

Production and composition of soybean seed enriched with cobalt, molybdenum, and nickel

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ABSTRACT

Cobalt (Co) and molybdenum (Mo) are essential for biological nitrogen fixation (BNF) in soybean and may be supplied through enriched seed. Nickel (Ni) is also essential to BNF; however, there are no reports of its use in association with Co and Mo for seed enrichment. This study aimed to assess whether soybean seeds can be enriched with Co, Mo, and Ni through foliar application to the parent plant, and to evaluate the effects of this application on both the parent plant and the resulting seeds. A study was carried out in a fully randomized design, with seven foliar-applied treatments at the R5.2 growth stage: control; CoMo at a fixed rate (FR) (40/400 g ha⁻¹ Co/Mo); CoMo in FR + 20 g ha⁻¹ Ni; CoMo in FR + 40 g ha⁻¹ Ni; CoMo in FR + 60 g ha⁻¹ Ni; CoMo in FR + 80 g ha⁻¹ Ni; CoMo in FR + 100 g ha⁻¹ Ni. The following variables were assessed: chlorophyll index, biometric and yield performance of the parent plant, and contents of chemical elements, oil, protein, and fatty acids in the seed. The results obtained in this study suggest that applying Co (40 g ha⁻¹), Mo (400 g ha⁻¹), and Ni (up to 100 g ha⁻¹) in a single application at the R5.2 growth stage tends to enrich the seeds produced with these three elements, without harming the growth and development of the parent plant. Seed enrichment altered the contents of other chemical elements in the seeds produced; however, it did not affect the oil, protein, or fatty acid contents.

Keywords: Fatty acids, *Glycine max* (L.) Merrill, Oil and protein, Seed enrichment, Seed yield.

Produção e composição de sementes de soja enriquecidas com cobalto, molibdênio e níquel

RESUMO

Cobalto (Co) e molibdênio (Mo) são elementos essenciais para a fixação biológica do nitrogênio (FBN) na soja e podem ser fornecidos através de sementes enriquecidas. O elemento níquel (Ni) também é essencial para a FBN, porém não há relatos de seu uso associado a Co e Mo para enriquecimento de sementes. Objetivou-se avaliar se é possível enriquecer sementes de soja com Co, Mo e Ni através de aplicação foliar na planta-mãe, e quais são os efeitos desta aplicação na planta-mãe e nas sementes produzidas. Foi conduzido um estudo em delineamento inteiramente casualizado, com sete tratamentos aplicados via foliar em R5.2: controle; CoMo em dose fixa (DF) (40/400 g ha⁻¹ Co/Mo); CoMo em DF + 20 g ha⁻¹ Ni; CoMo em DF + 40 g ha⁻¹ Ni; CoMo em DF + 60 g ha⁻¹ Ni; CoMo em DF + 80 g ha⁻¹ Ni; CoMo em DF + 100 g ha⁻¹ Ni. Foram avaliados: índice de clorofila, desempenho fitométrico e produtivo da planta-mãe; teor de elementos químicos, óleo, proteína e ácidos graxos nas sementes produzidas. Os resultados obtidos neste estudo são um indicativo de que ao utilizar as doses Co (40 g ha⁻¹), Mo (400 g ha⁻¹) e Ni (até 100 g ha⁻¹) em aplicação única no estágio fenológico R5.2, há tendência de enriquecimento das sementes produzidas com os três elementos, sem prejuízo ao crescimento e desenvolvimento da planta-mãe. Com o enriquecimento, houve alteração no teor de outros elementos químicos na semente produzida, porém não houve alteração nos teores de óleo, proteína e ácidos graxos.

Palavras-chave: Ácidos graxos, *Glycine max* (L.) Merrill, Óleo e proteína, Enriquecimento de sementes, Produtividade de sementes.



1. Introduction

The average soybean yield in Brazil has the potential to be doubled through the adoption of better management practices, among which seed inoculation with bacteria of the *Bradyrhizobium* genus stands out (Battisti et al., 2018). These bacteria are responsible for the BNF process, which supplies nitrogen, the chemical element required in the greatest amount by the crop, at about 80 kg N per ton of seed produced (Bender et al., 2015).

The BNF process is characterized by the conversion of N from its molecular form (N_2), which is highly stable due to its covalent triple bond, to ammonia (NH_3), a form assimilable by plants. During the BNF process, the bacteria should operate at maximum efficiency, which requires the presence of Co, Mo, and Ni to perform specific functions in specific metabolic steps (Prasad and Shivay, 2019; Rana et al., 2020; Hu et al., 2021).

