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SERUM CORTISOL CONCENTRATION IN DAIRY CATTLE FROM THE AMAZONIAN REGION

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ABSTRACT: *The study was carried out in the parish of El Triunfo, province of Pastaza, Ecuador, with the objective of evaluating variations in serum cortisol concentrations in dairy cattle. Fourteen Brown Swiss and Holstein Frisian cows were selected, which met the criteria of being healthy, free of ectoparasites, weighing between 350 and 450 kg, in or during their second lactation cycle, and without post-vaccination treatment at the time of sampling. Each cattle was sampled twice at 7:00 a.m. and 4:00 p.m., following biosecurity protocols. Samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which was analysed by Fluorescence Immunoassay (FIA) to quantify cortisol (ng/mL). Statistical analysis included the Shapiro-Wilks normality test, Student's t-test to compare cortisol concentrations between sampling times, and Pearson's correlation to assess the relationship between values obtained in the morning and afternoon. The results showed significantly higher cortisol levels in the morning (22.11 ng/mL) compared to the afternoon (16.94 ng/mL) ($P < 0.050$), indicating a circadian rhythm in cortisol secretion. This afternoon decrease reflected a characteristic pattern in dairy cattle from the Amazon region, suggesting that management and environmental factors influence cortisol variability, which is associated with a physiological response to moderate stress, common in management systems in the region.*

KEYWORDS: Stress, Cortisol, Circadian Rhythm, Lactation.

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INTRODUCTION

Cortisol, a glucocorticoid produced by the adrenal glands in response to stress, is one of the most widely used biomarkers to assess stress in animals (Arias, 2008). It can be measured in various biological samples such as blood, saliva, urine and faeces (Romero et al., 2011). In production animals, elevated cortisol levels have been associated with reproductive problems, growth retardation and increased susceptibility to disease (Sierra, 2019). In cattle, basal plasma cortisol levels are below 10 ng/ml, although under normal conditions they can fluctuate between 0 and 20 ng/ml (Sierra, 2019). However, in stressful situations, cortisol levels may exceed 96.18 nmol/L, and after approximately 7 hours may decrease to 45.49 nmol/L, increasing again upon re-exposure (Menares et al., 2019). Measurement of cortisol in blood serum is a less invasive technique that facilitates repeat sampling, without causing negative impacts on the animals' freedoms, such as access to food, water and a suitable environment (Plazas et al., 2018).

Milk production in this region faces a number of challenges, including climatic, nutritional and management factors (Terán, 2019). Measurement of cortisol not only allows assessment of the stress experienced by animals, but can also serve as an indicator of their productivity and quality of life (Broom & Fraser, 2015). Through analysis of cortisol levels in different management and environmental conditions, more effective strategies can be developed to improve cattle health and, therefore, dairy production in this rich and biodiverse region (Benavides et al., 2021). Thus, cortisol assessment becomes an essential tool for farmers seeking to optimise their practices and ensure the sustainability of dairy production in the Ecuadorian Amazon (López, 2021).

The relationship between cortisol, dairy cow health and the circadian cycle is a crucial area of study for understanding cattle physiology and welfare. Cortisol is a hormone released in response to stress, and its production is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Under normal conditions, cortisol levels fluctuate throughout the day, following a circadian pattern that is reflected in the animal's behaviour and physiology (Hernández et al., 2020). During the circadian cycle, cortisol levels tend to be highest in the early morning hours and gradually decrease towards evening. This fluctuation is important as cortisol not only affects the stress response, but also influences metabolic processes, immune function and milk production (Aldag et al., 2019). A chronic increase in cortisol, due to prolonged stressful situations, can lead to health problems such as infections, decreased fertility and reduced milk production (Gonzalez et al., 2020).

Dairy cows may respond differently to stress depending on the time of day, implying that management interventions should consider these biological rhythms to be most effective (López et al., 2021). Milking and feeding management could be adjusted to minimise stress at critical times of the circadian cycle, thus promoting better animal welfare and higher milk production (Lefcourt et al., 1993). The evaluation of cortisol in dairy cows from the Ecuadorian Amazon is a topic of growing interest in the veterinary and animal production field. Cortisol, a steroid hormone produced by the adrenal glands, plays a crucial role in the stress response in cattle (Van der Kolk et al., 1991). In the Amazon, where environmental conditions are unique and can generate significant levels of stress in animals, understanding how cortisol levels manifest themselves can provide valuable information on the health and welfare of dairy cows (Lefcourt et al., 1993). Therefore, the present research aims to evaluate serum cortisol concentration in dairy cattle in the Amazon region.

