Archives of Veterinary Science **ISSN 1517-784X**
v.27, n.1, p. 134-148, 2022 www.ser.ufpr.br/veterinary

EFFECTS OF OVER–DAY AND OVERNIGHT FASTING ON THE HEMATOLOGICAL, BIOCHEMICAL AND OXIDATIVE PROFILE OF HEALTHY DOGS

(Efeito do período de jejum diurno e noturno sobre os parâmetros hematológicos, bioquímicos e de estresse oxidativo de cães saudáveis)

Wennely Karoline Costa Souza[1], Mariana Orlandini Mendonça[2], Tainara Martins de Oliveira[2], Geovanna Santos Gonçalves[3], Susana Eduardo Vieira[3], Guilherme Stuqui Monzani[4], Paula Lima de Oliveira[6], Maria Rachel Melo Bosculo[5], Beatriz Perez Floriano[1], Breno Fernando Martins Almeida[1, 6]

[1]Centro Universitário das Faculdades Integradas de Ourinhos (Unifio), Ourinhos, São Paulo, Brasil; [2]Programa de Aprimoramento Profissional em Medicina Veterinária (PAPMV), Área de Laboratório Diagnóstico Veterinário, Centro Universitário das Faculdades Integradas de Ourinhos (Unifio), Ourinhos, São Paulo, Brasil; [3]Programa de Residência Multiprofissional em Área Profissional da Saúde, Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Unesp Campus Botucatu, Botucatu, São Paulo, Brasil; [4]Fundação Educacional de Andradina (FEA), Andradina, São Paulo, Brasil; [5]Hospital Veterinário Roque Quagliato, Centro Universitário das Faculdades Integradas de Ourinhos (Unifio), Ourinhos, São Paulo, Brasil; [6]Faculdade de Medicina Veterinária de Araçatuba (FMVA), Unesp Campus Araçatuba, Araçatuba, São Paulo, Brasil.

*Corresponding author: breno.fernando@unifio.edu.br

Editor: Julia Arantes Galvão

ABSTRACT - The influence of the circadian rhythm on canine laboratory parameters is yet to be elucidated. This study aimed to investigate possible fluctuations that occur on laboratory tests of healthy dogs following overnight and over–day fasting. For this purpose, 20 adult clinically healthy dogs were enrolled in the study. Venous blood samples were obtained 7:00 am (ONF, overnight fasting) and at 7:00 pm (ODF, over–day fasting) at the same day, following a 12–hour period of respectively overnight and over–day fasting for hematological, biochemical and oxidative stress assessment. The erythrogram showed significant reduction of red blood cells, hemoglobin, hematocrit and mean corpuscular volume (MCV) measured by the cell counter following ODF. The only change seen on the leukogram was a lower lymphocyte count following ODF. No significant changes were found on platelet count, although ODF was associated with decreased mean platelet volume (MPV) and total plasma protein (TPP). ODF caused a significant reduction of total cholesterol, ALP, phosphate and triglycerides. On oxidative stress parameters, ODF reduced total oxidant capacity (TOC) and increased lipid peroxidation. In conclusion, the time of fasting can partly influence hematological, biochemical and oxidative stress parameters in dogs significantly, which warrants standardization of fasting period prior to laboratory tests in dogs, especially during research.

Key words: canine; laboratory evaluation; circadian cycle.

RESUMO - Pouco se conhece a influência do ritmo circadiano nos parâmetros laboratoriais caninos. O presente estudo teve como objetivo investigar possíveis alterações que ocorrem nos testes laboratoriais de cães saudáveis após jejum noturno e diurno. Para tal, 20 cães adultos clinicamente saudáveis foram incluídos no estudo. Amostras de sangue venoso foram obtidas às 7h00 (ONF, jejum noturno) e às 19h00 (ODF, jejum diurno) no mesmo dia, após um período de 12 horas de jejum noturno e diurno, respectivamente, para avaliação hematológica, bioquímica e de estresse oxidativo. O eritrograma apresentou redução significativa de hemácias, hemoglobina, hematócrito e volume corpuscular médio (VCM) medido pelo contador de células após ODF. A única alteração observada no leucograma foi redução de linfócitos após ODF.

