



Murumuru (*Astrocaryum murumuru*) meal as an additive to elephant grass silage

Torta de murumuru (Astrocaryum murumuru) como aditivo en el ensilado de pasto elefante

Torta de murumuru (Astrocaryum murumuru) como aditivo em silagens de capim elefante

Claudia M Serra-Ferreira ; Agatha G Farias-Souza ; Rita C Almeida-Mendonça ; Melany Simões-Souza ;
Wagner R L Lopes-Filho ; Cristian Faturi ; Felipe Nogueira-Domingues ; Anibal C do-Rêgo* .

Study Group on Ruminants and Forage Production of the Amazon, Universidade Federal Rural da Amazônia.

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Abstract

Background: Tropical grasses, such as elephant grass, have high moisture content during its ideal phenological state for silage. High moisture content hinders proper preservation and reduces the nutritive value of silage due to secondary fermentation and production of effluents. Adding feed materials with high dry matter content, such as murumuru (*Astrocaryum murumuru*) meal, is a potential alternative to improve silage yield. **Objective:** To determine the effects of including murumuru meal (0, 7, 14, 21, and 28%) on the fermentative characteristics, microbiological activity, aerobic stability, and chemical composition of elephant grass silages. **Methods:** A completely randomized design with five treatments and five replicates was used. Elephant grass was collected at 60 d of age, minced, and homogenized with murumuru meal. The mass was placed in experimental 15-L silos. The silos were collected and analyzed 45 d later. **Results:** Effluent production decreased ($p < 0.05$) as the proportions of murumuru meal in silage increased. A quadratic effect ($p < 0.05$) was observed on dry matter recovery. An increase ($p < 0.05$) was observed in dry matter content, a decrease ($p < 0.05$) in the neutral detergent fiber content, and an increase ($p < 0.05$) in the non-fibrous carbohydrate content with the inclusion of murumuru meal. **Conclusions:** Addition of murumuru meal improves chemical composition and does not affect the fermentative characteristics of elephant grass silage, while it reduces effluent losses. Nevertheless, the inclusion of murumuru meal in the elephant grass silage decreased the time of aerobic stability.

Keywords: additives; *Astrocaryum murumuru*; by-product; conservation; elephant grass; effluents; fermentation; forage; fodder; murumuru meal; *Pennisetum purpureum*; silage; silo; stability.

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*Corresponding author. Avenida Presidente Tancredo Neves, nº 2501, Belém, Pará, 66077-830, Brazil. E-mail: anibalcr@gmail.com



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Resumen

Antecedentes: los pastos tropicales, tales como el pasto elefante, tienen alto contenido de humedad cuando están en su estado fenológico ideal para ensilar. Esto dificulta su adecuada preservación en el silo, reduciendo el valor nutritivo del ensilaje debido a fermentaciones secundarias y generación de efluentes. Una posible solución sería incluir materiales con alto contenido de materia seca, tales como la torta de murumuru (*Astrocaryum murumuru*). **Objetivo:** determinar el efecto de la inclusión de torta de murumuru (0; 7; 14; 21 y 28%) sobre las características fermentativas, microbiológicas, estabilidad aeróbica y composición química de los ensilajes de pasto elefante. **Métodos:** se utilizó un diseño completamente al azar, con cinco tratamientos y cinco repeticiones. El pasto elefante fue colectado a los 60 días de edad, luego picado y homogeneizado con la torta de murumuru. La masa fue colocada en silos experimentales con capacidad de 15 L. Luego de 45 días de ensilado, los silos fueron abiertos y las muestras fueron colectadas para su posterior análisis. **Resultados:** hubo reducción ($p < 0,05$) en la producción de efluentes a medida que se incrementó la proporción de torta de murumuru en el ensilado. Se observó un efecto cuadrático ($p < 0,05$) en la recuperación de materia seca. Hubo aumento ($p < 0,05$) en los contenidos de materia seca con la adición de torta de murumuru. Se observó disminución ($p < 0,05$) en el contenido de fibra detergente neutra y aumento ($p < 0,05$) de carbohidratos no fibrosos. **Conclusión:** La adición de torta de murumuru mejora la composición química, reduce las pérdidas por efluentes y no afecta las características fermentativas de ensilado de pasto elefante. Sin embargo, la inclusión de la torta de murumuru en el ensilado de pasto elefante disminuye el tiempo en estabilidad aeróbica.

