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Cannabis use on gingival bleeding and caries experience among students

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Aim: The present study aimed to investigate if cannabis use can be associated with gingival bleeding and caries experience among Brazilian students. **Methods:** A cross-sectional study was performed in 2016 with first-semester university students in Pelotas. Data was collected via a self-administered questionnaire. Self-reported gingival bleeding and caries experienced were collected. Cannabis use was assessed using a modified version of the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST). We define cannabis users as individuals who report using cannabis at least once a month. A Poisson regression model with robust variance was used to analyze the data. **Results:** A total of 2,058 (64.5% of eligible students) students were assessed. The prevalence of caries experience was 68.2% and gingival bleeding was 50.8%. The prevalence of cannabis use was 11.7%. After controlling (sex, skin color, age, family income, depressive symptoms, oral health self-perception tobacco use), individuals who use cannabis present a prevalence of gingival bleeding 2.51 (CI95%[1.42–4;53]) higher than individuals who did not use cannabis. The association was maintained even when the individuals who used tobacco were excluded from the sample (PR=2.24, CI95%[1.16 – 4.31]). Cannabis use did not show an association with the experience of dental caries in both crude (PR 0.91 CI95%[0.82 – 1.01]) and adjusted models (PR 0.93 CI 95%[0.83 – 1.05]). **Conclusion:** Cannabis use was associated with the presence of gingival bleeding among the students of a public university in the south of Brazil. However, the experience of dental caries did not present an association with cannabis use.

Keywords: Cannabis. Prevalence. Self report. Gingival diseases. Dental caries. Oral health.

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Introduction

Cannabis (or marijuana) is the most common recreational drug used worldwide, presenting a brain-psychoactive action[1-4]. About 13.1% of younger individuals in the United States of America reporting the use of this substance can be associated with social and health problems[5]. The highest prevalence of this drug seems to be among young individuals, of which university students represent an important part of this population[6] and the user tends to remain during adulthood[7]. The main pharmacological property of cannabis is due to the action of *delta 9-tetrahydrocannabinol* (THC)[8], which presents euphoriant properties. Moreover, this drug has shown important properties for its use in the control of chronic pain and as an anticonvulsant, increasing the interest in the medical use of cannabis and its synthetic pharmacy agonists[8,9]. Despite some studies have shown that synthetic cannabinoids might promote immunosuppressant and anti-inflammatory properties without the psychoactive properties[10] even as the effects above descript (anticonvulsant and analgesic effects)[11], the action of cannabis smoking in an oral environment has been associated with not desired effects in oral health[12,13].

Several modifications in the normal oral environment have been observed and reported in the literature, which the gingivitis, a decrease of salivary flow, leukoedema, and an increase in oral colonization by *C. Albicans*,[12] although the evidence about this topic is still incipient. In this context, some wide population-based studies[14-16] have investigated the association between the use of cannabis and periodontal disease showing that individuals who used cannabis present the highest prevalence of periodontal disease[14,15,17,18]. In a national examination survey, it was observed that individuals who present frequent consumption of cannabis (marijuana or hashish ≥ once per month for the last year) present an odds of 70% higher periodontitis prevalence than individuals who did not use the substance[14]. Moreover, the odds of disease were higher when the individuals who used tobacco were not considered[14]. Cannabis presents around 400 toxic compounds similar to tobacco as well as about 60 Cannabinoids[19,20]. These cannabinoids seem to be associated with alterations in the cell response, such as cell-T, macrophages, and lymphocytes[21]. Thus, influencing the periodontal response. On the other hand, the possible decrease of salivary flow reported with the use of cannabis could increase also the development of dental caries. Indeed, the decrease of salivary flow can predispose individuals to high dental caries risk[22, 23] although this relationship is not consistent in the literature[24,25].

Due to this high consumption and the possible effects related to oral and general health, this topic presents importance regarding public health[26], especially for younger individuals. Furthermore, there is a pressing need to expand the existing evidence base regarding the influence of cannabis on periodontal tissue and to enhance our understanding of its effects on dental caries. Therefore, the present study aimed to investigate if cannabis use can be associated with higher gingival bleeding and caries experience among Brazilian students. The present hypothesis is that a higher prevalence of gingivitis and caries experience will be observed in cannabis smokers.