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METHODOLOGY

Study Area

The study was carried out in the parish of El Triunfo, located in the province of Pastaza, Ecuador. With an area of 19,727 km², its main source of income is dairy cattle farming, reflecting the economic importance of this activity for its inhabitants (Vargas et al., 2013). El Triunfo is characterised by a humid and warm climate, with temperatures between 12 and 35 °C, which creates a favourable environment for agriculture and livestock (GAD Municipal Pastaza, 2021). This climate and its geographical conditions allow for a rich biodiversity (Herrera, 2021).

Experimental Design

For the development of this research, three farms were randomly selected, distributed in the study territory. Fourteen dairy cattle of Brown Swiss and Holstein Friesian breeds were selected and the animals were grouped in each farm: 5, 5, and 4. The selected animals met specific inclusion criteria: apparently healthy cows, free of pathogens and ectoparasites, weighing between 350 and 450 kg, in or past their second lactation period, and not in the post-vaccination process at the time of sampling. Two blood samples were taken from each bovine at different times (7:00 a.m. and 16:00 p.m.) to evaluate physiological variations in cortisol concentrations, following biosecurity protocols (UTE, 2021).

Blood Sampling

For blood sampling, the protocol of Pilla et al. (2022) was used, for which the blood collection tube was identified (OIE, 2018). The coccygeal vein, located in the ventral part of the animal's tail, was located. The area was disinfected with 70% alcohol, covering a diameter of 10 cm. Finally, 10 ml of blood was drawn with a 21G gauge needle, directly into a red capped tube without anticoagulant (Agrocalidad, 2018). Samples were kept away from sunlight, and stored in a transport cooler at 4–8 °C. Subsequently, it was transported to the laboratory of the Universidad Regional Amazónica Ikiam within 24 hours after extraction (Agrocalidad, 2018). At the laboratory, samples were centrifuged at 3000 rpm for 10 minutes to obtain blood serum, which was used for analysis of cortisol levels (Pilla et al., 2022).

Sample Analysis

For sample analysis, i-CHROMA TM Cortisol was used in conjunction with the iCHROMA TM reader which is a fluorescence immunoassay that quantifies Cortisol in serum/plasma/whole blood (BodiTech Med Incorporated, 2019). The immunoassay protocol was followed. 150 µL of detection diluent was taken with a pipette and dispensed into the detector tube containing a pellet. When the pellet was completely dissolved in the tube, it became a detection buffer. 30 µL of the sample (plasma) was taken using a transfer pipette and dispersed into the tube containing the detection buffer. The lid of the detection tube was closed and the sample was thoroughly mixed by shaking about 10 times. (The sample mixture should be used immediately.) 75 µL of a sample mixture was taken from the tube containing the detection buffer. 75 µL of a sample mixture was taken and loaded into the sample well of the cartridge. The loaded sample cartridge was then inserted into the iChamber slot or an incubator (25°C) for 10 minutes. To scan the sample loaded cartridge, it was inserted into the cartridge

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holder of the iChroma™ instrument. The test result was displayed on the iChroma™ instrument screen.

Statistical Analysis

A Shapiro-Wilks test was used to determine the normality of the data. Student's t-test was used to compare the cortisol values obtained in cattle, both in the morning and in the afternoon. Pearson correlation was performed to evaluate the relationship between morning and afternoon cortisol values.

RESULTS

Cortisol Levels

Cortisol levels in cattle were significantly higher in the morning (22.11 ng/mL) than in the afternoon (16.94 ng/mL) ($P < 0.050$), with 14 samples taken at each time (Table 1).

