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Heart rate variability indices in the time domain in healthy dogs supplemented with omega n-3

Índices da variabilidade da frequência cardíaca no domínio do tempo, em cães saudáveis suplementados com ômega n-3

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Abstract

The analysis of heart rate variability (HRV) in the time domain was performed by dynamic electrocardiographic monitoring for twenty-four hours (Holter method) in ten male and ten female healthy mixed-breed dogs before and after four weeks of oral supplementation with long-chain polyunsaturated fatty acids (PUFAs) derived from omega n-3 [497 mg docosahexaenoic acid (DHA) and 780 mg eicosapentaenoic acid (EPA)]. Males received an average dose of 34.9 mg DHA/kg and 22.2 mg EPA/kg, whereas females received an average of 27 mg DHA/kg and 43 mg EPA/kg. HRV indices calculated in the time domain were the following: NN, SDNN, SDANN, SDNN index, pNN50, rMSSD and heart rate, taking into account the effects of gender and supplementation. The results showed no significant effects of supplementation or gender on the indices; thus, dietary supplementation with omega PUFAs derived from n-3 over four weeks did not decrease heart rate and did not increase heart rate variability in healthy dogs.

Key words: Dog, HRV, PUFAs, holter

Resumo

A análise da variabilidade da frequência cardíaca (VFC) no domínio do tempo, foi realizada por meio da monitorização eletrocardiográfica dinâmica em vinte e quatro horas (Holter), em cães saudáveis, sem raça definida, sendo dez machos e dez fêmeas, antes e após suplementação oral, por quatro semanas com ácido graxos poliinsaturados de cadeia longa (PUFAs) derivados do ômega n-3 [497 mg ácido docosahexaenóico (DHA) e 780 mg ácido eicosapentanóico (EPA)], resultando em uma dose média de 34,9 mg/kg de EPA e 22,2 mg/kg de DHA em machos e média de 43 mg/kg de EPA e 27 mg/kg em fêmeas. Os índices da VFC calculados no domínio do tempo foram: NN, SDNN, SDANN, SDNN index, pnn50 e rMSSD e frequência cardíaca, verificando-se o efeito do sexo e da suplementação. Os resultados obtidos não demonstraram efeitos significativos da suplementação ou influência do sexo

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sobre os índices; a suplementação dietética com PUFAS derivados do ômega n-3 por quatro semanas não diminuiu a frequência cardíaca e não promoveu elevação da VFC em cães saudáveis.

Palavras-chave: Cão, VFC, PUFAS, holter

Introduction

The analysis of heart rate variability (HRV) is a non-invasive indicator of the influence of the autonomic nervous system on cardiac performance and is quantified by the fluctuation of the R-R intervals (MALIK, 1996).

Sinus rate variability is a result of variations in the autonomic signals to the sinus nodes; modifications in this variability may be used as reliable markers of such stimuli to the heart (CALVERT, 1998). The HRV can be determined over brief periods by electrocardiogram or, more frequently, during ambulatory electrocardiographic monitoring (24-hour Holter monitoring) (CALVERT, 1998; CALVERT; WALL, 2001a, 2001b; GRUPI, 2008; RASSI JUNIOR, 2010). As a research tool, HRV evaluation has allowed a better understanding of the contribution of the autonomic nervous system in different physiological (ABBOT, 2007; FARIA; NOGUEIRA; SOUZA, 2009; OLIVEIRA et al., 2011), pathological (CALVERT; WALL, 2001b; GUZZETTI et al., 2001) and therapeutic (CALVERT, 1998; CARARETO et al., 2007) settings.

The measurements for HRV analysis, grouped linearly, are analyzed in the time and frequency domains. In HRV analysis in the time domain, thus called because results are expressed in time units (milliseconds), each normal R-R interval is measured for a specific amount of time. The indices that reflect fluctuations are then calculated during the cardiac cycle (MINORS; GRADY, 1997; RASSI JUNIOR, 2010).