Materials and Methods

This study was reported according to recommendations of the STROBE statement (The Strengthening the Reporting of Observational Studies in Epidemiology Strobe guidelines)[27]. The study protocol was approved by the Human Research Ethics Committee of the Faculty of Medicine/Federal University of Pelotas, under protocol nº 49449415.2.0000.5317. A description of the methods of this survey was published elsewhere[28-32].

Setting and study design

Pelotas, situated in southern Brazil, boasts a population of approximately 400,000 inhabitants and is recognized as a significant hub for education within the state of Rio Grande do Sul. Within the city, there are five institutions of higher education, with only one being public—the Federal University of Pelotas (UFPel). Presently, UFPel comprises five campuses dispersed across the city, catering to around 13,000 undergraduate students. Each year, approximately 3,000 students are admitted through the selection process. Thus, in the first semester of 2016, we conducted a cross-sectional study, involving incoming students at UFPel who completed a self-reported questionnaire.

Sample size and power of the study

Given an estimated number of 3,000 student entrants in the first half of 2015 and an assumed prevalence of 50% (actual prevalence unknown) for the variables of interest, the study's margin of error would be 1.8 percentage points within a 95% confidence interval. The final sample size of 2,058 is adequate for analyzing associations, as it can detect a prevalence ratio of 1.4. This analysis assumes a prevalence of exposure of 50%, a prevalence of the outcome in exposed individuals of 5%, a statistical power of 80%, and a significance level (α) of 5%.

Participants

The eligible participants for this study comprised regular entrants during the first semester of 2016 at UFPel. These students were identified through a list provided by their respective academic units. Before their classes, the questionnaire was administered with the consent of their course instructors. Exclusions encompassed students unable to complete the questionnaire, those engaged in courses without formal enrollment at the institution, those enrolled in courses outside of Pelotas, and those partaking in distance learning programs.

Data collection

A self-administered questionnaire including demographic and socioeconomic characteristics, general habits and behaviors, and oral health questions was applied. Trained postgraduate and undergraduate students applied the questionnaires in the classrooms before the beginning of lessons. About the training process of interviewers, four hours of theoretical training were conducted and the questionnaire's questions were discussed. A pilot study was carried out with 100 university students in the second semester of graduate school, not eligible for the study, from five different academic units randomly selected.

Outcomes

Gingival bleeding and dental caries experience were considered the outcomes of this study. Gingival bleeding was assessed with the question: "Do your gum bleed when you brush your teeth?" Yes ("Always" or "Sometimes") or No ("Never"). The variable history of dental caries was constructed through three questions as follows: "Do you have or have you ever had any tooth affected by tooth decay?" (yes or no); "Do you have or have you ever had any tooth restored (filled) by tooth decay?"(yes or no); and "Do you have or have you ever had any tooth extract by tooth decay?"(yes or no). Individuals who answered "yes" to any of the questions were considered with experienced dental caries.

Independent variables

Demographic characteristics including sex and age were collected. Family income was collected categorically in Brazilian Reals (one American Dollar = 3.19 Brazilian Reals) (a) up to 500.00; b) 5,001.00 up to 1,000.00; c) 1,001.00 up to 2,500.00; d) 2,501.00 up to 5,000.00; e) 5,001.00 up to 10,000.00; and f) more than 10,001.00) and categorized in three categories: a) ≤ 1,000.00; b) 1,001.00 to 5,000.00 and c) ≥ 5,001.00.

Depression was also assessed by the Patient Health Questionnaire-2 (PHQ-2), an abbreviated version of the Patient Health Questionnaire Depression (PHQ-9) used for depression screening. The PHQ-2 is comprised of two questions about the frequency of depressed mood over the past two weeks. A PHQ-2 score ranges from 0-6 points[33]. A cutoff score of 3 was adopted.

Self-perception of oral health was measured through the question: "Compared to people of your age, how do you consider the health of your teeth, mouth, and gums?" Answers were dichotomized into Good (very good and good alternatives) and Bad (regular; bad and very bad alternatives).

The use of dental services in the last year was investigated through the question: "When was the last time that you went to the dentist?". The possible answers were: "1) Less than one year ago; 2) Between one and two years ago; 3) Between two and three years ago; and 4) More than four years ago. The answers were categorized into two groups: 0) During the last year, and 1) More than one year. Cannabis and tobacco use were assessed using a modified version of the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) recommended by the World Health Organization (WHO)[34]. It was asked about the frequency and the number of drugs used. We define cannabis users as individuals who report using cannabis at least once a month, and Tobacco users as those who report using tobacco weekly.

