

## Different methods of processing thermographic images to evaluate the carpal temperature of healthy calves

*Diferentes métodos de processamento de imagens termográficas para avaliação da temperatura do carpo em bezerros saudáveis*

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### Abstract

The aim of this study was to compare three methods of processing thermographic images for the evaluation of carpal temperature in healthy calves. Additionally, we sought to evaluate the thermal symmetry between the contralateral carpi of these calves. Thermographic images were obtained from both carpi of eight healthy calves aged between 20 and 45 days with a mean weight of  $38.65 \pm 2.27$  kg. Using GRAYESS® IRT Analyzer 7 software, the total temperature ( $T_{tot}$ ), region-of-interest temperature ( $T_{roi}$ ), and maximum mean temperature ( $T_{max}$ ) were determined. There was no difference between the temperatures obtained by the  $T_{tot}$  and  $T_{roi}$  methods.  $T_{max}$  showed higher temperatures ( $p < 0.01$ ) than the other methods. The three image processing methods showed high and significant positive correlations for the temperature of the right and left carpi of healthy calves.  $T_{max}$  presented a higher correlation coefficient ( $r = 0.99$ ) than the two other methods, which suggests a greater sensitivity for identifying thermal variations among the contralateral carpi. The 99% confidence interval for the difference between the temperatures of the right and left carpi ( $\Delta r-l$ ) was between 0.03 and 0.26°C; thus, a temperature variation within that range does not refer to pathologic processes.

**Key words:** cattle; joint; thermal symmetry; thermography

### Resumo

Este estudo objetivou comparar três métodos de processamento de imagens termográficas na avaliação da temperatura do carpo de bezerros saudáveis. Adicionalmente, buscou-se avaliar a simetria térmica entre os carpos contralaterais desses bezerros. Foram obtidas imagens termográficas de ambos os carpos de oito bezerros, hígidos, com idade entre 20 e 45 dias e peso médio de  $38,65 \pm 2,27$  kg. As imagens foram analisadas por meio do *software* GRAYESS® IRT Analyzer 7, que possibilitou a obtenção da temperatura total ( $T_{tot}$ ), temperatura da região de interesse ( $T_{roi}$ ) e temperatura máxima média ( $T_{max}$ ). Não houve diferença entre as temperaturas obtidas pelos métodos  $T_{tot}$  e  $T_{roi}$ . O  $T_{max}$  mostrou temperaturas mais elevadas ( $p < 0,01$ ) em comparação aos demais métodos. Os três métodos de processamento de imagens apresentaram correlações elevadas e significativas para a temperatura dos carpos direito e esquerdo de bezerros saudáveis, entretanto, o  $T_{max}$  apresentou maior coeficiente de correlação ( $r = 0,99$ ), o que sugere maior sensibilidade para identificação de variações térmicas entre os carpos contralaterais. O intervalo de confiança de 99% para a diferença de temperaturas entre os carpos direito e esquerdo ( $\Delta d-e$ ) foi de 0,03 a 0,26°C, o que indica que a variação de temperatura dentro desse intervalo não remete a alteração patológica.

**Palavras-chave:** articulação; bovino; simetria térmica; termografia

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## Introduction

Septic arthritis is a common cause of lameness in calves. When not treated early, it compromises the animal's welfare, causing significant economic losses. Therefore, techniques enabling early diagnosis are essential for the complete recovery of the animal<sup>(1)</sup>. Infrared thermography (IRT) is a technique that allows

for the early detection of increased temperature caused by inflammatory processes in a fast and non-invasive manner<sup>2</sup>. In cattle practice, IRT has been used to diagnose mastitis<sup>3</sup>, hoof disorders<sup>4</sup>, pneumonia<sup>5</sup>, and omphalitis<sup>6</sup>. Although IRT has a wide application field, several technical recommendations must be followed during the examination to minimize variations in the ambient temperature and to prevent other factors from interfering with the interpretation of the results<sup>(2,7,8)</sup>.