Thus, the HRV indices reflect the variation and activity of the sympathetic and parasympathetic autonomic nervous system on the heart (CALVERT, 1998). One such index is based on the measurements of the intervals between two individual R waves

(RR), determined by SDNN (standard deviation of normal to normal RR intervals). Other indices include the SDANN (standard deviation of the average NN intervals calculated over 5 seconds), the SDNN index (the mean of the 5-second standard deviations of NN intervals) and a comparison between two adjacent RR intervals; examples of the latter include the Pnn50 (the percentage of adjacent RR intervals in which the variability exceeds 50 milliseconds) and the rMSSD (root mean square successive difference in NN intervals) (MINORS; GRADY, 1997; CALVERT, 1998).

The rMSSD and pNN50 indices reflect the HRV across short periods and are predominantly influenced by the parasympathetic tone; in contrast, SDNN, SDANN and SDNN indices are influenced by the sympathetic and parasympathetic tone and reflect long-term HRV (BIGGER et al., 1989, 1992, MASSIN; DERKENNE; VON BERNUTH, 1999).

It is believed that the increase in sympathetic nervous system function contributes to the development of cardiovascular diseases, considering that the parasympathetic activity seems to protect against sudden death caused by cardiovascular diseases (GRASSI; ESLER, 1999; CHRISTENSEN et al., 2010).

Thus, the HRV is prognostic of the acute death risk associated with various cardiac diseases in man; however, its relationship with canine heart diseases is still uncertain. Few studies describe the HRV in the context of chronic degenerative valve diseases, cardiomyopathies, or heart failure (PASCON; CAMACHO, 2009) compared to the HRV in healthy individuals.

Recently, there has been a growing interest in the relationship between supplementation with polyunsaturated fatty acids (PUFAs) and the decrease in mortality due to coronary diseases. The

primary benefits of their use have been described in the prevention and treatment of cardiovascular diseases in humans and animals (BILLMAN; KANG; LEAF, 1997, 1999).

The omega 3 fatty acids docosahexaenoic acid (DHA) and eicosapentanoic acid (EPA) are obtained by diets rich in fish oil. They are incorporated into the phospholipid bilayer of the cellular membrane, especially in the brain and heart (BILLMAN; HALLAQ; LEAF, 1994).

The benefits of omega-3 fatty acid supplementation on the cardiovascular system surpass their anti-thrombotic effects, as they have also been implicated in the prevention of ventricular arrhythmias in animals (LEAF, 1995; LEAF et al., 2005).

The cardioprotective effects of the omega 3 acids were also observed in healthy humans after dietary supplementation with different concentrations of PUFAs for a three month period. The dietary intervention revealed a dose-dependent effect promoting an increase in heart rate variability in men, although this effect was not observed in women (CHRISTENSEN et al., 1996, 1999).

Ventricular arrhythmias represent a common and often fatal complication in dogs with cardiomyopathy, including the arrhythmogenic right ventricular cardiomyopathy (ARVC) common in Boxer dogs and humans. A six-week study evaluating the effect of PUFA supplementation in Boxer dogs with ARVC concluded that omega 3 fatty acids were capable of reducing arrhythmias and were thus useful in the treatment of this disease (SMITH et al., 2007).

The effects of dietary supplementation with omega 3 fatty acids on heart rate and HRV in induced myocardial ischemia and after physical stress in dogs was described by Billman and Harris (2011). In this study, supplementation with 1, 2 or 4 grams of PUFAs derived from omega 3 was performed for 12 weeks. The treatment caused a dose-dependent increase in PUFA levels in the

right atrium and a decrease in heart rate; however, the HRV increase was dose-dependent in resting dogs. The supplementation was therefore unable to attenuate the heart rate changes and HRV in dogs with myocardial ischemia induced by coronary occlusion, thereby failing to alter the cardiac response to physiological challenges, such as exercise.