Palabras clave: aditivos; *Astrocaryum murumuru*; conservación; efluentes; ensilaje; estabilidad; fermentación; forraje; harina de murumuru; pasto elefante; pienso; *Pennisetum purpureum*; silo; subproducto.

Resumo

Antecedentes: capins tropicais, como por exemplo o capim-elefante apresentam alto teor de umidade quando possuem produtividade compatível e estão no estágio fenológico adequado para ensilagem. Isso dificulta a adequada preservação no silo, ocasionando a redução do valor nutritivo da silagem devido a fermentações secundárias e a produção de efluentes. Uma possível solução seria o uso de aditivos com alto teor de matéria seca, como bolo de murumuru (*Astrocaryum murumuru*). **Objetivo:** determinar o efeito da inclusão da torta de murumuru (0; 7; 14; 21 e 28%) na ensilagem de capim-elefante sobre as características fermentativas, microbiológicas, estabilidade aeróbia e a composição química das silagens. **Métodos:** utilizou-se um delineamento inteiramente casualizado, com cinco tratamentos e cinco repetições. O capim-elefante foi colhido aos 60 dias de idade, o mesmo foi picado e homogeneizado à torta de murumuru. A massa foi ensilada em silos experimentais com capacidade de 15 L. Após 45 dias de ensilagem, os silos foram abertos e amostras foram coletadas para posteriores análises laboratoriais. **Resultados:** houve redução ($p < 0,05$) na produção de efluentes à medida que se elevaram as proporções da torta de murumuru na ensilagem. Observou-se efeito quadrático ($p < 0,05$) na recuperação de matéria seca. Houve aumento ($p < 0,05$) nos teores de matéria seca com a adição da torta murumuru. Observou-se diminuição ($p < 0,05$) nos teores de fibra em detergente neutro e aumento ($p < 0,05$) nos teores de carboidratos não fibrosos. **Conclusão:** torta de murumuru como aditivo melhora a composição química, reduz perdas principalmente por efluente e não afeta as características fermentativas de silagens de capim-elefante. Entretanto, a inclusão da torta de murumuru na ensilagem de capim-elefante diminui o tempo em estabilidade aeróbia.

Palavras-chave: aditivos; *Astrocaryum murumuru*; capim-elefante; conservação; efluentes; ensilagem; estabilidade; fermentação; forragem; *Pennisetum purpureum*; silagem; silo; subproduto; torta de murumuru.

Introduction

Effective preservation of tropical grass is a management strategy to ensure the availability of feed for animals throughout the year (Daniel *et al.*, 2019). Tropical grass silage species are easy to manage, have high biomass production, and high nutritional value during the wet period (Tomaz *et al.*, 2018). However, according to the same researchers, in this vegetative stage the grass has a high moisture content, which hinders its preservation in silage form and favors the growth of undesirable microorganisms (e. g., *Clostridia*), resulting in secondary fermentation and effluent production.

Among tropical grasses, elephant grass (*Pennisetum purpureum* Schum.) is one of the most widely used species in silage production because it is easy to handle and highly productive [approximately 80 tons of dry matter (DM)/ha/year] (Rocha *et al.*, 2017). However, similar to other tropical grass species, elephant grass has high moisture content (75–82%) at the ideal cutting stage, thereby strategies are needed to improve the fermentative profile of the silage (Santos *et al.*, 2013). Absorbent additives may be used to improve the fermentation process and reduce effluent losses in tropical grass silages. These additives act as moisture absorbing agents, increase the DM content of the ensiled material, produce minimal leachate, and indirectly inhibit the action of spoilage microorganisms (Daniel *et al.*, 2019). Thus, additives can improve silage quality of tropical grasses (Rêgo *et al.*, 2010).

The Amazon biome holds a high diversity of oilseeds that are used in agribusiness to produce cosmetics, fuels, food, and other products (Funasaki *et al.*, 2016). Among these, murumuru (*Astrocaryum murumuru*) stands out as a promising species for oil extraction, which generates a byproduct during this process (Mota *et al.*, 2015). Usually, such byproduct has a high DM (>85%) and satisfactory chemical composition, making it suitable for inclusion in ruminant diets (Menezes *et al.*, 2016).

