Inclusion of licuri cake in high-grain diets for steers: Intake, digestibility, carcass characteristics, and meat quality

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Abstract

A high-grain diet is essential to maximize growth and yield as well as to provide high-quality meat in beef cattle. This study evaluated the effects of including licuri cake in high-grain diets for steers on performance and carcass and meat quality. Forty-four crossbred (1/2 Bos taurus × 1/2 Bos indicus) castrated steers at 24 months of age, with an initial weight of 358.19 ± 41.57 kg, were used. The animals were distributed into four treatments that consisted of diets including licuri cake (0, 85, 170, or 255 g/kg, dry matter basis). Dry matter intake and digestibility decreased with the inclusion of licuri cake in the diet. However, the addition of the ingredient did not influence the intakes of total digestible nutrients (6.7 ± 0.5 kg/day) or crude protein. Licuri cake inclusion did not influence weight gain (1.5 ± 0.2 kg/day) or hot carcass weight (255.1 ± 24.9 kg). There was also no effect on carcass fat thickness, whereas the cholesterol content in the meat decreased with the addition of the cake. We recommend the inclusion of up to 255 g/kg licuri cake in the diet of feedlot steers fed high-concentrate diets.

Keywords: meat cholesterol, oilseed by-product, protein source, Syagrus coronata

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Introduction

The growing demand for quality meat has culminated in increased use of high-grain diets for feedlot cattle (Silvestre & Millen, 2021). However, in addition to being costlier than pasture feeding, the use of grains to feed ruminants competes with human food. In this respect, alternative ingredients that reduce the diet cost and do not cause this competition, such as agro-industrial by-products, are promising sources of energy and protein for high-grain diets for ruminants (Salami et al., 2019).

Licuri cake is a by-product of almond oil extraction. Its chemical composition includes (mean ± standard deviation) 235 ± 18 g/kg crude protein (CP), 112 ± 37 g/kg ether extract (EE), and 474 ± 97 g/kg neutral detergent fiber (NDF). In addition, licuri cake has an in vitro dry matter (DM) digestibility of ~81%, and ~71% of its CP consists of the B1+B2 fraction (Silva et al., 2014). Some studies have shown that the inclusion of 80–130 g/kg licuri cake (DM basis) in diets with a roughage content of 500 g/kg reduced weight gain and carcass weight in sheep (Santos et al., 2015; Costa et al., 2016a). Borja et al. (2010) recommended the inclusion of up to 450 g/kg licuri cake in the diet of feedlot goats, a value close to the 400 g/kg recommended by Ferreira et al. (2017) in the supplement of grazing dairy cattle. However, studies evaluating the inclusion of oilseed by-products, especially licuri, in high-grain diets for cattle are scarce.

We hypothesize that due to the high levels of NDF and EE present in licuri cake, the inclusion of this by-product in high-grain diets for cattle reduces the probability of metabolic disorders in animals. Moreover, its inclusion would reduce the use of maize grain and soybean meal in the cattle diet, reducing competition with human food, without compromising animal performance.
The objective of this study was to evaluate the effect of including licuri cake in high-grain diets for steers on intake, digestibility, performance, carcass traits, and meat lipid content.

**Material and methods**

This study was conducted in strict compliance with the Brazilian law on research involving the use of animals, adopted by the National Council for Experimental Control (CONCEA). The experimental procedures were undertaken according to the rules of the Ethics Committee on the Use of Animals at the State University of Southwest Bahia (CEUA-UESB; approval no. 147/2017).

The field study was carried out on a farm located in the municipality of Ribeiro do Largo, southwest region of the state of Bahia, Brazil (15°09'07" S, 40°15'32" W, 709 m above sea level). According to the Köppen classification, the region has a humid tropical climate, with average annual precipitation of 800 mm and average annual temperature of 27 °C. The experiment was conducted from March to June 2017, covering a period of 105 days, of which the first 14 days were used as a period of adaptation to the diets and facilities, followed by 91 days of data collection. Forty-four crossbred (1/2 Bos taurus × 1/2 Bos indicus), castrated steers at an average age of 24 months, with an average live weight of 358.19 ± 41.57 kg, were used. The animals were housed in collective stalls (11 animals/stall) with a usable area of 100 m², which was equipped with covered feeders (10 linear meters) and concrete drinking troughs with a capacity of 250 L of water. The steers were fed fresh rice husk and concentrate, at a roughage:concentrate (R:C) ratio of 15:85. The animals were fed fresh rice husk and concentrate, at a roughage:concentrate (R:C) ratio of 15:85. At the beginning of the experimental period, the steers were identified with numbered plastic ear tags, vaccinated, and weighed.