Despite the breadth of the research supporting the antiarrhythmic activity of omega 3 fatty acids in humans, animals and isolated heart cells, the effect of these fatty acids on heart rate variability in healthy dogs is still poorly understood. Based on the above, the objective of this study was to evaluate the effects of dietary supplementation for four weeks with PUFAs derived from omega 3 (EPA and DHA) and gender on heart rate variability in healthy dogs.

Materials and Methods

Twenty mixed breed, clinically healthy dogs between 2 and 3 years of age were enrolled in the study, including 10 males weighing between 22.3 and 10.2 kg and 10 females weighing between 18.1 and 7.9 kg. The animals, which belonged to the School of Veterinary Medicine and Animal Science – UNESP, were fed with good quality food and water *ad libitum*. The animals were clinically evaluated by physical examination (evaluation of the color of the mucosa, capillary refill time, peripheral lymph node palpation, heart rate, respiratory rate, abdominal palpation and rectal temperature), electrocardiogram, echocardiogram and Holter monitoring prior to their inclusion in the study.

The experimental design consisted of dynamic electrocardiographic monitoring (Holter monitoring) for 24 hours preceding and 4 weeks after daily oral supplementation with two capsules of one gram of omega 3 fatty acids. Each capsule contained 780 mg of EPA and 497 mg of DHA per unit, per animal; males received an average of 34.9 mg/kg EPA and 22.2 mg/kg DHA, whereas females received an average of 43 mg/kg EPA and 27 mg/

kg DHA. The fatty acid dose was based on previous studies of omega 3_a administration in dogs with dilated cardiomyopathy (FREEMAN et al., 1998; SMITH et al., 2007). The dogs received the omega 3 supplement each morning. A 24-hr period of Holter monitoring was performed, with continuous recording of three ECG channels in modified precordial derivations (V1, V3 and V5), using a digital device (Cardio Light), electromagnetic card (SD card) and computed analysis by decoding (Software CardioNet Client). As described by Calvert (1998), Yamaki et al. (2007) and Pascon and Camacho (2009), the recorder was directly attached to the back of the animal, offering freedom of movement to the animals while protecting the device and cables linked to the electrodes. The recording was standardized to the environment (location, temperature and noise), duration and the time when it was performed, thereby adapting the dogs to the procedure and environment.

The indices referring to heart rate variability according to the analysis in the time domain (NN, SDNN, SDANN, SDNN index, Pnn50 and rMSSD) were determined before and after the treatment. In addition, the minimum, medium and maximum heart rate as well as the rMMSD and pNN50 were determined at two different times of the day (wakefulness and sleep) to distinguish the possible influence of the supplementation on the vagal tone from its influence on supraventricular and ventricular arrhythmias.

For the statistical analysis, the dependent variables described above were analyzed by the PROC MIXED method of the SAS 9.2 software (SAS, Inst. Inc., Cary, NC, EUA). Sources of variation in the model including gender (male and female), time

(before and after PUFA supplementation) and first order interactions were considered fixed effects. The data were analyzed using animals (treatment) as a random effect. Wherever the ANOVA was significant, the means were separated using the LSD. The data were presented as a mean of the minimum squares and standard error. A significance level of 5% was used in all analysis.

Cholesterol and triglycerides were also measured in all animals after the supplementation with omega 3 fatty acids to assess if the dose used in the present study could have altered such laboratory values. According to Grundy et al. (2005), high EPA + DHA doses (2.4 g/d EPA + DHA) function in controlling severe hypertriglyceridemia.

Results and Discussion

There was no significant difference ($p < 0.05$) in the HRV indices between times (before and after the four weeks of supplementation) (Table 1) and gender (male and female), except for the rMSSD ($p < 0.001$); in this instance, the overall mean before and after four weeks of supplementation was higher in females than males. However, this difference was due to the higher values observed before the treatment, and therefore it is not clinically significant as it occurred only for this variable. This result is in contrast to the findings described by Christensen et al. (1999) in humans, in which the HRV indices in women were lower when compared to men. According to Sauder et al. (2013), the effect of a 3.5 g/d dose of omega 3 fatty acids on the HRV achieved statistical significance, reflecting a higher HRV after the indicated dose when compared to the placebo. The results for the HRV were not altered when women were excluded.