The objective of this study was to determine the effects of including increasing concentrations of murumuru meal on the fermentative characteristics, microbiological activity, aerobic stability, and chemical composition of elephant grass silage.

Materials and methods

Elephant grass was grown in a grassland area, located at 1°27'07"S, 48°26'14"W, at an altitude of approximately 15 m. The climate of the region, according to the Köppen-Geiger classification, is Af type. The grass was harvested manually near ground level at 60 d of growth and then processed in a forage chopper to obtain 1.0 cm theoretical particle size (Nogueira, Itapira, São Paulo, Brazil). The material was ensiled in 15 L plastic buckets, with sand and non-woven fabric (TNT) on the bottom to quantify effluent production. An average of 15 ± 2 kg of forage or forage natural matter (NM) was ensiled in each silo, at 600 kg/m³ density.

Murumuru meal was sourced from a local company in Ananindeua, Pará (Brazil). The meal is the byproduct of mechanical extraction of oil from murumuru seeds. Before ensiling, elephant grass and murumuru meal samples were collected for chemical characterization (Table 1). Several murumuru meal concentrations [0.0, 7.0, 14.0, 21.0, and 28.0%, depending on the NM of the ensiled mass] included in elephant grass ensiling were evaluated.

After processing, elephant grass and murumuru meal were weighed, homogenized, and compacted in the silos. Subsequently, the silos were sealed and weighed. After 93 d, the silos were re-weighed to quantify dry matter recovery (DMR; Jobim *et al.*, 2007). Then, the silos were opened and weighed. The visually deteriorated portion was weighted (WDM). Then, the sand and TNT were considered to determine the effluent losses (EL), and silage losses were estimated using equations proposed by Jobim *et al.* (2007), disregarding the deteriorated silage portion

from the total weight of the silo after opening. An 800 g sample was taken from each silo, part of which was used for microbiological evaluation; the remaining sample was packed in plastic bags and stored at -20 °C for further chemical composition analysis and evaluation of fermentative characteristics.

The microbiological evaluation was performed using an aqueous extract composed of 25 g of silage and 225 mL of peptone water (1 g/L of water), which was manually homogenized for 3 min in closed sterile bags. The extract was surface-plated on potato dextrose-agar (Sigma-Aldrich, Ribeirão Preto, São Paulo, Brazil). Yeast and mold colonies were counted after incubation at 26 °C for 2 and 5 d, respectively. The pH of the aqueous extract was measured at time zero using a potentiometer (Tekna, São Bernardo do Campo, São Paulo, Brazil). Ammonia nitrogen (N-NH₃) analysis was performed according to method No. 941.04 (AOAC, 1990).

For the organic acids (lactic, acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids) evaluation, 25 g samples were weighed, solubilized in 300 mL of deionized water, and processed in a stomacher-type homogenizer (Cole-Parmer, Madison, Wisconsin, USA). Subsequently, 1 mL of the supernatant liquid was removed for gas chromatography. An Agilent 7890A gas chromatograph equipped with a flame ionization detector (7683B) and a fused silica capillary column (J & W 19091F-112; Agilent Technologies, Santa Clara, CA, USA) was used. After that, total organic acids were determined summing all evaluated acids. The lactic acid and acetic acid ratio (LA:AA) was established by dividing lactic acid by acetic acid contents.

For evaluating aerobic stability after opening the silos, 500 g of silage was placed in 25 plastic buckets (9 L), which were covered with aluminum foil to prevent loss of silage moisture. The buckets and silages were kept in an air-conditioned room at 20 °C for 12 d, where ambient and silage temperatures were recorded every 30 min using data loggers (Escort, Buchanan, Virginia, USA). Aerobic stability was defined as the number of

hours the silage remained stable before reaching 2 °C above room temperature.

The samples were pre-dried in a forced-air circulation oven at 55 °C for 72 h and then ground in a mill fitted with a 1 mm sieve. The DM, ash, ether extract (EE), and crude protein (CP) content was determined according to the AOAC (1990) methodology (methods No. 934.01, No. 923.03, No. 920.39, and No. 978.04, respectively). The methods by Van Soest and Robertson (1985) were used to analyze neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADI) levels, which were determined by solubilization of cellulose with sulfuric acid. Non-fiber carbohydrates were quantified according to the method by Detmann and Valadares Filho (2010). For the determination of total soluble, the methodology described by Dubois *et al.* (1956) was used.