Statistical methods

Statistical analyses were performed using Stata 16.0 (Stata Corporation, College Station, TX, USA). For descriptive analysis, relative and absolutes frequencies were estimated. A crude and adjusted Poisson regression model with robust variance was used to assess the association between the covariables and the outcomes. The use of Poisson regression models for binary outcomes has been proposed and exten-

sively used in the last decade because provide the estimation of Prevalence or Risk ratios. This strategy allowed the estimation of Rate Ratio (RR) and 95% confidence intervals (CI). Exposure variables with p values of ≤ 0.20 in the crude analyses were included in the model fitting. A backward stepwise procedure was used to include or exclude explanatory variables in the model fitting. Associations were considered significant if they had a p-value of ≤ 0.05 after adjustments. A sensitivity analysis was performed excluding participants who use tobacco in the model with the outcome of gingival bleeding.

Results

A total of 3,237 students were considered eligible for this study. Losses corresponded to 34.6% (n=1,119) and were due to the low classroom frequency of students, who were not found after three searches in the classroom. Thus, 2,118 (65.4%) were invited to participate in the present study and 2,058 (64.5%) answered the questionnaire. Regarding refusals, only 1.4% (n=29) did not agree to participate in the study. The majority of participants were females (52%), with age-olds ranging from 18 to 24 (66%).(Table 1). The prevalence of cannabis use was 11.7% among the students, of which almost 77% presented between 18 and 24 years old. Regarding the prevalence of oral health variables, the caries experience was reported by 68.2% of participants, and gingival bleeding was reported by 50.8% of participants.

Table 1. Description of the general characteristics of the sample according to gingival bleeding and dental caries experienced.

Variable/Category	Gingival bleeding			Dental caries experience		
	No	Yes	p-value*	No	Yes	p-value*
Sex						
Male	945 (95.9)	40 (4.1)	0.224	358 (36.3)	629 (63.7)	<0.001
Female	1,023 (94.8)	56 (5.2)		299 (27.7)	782 (72.3)	
Skin Color						
White	1,449 (96.3)	56 (3.7)	<0.001	477 (31.6)	1,035 (68.4)	0.894
Non-white	486 (92.8)	38 (7.25)		166 (31.9)	355 (68.1)	
Age (years)						
16 to 17	298 (95.8)	13 (4.2)		135 (43.6)	175 (56.4)	
18 to 24	1,291 (94.8)	71 (5.2)	0.539	481 (35.2)	887 (64.8)	<0.001
25 to 34	208 (96.7)	7 (3.3)		40 (18.6)	175 (81.4)	
35 or more	169 (96.0)	7 (4.0)		4 (2.3)	171 (97.7)	
Family Income						
Low	255 (92.4)	21 (7.6)	0.020	55 (19.9)	222 (80.1)	<0.001
Middle	1,001 (95.2)	50 (4.8)		331 (31.5)	721 (68.5)	
High	369 (97.1)	11 (2.9)		142 (37.5)	237 (62.5)	

Continue

Continuation						
Body mass index						
Underweight	111 (92.5)	9 (7.5)		31 (26.0)	88 (74.0)	
Normal	1,209 (96.0)	51 (4.0)	0.016	425 (33.7)	838 (66.3)	0.092
Overweight	435 (95.6)	20 (4.4)		130 (28.6)	324 (71.4)	
Obesity	150 (90.9)	15 (9.1)		49 (29.2)	119 (70.8)	
Depressive symptoms						
No	1,659 (96.1)	68 (3.9)	<0.001	551 (31.8)	1,184 (68.2)	0.890
Yes	309 (91.1)	30 (8.9)		108 (32.1)	228 (67.9)	
Oral health self-perception						
Good	1,425 (96.7)	49 (3.3)	<0.001	522 (35.3)	959 (64.7)	<0.001
Bad	537 (91.6)	49 (8.4)		127 (23.1)	456 (76.9)	
Last dentist appointment						
≤ 1 year	1,337 (96.2)	53 (3.8)	0.003	434 (31.2)	957 (68.8)	0.627
>1 year	590 (93.2)	43 (6.8)		205 (32.5)	430 (67.7)	
Tobacco use						
No	1,721 (95.6)	79 (4.4)	0.074	591 (32.8)	1,211 (67.2)	0.031
Yes	195 (92.9)	15 (7.1)		54 (25.5)	158 (74.5)	
Cannabis use						
No	1,687 (96.0)	70 (4.0)	<0.001	551 (31.3)	1,207 (68.7)	0.044
Yes	210 (90.5)	22 (9.5)		89 (37.9)	146 (62.1)	

*Chi-square test.