The 44 steers were distributed randomly into four treatment groups (diets containing 0, 85, 171, or 255 g/kg licuri cake, on a total diet dry matter [DM] basis) in a completely randomized design. Each animal was considered an experimental unit. The diets were formulated according to the NASEM (2016), to meet the nutritional requirements for a gain of 1.4 kg/day (Tables 1 and 2). The animals were fed ad libitum, with the feed divided into two daily supplies (07h00 and 16h00), allowing ~10% ors.

**Table 1 Chemical composition of the feedstuffs used in the experimental diets (g/kg DM)**

<table>
<thead>
<tr>
<th>Feedstuffs</th>
<th>Rice husk</th>
<th>Grain maize</th>
<th>Grain sorghum</th>
<th>Licuri cake</th>
<th>Soybean meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (g/kg FM)*</td>
<td>899</td>
<td>905</td>
<td>901</td>
<td>899</td>
<td>910</td>
</tr>
<tr>
<td>Crude protein (g/kg DM)*</td>
<td>35</td>
<td>101</td>
<td>94</td>
<td>248</td>
<td>506</td>
</tr>
<tr>
<td>Ether extract (g/kg DM)*</td>
<td>25</td>
<td>50</td>
<td>30</td>
<td>70</td>
<td>22</td>
</tr>
<tr>
<td>NDFap (g/kg DM)*</td>
<td>831</td>
<td>168</td>
<td>118</td>
<td>567</td>
<td>120</td>
</tr>
<tr>
<td>NFC (g/kg DM)*</td>
<td>41</td>
<td>666</td>
<td>744</td>
<td>61</td>
<td>295</td>
</tr>
<tr>
<td>Lignin (g/kg DM)*</td>
<td>121</td>
<td>17</td>
<td>18</td>
<td>134</td>
<td>50</td>
</tr>
<tr>
<td>Ash (g/kg DM)*</td>
<td>66</td>
<td>13</td>
<td>12</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>NDIN*</td>
<td>7.9</td>
<td>4.2</td>
<td>8.0</td>
<td>12.1</td>
<td>2.7</td>
</tr>
<tr>
<td>ADIN*</td>
<td>9.0</td>
<td>1.9</td>
<td>7.6</td>
<td>25.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*Fresh matter; *Dry matter; *Neutral detergent fibre corrected for ash and protein; *Non-fibrous carbohydrates; *Neutral detergent insoluble nitrogen; *Acid detergent insoluble nitrogen; *Value expressed in % of total nitrogen

Samples of feed, ors, and faeces were evaluated for the contents of DM (method INCT-CA G-003/1); mineral matter (method INCT-CA M-57 001/1); crude protein (CP, method INCT-CA N-001/1); ether extract (EE, INCT-CA method G-004/1); neutral detergent fibre corrected for ash and protein (NDF, methods INCTCA F-002/1, INCT-CA M-002/1, and INCT-CA N-004/1); acid detergent fibre (ADF, method INCT-CA F-004/1); and lignin (method INCTCA F-005/1), according to techniques described by Detmann et al. (2012). Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined according to the methodology proposed by AOAC (2000).

Non-fibrous carbohydrates corrected for ash and protein (NFC) were calculated according to the formula described by Detmann et al. (2010):

\[
\text{NFC} = 100 - (\%\text{CP} + \%\text{EE} + \%\text{MM} + \%\text{NDFap})
\]

where \%\text{CP} = crude protein content; \%\text{EE} = ether extract content; \%\text{MM} = ash content; and \%\text{NDFap} = neutral detergent fibre content corrected for ash and protein.