Received in 06/17/2020
Approved in 01/19/2021

Nenhuma alteração significativa foi observada na contagem de plaquetas, embora diminuição do volume plaquetário médio (VPM) e proteína plasmática total (TPP) tenha sido observada após ODF. ODF causou uma redução significativa do colesterol total, FA, fósforo e triglicerídeos. Nos parâmetros de estresse oxidativo, o ODF reduziu a capacidade oxidante total (COT) e aumentou a peroxidação lipídica. Em conclusão, o tempo de jejum pode influenciar parcialmente os parâmetros hematológicos, bioquímicos e de estresse oxidativo em cães de forma significativa, o que garante a padronização do período de jejum antes dos testes laboratoriais em cães, especialmente em amostras destinadas à pesquisa.
Palavras-chave - canino; avaliação laboratorial; ciclo circadiano.

INTRODUCTION

The circadian rhythm comprises a biological period of 24 hours that allows living beings to adapt to external environmental events (Pittendrigh, 1981). It is responsible for the rhythmicity of the organism's physiological and behavioral activities, such as eating, sleeping and waking time, known as the sleep-wake cycle, as well as the light and dark cycle, external temperature, among others (Rotenberg et al., 1997). Although several pre-analytical factors are known to affect laboratory analyses, little is known about how the circadian cycle might influence laboratory parameters of healthy dogs. It is known, for example, that failure to follow the recommended fasting time for laboratory tests can result in lipemic blood samples (Radin, 2012; Silva et al., 2019), thus leading to errors in biochemical (Bonatto et al., 2021; Oliveira et al., 2020a) and even hematological analyses (Costa et al., 2020; Oliveira et al., 2020b). The same happens with hemolyzed samples submitted to biochemical analyses (Almeida et al., 2011). However, little is known regarding how circadian variations related to overnight or over−day fasting can influence hematological, biochemical and oxidative stress parameters in dogs.

Hematological and biochemical analyses are highly requested in veterinary medicine, since they are essential for diagnosing diseases, establishing a prognosis and monitoring treatments (Nilsen et al., 2010). Both analyses are usually unspecific, although they could be altered due to the most diverse pathological conditions. Serum biochemistry can assist in the diagnosis of metabolic disorders (Nelson; Couto, 2001), reflecting changes on hepatic, renal, digestive and endocrine systems, among others (Meyer; Harvey, 2004; Nelson; Couto, 2001). The blood count provides quantitative information, including red blood cell (RBC), white blood cell (WBC) and platelet (PLT) counts, differential leukocyte count, hematocrit (HCT) or globular volume (GV), hemoglobin concentration, hematimetric indices such as mean corpuscular volume (MCV), mean platelet volume (MPV), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW). In addition, qualitative evaluations provide

information on the morphology of RBC, WBC and PLT, in addition to detecting the presence of hematozoa, and can be performed on blood smears (Rebar et al., 2003).

Oxidative stress (OS) occurs due to an imbalance between oxidizing substances and antioxidants, either related to excessive production of oxidants or decreased antioxidant defense (Almeida et al., 2017; Almeida et al., 2013; Ferreira; Matsubara, 1997). Oxidants are produced during metabolization of oxygen and are called reactive oxygen species (ROS) (Barreiros et al., 2006). In humans, the involvement of the circadian rhythm in the development of OS has been demonstrated both in physiological and pathological conditions (Brian; Musiekb, 2020; Kanabrocki et al., 2002; Wilking et al., 2013). In veterinary medicine, a few studies have shown that the increase in oxidizing substances and the reduction in antioxidants are related primarily or secondarily to the development of disease, leading to excessive cellular injury by oxidizing substances (Curtis, 2013). In dogs, oxidative stress has been widely investigated, including pathological conditions such as uremia (Almeida et al., 2013; Bosco et al., 2016; Silva et al., 2013), leishmaniasis (Almeida et al., 2013; Almeida et al., 2017), sarcoptic mange (Beigh et al., 2016), lymphoma (Bottari et al., 2015), breast carcinoma (Machado et al., 2015), monocytic ehrlichiosis (Rubio et al., 2017a), periodontal disease (Silva et al., 2018), reproductive disorders (Andrade et al., 2010), inflammatory bowel disease (Rubio et al., 2017b), among others. However, despite the abundance of studies investigating OS in dogs, there are currently no studies that have determined possible circadian variations resulting from fasting time on canine OS parameters.