Table 1 shows the distribution between the general characteristics of the sample and the outcomes. In the bivariate analysis, gingival bleeding was more prevalent in individuals who use cannabis. Moreover, the prevalence of gingival bleeding was higher in students with non-white skin color, low family income, obese body mass index, depressive symptoms, bad oral health perception, and did not visit dental service in the last year. On the other hand, dental caries experience was lower in individuals who smoke cannabis in bivariate analysis. Male individuals, 35 years old or more, with low family income, bad oral health perception, and tobacco smokers also presented a high experience of dental caries.

The crude and adjusted models investigating the influence of cannabis use and gingival bleeding are displayed in Table 2. After controlling for possible confounders (sex, skin color, age, family income, depressive symptoms, oral health self-perception tobacco use), individuals who use cannabis present a prevalence of 2.51 CI95% [1.42 – 4;53] higher than individuals who did not use cannabis. The association was maintained even when the individuals who used tobacco were excluded from the sample, although the effect showed a decrease (PR 2.24 CI 95% [1.16 – 4.31]).

Table 2. Crude ([c]) and adjusted ([a]) prevalence ratio (PR) of independent variables for gingival bleeding in university students. Pelotas, RS, Brazil. Poisson Regression (*n*=1576).

Variable/Category	PR[c] (CI95%)	p-value	PR[a](CI95%)	p-value
Sex(ref=male)		0.225		0.238
Female	1.28 (0.86 – 1.90)		1.33 (0.83 – 2.12)	
Skin Color(ref=white)		<0.001		0.165
Non-White	1.95 (1.31 – 2.91)		1.42 (0.87 – 2.32)	
Age (yrs)(ref=16 to 18)		0.549		-
18 to 24	1.15 (0.70 – 2.23)		-	
25 to 34	0.78 (0.32 – 1.92)			
35 or more	0.95 (0.39 – 2.34)			
Family income(ref= low)		0.006		0.017
Middle	0.63 (0.38 – 1.02)		0.64 (0.37 – 1.08)	
High	0.38 (0.19 – 0.78)		0.40 (0.18 – 0.87)	
Body mass index (ref=underweight)		0.225		-
Normal	0.54 (0.27 – 1.07)		-	
Overweight	0.59 (0.27 – 1.25)			
Obesity	1.21 (0.55 – 2.68)			
Depressive symptoms (ref=No)		<0.001		0.022
Yes	2.25 (1.49 – 3.40)		1.80 (1.09 – 2.98)	
Oral health self-perception (ref=good)		<0.001		0.006
Bad	2.52 (1.71 – 3.70)		1.92 (1.21 – 3.05)	
Last dentist appointment (ref ≤ 1 year)		0.004		-
> 1 year	1.78 (1.21 – 2.64)		-	
Tobacco use (ref=No)		0.073		0.214
Yes	1.63 (0.96 – 2.77)		0.61 (0.27 – 1.34)	
Cannabis use (ref=no)		<0.001		0.002
Yes	2.38 (1.50 – 3.77)		2.54 (1.42 – 4.53)	
Cannabis use excluding tobacco smokers* (ref=no)		0.029		0.016
Yes	1.98 (1.07 – 3.65)		2.24 (1.16 – 4.31)	

* Analysis performed restricted to participants that no use tobacco (n=1434 individuals). In the adjusted model, the values of variables displayed are referent to cannabis use including tobacco smokers.

Table 3 displays the models investigating the use of cannabis and the dental caries experience. Both in the crude (PR 0.91 CI95%[0.82 – 1.01]) and in adjusted models (PR 0.93 CI 95%[0.83 – 1.05]) by confounders (sex, age, family income, oral health self-perception, tobacco use), the cannabis use was not associated with the experience of dental caries.

Table 3. Crude ([c]) and adjusted ([a]) prevalence ratio (RR) of independent variables for caries experience in university students. Pelotas, RS, Brazil. Poisson Regression (*n*=1615).

