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A new proposal for the use of the focal animal technique in buffaloes in the Eastern Amazon

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This study aims to present a proposal for using the focal animal recording technique to evaluate the welfare of buffaloes and to verify the association between each behavior and thermal comfort indices. The study was conducted in an experimental paddock located in Santarém, Pará, Brazil. A total of 10 female Murrah animals were used. The behavior of the animals was recorded during the day, with the use of three trained observers, for 72 consecutive h. Climatic variables were collected, and the Temperature-Humidity Index (THI) and the practical Buffalo Comfort Climatic Conditions Index (BCCCIp) were determined. The multivariate technique of principal components and Spearman's correlation were employed. BCCCIp and THI were outside the thermal comfort zone at different times of the day. Grazing (P) was more frequent in the coldest hours of the day, while rumination occurred at different periods, mainly during the daytime and frequently in a lying position. There was a positive correlation between idle lying behavior and average temperature—Tmed (r = 0.583; p < 0.028), THI (r = 0.432; p < 0.034), and BCCCIp (r = 0.554; p < 0.049). There was a positive correlation between grazing and Tmed (r = 0.665; p < 0.0004) and BCCCIp (r = 0.583; p < 0.036). The standing idle behavior negatively correlated with Tmed (r = -0.718; p < 0.0001), THI (r = -0.522; p < 0.008), and BCCClp (r = -0.8076; p < 0.0008). The lying ruminating behavior had a positive correlation with Tmed (r = 0.586; p < 0.002), THI (r = 0.477; p < 0.018), and BCCCIp (r = 0.8033; p < 0.0009). Furthermore, ruminating while standing correlated positively with Tmed (r = 0.680; p < 0.0003). The adaptation of the focal animal technique, with six observers evaluating each animal for 6 min through filming, proved to be efficient in pointing out the different behaviors of buffalo raised in Eastern Amazon fields under heat stress at different times of the day.

KEYWORDS

observation technique, animal behavior, snapshot recording, buffalo, ethogram

1. Introduction

The focal animal method described by Altmann (1) involves recording the behavior of an individual or a group of animals. For this method to be effective, a predetermined observation time to observe the sample subject must be set and recorded for each session. The length of each sampling session depends on the observer's fatigue and physical readiness. This information is important to determine the appropriate experimental methodology.

The focal animal technique is a cost-effective and less laborious approach to identifying behavioral parameters. It requires fewer animals and observations to represent the entire group. The former factor helps to identify abnormal behaviors both inside and outside the natural environment, such as in pastures. This method is useful in recognizing any unusual behavior patterns (1).

Some studies were conducted to identify the behavior of cattle in different environments, such as in the intervals between milking dairy cows (2-4) or in the field (5), which contributes to the proper management of individuals by favoring animal welfare (AW) and enhancing herd productivity (6-10).

Different studies were carried out to observe the behavior in different environments, including their access to shade and water for immersion. The results showed that the buffaloes preferred to stay protected from solar radiation during the hottest hours of the day and that they used water as their preferred environment for cooling. For example, water buffaloes were observed grazing in Lake Kerkini National Park in northern Greece (11).

In general, observations are carried out by taking into account different behaviors that could signal the degree of AW in cattle, pigs, buffaloes, sheep, and goats. When coded correctly, these behaviors can provide information that will help in the identification of abnormalities, with the goal of adopting management techniques that favor the reduction of the expression of undesirable behaviors to favor AW.

In this context, some indices help to determine the influences resulting from these production systems, such as the Temperature–Humidity Index (THI) and the Practical Buffalo Comfort Climatic Conditions Index (BCCCIp), calculated according to meteorological variables and physiological parameters of buffaloes (2), signaling stress in animals (6, 7).

Based on this information, this study aims to present a proposal for using the focal animal recording technique to evaluate the welfare of buffaloes and to verify the associations between each behavior and thermal comfort indices. The animals were kept in an experimental paddock in Santarém, Pará, Brazil.

2. Materials and methods

2.1. Observation environment

The experiment took place in a paddock of rural property in Santarém, Pará, Brazil, with an area of 2.5 ha of *Brachiaria decumbens*, with trees homogeneously distributed for natural shadings, such as Jatobá (*Hymenaea courbaril*), with approximately 10 m in height, and other species, with a height of less than 5 m, with a lake of 50 m wide and 2 m deep, and with free access given to the animals for immersion and a source of hydration for both the individuals and the animals (Figure 1).