Total digestible nutrients were calculated as:

\[
\text{TDN} = (\text{DCP} + (\text{DEE} \times 2.25) + \text{DNDF} + \text{DNFC})
\]

where DCP = digestible crude protein; DEE = digestible ether extract; DNDF = digestible neutral detergent fibre; and DNFC = digestible non-fibrous carbohydrates.
To estimate faecal output, titanium dioxide (TiO$_2$) was used as an external marker, which was provided daily at 07h00, in a single dose of 10 g per animal/day, inside paper cartridges that were introduced orally for a period of 11 days. The first seven days were used to regulate the marker’s flow in the gastrointestinal tract of the animals and to adapt them to the management, and the other five days for faecal collection (Smith & Reid, 1955). Faeces were collected directly in the stalls, once daily, at five pre-established times (08h00, 10h00, 12h00, 14h00, and 16h00), for five consecutive days. Subsequently, the faeces were stored in plastic bags and frozen at -10 °C. The five samples from each animal were placed in a single composite sample for further analysis. Titanium dioxide was analysed according to the methodology described by Detmann et al. (2012). Subsequently, faecal output was calculated as proposed by Detmann et al. (2012), using the following formula:

$$\text{FO} \ (\text{kg/days}) = (\text{PurTiO}_2\text{Sup/TiO}_2\text{Pre}) \times 100,$$

where FO: faecal output (kg/day); PurTiO$_2$Sup: purity of the titanium dioxide supplied (g); and TiO$_2$Pre: titanium dioxide present (% DM).

The daily DM intake of each animal was estimated using indigestible neutral detergent insoluble fibre (iNDF), after in situ incubation of samples of feed (supplied and orts) and faeces inside non-woven fabric (“TNT”) bags (5 × 5 cm) for 288 h, according to the method described by Detmann et al. (2012). After obtaining the above data, the following formula was used to determine the total individual DM intake:

$$\text{TDI} \ (\text{kg/days}) = \left( \text{FO} \times \text{iNDFfaeces}\right) / \text{iNDFDiet},$$

where FO: faecal output (kg/days), obtained using titanium dioxide; iNDFfaeces: indigestible neutral detergent fibre in the faeces (kg); and iNDFDiet: indigestible neutral detergent fibre in the diet (kg).

The apparent digestibility of nutrients (D) was determined using the formula:

$$D = \left( \frac{\text{kg nutrient intake} - \text{kg nutrient output}}{\text{kg nutrient intake}} \right) \times 100.$$
FC = (DMI/ADG),
where DMI: daily total dry matter intake in kg; and ADG: average daily gain in kg.

At the end of the experiment, the animals were slaughtered in a commercial abattoir in the region, according to rules set forth in Normative Instruction No. 3, of January 17, 2000, by the Ministry of Agriculture, Livestock and Supply, Brazil. The carcass of each animal was identified and sawn lengthwise along the sternum and spine, producing two similar halves, which were weighed to determine hot carcass weight (HCW). Hot carcass yield (HCY) was calculated as the ratio between hot carcass weight and final body weight:

\[
\text{HCY} = \frac{\text{HCW}}{\text{FBW}} \times 100,
\]
where HCY: hot carcass yield (%); HCW: hot carcass weight (kg); and FBW: final body weight (kg).

On the right side of the carcass, a cross-section was made between the 12th and 13th ribs, exposing the *Longissimus dorsi*. The muscle was cut and the samples were later packed (first in plastic wrap; then in aluminium foil; and then in plastic bags, previously marked with animal and treatment). These were immediately stored at a temperature of -10 °C until laboratory analysis, which took place in the Laboratory of Chemical Methods and Separations (LABMESQ-UESB).

Cholesterol in the *Longissimus dorsi* muscle was extracted, detected, identified, and quantified according to the methodology described by Saldanha et al. (2006). To determine the total lipid content in the muscle, meat samples were processed and later analysed following the method proposed by Bligh & Dyer (1959) and Saldanha et al. (2006).

Results were interpreted statistically using analysis of variance and orthogonal contrasts, by applying the following mathematical model:

\[
Y_{ijk} = m + T_i + e_{ijk},
\]
where \(Y_{ijk}\): observed value of the variable; \(m\): general constant; \(T_i\): effect of treatment \(i\); and \(e_{ijk}\): error associated with each observation.