Table 1: Cortisol levels in dairy cattle from the Amazon region

Cortisol / Bovine	Media ng/mL	E.D.	n
Morning	22.11	7.89[a]	14
Afternoon	16.94	4.38b	14

Different letters show significance ($P < 0.050$)

Cortisol Variation

This indicates that, on average, cortisol levels were highest in the morning and decreased significantly in the afternoon. This result supports the presence of a typical circadian rhythm in cortisol secretion in Amazonian cows (Figure 1).

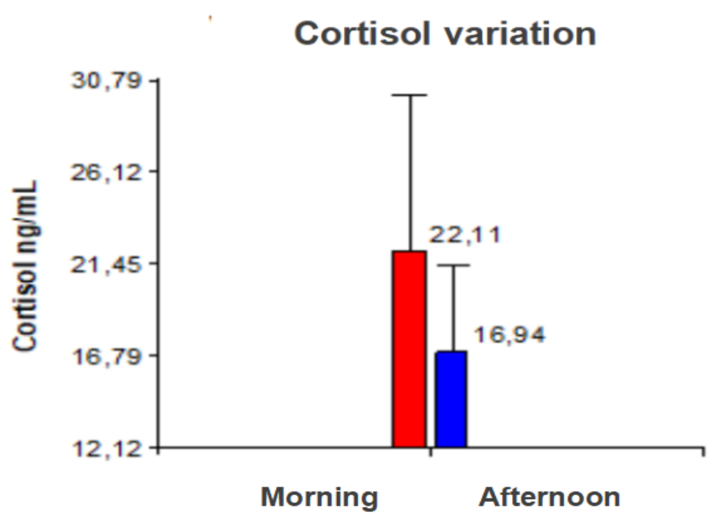

Figure 1: The Student's t-test showed a significant difference between morning and afternoon cortisol levels ($p = 0.001$)

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In Figure 2, these results suggest that animals with higher cortisol levels at the start of the day tend to experience more marked declines towards the evening, reflecting an active circadian rhythm.

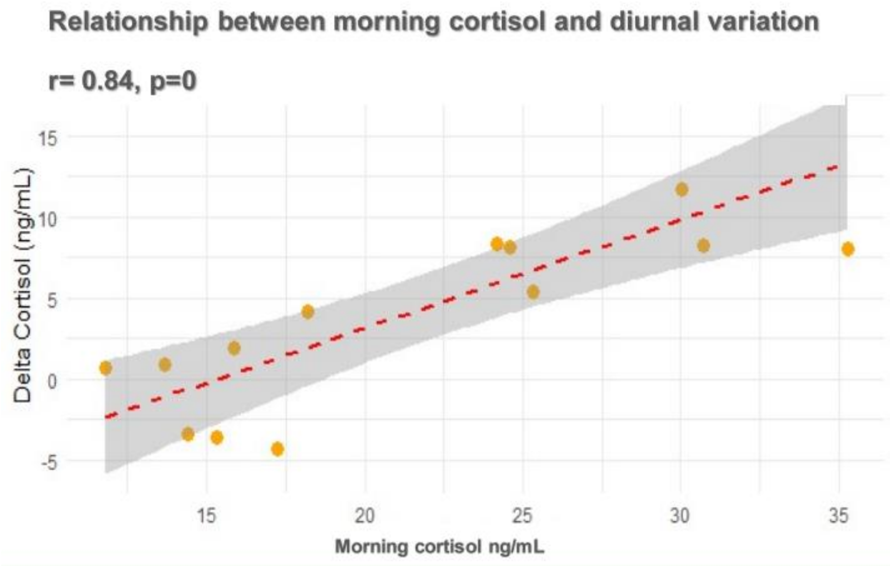

Figure 2: Strong positive correlation (r = 0.84, p < 0.001) between morning cortisol levels.

DISCUSSION

The results obtained in this study, showing a significant difference in cortisol levels between morning (22.11 ng/mL) and afternoon (16.94 ng/mL) (Table 1), agree with previous research reported by Koolhaas et al. (2011), which indicates that cortisol levels in cattle can reach values close to 20 ng/mL in the morning, which is consistent with the 22.11 ng/mL found in this study. This pattern, in which cortisol levels are highest in the morning and decrease in the afternoon (Romero Peñuela et al., 2020), suggest that this is a typical behaviour in cortisol secretion in dairy cattle, decreasing to lower levels, such as 5–10 ng/mL, in the afternoon (Koolhaas et al., 2011). General blood cortisol values in cattle under normal welfare conditions (no stress) are usually in the range of 2–5 ng/mL, adequate to maintain homeostasis (Cooke et al., 2017).