Statistical analysis

A completely randomized experimental design with five replicates per concentration was used. Before statistical analysis, the molds count data were log₁₀-transformed. The fermentation, microbiological characteristics, aerobic stability, and chemical composition data of elephant grass silage was submitted to analysis of variance and polynomial and simple linear regression analysis to study the effects of murumuru meal concentrations. For this, we used the SAS[®] Statistical Analysis Program, Version 6 (Statistical Analysis System, 2001), with 5% as the significance level.

The mathematical model was as follows: $Y_{ij} = \mu + T_i + e_{ij}$. Where: Y_{ij} = observation j , referring to each inclusion level of murumuru meal (i); μ = overall average; T_i = effect of the inclusion level of murumuru meal; and e_{ij} = error associated with each observation.

Results

Chemical composition of elephant grass fresh and murumuru meal are presented in Table 1.

Table 1. Chemical composition of fresh elephant grass and murumuru meal.

Item (% DM)	Elephant grass	Murumuru meal
DM % FM	17.8	89.7
OM	95.3	97.4
Ash	4.7	2.7
CP	10.6	8.8
EE	1.8	15.1
NDF	72.5	37.9
ADF	40.2	26.1
HEM	32.3	11.8
CEL	36.5	12.4
LIG	3.7	13.7
NFC	10.4	35.5
SC	2.7	10.5

DM = dry matter, FM = fresh matter, OM = organic matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, HEM = hemicellulose, CEL = cellulose, LIG = lignin, NFC = non-fibrous carbohydrate, and SC = soluble carbohydrates.

There was a reduction ($p < 0.05$) in the effluent production of elephant grass silage with the inclusion of murumuru meal (Table 2). With the inclusion of murumuru meal, a quadratic effect ($p < 0.05$) on the dry matter recovery of silages (DMR%) was observed along with an increase ($p < 0.05$) in the portion of deteriorated material in the silages that included the additive. An increase ($p < 0.05$) in mold count was noted with the inclusion of murumuru meal to elephant grass silage. Yeast was not detected ($p > 0.05$) in the silage, that is, the count was below 2.0 cfu g^{-1} of forage.

A quadratic effect ($p < 0.05$) was observed in the pH value with the inclusion of murumuru meal in elephant grass silage (Table 3). Ammonia nitrogen (N-NH_3) content of silages increased linearly ($p < 0.05$) with the inclusion of murumuru meal. There was no effect ($p > 0.05$) of the additive on the lactic acid concentration of the silages.

Table 2. Fermentative losses and microbial composition of elephant grass silage containing murumuru meal.

Item	Inclusion of murumuru meal (%)					Regression equation	R ²
	0	7	14	21	28		
Effluents (kg/ton NM)	72.4	9.2	1.3	1.3	1.2	$Y = 65.54 - 15.71x$	0.62
DMR (%)	95.8	84.8	82.3	85.3	84.1	$Y = 94.65 - 1.35X + 0.04x^2$	0.85
PDM (g)	408	875	1280	1098	1129	$Y = 450.50 + 170.50x$	0.43
Molds ($\log_{10} \text{ cfu g}^{-1}$)	2.63	2.83	2.78	3.22	3.58	$Y = 2.30 + 0.23x$	0.62

NM = Natural matter; DMR = Dry matter recovery; PDM = Portion of deteriorated material.

Table 3. Fermentative characteristics and aerobic stability of elephant grass silages containing murumuru meal.