The first studies addressed the supply of Co and Mo via seed treatment; however, there was concern that the elements could harm the fixing bacteria due to their salinity. In this sense, alternatives to this practice have been sought by attempting to enrich soybean seeds in seed production fields through foliar application to the parent plant, first with Mo (Campo et al., 2009) and later with Co and Mo together (Cortese et al., 2019), with positive results and no negative effects on yield.

Although it is possible to enrich soybean seeds, providing these elements through seed treatment is still a current practice. Attention must be paid to the fact that the element Ni has also been employed along with Co and Mo in seed treatment, which might potentiate the saline effect on the bacteria. Based on that, the possibility of enriching soybean seed with Ni, along with Co and Mo, through foliar application to the parent plant was raised, as already shown to be possible only with Ni by Barcelos et al. (2018).

However, there are no reports in the literature on the implementation of this practice; therefore, determining the optimal Ni dose is needed, which requires assessing the parent plant and the seed produced after enrichment application. According to Marcos Filho (2015), alterations in the production environment may alter the composition of the seed, thereby compromising its metabolism and, consequently, the physiological potential of the seed produced. In this context, it is important to quantify alterations in soybean seed composition following enrichment application.

Based on the hypothesis that soybean seeds can be enriched with Co, Mo, and Ni through foliar application to the parent plant, this study aimed to verify the effects of this application on the parent plant and on the seed produced.

2. Material and Methods

The study was carried out in greenhouse conditions, with the soil used to fill the pots classified as Latossolo Vermelho (Santos et al., 2018). The chemical attributes of the soil were: $pH_{(CaCl_2)} = 5.01$; $H+Al = 5.11 \text{ cmol}_c \text{ dm}^{-3}$; $Al^{+3} = 0.00 \text{ cmol}_c \text{ dm}^{-3}$; $Ca^{+2} = 1.58 \text{ cmol}_c \text{ dm}^{-3}$; $Mg^{+2} = 0.59 \text{ cmol}_c \text{ dm}^{-3}$; $K^+ = 0.03 \text{ cmol}_c \text{ dm}^{-3}$; $P = 1.42 \text{ mg dm}^{-3}$; $Cu^{+2} = 4.72 \text{ mg dm}^{-3}$; $Fe^{+2} = 122.14 \text{ mg dm}^{-3}$; $Mn^{+2} = 82.85 \text{ mg dm}^{-3}$; $Zn^{+2} = 0.38 \text{ mg dm}^{-3}$; $Mo = 0.00 \text{ mg dm}^{-3}$; $CEC_{(effective)} = 2.21 \text{ cmol}_c \text{ dm}^{-3}$; $CEC_{(pH7.0)} = 7.32 \text{ cmol}_c \text{ dm}^{-3}$; $SB = 2.20 \text{ cmol}_c \text{ dm}^{-3}$; $V\% = 30$.

A fully randomized design with five replications was adopted, comprising seven treatments: T1 (control), T2 (40/400 g ha^{-1} Co/Mo), T3 (40/400 g ha^{-1} Co/Mo + 20 g ha^{-1} Ni), T4 (40/400 g ha^{-1} Co/Mo + 40 g ha^{-1} Ni), T5 (40/400 g ha^{-1} Co/Mo + 60 g ha^{-1} Ni), T6 (40/400 g ha^{-1} Co/Mo + 80 g ha^{-1} Ni), and T7 (40/400 g ha^{-1} Co/Mo + 100 g ha^{-1} Ni). The following sources of elements were used: cobalt sulfate (21%), sodium molybdate (39%), and nickel sulfate (22%).

Once the soil chemical attributes were analyzed, liming and base fertilization were carried out in a predetermined volume of soil for the total number of pots to be utilized, considering five-liter pots. For liming, calcitic limestone was utilized, aiming to raise base saturation to 60%. For fertilization, a target yield of 4.0 Mg ha^{-1} was used as a reference (Pauletti and Motta, 2017), and the quantities applied were: 25 kg ha^{-1} N and 120 kg ha^{-1} P_2O_5 , using monoammonium phosphate fertilizer (11-52-00); and 130 kg ha^{-1} K_2O , utilizing the potassium chloride fertilizer (60% K_2O).