The use of IRT in clinical practice is limited by the challenge of obtaining a mean temperature that is representative of the evaluated area as a thermographic image can comprise more than  $1280 \times 1024$  pixels, each with a temperature value<sup>(9)</sup>. Computer programs support different methods for choosing the anatomical region of interest (ROI), with each evaluation method generating different temperature means. Therefore, the choice of the method used to obtain the mean temperature of the evaluated region is fundamental for an accurate examination<sup>(2,10,11)</sup>. Methods of thermographic image analysis for the evaluation of the human skin temperature have been studied<sup>(9,10)</sup>; however, we did not find studies that compared methods for assessing the temperature of cattle. Moreover, there have been no studies on the thermal symmetry of the limbs of healthy bovines. This parameter can be characterized as the temperature difference between two contralateral regions of the body, which are identical in shape, size, and position<sup>(11)</sup>.

This study aimed to compare three thermographic image processing methods in evaluating the carpal temperature of healthy calves. Furthermore, we aimed to evaluate the thermal symmetry between the contralateral carpi in healthy calves.

## Material and methods

### Animals

This study was conducted after approval by the Ethics Committee on the Use of Animals at Federal University Goiás (UFG) (approval number, 015/18). Eight healthy male Holstein calves aged between 20 and 45 days, without lameness, and weighing  $38.65 \pm 2.27$  kg were used in this study. The animals were kept in individual stalls at the Experimental Farm of the Dairy Cattle of the UFG, in the municipality of Goiânia (Goiás, Brazil) and fed 2 L of milk twice daily, with access to bran concentrate and water *ad libitum*. The health of the calves was evaluated prior to thermographic examination through general and specific clinical examinations of the locomotor system<sup>(12)</sup>.

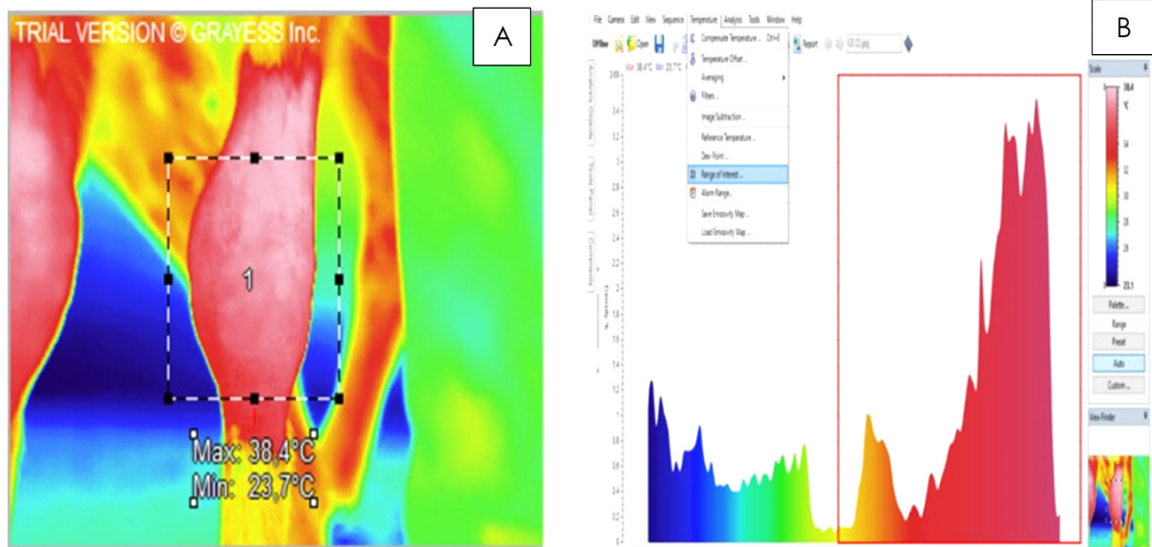
### Thermographic examination

Twenty-four hours prior to the thermographic examination, bilateral carpus trichotomy was performed using an Oster Agc<sup>®</sup> shearing machine and a No. 40 blade. Thermographic examination was performed using a FLIR<sup>®</sup> T440 thermographic camera T440 (Flir<sup>®</sup> Systems Inc., Wilsonville, Oregon, USA), with a spatial resolution of  $320 \times 240$  pixels and sensitivity to detect thermal differences of  $0.04^\circ\text{C}$ , configured with the following parameters: an emissivity of 0.98 (), ambient