Variable/Category	PR[c] (CI95%)	p-value	PR[a](CI95%)	p-value
Sex(ref=male)		<0.001		0.002
Female	1.14 (1.07 – 1.21)		1.11 (1.04 – 1.18)	
Skin Color(ref=white)		0.894		-
Non-White	1.00 (0.93 – 1.07)		-	
Age (yrs)(ref=16 to 18)		<0.001		<0.001
18 to 24	1.15 (1.03 – 1.28)		1.13 (1.00 – 1.28)	
25 to 34	1.44 (1.28 – 1.62)		1.42 (1.24 – 1.63)	
35 or more	1.73 (1.57 – 1.91)		1.63 (1.45 – 1.84)	
Family income(ref=low)		<0.001		0.006
Middle	0.86 (0.80 – 0.92)		0.89 (0.83 – 0.96)	
High	0.78 (0.71 – 0.86)		0.87 (0.78 – 0.96)	
Body mass index (ref=underweight)		0.280		-
Normal	0.90 (0.80 – 1.01)		-	
Overweight	0.97 (0.86 – 1.09)			
Obesity	0.96 (0.83 – 1.11)			
Depressive symptoms (ref=No)		0.890		-
Yes	0.99 (0.92 – 1.08)		-	
Oral health self-perception (ref=good)		<0.001		<0.001
Bad	1.19 (1.12 – 1.26)		1.18 (1.11 – 1.21)	
Last dentist appointment (ref ≤ 1 year)		0.629		-
> 1 year	0.98 (0.92 – 1.05)		-	
Tobacco use (ref=No)		0.017		0.042
Yes	1.11 (1.02 – 1.21)		1.10 (1.00 – 1.21)	
Cannabis use (ref=no)		0.061		0.260
Yes	0.91 (0.82 – 1.01)		0.93 (0.83 – 1.05)	

Discussion

The present study showed that the use of cannabis by university students was strongly associated with the prevalence of gingival bleeding, although not associated with the caries experience. These results reinforce previous observations[14,15,17,18] that have indicated a significant influence of cannabis use in the gingival tissue, which may result in a higher risk of periodontal disease[17,18]. However, the association between cannabis use and caries experience was not observed in the present sample. Moreover, is important to highlight that we observed high cannabis consumption in the sample investigated, reinforcing the importance and need to conduct investigations concerning this topic.

A prevalence 2.5-fold higher of gingival bleeding was observed in individuals who reported the use of cannabis, even after control by confounder variables - including

tobacco. Due to the high influence of tobacco on the periodontal tissues, recent studies[14,35] have suggested performing the analysis restricted to participants who had not used tobacco, in view to improving the strength of the results. Therefore, we also perform additional analysis to confirm the effect of cannabis on gingival bleeding. Similar to those observed in other studies[14,35], we confirm the association even in restricted non-tobacco smokers, improving the robustness of the association. Therefore, the results of the present corroborate with previous literature, which shows the influence of cannabis in the periodontal tissues both in humans[14,35] and in animal studies[36,37]. Besides, this effect seems to be independent of tobacco[14,35].

Regarding the physiological mechanism that explains these results, the literature has reported the potential influence of the cannabinoids in the cell response, such as Cell-T and Macrophages[38,39]. This alteration could change the inflammatory response of periodontal tissues. Moreover, recent studies have observed that cannabinoids play a role also in bone remodelation[40]. Although we did not measure periodontal disease, we asked the individuals about the presence of gingival bleeding, which represents the objective parameter for inflammation of periodontal tissues[41]. Although we did not differ between the occurrence of gingivitis and periodontitis due to the methodology adopted in the present study, with the use of the validated question to investigate gingival bleeding[42]. An *in vivo* study with rats has shown that sites with induced periodontitis present a higher increase of disease when the rats were exposed to cannabis[43]. However, statistical differences were not observed in sound sites concerning the rats exposed and not exposed to cannabis[43].