2.2. Experimental animals

From a group of 15 buffaloes, 10 clinically healthy, non-lactating Murrah females were selected, with an average weight of $550\pm26\,\mathrm{kg}$ and an average age of 24 ± 2.3 months who had lived together for more than a year. The paddock capacity was 7.33 animal units (AU)/hectare.

2.3. Ethogram

The animals were filmed during the day using a cell phone video camera, Sony®, model: Cyber-shot, DSC-TF1, resolution: 16.1 MP. Filming was carried out during both the day and night in three different periods, specifically in the morning from 6:00 a.m. to 9:00 a.m., midday from 9:01 a.m. to 2:00 p.m., and in the afternoon from 2:01 p.m. to 6:00 p.m. The animals were given a 5-day adaptation period to get used to the presence of the observers. The camera was positioned approximately 3 m away from the animals; however, this variation may be greater due to the animals walking in the paddock, which did not prevent the animals from being viewed.

Observations were carried out over 3 consecutive days. Each focal animal was observed every hour for 6 min per hour. This time was adopted, as 6 min per animal was defined since an hour has 60 min. Consequently, these were equally distributed among the 10 experimental animals (60 min/10 animals = 6 min/animal). In total, six specialized observers alternated every 6 min per hour to reduce possible influences on confirming behavior.

In the analysis of the footage, the images were transferred to the computer, and three trained observers analyzed them separately using Cowlog 3.0 software (12).

The predefined behavioral variables, according to the literature, were posture (standing or lying down), activities performed (grazing, rumination, and idleness) (state type), tail wagging, scratching, and licking (instant) (Table 1), with the state type described as behaviors greater than 5 s and instantaneous (less than 5 s), as described by Lehner (15). It was also observed whether the animals were immersed in the lake to cool off. The number of times the behavior was performed in a given period of time was recorded.

2.4. Climate variables

The climatic variables, including air temperature (AT), relative humidity (RH), and dew point, were measured using a thermohygrometer, Incoterm® 5203.03.0.00. The device was installed at a fixed wooden point in the paddock in which the animals were conditioned, and the data were recorded every 15 min, and these data were presented in the tables on average every hour.

2.5. Temperature-Humidity Index (THI)

The THI was calculated by taking into account temperature and relative humidity. The THI value up to 72 was considered thermal comfort; between 72 and 78 signals mild or mild thermal stress; between 79 and 88 signals moderate thermal stress; and between 89 and 98 signals severe stress (16).

THI was obtained through Equation 1, adapted from Thom (16).



TABLE 1 Ethogram: pre-defined behaviors according to the literature and its definitions.

Behavior	Definition
Standing	Leaning on its limbs, moving or standing still.
Lying down	Animal with four-flexed legs and with the abdomen wholly or partially in contact with the earth.
Grazing	Act of feeding on pasture, always on foot.
Ruminating	Animal chewing, swallowing, regurgitating, and re-chewing with the presence of bolus
Idleness	Looking in any direction without visible appearance, lying, or standing.
Wagging the tail	Flank of the cheek, which can be performed
Scratching the body	Standing or lying down.
Licking	Looking inattentively in any direction

Source: Adapted from Coimbra et al. (13), Agudelo et al. (14), and Silva et al. (4).

THI =
$$(0.8T) + (RH/100) * [(T-14.4) + 46.4]$$

where T is the dry bulb air temperature ($^{\circ}$ C) and RH is the relative air humidity (%).

2.6. Practical Buffalo Comfort Climatic Condition Index (BCCCIp)

The practical Buffalo Comfort Climatic Conditions Index (BCCCIp) was calculated by taking into account the temperature and relative humidity of the air. The following bands were considered: — comfort (\leq 34.65), danger (34.66–38.02), stress (38.03–41.39), and emergency (\geq 41.40) (17).

The BCCCIp was calculated using the formula:

BCCCIp = 0.0571*RH + 1.0480*AT,

where RH is the relative humidity of the air (%) and AT is the air temperature (°C).

