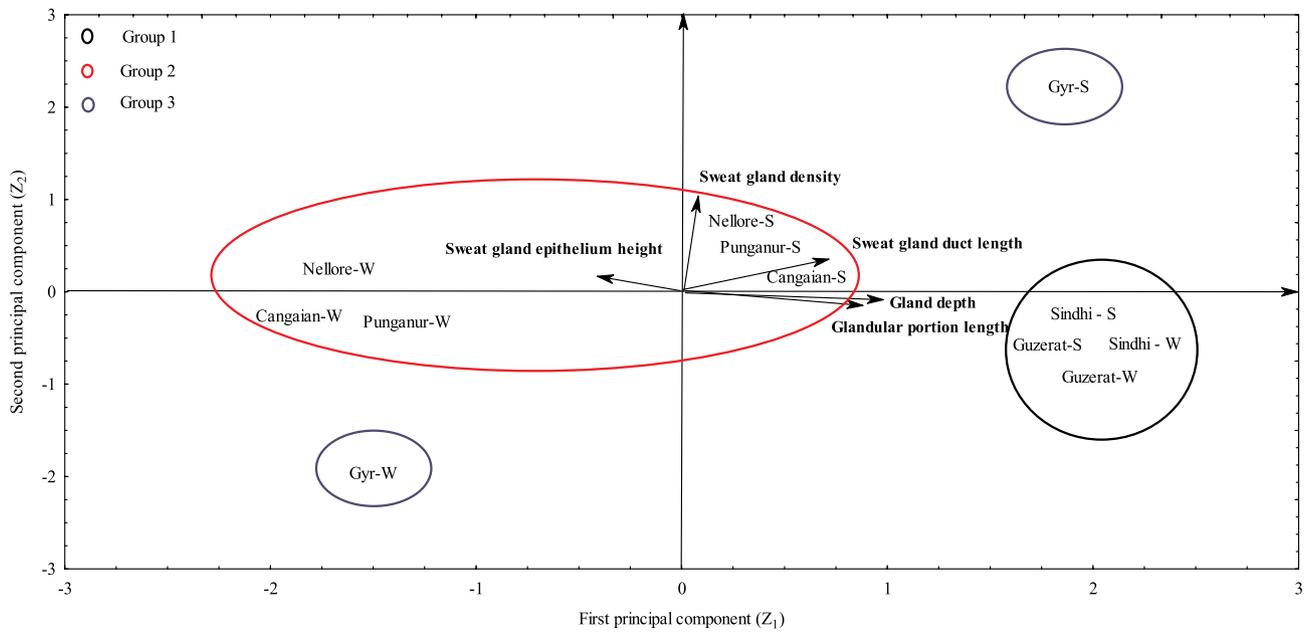


Fig. 1. Bi-plot of the sweat gland traits measured during summer (S) and winter (W) and *Bos indicus* cattle breeds in the first two principal components (Z₁ and Z₂).