Table 1. Means and standard deviations of the HRV indices and the minimum, medium and maximum heart rate of healthy dogs before and after supplementation with PUFAs derived from omega 3.

Gender	Females		Males	
	Before	After	Before	After
Times				
Indices				
NN	749.7±130.6	767.0±107.0	742.1±106.8	759.3±97.6
SDNN	307.9±81.5	314.9±77.2	331.2±99.4	318.5±80.1
SDNN index	246.3±80.2	247.6±63.7	265.5±88.2	255.5±80.5
SDANN	176.5±31.6	188.1±50.3	197.4±61.4	184.6±38.7
rMSSD	162.0±27.3A*	147.7±54.3A	112.2±42.4B	122.2±63.6B
pNN50%	60.1±15.8	61.8±9.0	59.8±14.0	60.4±13.8
FC Minimal	39±1.6	38.5±1.6	38.7±1.2	37.3±1.2
FC Medium	89.3±1.6	88.8±1.6	91.8±1.6	87.8±1.6
FC Maximum	248.5±1.1	249.5±1.1	249.0±1.1	246.2±1.1

Source: Elaboration of the authors.

There was no significant difference in the heart rate of the dogs supplemented with PUFAs derived from omega 3 before and after the four weeks of supplementation. Contrary to the findings by Billman and Harris (2011), who performed similar supplementations but only for seven weeks, the current study found the concentration of the PUFAs derived from omega 3 in the heart tissue to be significantly increased after supplementation compared to the placebo group; consequently, this increase favorably altered the autonomic heart tone by lowering of the heart rate, elevation of HRV for resting dogs.

Likewise, the rMMSD and pNN50 indices at two daily time points (wakefulness and sleep) were not significantly influenced by supplementation or gender.

Such indices were chosen for analysis because they predominantly reflect the vagal tone; parasympathetic stimulation results in a quick and short-term response, and thus is reflected in the subsequent heart beats (RASSI JUNIOR, 2010).