temperature of  $27^\circ\text{C}$ , relative humidity of 59%, image acquisition distance of 0.5–1 m, and the rainbow option selected as the color palette. The temperature and relative humidity of the environment were measured using an Incoterm<sup>®</sup> TH50 (Incoterm<sup>®</sup>, Porto Alegre, RS, Brazil) digital thermohygrometer. The reflected temperature was determined and adjusted in each obtained image using aluminum foil, as described by Usamentiaga *et al.* (2014)<sup>(13)</sup>. The animals were transferred from the stalls to an area without direct sunlight and wind, after which the carpus region was cleaned with a dry, soft cloth. The calves were kept standing for 30 min prior to examination to avoid temperature changes associated with the possible presence of moisture and with the handling during cleaning of the carpal region. Individual thermographic images of the cranial aspect of each carpus were obtained 60 cm from the camera, which was positioned  $90^\circ$  to the carpus. All thermographic examinations were performed sequentially over a short period of time during the morning (08:00 – 09:00 AM) to avoid the influence of sudden temperature variations and circadian cycle effects.

### Thermographic image processing

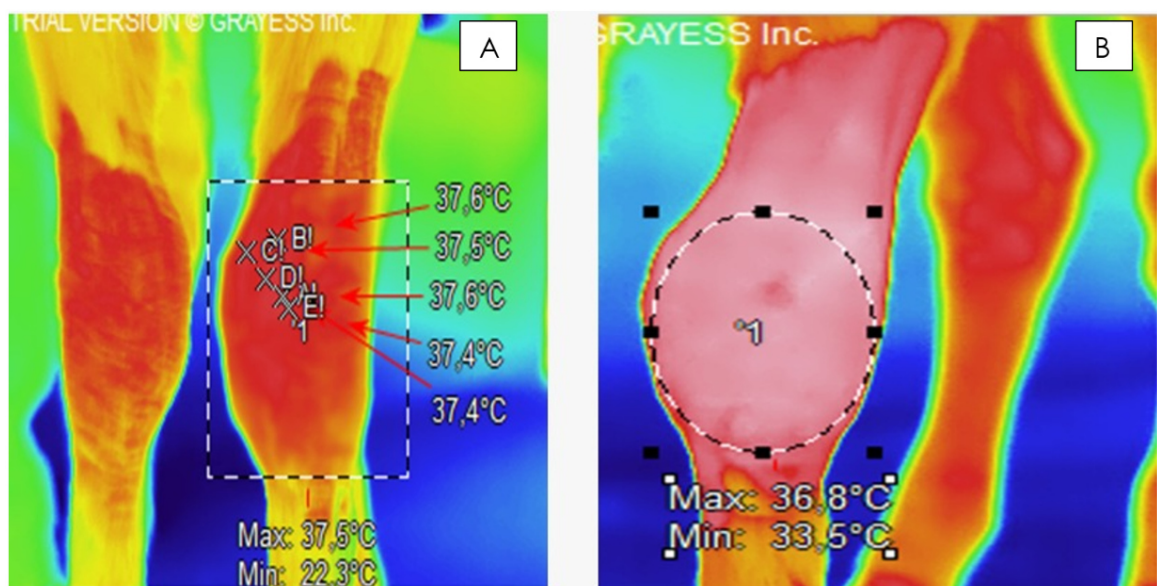
Thermographic images were analyzed using the GRAYESS<sup>®</sup> IRT Analyzer 7 (Grayess<sup>®</sup> Inc., Bradenton, Florida, USA) software. The total temperature ( $T_{\text{tot}}$ ), region-of-interest temperature ( $T_{\text{roi}}$ ), and mean maximum temperature ( $T_{\text{max}}$ ) were determined according to the methodology proposed by Ludwig *et al.* (10). The demarcation of the areas to be included in each thermographic image was based on the anatomy of the region, eliminating the need to use marker instruments. For the  $T_{\text{tot}}$  method, the geometric figure of a rectangle was applied to an area wider than the carpal region, extending from the distal radial epiphysis to the proximal metacarpal epiphysis (Figure 1A). A histogram was generated containing the temperatures identified inside the rectangle, which were represented by a colorimetric scale. In this scale blue represented lower temperatures and red represented higher temperatures. Subsequently, the temperatures corresponding to those observed in the carpus (ROI) (Figure 1A) were selected on the histogram, according to the colorimetric scale (Figure 1B). Colors that did not represent the carpal temperature (blue and green) were excluded for the  $T_{\text{tot}}$  calculation (Figure 1B).  $T_{\text{tot}}$  was determined through the arithmetic mean of the temperatures selected in the histogram.