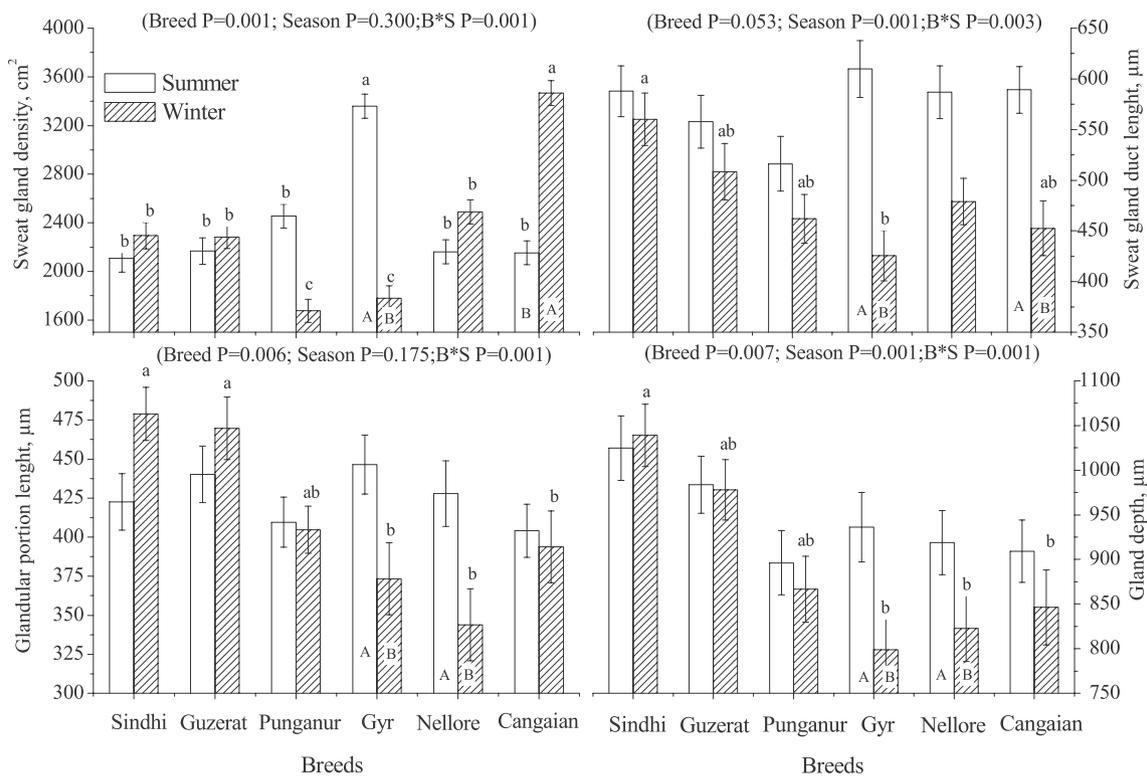


Fig. 2. Least square means (±SEM) of the sweat gland traits in *Bos indicus* cattle breeds during summer and winter. Uppercase letter correspond to the effect of season, while lowercase letters correspond to the breed's differences. B*S = interaction between the fixed factors (breed and season).

opposite occurred with sweat gland density, which was higher ($P < 0.05$) during the winter (Fig. 2). Clearly, the breed group structure under the two principal components revealed that the most visible changes in the sweat gland traits between summer and winter were observed in Gyr cattle (Group 3). Indeed, the hypothesis test showed that glandular portion length, sweat gland duct length, gland depth, and sweat gland density of Gyr was higher ($P < 0.05$) in summer (Fig. 2). The sweat gland density was the highly ($r = 0.92$) correlated with the Z₂; therefore, even though the total variance (21.56%) represented in the Z₂

was lower in comparison to the Z₁, the sweat gland density was the most important variable that separated Gyr from the other five cattle breeds. In quantitative terms, the Gyr least squares mean for sweat gland density in summer (3359.8 sweat glands cm⁻²) was twice as large ($P < 0.05$) than in winter (1779.3 8 sweat glands cm⁻²; Fig. 2).

4. Discussion

This study addresses the hypothesis that *Bos indicus* cattle breeds can

be discriminated by the changes that occur in the sweat gland traits between the summer and winter seasons in tropical conditions. To test this hypothesis, parameters including sweat gland epithelium, glandular portion length, sweat gland duct length, sweat gland depth and density were determined during the summer and winter seasons for Nellore, Cangaian, Gyr, Guzerat, Punganur, and Sindhi non-lactating adult cows. The cattle were exposed to high levels of ambient air temperature and solar radiation during the summer, and a mean of 76 and 67 for the temperature humidity index (THI) was registered during the summer and winter seasons, respectively. According to Zimbleman et al. (2009), for cattle, such values would be considered moderately stressful in summer and thermoneutral in winter. The main findings of our investigation were: (1) that different patterns of phenotypic plasticity in the sweat gland parameters across the year can discriminate *Bos indicus* cattle breeds; (2) that the Guzerat, Sindhi, and Punganur cattle breeds did not show significant differences in their sweat gland parameters in summer and winter; and (3) the Gyr cattle showed the visible changes, reflected by the higher values of glandular portion length, sweat gland duct length, gland depth and sweat gland density during the summer season.

The phenotypes of the cattle breeds investigated in this study were the product of artificial and natural selection for different purposes (meat or dairy), likely resulted in different ways to cope with thermal environmental constraints (Mariante and Egito, 2002; da Silva and Maia, 2013). In cattle managed under tropical conditions, heat balance with a rise in the ambient air temperature is attained mainly by the evaporation of water at the cutaneous surface via sweating (Finch, 1985; Hansen, 2004; Maia et al., 2005; Fonsêca et al., 2016; deMelo Costa et al., 2018). One would expect a proportional relationship between anatomical aspects of sweat gland parameters and sweating activity. The literature provides abundant findings demonstrating that the more heat tolerant traits attributed to *Bos indicus* cattle in comparison to *Bos taurus* are related to morphological characteristics of the skin. *Bos indicus* cattle have larger and more numerous sweat glands, and consequently greater capacity to maintain their thermal equilibrium by evaporation of sweat (Hansen, 2004; McManus et al., 2011; Jian et al., 2014). Jian et al. (2014) studying differences in skin morphology of Sahiwal (*Bos indicus*), Holstein (*Bos taurus*), and their crossbreds determined the following values for sweat gland density: Sahiwal (1058 sweat gland cm^{-2}), Holstein Friesian (920 sweat gland cm^{-2}), and crossbred (709 sweat gland cm^{-2}). Overall means for sweat gland density in all *Bos indicus* breeds investigated in the present study were higher than those reported by Jian et al. (2014). Indeed, Allen (1962) showed that cutaneous evaporative heat loss was greater in *Bos indicus* than in *Bos taurus* due to a larger number of sweat glands per unit of skin surface.