Item	Inclusion of murumuru meal (%)					Regression equation	R ²
	0	7	14	21	28		
pH	3.50	3.30	3.30	3.40	3.48	$Y = 3.47 - 0.026x + 0.01x^2$	0.85
N-NH_3 (% N total)	2.94	3.15	3.35	3.88	4.09	$Y = 2.87 + 0.04x$	0.77
Lactic acid (%DM)	1.57	1.75	2.55	1.70	1.60	$Y = 1.83$	-
Acetic acid (%DM)	2.80	1.80	0.80	0.50	1.10	$Y = 2.34 - 0.07x$	0.45
LA:AA (%DM)	0.56	0.97	3.19	3.4	1.45	$Y = 1.86 - 0.16x + 0.004x^2$	0.44
OA-total (%DM)	4.37	3.55	3.35	2.20	2.70	$Y = 4.38 - 0.13x + 0.002x^2$	0.37
AE	147.4	122.8	120.6	97.8	110.	$Y = 140.1 - 148x$	0.22

$\text{N-NH}_3/\text{NT}$ = ammonia nitrogen (% total nitrogen); DM = Dry matter; OA-total = Total organic acids; AE = aerobic stability; LA:AA = lactic acid/ acetic acid.

However, there was a linear decrease ($p < 0.05$) in the acetic acid content of silages with the inclusion of murumuru meal. A quadratic effect ($p < 0.05$) was observed in the LA:AA ratio and the total organic acid concentration with the inclusion of murumuru meal in the elephant grass silage. Butyric, propionic, isobutyric, valeric, and isovaleric acids were not present in the silages. There was a linear reduction ($p < 0.05$) in the aerobic stability of silages with the inclusion of murumuru meal.

The addition of murumuru meal in elephant grass silage linearly increased ($p < 0.05$) the DM content of the silage (Table 4). However, it

did not change ($p > 0.05$) the OM or ash content of the silages. There was a reduction ($p < 0.05$) in the CP content of silages with the inclusion of murumuru meal, while a quadratic effect ($p < 0.05$) was noted on the EE levels.

A linear ($p < 0.05$) reduction in the NDF content of elephant grass silages was observed with the inclusion of murumuru meal. Regarding the ADF, cellulose, and lignin levels, a quadratic effect ($p < 0.05$) was observed with the inclusion of the additive. The hemicellulose content decreased linearly ($p < 0.05$), and the NFC levels increased ($p < 0.05$) with the inclusion of the additive to the silage.

Table 4. Chemical composition of elephant grass silage with the addition of murumuru meal.

Item	Inclusion of murumuru meal (%)					Regression equation	R ²
	0	7	14	21	28		
DM	18.06	21.62	26.39	31.62	36.08	$Y = 17.549 + 0.658x$	0.98
OM	95.41	95.36	95.99	95.53	95.66	95.57	-
Ash	4.59	4.63	4.00	4.47	4.34	4.42	-
CP	10.68	10.40	10.23	9.83	9.78	$Y = 10.660 - 0.034x$	0.57
EE	1.96	3.42	5.08	5.74	5.68	$Y = 1.775 + 0.336x - 0.007x^2$	0.71
NDF	65.28	57.10	53.90	47.98	45.82	$Y = 63.620 - 0.686x$	0.85
ADF	40.03	36.07	33.33	29.08	29.81	$Y = 40.271 - 0.712x + 0.011x^2$	0.79
HEM	25.25	21.02	20.57	18.90	16.02	$Y = 24.468 - 0.294x$	0.71
CEL	32.81	25.82	21.58	17.38	19.14	$Y = 33.010 - 1.227x + 0.025x^2$	0.77
LIG	7.22	10.25	11.75	11.70	10.67	$Y = 7.263 + 0.514x - 0.014x^2$	0.52
NFC	18.64	26.22	29.33	34.01	36.52	$Y = 20.230 + 0.622x$	0.76

DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, NDF = neutral detergent insoluble fiber, ADF = acid detergent fiber, HEM = hemicellulose, CEL = cellulose, LIG = lignin, NFC = non-fibrous carbohydrate.

Discussion

Absorbent additives can increase DM content of the silage (>85%), allowing them to act as moisture scavengers and increase the DM content of the ensiled material, reducing EL in high humidity silages (Razak *et al.*, 2012). The murumuru meal used in this study had DM content above the minimum values suggested for a moisture sequestering additive. This characteristic allowed it to decrease effluent production in the grass silage (Table 2). This reduction has a direct effect on reducing the

potential environmental impact of the effluent. Silage effluent is a pollutant that can cause eutrophication of water bodies (Gebrehanna *et al.*, 2014).