For sowing, seeds of the cultivar 64I63 RSF IPRO, which has an indeterminate growth habit and maturity group 6.4, were used. The seeds were previously treated [(CS of clorantraniliprole - 625 g L^{-1} - dose of 1 mL kg^{-1}) + (CS of Fludioxonil - 25 g L^{-1} + Metalaxyl - 10 g L^{-1} - dose of 1 mL kg^{-1}) + (CS of Thiamethoxam - 350 g L^{-1} - dose of 2 mL kg^{-1})]. At sowing time, the seeds were inoculated with liquid inoculants containing *Bradyrhizobium japonicum* bacteria (5.10^9 CFU mL^{-1}), using 3 mL kg^{-1} of seed (twice the dose for the first year of growth in the soil).

According to the phenological scale of Ritchie et al. (1977), after plant emergence and establishment, in the VC growth stage, thinning was carried out, leaving three plants per pot. At the R5.2 growth stage, foliar applications of treatments were carried out. An electric backpack sprayer was used, comprising a spraying bar with four nozzles spaced at 0.32 m, set to a spraying volume of 175 L ha^{-1} . Along with treatments, an adhesive spreader was added at a dose of 1 mL L^{-1} . Pests and diseases were monitored, and management practices were implemented when needed.

The chlorophyll index was assessed on the ninth day after treatment application to evaluate, in addition to the index, possible phytotoxicity. In the three plants per replication, the third fully expanded trifoliolate leaf from the top was considered, with assessment performed on the central leaflet. For assessment, a Falker electronic chlorophyll meter (ClorofiLOG – CFL1030) was used.

Stem diameter and plant height were measured on the three plants per replication after a cut close to the soil at harvest. Stem diameter was measured right under the first visible node, in the direction of the smallest diameter. The measurement was carried out using a digital caliper. Plant height was measured from the top down on the main stem, using a measuring tape.

For the assessment of yield components, all pods from the three plants in each replication were harvested and quantified. Then, these were threshed, and the total number and mass of seeds were quantified, with results expressed per plant.

The content of chemical elements (macro and micronutrients and other elements) in the seed were determined in composite samples. To obtain each composite sample, the replications for each treatment had to be grouped due to the limited number of seeds per replication, resulting in a single composite sample per treatment. These composite samples were analyzed according to the methodology proposed by the Brazilian Agricultural Research Corporation, using ICP-OES technology, which is characterized by high sensitivity and adequate accuracy and precision of results (Silva, 2009).

The contents of oil, protein, and fatty acids were determined in whole seeds by Near Infrared Reflectance Spectroscopy (NIR), as described by Heil (2010). Whole, clean seeds were scanned using Thermo equipment, model Antaris II, equipped with an integrating sphere with a resolution of 4 cm², an average of 32 scans, with a background scan for each reading. For prediction, mathematical models developed by Embrapa Soybean were used.

The control was not compared with the subsequent treatments; it was included in the tables solely for observation. For biometric performance, yield, oil, protein, and fatty acid contents, the data were tested for normality of errors (Shapiro-Wilk test), homogeneity of variances (Bartlett test), and independence of errors (Durbin-Watson test); then, they were submitted to analysis of variance for a fully randomized design. For the analysis of chemical element content in the seed as a function of grouping by replication, the data were plotted. Then, regression analysis was carried out, selecting the mathematical model that best fitted the data set based on the *p* value and the highest R². In all

analyses, the 5% probability level was adopted, except for the analysis of chemical elements in the seed, for which the 10% level was adopted. All analyses were run in R using the AgroR package (Shimizu et al., 2024).

3. Results and Discussion

As shown in Table 1, the chlorophyll index was not significantly affected by the treatments employed. Chlorophyll is a photosynthetic pigment of pivotal importance to photosynthesis, responsible for converting solar energy into chemical energy, which is utilized for the synthesis of carbohydrate molecules, which are used as a source of energy by plants (Pareek et al., 2017). Based on these results, the application of these elements had neither beneficial nor detrimental effects on photosynthetic activity. Einhardt et al. (2021), when testing foliar-sprayed Ni doses at the V4 growth stage, the growth stage preceding that used in the present study, observed that the chlorophyll index was higher than that of the control up to the dose of 60 g ha⁻¹ Ni, but observed toxicity from 120 g ha⁻¹ Ni, indicating damage to photosynthesis.

For stem diameter, plant height, number of pods, and seeds per plant, no significant differences were observed across treatments; i.e., the elements did not affect soybean development, which, in this case, had an indeterminate growth habit. The application was carried out in the R5.2 growth stage. At this stage, there is little potential for a positive effect on plant structure and yield components, as soybean reaches its maximum height, number of nodes, and leaf area near the R5.5 growth stage (Ritchie et al., 1977). Regarding yield, assessed as the mass of seeds per plant, no effect of the treatments was observed, indicating that seed filling was not affected and confirming the absence of phytotoxicity as a function of nickel dose (Table 1).