The focus of the present investigation was to find possible differences between tropical *Bos indicus* cattle breeds regarding changes in the anatomical aspects of sweat gland parameters during the summer and winter seasons. For *Bos taurus* cattle breeds during thermal stress, Pereira et al. (2014) observed that Alentejana, Mertolenga, Limousine and Holstein Friesian cattle triggered thermoregulatory responses that increased the latent heat flow by the respiratory tract and cutaneous surface, but with different magnitudes. We show here that the pattern of modifications in anatomical aspects of sweat gland parameters of *Bos indicus* cattle breeds varied with different magnitudes across two seasons that differed in air temperature, relative humidity and solar radiation. For instance, density of sweat glands, glandular portion length, sweat gland duct length, and gland depth of Gyr cattle were significantly higher during the summer season than during winter. The question that arises is whether such changes can be related to a greater capacity for sweat evaporation in summer? Unfortunately, we cannot indicate if these changes are related to a greater capacity for sweat evaporation in summer because sweating activity was not simultaneously recorded in this study.

However, there is evidence in the literature supporting a relationship between changes in anatomical aspects of sweat gland parameters and

increase in capacity of sweating evaporation in Gyr cattle during the summer. In cattle, the number of sweat glands is associated with the primary hair follicles, whose number remains stable in adult animals (Nay and Hayman, 1956). However, there is a positive relationship between the length/diameter of the sweat gland and sweating activity, which can lead to modifications in the number of sweat glands per unit of skin surface. Although we didn't measure the diameter, the increase observed in glandular portion length, sweat gland duct length, and gland depth may have resulted in an increase in the length/diameter of the sweat gland, consequently increasing their density. Gyr cattle are known for their potential to produce milk in tropical regions, likely aided by genetic evaluation and selection programs in Gyr populations; indeed, genes have been selected in breeding programs to improve the milk yield in Gyr cattle population (Berman, 2011). According to our findings, it seems that this breed may be more sensitive to thermal variations in the environment because of the positive relationship between the capacity to produce milk and resting metabolic heat production. Perhaps, this relationship may explain why the Gyr cattle expressed the noticeable changes in sweat gland parameters between summer and winter.

Our results also demonstrate that breeds such as Sindhi, Guzerat, Nellore, Cangaian and Punganur cattle did not show apparent changes in their sweat gland parameters between summer and winter seasons, particularly Sindhi and Guzerat cattle. Only the glandular portion length was higher in Nellore cattle during the summer season, while the sweat gland density of Punganur cattle was higher during the winter season. Fonsêca et al. (2016) did not observe differences in sweating activity between Sindhi and Guzerat heifers at an ambient temperature near 32 °C; when the ambient temperature rose from 26 to 32 °C, sweating activity was moderately higher in both breeds. Similarly, de Melo Costa et al. (2018) demonstrated that the sweating activity of Nellore cattle at ambient air temperatures ranging from 20 to 35 °C was practically stable. Based on these findings, it seems that these breeds are less sensitive to changes in ambient air temperature in tropical regions than Gyr. To the best of our knowledge, this is the first report to present data about sweat gland parameters of Cangaian and Punganur cattle. The most curious result was an increase in sweat glands density during the winter season for Punganur cattle, a pattern we are not yet able to explain.

5. Conclusion

Tropical *Bos indicus* cattle breeds can cope with thermal constraints in different ways. We were able to discriminate between *Bos indicus* cattle breeds by assessing changes in morphological traits of the skin and sweat glands associated with seasonal changes in tropical conditions. Nevertheless, more research and empirical evidence for the relationship between sweat gland parameters, sweating activity, and heat tolerance of tropical *Bos indicus* cattle breeds is needed to better understand the phenotypic plasticity of such breeds raised in hot conditions, especially in the face of increasing air temperatures associated with global climate change.

Compliance with ethical standards

All procedures described in this study were approved by the Ethics, Bioethics, and Animal Welfare Committee at the Federal University of Uberlândia (Protocol number: 162/13).

Author contributions

Conceptualization: V.F.C.F.; C.C.N.N.; M.R.B.M.N. Methodology: C. C.N.N.; V.F.C.F.; M.R.B.M.N.; M.E.B.; Software: V.F.C.F.; C.C.N.N. Validation: V.F.C.F.; C.C.N.N.; A.F. Formal analysis: V.F.C.F.; C.C.N.N. Data collection: C.C.N.N., M.R.B.M.N., M.E.B. Writing original draft: C. C.N.N.; V.F.C.F., C.C.M.C. Writing – review and editing: A.F., V.F.C.F., C. C.M.C. Visualization: V.F.C.F., C.C.N.N., A.F.

Declaration of competing interest

We declare that no competing interests exist that are of influence on this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2019.102443>.

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