**Figure 1.** (A) Thermography image processed by the total temperature ( $T_{tot}$ ) method. The geometric figure of a rectangle was applied to an area wider than the carpal region (region of interest), extending from the distal radial epiphysis to the proximal metacarpal epiphysis. (B) Histogram of the temperatures identified in Figure 1A. The temperatures selected in the red rectangle correspond to those observed in the carpus.

For the  $T_{max}$  method, a rectangle was applied to an area wider than the carpus (ROI), as described for the  $T_{tot}$ . The software was programmed to detect the five pixels of the highest temperature in the rectangle at a minimum distance of five pixels from each other. The representative carpal temperature was calculated by averaging the temperature over an area of  $5 \times 5$  pixels around the hottest pixels identified by the software (Figure 2A). For the  $T_{roi}$

method, an elliptical figure was applied and adjusted to the carpal region (ROI), with the upper margin limited to the distal region of the radius, the lower margin limited to the proximal region of the metacarpal, and the lateral and medial margins limited to the lateral and medial borders of the carpus, respectively (Figure 2B).  $T_{roi}$  final temperature was determined as the arithmetic mean of the temperature within the elliptical figure.



**Figure 2.** (A) Thermographic image processed by the mean maximum temperature ( $T_{max}$ ) method showing the five hottest carpal spots selected automatically by the analysis software. (B) Thermographic image processed by the region-of-interest temperature ( $T_{roi}$ ) method demonstrating the use of an ellipse to delimit the carpus as the region of interest.

*Statistical analysis*

The obtained data were tabulated and tested for homoscedasticity and normality using Levene's and Shapiro-Wilk tests, respectively, with SAS software (version 8.0; SAS Institute, Inc., Cary, NC). The effect of the thermographic image processing method on the carpal temperature and the difference between the temperatures of the right and left carpi ( $\Delta r-l$ ) were evaluated by analysis of variance in a general linear model using Proc GLM in the SAS software, followed by the Tukey test;  $p$ -values  $< 0.01$  were considered significant. The Pearson's correlation coefficient ( $r$ ) between the right and left carpal temperatures was also obtained for each of the three processing methods;  $p$ -values  $< 0.01$  were considered significant. To classify the degree of correlation, the

following parameters were used: weak when  $0 < r < 0.4$ , moderate when  $0.4 < r < 0.7$ , and strong when  $0.7 < r < 1.0$ . Additionally, the confidence interval for the thermal symmetry between the contralateral carpus at the 99% level was calculated based on the mean and standard deviation of  $\Delta r-l$ .