2.7. Statistical analysis

With the objective of evaluating the behavior of the animals throughout the shifts of the day and facilitating the understanding of the behaviors (morning from 6:00 a.m. to 9:59 a.m., midday from 10:00 a.m. to 1:59 p.m. and afternoon from 2:00 p.m. to 6:00 p.m.), the multivariate technique of principal components was used to group the behaviors and to identify which activities the animals perform most in these periods. In addition, the behaviors were also evaluated using Spearman's correlation (at a significance level of 5%) with the values of THI, Tmed, and BCCCIp to identify positive and negative correlations that may influence the activities that the animals perform throughout the day. Data were also initially analyzed by comparing the activities in each shift for both sun and shade treatments using the chi-squared test and a subsequent comparison of pairs of values using the chi-squared test with Bonferroni correction. Subsequently, the sun and shade treatments were compared for each shift using the Wilcoxon test. All analyses consider a significance level of 5% and were performed using the R-Studio software, version 1.1.463 3 (18).

3. Results and discussion

When analyzing the trends in average air temperature over 3 days, it became evident that temperatures were consistently higher during the day. This was evidenced by the temperature readings that showed 30.2°C in the morning and 32.5°C in the afternoon. However, 27.5°C was observed at night (as indicated in Table 2). The temperatures considered favorable for breeding (including reproductive and productive aspects) of buffaloes ranged from 13°C to 18°C; thus, the

TABLE 2 Meteorological indices and animal thermal comfort of buffalo raised in the paddock with the availability of shade in Santarém-PA, Brazil.

Period	Time	AT (°C)	THI	BCCCIp
Morning	06:00	27.5	79.8	33.27
	07:00	27.5	79.8	33.01
	08:00	27.2	79.6	32.80
	09:00	27.5	79.8	33.37
Midday	10:00	28	80.8	33.47
	11:00	32	86.3	34.69
	12:00	30.2	83.7	36.07
	13:00	32	86.3	37.79
Afternoon	14:00	31.7	79.2	37.66
	15:00	32.5	87.3	38.03
	16:00	31.3	79.8	35.06
	17:00	28.5	81.8	33.73
	18:00	26	77.5	32.16

AT, Average temperature; THI, Temperature–Humidity Index; BCCCIp, practical Buffalo Comfort Climatic Conditions Index (17).

animals were able to express their normal characteristics and well-being on pasture (19).

During the intermediate period between 1:00 p.m. and 3:00 p.m., the highest temperature, THI, and BCCCIp were recorded, which could cause heat stress in animals (20). Animals develop heat stress due to an imbalance between high environmental temperatures and metabolic heat production. Furthermore, when thermoregulatory mechanisms such as behavioral, endocrine, and metabolic responses are not capable of restoring thermoneutrality or when they are not sufficient, heat stress can be triggered. By including this at the beginning of the Discussion section, the reader will be able to easily understand why all the variables evaluated in the present study are associated (21, 22). Therefore, environmental variables have a direct influence on the thermoregulation of buffaloes, which can lead to problems in growth and reproduction (23, 24).

The temperatures mentioned above were observed during the study of buffalo behavior. It is an important factor to consider as buffaloes have peculiar characteristics, such as a low number of sweat glands and a high concentration of melanin. This makes them more susceptible to heat and solar radiation (25–27) and heat stress, causing them to spend more time grazing when temperatures are lower (28–30).

The relative humidity of the air was high at some hours of the day, reaching values between 87 and 100%. According to Azevedo and Alves (31), animals begin to express their productive potential when the humidity levels are between 60 and 70%, which are considered adequate for the species.

The THI determines and quantifies the intensity of heat stress in domestic species (8). Thus, it was observed that the THI values for buffalo breeding were higher than the recommended values (32). For the animals to be considered within the thermoneutrality zone, the THI must remain up to 72. If the THI is between 72 and 78, it indicates mild stress or mild thermal stress. If the THI ranges between 79 and 88, it suggests moderate stress, and between 89 and 98, it indicates

severe stress. Similar results were reported in the study by Moraes et al. (33), Pantoja et al. (34), and Almeida et al. (30).

The BCCCIp, which is used for diagnosing cases of heat stress in buffalo herds, indicated that between $12:00\,h$ and $13:00\,h$, the buffaloes were in the danger zone (34.66-38.02), and at $15:00\,h$ they were under heat stress (38.03-41.39) (17). This may be related to the environment, as the behavior and physiology of buffaloes have a direct influence on this variable, resulting in negative effects on animal welfare (6,35-37).