In the case of the levels found in this study, both morning and afternoon values are considerably higher than typical cortisol levels under welfare conditions, indicating that, at the time of measurement, the animals were experiencing some form of stress. Although the values do not reach the extreme levels observed in severe stress situations (such as prolonged transport or prolonged isolation, which can exceed 30 ng/mL), noting that the cortisol levels recorded in this study are within a range that could be associated with moderate stress (Cooke & Bohnert, 2011).

The variability in cortisol levels (Figure 1), throughout the day is linked to a number of physiological, metabolic and behavioural conditions that occur depending on the time of day, such as the body's preparation for the physical and metabolic demands of the day (Koolhaas et al., 2011). According to Mormède and Lemaire (2007), and Cheng and Yang (2006), cortisol releases peaks in the morning due to activation of the hypothalamic-pituitary-adrenal (HPA)

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axis, such as grazing and socialisation. This circadian pattern is key to understanding the mobilisation of energy and metabolic resources during the early hours of the day. Furthermore, these circadian cortisol rhythms influence the ability of cattle to adapt to different environments, highlighting the importance of considering these patterns in animal welfare management (Lima & Costa, 2020).

Studies in tropical areas, such as the Ecuadorian Amazon, have documented that various environmental factors, such as temperature and humidity, as well as management conditions, also affect cortisol levels, along with circadian rhythms. These factors can modify the hormonal response of animals as heat stress and interactions with the environment, such as milking or grazing, can alter hormonal regulation in cattle (Yánez et al., 2021). High temperatures and humidity in the Amazon region have been associated with increased cortisol levels, which may generate additional variations in hormonal patterns observed in other regions with more temperate climates (Solano & Muñoz, 2019; Lima & Costa, 2020). Stress may also be related to the presence of disease or infection; the cow's body may produce more cortisol in response to physical discomfort. Sick animals, especially those with infections such as mastitis, often have elevated cortisol levels due to inflammation and immune response (Hemsworth et al., 2011).

These patterns, with higher cortisol levels in the morning and a decrease in the afternoon, are consistent with what has been observed in previous research in cattle in various geographical areas (Figure 2), suggesting that, despite the particular environmental conditions of the Amazon region, cattle follow a similar pattern of cortisol secretion (Pargas et al., 2014; Cheng et al., 2006). These findings reinforce the hypothesis that cortisol circadian rhythms are fundamental in the biology of animals and allow them to adapt and regulate their physiological responses to daily demands. The results obtained in this study are consistent with what is observed in other works where elevated cortisol levels have been recorded in cattle in response to stressful conditions, such as, in milking or moderate confinement situations; blood cortisol levels can increase to values of 10 to 20 ng/mL (Lay et al., 1992). Roman et al. (1978) have found that cortisol levels can reach around 13 ng/mL during isolation or restricted management, with slight reductions depending on the duration and intensity of stress. These values reflect a slight response of the animals' hormonal system to the stress associated with isolation or movement limitations, which increases the release of cortisol, a hormone related to the stress response (Lay et al., 1992).

The results of this study contribute to the understanding of the biological rhythms of cattle in the Ecuadorian Amazon, indicating that, although factors such as heat stress may influence cortisol levels, circadian patterns continue to play a crucial role in hormone regulation. These findings are valuable for the design of management strategies to optimise animal welfare in the region (Lima & Costa, 2015). Management of cattle during the early hours of the day, when cortisol levels are highest, could be reduced or adapted to minimise stress. In addition, lower cortisol levels in the afternoon offer an opportunity to implement more relaxed management practices during those hours, favouring productivity and animal welfare (Mormède et al., 2007).

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CONCLUSION

The results show a circadian pattern in cortisol secretion in dairy cattle, with higher levels in the morning (22.11 ng/mL) and a significant decrease in the afternoon (16.94 ng/mL). This behaviour points to the influence of management and environmental factors on cortisol variability, reflecting a physiological response to moderate stress, typically observed in management systems in the Ecuadorian Amazon.

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