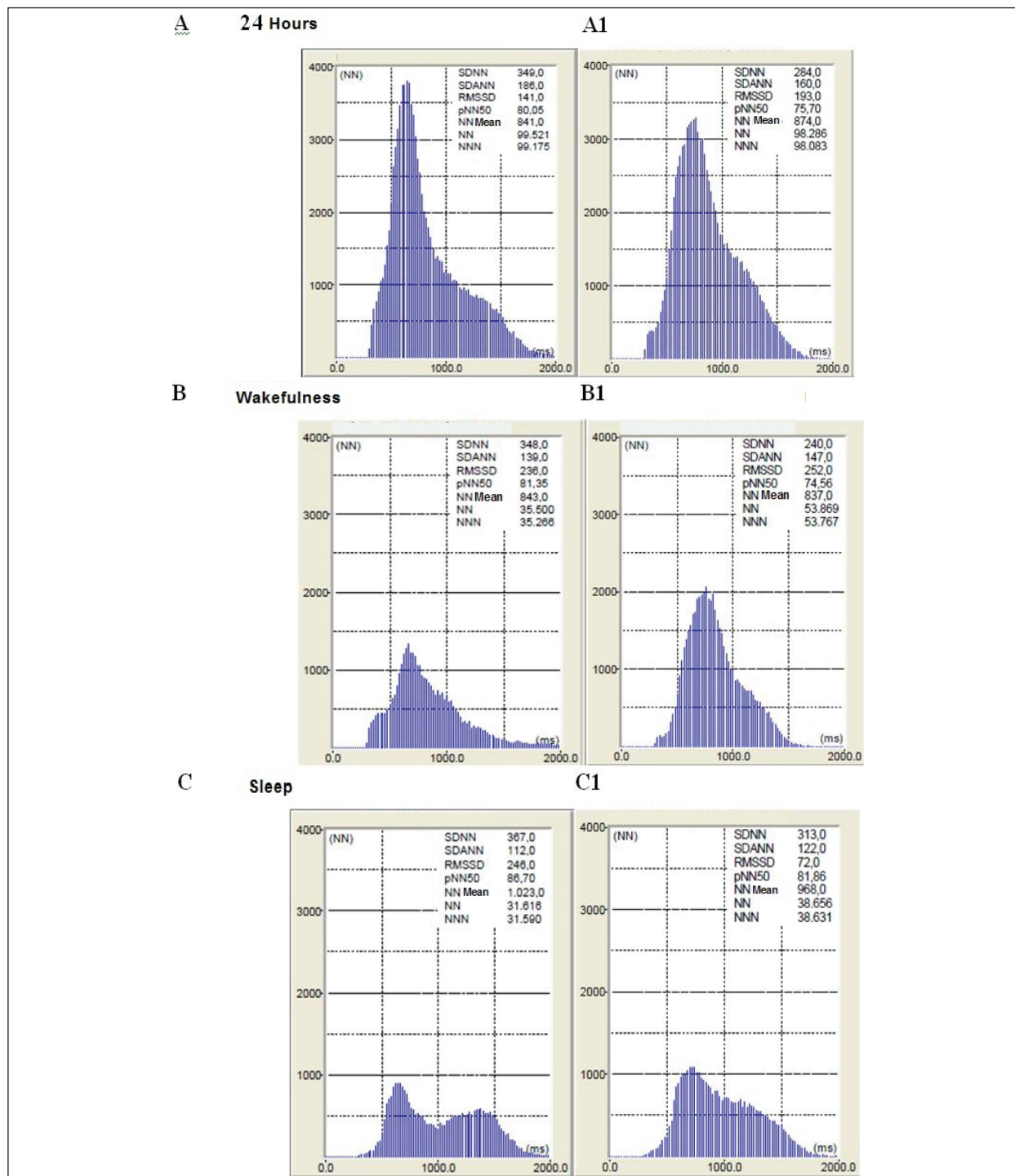
The HRV histogram (Figure 1) takes into account periods of wakefulness and sleep across the 24 hours of Holter monitoring. In the present study,

wakefulness coincided with an increase in the heart rate and a decrease in its variability. During sleep, the heart rate slowed but became more variable as a consequence of the circadian cycle; however, there was no significant difference between males and females or before and after supplementation with omega 3 fatty acids in healthy dogs.

Supplementation with omega 3 fatty acids did not significantly lower supraventricular or ventricular arrhythmias ($p < 0.33$ and $p < 0.31$, respectively). This outcome was unexpected, as the PUFAs derived from omega 3 have antiarrhythmic properties described by many authors (MCLENNAN, 1996; BILLMAN; HALLAQ; LEAF, 1994; BILLMAN; KANG; LEAF, 1997, 1999; SMITH et al., 2007).

It is possible that four weeks of supplementation were not enough to influence the autonomic heart balance of healthy dogs. According to Hansen et al. (1998) and Hall et al. (2006), the plasma concentrations increase significantly after one week of supplementation with omega 3; however, four to six weeks of continued supplementation are needed for this concentration to be maintained in dogs. Consistent dosing of n-3 fatty acids over long periods is also necessary to maintain the total plasma levels of EPA and DHA.

Figure 1. Histogram of the variable NN in the time domain during a 24 Holter evaluation of a female, healthy dog; A is before supplementation and A1 is after supplementation; B and B1 are during wakefulness; and C and C2 are during sleep.



NN: mean of all normal RR intervals; SDNN: standard deviation of all normal RR intervals of the exam; SDANN: standard deviation of the mean of the normal RR intervals taken every five minutes; SDNN index: standard deviation means calculated for the normal RR intervals taken every five minutes; pNN50: percentage of differences greater than 50 ms between adjacent normal RR intervals of the exam over 24 hours; rMSSD: square root of the mean squared differences in NN intervals. *Uppercase letters compare general means for gender ($p < 0.05$)

Source: Elaboration of the authors.