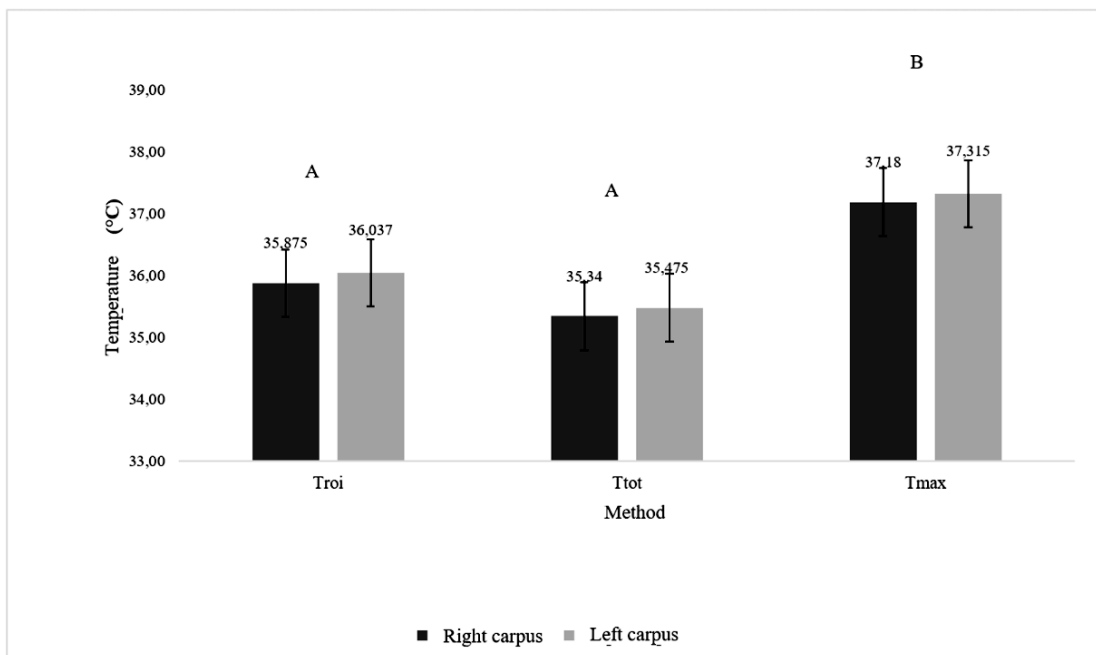
**Results and discussion**

There were no differences between temperatures obtained by the  $T_{roi}$  and  $T_{tot}$  methods; however, the  $T_{max}$  method resulted in significantly higher temperatures ( $p < 0.01$ ) compared to the other methods (Table 1 and Figure 3). There were no differences between the temperatures of the right and left carpi within each evaluated method ( $T_{tot}$ ,  $p = 0.31$ ;  $T_{roi}$ ,  $p = 0.58$ ;  $T_{max}$ ,  $p = 0.45$ ) (Table 1).

**Table 1.** Mean temperature, thermal symmetry between right and left carpi ( $\Delta r-l$ ), and Pearson's correlation coefficient ( $r$ ) between the temperatures of the right and left carpi in healthy calves according to the thermographic image processing method

Method	Carpal temperature (°C)		Thermal symmetry ( $\Delta r-l$ )	Pearson's correlation coefficient ( $r$ )
	Right	Left		
$T_{max}$	37.180 ± 0.711 <sup>Ba</sup>	37.315 ± 0.677 <sup>Ba</sup>	0.1350 ± 0.115 <sup>A</sup>	0.99*
$T_{roi}$	35.875 ± 0.604 <sup>Aa</sup>	36.037 ± 0.545 <sup>Aa</sup>	0.1625 ± 0.283 <sup>A</sup>	0.88*
$T_{tot}$	35.340 ± 0.580 <sup>Aa</sup>	35.475 ± 0.550 <sup>Aa</sup>	0.1375 ± 0.256 <sup>A</sup>	0.90*

$T_{roi}$ : temperature of the region of interest;  $T_{tot}$ : total temperature;  $T_{max}$ : mean maximum temperature  
 $\Delta r-l$ : difference between the temperatures of the right and left carpi  
<sup>A, B</sup> Different capital letters in the same column indicate statistical difference ( $p < 0.01$ ) by Tukey's test.  
<sup>a, b</sup> Different lowercase letters in the same row indicate statistical difference ( $p < 0.01$ ) by the Tukey test.  
\* Significant Pearson correlation coefficient ( $r$ ) ( $p < 0.01$ ).



**Figure 3.** Means and standard errors of the right and left carpal temperatures in healthy calves according to thermographic image processing method.

<sup>AB</sup> Different capital letters indicate a statistical difference by the Tukey test ( $p < 0.01$ ).



The absence of a difference between  $T_{roi}$  and  $T_{tot}$  indicates that the two methods are equivalent for evaluating calves' carpal temperature. This is due to the two methods using the arithmetic mean of the temperature of all pixels in the selected area. In the methodology used for  $T_{tot}$ , the exclusion of pixels outside the anatomical ROI was performed using the histogram of image temperatures (Figure 1B), resulting in mean temperatures matching those found for  $T_{roi}$ . As expected, the  $T_{max}$  method showed significantly higher mean temperatures compared to those for  $T_{tot}$  and  $T_{roi}$ , as the temperature of the ROI was calculated by the arithmetic mean of the five hottest points in the evaluated region.

Our results are similar to those of Ludwig et al.<sup>(10)</sup>, who compared the performance of these three methods in the thermographic evaluation of calf skin in humans. According to these authors, the  $T_{roi}$  method allows for the assessment of the temperature from large areas of the body, which may have anatomical differences that influence skin temperature, such as superficial irregularities and the presence of underlying fat or muscle tissue. Consequently, large thermal variations can occur within the area evaluated in the  $T_{roi}$  method, generating temperature distribution outside the normality curve (Gauss curve)<sup>(10)</sup>. This large thermal variation in large areas can be minimized using the  $T_{tot}$  method, which allows the removal of temperatures outside the area of interest, as shown in Figure 1. In clinical practice, the use of the  $T_{tot}$  method can be advantageous when the region to be evaluated has not been adequately prepared, such as the presence of dirt in the ROI.