The most expressive behavior observed in buffaloes raised on pasture was grazing, followed by rumination and minimal idling (Figure 2). The low idleness rate may be due to good-quality pasture, which provides the animals with a greater ability to perform the activity (38). In a study evaluating behavioral responses relating to the thermoregulation capacity of buffaloes, grazing was the most frequent behavior observed, regardless of access to water for immersion (11).

In relation to the morning shift, it was noted that the animals graze in a shaded area, possibly due to stress from high temperatures and intense solar radiation (26, 37). These results support the findings of Almeida et al. (30), who found no significant differences; that is, the presence of shading did not interfere with the grazing of the animals. This is likely due to the colder air temperature during the experiment.

Rumination was more frequent during the hottest hours of the day, mainly in the lying position and in a shady environment. This is possibly due to the fact that the animals sought shade to cool off and eat more during the night when temperatures were lower. During the day, when temperatures are high, animals tend to ruminate on food consumed at night. With similar results, Santos et al. (38), in the Amazon, observed that buffalo heifers sought shaded areas, especially during the hottest hours of the day, to ruminate, both standing and lying down, in search of a more suitable place for their well-being.

The standing and lying positions were evidenced according to the behavior or activity that the individual performed: the first position, standing, was observed in most situations, mainly during grazing; however, the second position, lying, was less adopted by buffaloes while ruminating, mostly in the shade. This fact was observed in studies by Santos et al. (39). It is noteworthy that this position is more commonly used in the rumination process. Corroborating the studies carried out by Ablas et al. (11) and Marques et al. (40), it was identified that the lying position was the most used position by animals in the rumination process.

The rumination time was lower than the grazing time in all shifts; however, rumination is more common in warmer periods of the day, being carried out mainly in a lying position and in shaded areas (p<0.05) (Table 3), as the canopy of trees prevents the exacerbated passage of solar radiation, promoting a reduction in the temperature of 2 to 3°C when compared to places without trees. With this decrease in temperature, there is a reduction of heat in the animals, which consequently provides them with a more thermally comfortable environment (41, 42).

During the morning shift, the predominant behavior of the animals was grazing in the sun. As for the afternoon shift, the primary activities observed were animals ruminating while standing and lying down in sunny areas. During the midday shift, activities such as walking, grazing, and ruminating while standing and lying down were notably observed in the shaded areas.

The animals spend most of their time in a lying position, showing a good AW index (43, 44).

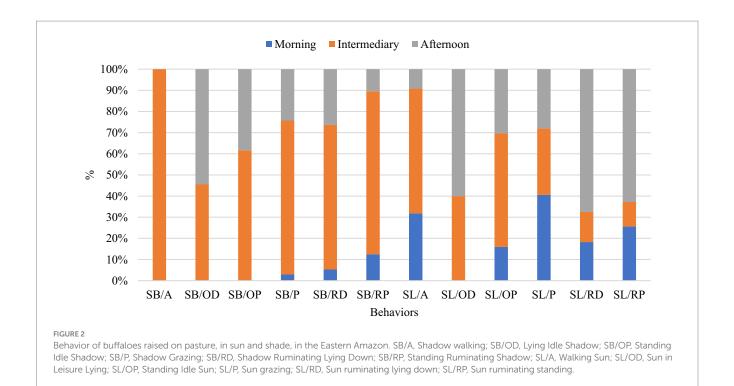


TABLE 3 Behavior of buffaloes raised on pasture, in sun and shade, in the Eastern Amazon.

Shadow (Minute)						
Shift	A	OD	OP	P	RD	RP
Morning	0a	0a	0a	6b	30c	6b
Midday	10a	5a	45b	150c	389d	37b
Afternoon	0a	6b	28c	50d	150e	5b

Sun (Minute)						
Shift	A	OD	OP	P	RD	RP
Morning	21b	0a	11b	1246d	59c	11b
Midday	39b	2a	37b	968c	46b	5a
Afternoon	6a	3a	21b	860d	218c	27b

A, walking; OD, leisurely lying down; OP, idle standing; P, grazing; RD, ruminating lying down; RP, ruminating while standing. Different letters (a, b, c, d) in the line indicate statistical differences (p < 0.05).

The behavior of lying down in buffaloes is associated with thermoregulation because lying on a cold surface can promote heat dissipation by convection, just as lying down reduces movement and, therefore, reduces the amount of heat produced by the body (6).