Di Stasi et al. (2004) previously evaluated the incorporation of n-3 PUFAs over time in human plasma and blood cells as a biomarker for other cellular membranes (such as myocytes) and determined that one gram per day of supplementation was capable of inducing significant changes in the composition of the polyunsaturated fatty acids in the blood, even after one week. This rapid uptake explains its beneficial effects against cardiovascular diseases.

Although the incorporation in the plasma and cells is rapid in both humans and dogs, the interaction of PUFAs with the autonomic tone of healthy dogs, and consequently the HRV and heart rate, most likely requires longer supplementation. These findings suggest that observable benefits are not only dose-dependent, as described by other authors but also time-dependent.

The supplementation for four weeks was capable of reducing the serum levels of cholesterol and triglycerides (ARAUJO et al., 2012); however, it was not enough to influence the autonomic tone or induce changes in the intrinsic activity of the cells of the cardiac pacemaker 24 (BILLMAN; HARRIS, 2011).

According to Albert et al. (2002) and Smith et al. (2007), intracellular concentrations of omega 3 of approximately 6.9% were associated with a 90% reduction in the risk of sudden cardiac death, which suggests that a threshold of PUFAs derived from omega 3 must be achieved in order for the significant changes in heart rate, HRV and arrhythmias to become detectable.

In the present study, the mean omega 3 dose used for males was 34.9 mg/kg EPA and 22.2 mg/kg DHA, whereas a mean of 43 mg/kg EPA and 27 mg/kg DHA was used for females. These doses were, based upon recent studies; however, according to Freeman et al. (1998), the dose required to obtain cardiovascular effects is 40 mg/kg of EPA and 25 mg/kg of DHA, which may explain the absence of statistically significant variations in the current study.

The goal of studying the cardiac autonomic response prior to supplementation with n-3 PUFAs has been to understand the effect of dietary changes on clinically healthy animals. The knowledge not only allows for an evaluation of dietary benefits in healthy animals but also permits a better interpretation of the interactions between diet and disease. Additional studies are necessary to evaluate the effect of fatty acids derived from omega 3 to make it routinely recommended to treat arrhythmias. In addition to determining an optimal dose, which may itself be problematic when comparing species and breeds, the duration of the treatment required to observe clinical benefits must be further investigated.

Conclusions

There are no differences in heart variability indices in the time domain between male and female dogs.

The supplementation with PUFAs derived from omega 3 [497 mg of docosahexaenoic acid (DHA) and 780 mg of eicosapentanoic acid (EPA)] in the diet of healthy dogs for four weeks was insufficient to provide an increase in HRV or a decrease in heart rate.

As a result of the present study, a longer period of supplementation (beyond four weeks) is suggested to obtain the desired effect on the autonomic heart tone, specifically a decrease in heart rate and increase in HRV indices.

Acquisition Sources

a. Fórmula Médica Compounding Pharmacy, Responsible Pharmacist Cintia Graziela Squissato Basqueira CRF-SP: 27.191 Av. Moema, 678 Moema SP Telefax: (11) 5055.7380.

b. Equipment certified by the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária – ANVISA), protocol number:

10361050006 – Light Cardios Digital Cardio Recorder – Advanced Equipments in Ambulatorial Electrocardiography and MAPA. Av. Paulista 509, São Paulo –SP – F.11-3883-3000.

c. Equipments certified by the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária – ANVISA), protocol number: 10361050002, from 11/02/00 –Holter Analyzer.

Ethics and Biosafety Committee

The present study was approved by the Animal Ethics Committee (Comissão de Ética no Uso de Animais – CEUA), under the protocol 69/2010. School of Veterinary Medicine and Animal Science - State University of São Paulo, Botucatu.

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