On the other hand, we did not confirm the second hypothesis concerning the association between the use of cannabis and dental caries experience. This result corroborates with the studies that investigated this association[25 44]. A sample of forty-three individuals was investigated and no differences were observed between decayed and filled surfaces as well as salivary flow with the use of cannabis[44]. Although the choice of using caries experience could overestimate the presence of disease, since those individuals may present an important part of filled teeth, we also perform the analysis considering only the individuals that reported having decayed teeth. However, the results did not present any changes rejecting the initial hypothesis. While cannabis seems to directly influence inflammation, its impact on dental caries is only through mediation for salivary flow or dietary habits[45,46]. Therefore, there appears to be no biological plausibility for a direct effect of cannabis on caries. Changes in salivary flow and dietary habits could potentially exacerbate the progression of caries[45,46]. However, unlike the straightforward effects on inflammation, the effect of cannabis on caries appears to be small and indirect and seems not to be detectable in epidemiological studies. Saliva has an important protective effect against caries[47], and changes in the quantity or quality pH of the saliva could result in xerostomia and affect the prevalence of caries. Moreover, some studies[25,45] speculated that marijuana could influence dietetic behaviors, which are the main risk factors for caries. However, the results of studies have demonstrated that these mechanisms are very subtle and do not seem to be observed at an epidemiological level[25,45]. Furthermore, no other mechanism is known by which cannabis could affect the etiology of dental caries[25,45].

The principal strength of this report is the large sample investigated and the low rate of refusal, although a part of the students cannot be accessed. However, this response rate is similar to previously published papers with interview methodologies[48,49]. Moreover, we construct a possible model to control and investigate the possible confounding variables reported in the literature. Thus, the control in the final models by several confounders, such as socioeconomic, psychosocial, and behavioral, provide thus, the robustness of our results. In addition, complementary analyses restricted to individuals who do not smoke tobacco highlight the strength of our present results. In this way, besides using cannabis we asked the students about the frequency of drug use. In this way, we can quantify the amount of substance used and classify a minimum frequency.

However, the extrapolation of the present study is very limited, because of the high educational level and the low age of the participants. In this way, direct comparisons with general populations were not possible. Overall, students are a low-investigated population that presents peculiar characteristics, which are poorly explored by epidemiological studies and should be further investigated. Most of them are adolescents and young adults who are still in constant change in life. An elevated part of students start to initiate their own lives, some away from their families and present still in a stage of personality development and formation[50]. Thus, the habits developed in this stage of the life cycle can persist for the entire life. In this context, we observed that the greater part of students who use cannabis are between 18 and 24 years old. This finding is important data to stimulate public health policies to decrease the use of cannabis and other drugs also among university students.

To reduce the possible decrease in the response rate of the participants regarding the use of cannabis and tobacco were taken in a confidential survey. We used a separate questionnaire for these questions, which was deposited in a closed urn, separately from the general questionnaire. An important limitation that must be considered is the use of self-perceived parameters. Although clinical examinations are the gold standard for oral examinations, the use of self-reporting is a valid tool employed in epidemiologic studies due to its efficient and simple method, presenting a low cost and a reduced time to examination[51-53]. However, the utilization of self-reported data on gingival bleeding and caries experience can introduce important limitations of this study. Self-reported measures can inherently introduce the potential for recall bias and subjective interpretation by participants. Individuals may inaccurately recall or misinterpret their experiences regarding gingival bleeding and caries, leading to data that may not fully capture the true prevalence or severity of these oral health conditions. Moreover, reliance on self-reported data may overlook asymptomatic cases or variations in individuals' awareness and perception of their oral health status. Thus, these limitations must be taken into the interpretation of the findings. Additionally, the study's cross-sectional design limits its ability to establish causal relationships between cannabis use and oral health outcomes. While the study adjusted for potential confounders, residual confounding cannot be ruled out due to unmeasured or inadequately measured variables. In conclusion, the use of cannabis was associated with the presence of gingival bleeding among the students at a public university in the south

of Brazil. However, the experience of dental caries did not present an association with cannabis use.

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Data availability

Datasets related to this article will be available upon request to the corresponding author.

Competing interests

The authors declare no competing interests.

Author contribution

Luiz Alexandre Chisini: conceived the idea, collected the data, performed the data analyzes, and wrote the manuscript. Flávio Fernando Demarco and Francine Dos Santos Costa: conceived the idea. Luana Carla Salvi, André Luiz Rodrigues Mello, Laylla Galdino dos Santos, Luiza Gioda Noronha, Kaila Andressa Dos Santos Oliveira, and João Luiz Dalmaso: wrote the manuscript. All authors discussed the findings, revised and approved the final version of the manuscript.

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