There was a positive correlation between lying down and AT (r=0.583; p<0.028), THI (r=0.432; p<0.034), and BCCCIp (r=0.554; p<0.049) (Table 4). This occurs because the hottest hours of the day are used to practice this activity, usually in the shade, avoiding an increase in body temperature, which is a thermoregulatory behavior of buffaloes (27, 33). Research evaluating the thermoregulatory behavior of buffaloes indicates that shade is an important component to help dissipate heat under different conditions (6, 37, 38, 45, 46). Furthermore, heat stress tends to reduce grazing activity (47).

There was a positive correlation between grazing and AT (r=0.665; p<0.0004) and BCCCIp (r=0.583; p<0.036). This indicates that when the temperature increases, grazing also tends to increase; however, in general, this happens in shaded areas (37, 48), and, as previously shown, this is a common practice in the morning shift.

Standing idle behavior was negatively correlated with AT (r=-0.718; p<0.0001), THI (r=-0.522; p<0.008), and BCCCIp (r=-0.8076; p<0.0008). This can possibly be explained as a result of this activity being performed when the animals seek to reduce body heat, such as foraging, ingesting and digesting food, and absorbing nutrients, which are processes that generate heat. In addition, therefore, reducing food consumption provides thermoregulation (49). Thus, heat stress stimulates peripheral heat receptors to transmit suppressor nerve impulses to the appetite center present in the hypothalamus, causing a decrease in food consumption (50) and consequently performing idle behavior. Similar results on the reduction of food intake during heat stress were evidenced in the studies by Habeeb et al. (51), Khongdee et al. (52), and Savsani et al. (53).

The lying ruminating behavior had a positive correlation with AT (r=0.586; p<0.002), THI (r=0.477; p<0.018), and BCCCIp (r=0.8033; p<0.0009). Furthermore, ruminating while standing correlated positively with AT (r=0.680; p<0.0003). This fact stems from this activity being carried out in the shade, providing animal well-being, as well as because this is an evolutionary adaptation of buffaloes that express their thermoregulatory behavior lying under the shade of trees (52). In this situation, we recommend taking wind speed into account, as other studies used fans in the summer and, for this reason, identified a greater adoption of the lying position in buffaloes, as described in the studies by Ahmad et al. (54).

The animals spent an average of 3 h in the lake, usually in the afternoon shift from 1 p.m. to 3 p.m. Buffaloes have only one-sixth of sweat glands (55) and fewer hairs than cattle (56, 57). In addition,

TABLE 4 Positive and negative correlations between the variables, average temperature, THI, and BCCCIp, were equally observed in all shifts.

Behaviors	R2	Indices			
	strength and <i>p</i> value	AT	THI	BCCClp	
A	\mathbb{R}^2	0.42979	0.36827	0.48262	
	p value	0.1427	0.2157	0.0948	
OD	R ²	0.64498	0.56337	0.55479	
	p value	0.0173	0.0450	0.0491	
P	\mathbb{R}^2	0.63436	0.51262	0.58322	
	p value	0.0199	0.0733	0.0364	
OP	R ²	-0.87692	-0.68348	-0.80769	
	p value	< 0.0001	0.0100	0.0008	
RD	R ²	0.77841	0.57268	0.80330	
	p value	0.0017	0.0408	0.0009	
RP	R ²	-0.07961	-0.14789	-0.21083	
	p value	0.7960	0.6297	0.4893	

A, walking; OD, idle lying down; P, grazing; OP, idle standing; RD, ruminating lying down; RP, ruminating standing up; AT, average temperature; THI, Temperature–Humidity Index; BCCCIp, practical Buffalo Comfort Climatic Condition Index. A value of p of < 0.05 indicates a statistical difference.

TABLE 5 List of instantaneous behaviors observed in buffaloes grazing in the municipality of Santarém, Pará, Brazil.

Behavior	Quantity (movements)	Duration (amount of time per behavior)
Wagging the tail	721a	3-5 s
Scratching the body	142b	15-30 s
Licking	82c	30-37 s

Different letters in column (a, b) indicate statistical differences (p < 0.05 – chi-squared test).

buffaloes have difficulty thermoregulating at high temperatures due to their dark skin and thick epidermis (58), which can often present lesions depending on the region of the country (59), where the presence of solar radiation can be more intense. Thus, these individuals suffer from thermal stress when exposed to solar radiation and consequently immerse themselves in water to minimize stress under these environmental conditions (37) or even tend to ingest more water (60–62). Water intake by buffaloes is linked to dehydration due to massive sweating, aiming to maintain the thermoregulation that induces the thirst center in the hypothalamus during excessive heat load (61).