Table 2 shows that the treatments did not influence the contents of oil, protein, palmitic, stearic, oleic, linoleic, or linolenic acids. Alterations in these characteristics are more closely related, for example, to exposure of plants to different temperature intervals during seed formation (Alsajri et al., 2020). Therefore, it is noted that enrichment with the elements up to the contents obtained did not negatively affect the characteristics analyzed. In addition, the importance of assessing these characteristics, as they may influence seed performance, needs to be confirmed in future studies. Capelin et al. (2021) observed a positive, significant correlation between germination and accelerated aging and protein content, and a negative, significant correlation with oil content.

Table 1. Values of treatments and summary of the analysis of variance comprising significance levels (*p* value) for chlorophyll index, biometric performance, yield components, and seed yield of soybean following foliar application of treatments aiming to enrich the seed during its production.

TREAT ⁽¹⁾	CHLOR	SD	PH	NPPL	NSPL	MSPL
-----	-----	---mm---	---cm---	---n°---	---n°---	---g---
Control	38.3	2.50	64	18	37	5.89
T1	37.6	2.48	61	15	33	5.02
T2	37.7	2.59	64	18	37	5.28
T3	38.9	2.45	62	15	32	5.22
T4	41.7	2.85	63	19	39	5.72
T5	39.3	2.45	60	14	29	4.80
T6	38.3	2.55	68	19	40	6.35
<i>p</i> value	0.0847	0.1212	0.2895	0.1689	0.2678	0.3862

⁽¹⁾TREAT: treatments; T1, T2, T3, T4, T5, and T6: treatments 1, 2, 3, 4, 5, and 6, respectively; CHLOR: chlorophyll index; SD: stem diameter; PH: plant height; NPPL: number of pods per plant; NSPL: number of seeds per plant; MSPL: mass of seeds per plant. Significant at 1 and 5% of probability ($p < 0.01$ and $p < 0.05$, respectively).

Table 2. Values of treatments and summary of the analysis of variance comprising significance levels (*p* value) for the contents of protein, oil, and fatty acids in soybean seed, following foliar application of treatments aiming to enrich the seed during its production.

TREAT ⁽¹⁾	Protein	Oil	PA	EA	OA	LA	LLA
-----	%	%	%	%	%	%	%
Control	39.90	21.59	12.66	2.64	19.18	64.77	8.31
T1	38.15	22.75	12.70	2.79	18.37	64.48	8.65
T2	38.73	22.36	12.57	2.70	18.30	65.45	8.63
T3	37.94	22.80	12.45	2.69	20.26	62.44	8.49
T4	40.71	21.47	11.73	2.58	23.04	61.94	8.65
T5	39.58	22.06	12.45	2.71	22.75	63.11	8.31
T6	39.10	22.29	12.57	2.82	20.19	65.12	7.88
<i>p</i> value	0.0716	0.2498	0.1606	0.7224	0.6648	0.2576	0.8313

⁽¹⁾TREAT: treatments; T1, T2, T3, T4, T5, and T6: treatments 1, 2, 3, 4, 5, and 6, respectively; PA, EA, OA, LA, LLA: palmitic, stearic, oleic, linoleic, and linolenic acids, respectively. Significant at 1 and 5% of probability ($p < 0.01$ and $p < 0.05$, respectively).

It is noted that the contents of Co and Mo, when applied together (CoMo), were higher than the control without application, suggesting that enrichment with both elements is likely. The content of Co increased from 0.16 to 1.25 mg kg⁻¹ of dry matter (DM), while the content of Mo increased from 1.68 to 24.85 mg kg⁻¹ DM. Cortese et al. (2019) and Abreu-Junior et al. (2023) also reported the possibility of enriching soybean seeds with Co and Mo. When Co and Mo were applied along with Ni, enrichment of both elements continued. At the same time, there was a tendency for enrichment with Ni, with its content in the seed increasing linearly as the applied element doses increased. The control had a Ni content of 2.00 mg kg⁻¹ DM, while the application of the maximum Ni dose resulted in a content of 10.91 mg kg⁻¹ DM (Table 3). Barcelos et al. (2018) observed Ni accumulation of 18 mg kg⁻¹ DM in the seed when applying a Ni dose of 100 g ha⁻¹ in combination with a fungicide.