**Results**

The intakes of DM and non-fibrous carbohydrate (NFC) by the steers decreased linearly \((P<0.05)\) with the inclusion of licuri cake in the diet. However, the addition of the ingredient did not influence \((P>0.05)\) the intakes of CP or NDF. Ether extract intake showed a quadratic response \((P<0.05)\), with a maximum point occurring at the licuri cake level of 170 g/kg in the diet (Table 3).

**Table 3** Nutrient intake and apparent digestibility in feedlot steers fed high-concentrate diets with licuri cake

<table>
<thead>
<tr>
<th>Intake (kg/day)</th>
<th>Licuri cake level (g/kg DM)</th>
<th>SEM*</th>
<th>(P) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>85</td>
<td>170</td>
</tr>
<tr>
<td>Dry matter</td>
<td>10.44</td>
<td>10.24</td>
<td>9.11</td>
</tr>
<tr>
<td>Crude protein</td>
<td>1.67</td>
<td>1.52</td>
<td>1.33</td>
</tr>
<tr>
<td>Ether extract</td>
<td>0.31</td>
<td>0.37</td>
<td>0.44</td>
</tr>
<tr>
<td>NDFap&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>3.51</td>
<td>3.56</td>
<td>3.48</td>
</tr>
<tr>
<td>Non-fibrous carbohydrates</td>
<td>5.02</td>
<td>5.14</td>
<td>4.26</td>
</tr>
<tr>
<td>Total digestible nutrients</td>
<td>7.59</td>
<td>7.56</td>
<td>6.21</td>
</tr>
<tr>
<td>Digestible (g/kg)</td>
<td>731</td>
<td>730</td>
<td>691</td>
</tr>
<tr>
<td>Dry matter</td>
<td>574</td>
<td>527</td>
<td>461</td>
</tr>
<tr>
<td>Ether extract</td>
<td>616</td>
<td>641</td>
<td>644</td>
</tr>
<tr>
<td>NDFap&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>537</td>
<td>559</td>
<td>486</td>
</tr>
<tr>
<td>Non-fibrous carbohydrates</td>
<td>856</td>
<td>823</td>
<td>767</td>
</tr>
</tbody>
</table>

<sup>*</sup>Neutral detergent insoluble fibre corrected for ash and protein. <sup>†</sup>Standard deviation of the mean.  
<sup>‡</sup>Significance of P for contrast of linear (L) or quadratic (Q) order. Regression equations: \(y = -0.0876 + 10.619, R^2 = 0.943\); \(y = -0.000625x^2 + 0.017x + 0.301, R^2 = 0.82580\); \(y = -0.053x + 5.216, R^2 = 0.8385\); \(y = -0.2946x + 74.03, R^2 = 0.9118\); \(y = -0.0457x^2 + 0.8599x + 613.14, R^2 = 0.9512\); \(y = -0.3094x + 552.85, R^2 = 0.6728\); \(y = -0.525x + 858.8, R^2 = 0.9865\).

The apparent digestibility coefficients of DM, NDF, and NFC decreased linearly \((P<0.001\) and \(P<0.05\)) with the inclusion of licuri cake in the diet. The apparent digestibility coefficient of CP did not differ \((P>0.05)\) between the treatments.
The inclusion of licuri cake in the steer diet did not influence \((P > 0.05)\) FBW, ADG, HCW or backfat thickness. However, HCY decreased linearly \((P < 0.05)\) and ribeye area showed a quadratic response \((P < 0.05)\) to the dietary inclusion of the ingredient, with maximum values attained at the level of 85 g/kg (Table 4).

**Table 4** Performance and carcass traits of steers fed high-concentrate diets with inclusion of licuri cake

<table>
<thead>
<tr>
<th>Licuri cake level (g/kg DM)</th>
<th>SEM(^2)</th>
<th>(P) value(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>85</td>
<td>170</td>
</tr>
<tr>
<td>Initial weight (kg)</td>
<td>361.2</td>
<td>343.6</td>
</tr>
<tr>
<td>Final weight (kg)</td>
<td>496.4</td>
<td>499.1</td>
</tr>
<tr>
<td>Average daily gain (kg/day)</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Feed conversion (kg/kg)</td>
<td>7.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Hot carcass weight (kg)</td>
<td>257.1</td>
<td>260.6</td>
</tr>
<tr>
<td>Hot carcass yield (%)</td>
<td>51.8</td>
<td>52.2</td>
</tr>
<tr>
<td>Arroba (animal weight unit: (\pm) 15 kg)</td>
<td>16.5</td>
<td>17.3</td>
</tr>
<tr>
<td>Backfat thickness (mm)</td>
<td>5.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Ribeye area (cm(^2))</td>
<td>62.1</td>
<td>71.8</td>
</tr>
</tbody>
</table>