The  $T_{max}$  method has the advantage of calculating the representative temperature of the region based on the same number of pixels, regardless of anatomical variations and temperature heterogeneity in the region evaluated<sup>14</sup>. This last aspect is especially important in the evaluation of ROIs in warmer areas due to the distribution of cutaneous blood vessels or inflammation<sup>9</sup>. Therefore, the  $T_{max}$  method seems to be more suitable for evaluating temperatures during physical activity or pathological thermal patterns<sup>(10)</sup>.

For each of the three image processing methods, there was a significant and strong correlation ( $p < 0.01$  and  $0.7 < r < 1.0$ ) between the temperatures of the right and left carpi in healthy calves. This result can be explained by the low variation in the age of animals (20 to 45 days) and the similarity of physical effort, which preclude the occurrence of unilateral temperature variations in the limbs<sup>(10)</sup>.  $T_{max}$  presented the highest correlation coefficient ( $r = 0.99$ ), indicating that this method has greater sensitivity to compare temperatures of healthy contralateral anatomical regions, allowing the identification of small thermal variations. In clinical practice, use of the  $T_{max}$  method may represent an interesting technique for the early diagnosis of

inflammatory processes. Contrary to the results of the present study, Ludwig et al.<sup>(10)</sup> found a lower correlation coefficient for the  $T_{max}$  method than for the  $T_{roi}$  and  $T_{tot}$ . This difference may be associated with the population and anatomical region evaluated, since that study<sup>(10)</sup> compared the calf skin temperature of men and women aged between 15 and 45 years old, who performed different levels of physical activity. Therefore, these authors may have found a higher thermal difference between the skin of the contralateral calves due to unilateral blood flow changes caused by injuries, pathological conditions<sup>(15)</sup> and training level<sup>(16)</sup>.

There were no differences among the evaluated processing methods in terms of the difference in temperature between the right and left carpi ( $\Delta r-l$ ) (Table 1). Moreover, it was found that the 99% confidence interval for  $\Delta r-l$  was 0.03 to 0.26 °C, which represents the variation in thermal symmetry between the contralateral carpi of healthy calves under the conditions of this study. In this context, no studies were found in cattle that establish physiological patterns of temperature variation between contralateral joints. It is expected that healthy contralateral anatomical structures under similar environmental conditions present small temperature variations, as observed in humans<sup>(9,11)</sup>, dogs<sup>(17)</sup> and horses<sup>(18)</sup>. Knowing the thermal symmetry between contralateral joints is essential to identify temperature variations that exceed these limits, which may indicate pathological processes<sup>(11)</sup>. Therefore, this result can be useful for future research on thermal symmetry of the carpal joint of healthy calves for the early diagnosis of joint pathological processes.

## Conclusions

The  $T_{roi}$  and  $T_{tot}$  thermographic image processing methods generate similar temperatures in the evaluation of the carpus in healthy calves; however, the  $T_{max}$  method generates higher temperatures. The three methods provide representative temperatures and high correlation between the right and left carpi in these animals. However,  $T_{max}$  has greater accuracy to detect small temperature variations between the contralateral carpus, due to its higher correlation coefficient. Furthermore, the thermal symmetry between the contralateral carpi of healthy calves varies between 0.03 and 0.26°C.

## Conflict of interest

The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

## Author contributions

*Conceptualization:* P.A.C. Borges, D.C. Silva, N.A.A.

da Silva, N.C. Borges, P.J.B. Queiroz, L.A.F. Silva; *Data curation*: P.A.C. Borges; *Formal analysis*: P.A.C. Borges, D.C. Silva; P.J.B. Queiroz; *Acquisition of financing*: L.A.F. Silva; *Research*: P.A.C. Borges, D.C. Silva, N.A.A. Silva, V.H. Lima; *Supervision*: L.A.F. Silva, N.C. Borges; *Writing (outline and original)*: P.A.C. Borges; *Writing (review and editing)*: P.A.C. Borges, D.C. Silva, P.J.B. Queiroz.

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