The quantities of the elements when employed as seed treatment are 2 to 3 g ha⁻¹ Co, 12 to 25 g ha⁻¹ Mo, and 2.5 g ha⁻¹ Ni (Sfredo and Oliveira, 2010; Rodak et al., 2024). In this study, the maximum contents of Co, Mo, and Ni in the enriched seed, considering the maximum Ni dose, were 1.29, 27.31, and 10.91 mg kg⁻¹ DM, respectively (Table 3). Considering the maximum contents of these elements observed in the enriched seed as a reference, the quantity of these elements achieved

per hectare depends on the amount of seed used per hectare, so this is an important detail to consider in crop planning.

Although foliar application was performed, the accumulation of the observed elements should be discussed in light of Andresen et al. (2018), who stated that, because plants are sessile organisms, they cannot escape high metal contents in the soil. A common mechanism they adopt to circumvent such situations is the sequestration and storage of toxic metals in compartments such as vacuoles, where they interfere as little as possible with metabolic processes, such as respiration and photosynthesis. This is a common feature of hyperaccumulating plants, which accumulate one or more metals in their tissues to protect themselves against pathogen attack. Ferreira et al. (2020) observed that soybean plants derived from Ni-enriched seeds showed greater tolerance to Asian soybean rust.

As Ni contents increased through foliar application, there was a tendency toward an increase in the contents of Mo and sodium (Na), and a reduction in the contents of Fe, Cu, Si, and Al in the seed (Table 3). Na, Si, and Al are classified as beneficial elements to plants, as well as Co, i.e., they stimulate growth, but are not essential to development or are essential only for some plant species. Fe and Cu are classified as essential elements, i.e., plants are not capable of completing their life cycle in their absence; they cannot be replaced by another

element that performs the same function; and they must be directly involved in plant metabolism (Kirkby, 2012).

Table 3. Values of treatments and regression fitting comprising significances (p value) for the chemical elements analyzed, following foliar application of treatments aiming to enrich the seed during its production.

TREAT ⁽¹⁾	Co	Mo	Ni	Fe	Cu	Si	Al	Na
mg kg ⁻¹ DM								
Control	0.16	1.68	2.00	130.41	26.12	65.40	54.77	16.26
T1	1.25	24.85	1.72	84.24	11.39	37.01	18.24	9.30
T2	1.29	23.98	4.20	81.27	8.39	30.45	10.97	9.31
T3	1.26	23.38	5.18	75.30	7.10	30.63	9.72	8.75
T4	1.60	23.56	7.90	79.69	7.86	30.67	8.51	9.72
T5	1.17	25.30	9.26	67.36	7.23	27.30	7.94	10.32
T6	1.29	27.31	10.91	69.48	6.92	29.82	7.87	10.20
p value	ns	0.003 ^(Q)	0.000 ^(L)	0.020 ^(L)	0.055 ^(Q)	0.083 ^(L)	0.018 ^(Q)	0.079 ^(L)

⁽¹⁾TREAT: treatments; T1, T2, T3, T4, T5 and T6: treatments 1, 2, 3, 4, 5 and 6, respectively; ns: not significant; significant at 1, 5 and 10% of probability ($p < 0.01$, $p < 0.05$ and $p < 0.10$, respectively); (Q): quadratic fitting; (L): linear fitting; Mo: $0.001x^2 - 0.082x + 24.97 / R^2 = 0.98$; Ni: $0.091x + 1.967 / R^2 = 0.98$; Fe: $-0.158x + 84.16 / R^2 = 0.77$; Cu: $0.000x^2 - 0.107x + 10.89 / R^2 = 0.85$; Si = $-0.064x + 34.22 / R^2 = 0.568$; Al: $0.001x^2 - 0.261x + 17.28 / R^2 = 0.93$; Na: $0.012x + 8.992 / R^2 = 0.57$.

The iron content in the control was 130.41 mg kg⁻¹ DM; with CoMo application, it decreased to 84.24 mg kg⁻¹ DM; and with CoMo application associated with the maximum Ni dose, it decreased to 69.48 mg kg⁻¹ DM (Table 3). In the BNF process, both leghemoglobin and nitrogenase need iron. Iron binds to oxygen and transports it to leghemoglobin, and in nitrogenase, it acts as an electron carrier. In this way, BNF cannot be carried out in the absence of iron (Merry et al., 2022).