The increase in PDM with the inclusion of murumuru meal was probably a reflection of the rise on NFC content on these silages. It is possible that the chemical composition of murumuru meal in elephant grass ensiling provided a greater input for the proliferation of undesirable microorganisms, which deteriorated the ensiled mass (Monteiro *et al.*, 2011).

This was confirmed by the high mold count due to the inclusion of the additive. The fact that no yeast was observed in the silages does not necessarily mean that there was no presence of this group of microorganisms. Yeast may have been present in the culture medium used; however, the latter may not have been ideal for cultivating such microorganisms. The presence of mold in the silages may be associated with the deleterious effects of oxygen, especially in the upper portion of the silo due to the difficulty of compacting this region during ensiling (Borreani *et al.*, 2018), reflecting the high PDM levels. This result contributed to the low DMR levels in murumuru meal silages.

Although there was an increase in the N-NH₃ content with murumuru meal inclusion, the highest content observed was still below the range considered desirable (<12%) for quality silage (Lopes and Evangelista, 2010). However, our results showed that the proteolysis that occurred during fermentation was not enough to alter CP levels, which varied below the 1.5% level among silages. That indicates no excessive protein degradation at the levels studied.

Lactic acid content observed in the silages indicate that fermentation occurred adequately as it was mainly responsible for the reduction in pH during ensiling and for maintaining acidification of the mass until the silo was opened. This response was evidenced by the pH values observed at this time, as well as by their similarity irrespective of murumuru meal inclusion. Reduction in acetic acid concentration with the addition of murumuru meal was probably a limiting factor in inhibiting mold growth in elephant grass as this acid has antifungal properties. The LA:AA ratio and total organic acid concentration reflected the aforementioned acid concentrations.

Reduction of aerobic stability of the silages with inclusion of murumuru meal occurred due to increase in NFC with meal addition (Table 4). The NFC are substrates used by spoilage microorganisms in the silage. The high substrate input evidenced by NFC

content, high mold count, and low acetic acid concentration results in less stable silages when the additives are included (McDonald *et al.*, 1991).

Increase in DM content of the silage with inclusion of murumuru meal was caused by high DM content of the additive, thus, demonstrating its potential as an absorbent additive for grass silage. The similarity in OM and ash concentrations among silages was probably caused by the similarity of these constituents in the meal and elephant grass.

Reduction in NDF, ADF, HEM, and CEL content with inclusion of murumuru meal in grass silage was probably caused by the lower content of these components in murumuru meal than in elephant grass. By adding murumuru meal to elephant grass silage, the concentrations of these components decreased proportionally. Despite reduction of NDF, ADF, HCEL, and CEL parameters in elephant grass silage with the inclusion of murumuru meal, lignin content increased. Lignin is a component that can interfere with feed digestibility. Its increase was anticipated as murumuru meal has higher lignin content (13.7%) than that of elephant grass (3.7%). High lignin levels may compromise the use of food by ruminal microorganisms and affect DM digestibility as they make part of unavailable cell wall components, thus resulting in fiber degradation (Wilson and Hatfield, 1997).

Increase in the NFC content of the silages with inclusion of murumuru meal was probably caused by high NFC content in the meal. Non-fibrous carbohydrates are considered mostly soluble and highly digestible, thereby increasing the nutritional value of the feed. In the case of silages, this increase in nutritional value depends on the microorganisms (homofermentative or heterofermentative), and products such as water, heat, carbon dioxide, lactic acid, alcohol, and volatile fatty acid generated (Muck, 2010).

In conclusion, including up to 28% murumuru meal in elephant grass silage improves the chemical composition of the silage, but it makes it more susceptible to deterioration when exposed to oxygen. Based on these results, we recommend to offer elephant grass-murumuru meal silage to the animals shortly after it is removed from the silo to avoid significant losses from aerobic deterioration.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Claudia M Serra-Ferreira and Agatha G Farias-Souza participated of the formal analysis and writing-original draft. Rita C Almeida-Mendonça and Wagner R L Lopes-Filho were responsible for methodology and investigation. Melany Simões-Souza wrote, revised and edited the text. Cristian Faturi were responsible for statistical analyses. Felipe Nogueira-Domingues was supervisor. Aníbal C do-Rêgo participated in supervision and administration of the project.

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