\(^1\)Standard error of the mean. \(^*\)Significance of \(P\) for contrast of linear (L) or quadratic (Q) order. Regression equations:

1. \(y = -0.0786x + 52.331\), \(R^2 = 0.6284\);
2. \(y = -0.0454x^2 + 1.1171x + 63.046\), \(R^2 = 0.6739\)

The total fat content in the steers’ meat responded quadratically \((P < 0.05)\), with maximum fat observed at the licuri cake inclusion level of 170 g/kg in the diet. The cholesterol content of the meat decreased linearly \((P < 0.05)\) with the inclusion of the ingredient (Figure 1).

**Figure 1** Total lipid and cholesterol contents in the meat of steers fed high-concentrate diets with the inclusion of licuri cake. Total lipids (g/kg), standard deviation of the mean (SEM) = 1.168. \(P\) value = 0.043. Equation: \(y = -0.0005x^2 + 0.1346x + 22.29\), \(R^2 = 0.7697\); Cholesterol (mg/100 g). SEM = 1.968. \(P\) value = 0.036. Equation: \(y = -0.0069x + 38.017\), \(R^2 = 0.8499\)

**Discussion**

The decreasing DM intake of the steers in response to the inclusion of licuri cake can be attributed to higher NDF and EE contents of the experimental diets provided by the inclusion of the by-product. The greater presence of NDF derived from licuri cake, in association with EE, possibly reduced the rate of passage and disappearance of rumen DM (Silva *et al*., 2015), thereby reducing DM intake by the animals. Santos *et al.* (2020) also ascribed the decrease in DM intake of cattle to increases in the NDF and EE contents of diets including licuri cake.

Even with the total replacement of soybean meal with licuri cake, there was no influence on DM intake, reinforcing the potential of licuri cake as a protein concentrate for ruminants, as described by Silva *et al.* (2014). According to the Brazilian system (BR-Corte, 2016), the protein requirements of male crossbred zebu with a body weight of 350–450 kg and weight gain of 1.40 kg/day ranges between 1.05 and 1.09 kg/day. Considering
that the animals in this study consumed between 1.20 g and 1.67 kg of protein/day, there was no loss in rumen microorganism activity, suggesting it is possible to meet the protein requirement of cattle.

The maximum EE intake seen at the level of 170 g/kg represented approximately 4.8% DM of the ingested diet, which is within acceptable limits (5–6% in the diet DM) that do not cause toxic effects for ruminal microorganisms. However, the increased intake of the medium-chain saturated fatty acids, C12:0 and C14:0, present in the licuri cake (Silva et al., 2021), may be related to DM intake depression mechanisms of cattle, induced by the production of cholecystokinin and ghrelin, peptides that inhibit feed intake (Bradford et al., 2008; Fukomori et al., 2013).

It is likely that the reduction of around 20% in the amount of maize grain in the experimental diets is the main responsible for the decreased intake of NFC from the experimental diets. Moreover, the full replacement of soybean meal (295 g/kg NFC, DM basis) with licuri cake (61 g/kg NFC, DM basis) also caused the NFC content of the experimental diets to decrease. Borja et al. (2015) observed a 63% reduction in the A+B1 carbohydrate fractions of the diets when licuri cake was included in the diet of goats. Non-fibrous carbohydrates are highly degradable constituents and important sources of energy for ruminants; thus, their lower intake stemming from the increasing inclusion of licuri cake in the diet reduced DM degradability (Hall & Mertens, 2017).

The apparent digestibility of DM declined with the inclusion of licuri cake in the cattle feed due to the increase in the NDFap and lignin contents and the reduction in the NFC contents of the experimental diets. These results concur with those observed by Costa et al. (2016a), who reported a decrease in the digestibility of DM from diets containing licuri cake, which the authors ascribed to the high concentrations of NDF, ADF, and lignin in the diets.