Copper content was 26.12 mg kg⁻¹ DM in the control treatment, decreasing to 11.39 mg kg⁻¹ DM with CoMo application and to 6.92 mg kg⁻¹ DM when CoMo was associated with the maximum Ni dose (Table 3). In the BNF process, copper is a cofactor of some high-affinity cytochromes that mediate energy transduction in bacteroids. Additionally, it is part of several superoxide dismutases that detoxify free radicals, which are byproducts of the BNF process (González-Guerrero et al., 2014).

The explanation for these elements' behavior is that they are transported across the cell membrane by highly specific transport proteins; however, not all these proteins are highly specific, so that ions with similar chemical properties share some transport routes (Andresen et al., 2018). Solubilized Ni compounds may be transported by several cation transporters, including those for Fe²⁺, Mg²⁺, Cu²⁺, and Zn²⁺ (Vatansever et al., 2017). In this sense, when ions have similar properties, their uptake is determined by their relative content: the ion present at a higher content is preferentially taken up, whereas the ion present at a lower content is inhibited. This phenomenon is known as competitive inhibition (Silva and Trevizam, 2015).

It is highlighted that when exposed to high levels of heavy metals, plants may exhibit phytotoxicity symptoms, such as delayed growth, chlorosis, and even death (Ghori et al., 2019); however, such symptoms

were not observed following the application of elements for enrichment. As already highlighted, alterations in the contents of some elements were observed in the analyzed seed (Table 3). These alterations are related to synergistic and antagonistic interactions among elements (Rietra et al., 2017), and it is important to verify, in future studies, the balance of elements in the parent plant's leaves following application during the maturation phase, aiming to establish dose limits to be applied.

It is also important to verify whether alterations in the contents of elements influence the physiological quality of the seed produced, whether they cause deficiency of any element in newly emerged seedlings, whether they influence BNF, and whether they influence the performance of plants derived from these seeds. In addition, it is important to verify whether these plants will produce seeds containing traces of these elements, so that the effectiveness of adopting this practice in seed production fields can be assessed. As an additional point, it is important to verify whether these plants will be more tolerant to diseases that affect the crop as a function of Ni application, as highlighted in the studies by Barcelos et al. (2018) for powdery mildew, Ferreira et al. (2020) for rust, and Fontes et al. (2024) for white mold. In the case of a satisfactory result, it is necessary to determine the extent to which the content of each element in the seed results in returns in both phytotechnical and economic performance.

The enrichment of soybean seed with Co, Mo, and Ni through a single foliar application during seed maturation, which appears likely based on the results obtained in the present study, appears to be an additional alternative to supplying these elements via seed treatment, which would decrease damage or even death of bacteria delivered through inoculants, due to a reduction in the saline effect. In addition, it might be a

more efficient way to supply these elements, as they are already present in the seed and readily available when required during the BNF process.

4. Conclusions

The results obtained in this study suggest that applying cobalt (40 g ha⁻¹), molybdenum (400 g ha⁻¹), and nickel (up to 100 g ha⁻¹) in a single application at the R5.2 growth stage tends to enrich the seeds produced with the three elements, without harming the growth and development of the parent plant. Additionally, seed enrichment altered the content of other chemical elements in the seeds produced; however, it did not alter the contents of oil, protein, and fatty acids.

Authors' Contribution

Conceptualization: Paulo Henrique Cazarim, Claudemir Zucareli, Inês Cristina de Batista Fonseca; Data curation: Paulo Henrique Cazarim, André Sampaio Ferreira; Formal analysis: Paulo Henrique Cazarim, André Sampaio Ferreira, Inês Cristina de Batista Fonseca; Funding acquisition: Claudemir Zucareli, Inês Cristina de Batista Fonseca; Investigation: Paulo Henrique Cazarim, Marcos Vinicius Knorr; Methodology: Paulo Henrique Cazarim, André Sampaio Ferreira, Claudemir Zucareli, Inês Cristina de Batista Fonseca; Project administration: Paulo Henrique Cazarim, André Sampaio Ferreira; Resources: Paulo Henrique Cazarim, André Sampaio Ferreira; Software: Paulo Henrique Cazarim, André Sampaio Ferreira, Inês Cristina de Batista Fonseca; Validation: Paulo Henrique Cazarim, André Sampaio Ferreira; Visualization: Paulo Henrique Cazarim, André Sampaio Ferreira; Writing - original draft: Paulo Henrique Cazarim; Writing - review & editing: Paulo Henrique Cazarim, André Sampaio Ferreira, Claudemir Zucareli.

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