Note that tail wagging was routinely observed (721 times), differing from the other behaviors, in the period of study in hours, and each movement lasted a maximum of 5 s (Table 5), according to the methodology of Almeida et al. (63). This can be considered an important parameter, which demonstrates a degree of AW classified as good, if we consider that the environment had leaking places, such as, for example, shade and water (64). However, if we observe the CI and THI, these are above the reference value, which can cause heat stress but is generally mitigated by the presence of trees. Corroborating with Albright and Arave (65), it was revealed that tail movements

observed in cattle were reported as a strong indicator of mood and well-being.

Scratching body parts using a limb was recorded 142 times, lasting from 15 to 37 s per execution. Licking one's own body or that of another animal was an uncommon practice among buffaloes, observed only 82 times with a duration of 30–37 s per behavior. Scratching and licking are commonly identified in the relationship between mother and calf; however, as observed in this study, these behaviors are positive and are observed in buffaloes over 20 months of age and not restricted to maternal coexistence. Scratching and licking are presented as expressions of social behavior when practiced through interaction between animals and are considered positive and/ or appropriate behaviors for dairy buffaloes (66).

However, interpretations of this behavior must be considered, particularly when associating this behavior with others. Degasperi et al. (66), when studying the behavior of cattle, found that the practice of licking oneself or another animal is a behavior inherited from wild ancestors, which had a function linked to cleaning and extracting salt from the animals' fur. According to the author, domestic cattle practice this behavior, which is relevant to social dynamics and hygiene, and another factor linked to this behavior is physiological, which signals a reduction in heart rate, giving a sense of calmness.

In this way, this study provides insights into the use of focal animal recording techniques to assess the welfare of buffaloes and their association with thermal comfort indices. The results have several implications for future research and practical applications:

- Improved welfare assessment: Focal animal recording techniques have proven to be effective in capturing and analyzing buffalo behavior under different climatic conditions. This approach offers a more detailed and complex understanding of buffalo welfare, enabling more informed management practices.
- Identification of thermal stress patterns: The study's results highlight the relationship between buffalo behavior and thermal comfort indices (THI and BCCCIp). This information can be crucial for identifying specific patterns of thermal stress and discomfort in buffaloes and guiding the development of targeted interventions.
- 3. Applicability to different climate zones: The success of the study in the Eastern Amazon region, which experiences thermal stress at different times of the day, suggests that focal animal recording techniques can be adopted and applied in various climatic conditions, making it a valuable tool for assessing buffalo welfare in different geographical regions.
- 4. Enhanced animal management: By understanding how buffalo behavior correlates with environmental factors such as temperature and humidity, farmers and animal welfare professionals can implement more effective management strategies. For example, adjusting feeding and resting schedules based on temperature fluctuations can help improve buffalo comfort and productivity.
- 5. Directions for future research: This study opens avenues for further research into buffalo welfare and the mitigation of thermal stress. Future studies can build upon these results to develop real-time monitoring systems or decision-support tools that assist farmers in making timely interventions to ensure the well-being of their buffalo herds.

4. Conclusion

The adaptation of the focal animal technique, with six observers evaluating each animal for 6 min through filming, proved to be efficient in identifying different behaviors of buffaloes raised in the fields of the Eastern Amazon under heat stress at different times of the day. Furthermore, it is currently possible to highlight a variation in behavior between morning and afternoon shifts, with grazing being more prevalent in the morning and rumination in shaded areas being more common in the afternoon. Therefore, the technique adequately reflects the behavioral repertoire of the species.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The experimental procedures were carried out in accordance with Brazilian law and were previously approved by the Animal Ethics Committee (CEUA/UFPA), under protocol number 9307300720. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

WS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Writing – review & editing. JS: Investigation, Methodology, Writing – review & editing. AG: Investigation, Methodology, Writing – original draft. AA: Investigation, Methodology, Writing – original draft. AB: Investigation, Methodology, Writing – original draft. ÉS: Investigation, Methodology, Writing – original draft. MP: Investigation, Methodology, Writing – original draft. RC: Investigation, Methodology, Writing – original draft. RC: Investigation, Methodology, Writing – original draft. AS:

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Conflict of interest

WS, JL-J, RC, and AS were employed by Brazilian Agricultural Research Corporation.

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