Dietary inclusion of licuri cake did not influence the apparent digestibility of CP, possibly due to the similar quality of the protein that arrived in the small intestine for digestion between the different experimental diets (Schwab & Broderick, 2017). We propose that despite being richer in NDIN and ADIN than soybean meal, the use of licuri provided a lower rate of passage of fermentable organic matter due to its higher NDF content, resulting in greater synthesis of rumen microbial protein. Daza et al. (2020) also did not observe an effect of the inclusion of licuri nut on the apparent digestibility of CP in a sheep diet.

The decrease in dietary NDF digestibility was likely due to the increase in the levels of lignin and EE derived from the inclusion of licuri cake in the steer diets. Both lignin and EE affect the rumen degradability of NDF by hindering the access of microorganisms to the most degradable fractions of the cell wall. In a meta-analysis, Weld & Armentano (2017) showed the depressant effect of medium-chain fatty acids (C12:0 and C14:0), prevalent in licuri cake, on the digestibility of NDF in cattle. In this respect, Bagaldo et al. (2019) also found a decrease in NDF digestibility with the inclusion of licuri cake in sheep concentrate.

The lack of effect of the inclusion of 255 g/kg licuri cake on steer performance (weight gain, FBW, FC, and HCW) can be attributed to the similar digestible protein (0.74 ± 0.17 kg/day) and metabolizable energy (2.59 ± 0.13 Mcal/kg DM) contents ingested by the animals. Even with a decrease in DM intake and digestibility, the increase in fat intake possibly supported the nutrient flow that allowed tissue deposition and performance within the estimated range (1.4 kg/day) for the steers. Lisboa et al. (2021) also found that the inclusion of up to 240 g/kg palm kernel cake does not interfere with the weight gain of steers, despite reducing intake and the digestibility of the dietary DM.

Hot carcass yield decreased with the inclusion of licuri cake, possibly due to the increase in offal weight induced by the higher NDF content of the by-product. In addition, the gastrointestinal content of the animals that consumed more licuri cake may have remained longer in the rumen—reticulum during the pre-slaughter fast, which also resulted in a lower HCY. Costa et al. (2016a) and Silva et al. (2021) also observed a decrease in carcass yield following the inclusion of licuri cake in the diet of sheep and goats.

In steers fed high-concentrate diets, lipid levels in the meat are more influenced by EE intake in comparison to animals fed diets with a higher roughage content. In the present study, the peak of fat in meat (170 g/kg licuri cake) was close to the peak of EE intake (170 g/kg licuri cake). Costa et al. (2018) also observed an increase in the fat content of steers with the addition of licuri cake to the animal diet.

The decrease in the cholesterol level of the steers’ meat can be attributed to a reduction in NDF digestibility. Rumen fermentation of NDF releases mainly acetate, the main precursor of acetyl units that are used for cholesterol synthesis in bovine tissues. Thus, the decrease in NDF digestibility possibly induced a reduction in the availability of acetyl units for endogenous cholesterol synthesis. Complementing this, Costa et al. (2016b) found no effect of licuri cake inclusion on the plasma cholesterol of sheep.

**Conclusion**

Dietary inclusion of licuri cake reduced the intake and digestibility of the diet dry matter, but did not influence weight gain or carcass weight in confined steers. In addition, the cholesterol content in the meat of
steers decreased with the dietary inclusion of licuri cake. Therefore, we recommend the inclusion of up to 255 g/kg licuri cake in the diet of feedlot steers fed high-concentrate diets.

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Authors’ Contributions

A. B. de Oliveira, F. F. da Silva, J. W. D. da Silva, and G. G. P. de Carvalho participated in designing the study, laboratory analysis, and manuscript writing. L. V. Santos, T. R. Paixào, and A. P. G. da Silva were involved in drafting and revising the manuscript for important intellectual content. S. O. de Souza, C. Soares, D. M. de Lima Júnior, and R. R. Silva were responsible for data analysis and interpretation. D. M. de Lima Júnior, L. V. Santos, and R. R. Silva were involved in the preparation and revision of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References