In view of the scarcity of studies in veterinary medicine evaluating the effect of the fasting period on canine hematological, serum biochemical and oxidative stress parameters, the present study aimed to identify changes on blood count, biochemistry and oxidative stress determinations in healthy dogs undergoing over–day and overnight fasting.

MATERIAL AND METHODS

Approval by the Ethics Committee

The research project was approved by the Ethics Committee for Animal Usage of the Centro Universitário de Ourinhos (Protocol No. 022/2020). Participation of all dogs in the study involved signing an informed consent form by their guardian.

Animal selection

Twenty adult dogs of both sexes (12 females and 8 males) aged 2 to 6 years and weighing 12.6 ± 8 kg were enrolled in the study. Subjects were deemed healthy based on clinical examination and laboratory tests performed three days before inclusion in the study (blood count, ALT, albumin, creatinine, FA, total protein and urea).

Experimental design

Subjects had venous blood samples taken twice on the same day and at the same time, to comprise the following experimental groups:

Overnight fasting (ONF): the day before the first blood sample, dogs were fed precisely at 7:00 pm and only dogs that ate all the food provided were included in the study. Following 12 hours of overnight fasting, the blood sample was obtained at 7:00 am. Immediately after blood collection, food was again provided and over−day fasting was initiated following the same criteria.

Over−day fasting (ODF): the blood sample was obtained precisely at 7:00 pm following a 12−hour over−day fasting period.

All dogs received exclusively commercial food, at the amount indicated by each manufacturer. Each dog received its own food without changing the product and only dogs who ate all the food prior to each fasting period were included.

Sample collection and laboratory tests

Blood samples were collected through jugular venipuncture and conditioned in K_2EDTA tubes (BD Vacutainer®, Becton-Dickson, New Jersey, USA) to perform complete blood count (CBC) and in clot activator tubes (BD Vacutainer®, Becton-Dickson, New Jersey, USA) for serum biochemical and OS analyses. For serum samples, tubes were centrifuged 20 minutes following blood collection at 3000 rpm during 5 minutes and serum was stored at −20 ºC protected from light until determinations were performed for a maximum period of 10 days.

CBC was performed by an automated veterinary cell counter (ABX Micros ESV 60, Paris, France) previously calibrated and verified with low, normal and high-level controls (ABX Minotrol 16, Paris, France), for determination of RBC, WBC and PLT counts, HCT, hemoglobin, RDW, MPV, MCHC and MCV. Additionally, assessment of HCT was also performed using the Strumia microcapillary method by centrifugation at 11400 rpm for 5

minutes. Differential leukocyte count, along with cellular morphological assessments, as well as platelet estimation per 1,000× field, were performed on a blood smear stained with commercial hematological dye (Panótico Rápido, Laborclin, Pinhais, PR, Brazil). Total plasma protein (TPP) was determined using a portable refractometer (ATAGO, Mod. Master-SUR-NM, Tokio, Japan).

Serum biochemical analyses were performed by a semi-automated photocolorimeter (BIO 2000, BioPlus, São Paulo, Brazil) using a set of commercial reagents (Labtest Diagnóstica SA, Minas Gerais, Brazil) according to manufacturer's recommendations. Biochemical determinations were performed in duplicate at 37 ºC after calibration with a calibrator (Calibra H, Labtest Diagnóstica SA, Minas Gerais, Brazil) and verification with commercial controls level I (Qualitrol 1H, Labtest Diagnóstica SA, Minas Gerais, Brazil) and II (Qualitrol 2H, Labtest Diagnóstica SA, Minas Gerais, Brazil). Total cholesterol and triglycerides levels were determined by the enzymatic-Trinder method, glucose by the glucose oxidase-Trinder method, ALT and AST by the ultraviolet (UV) kinetic methodology, albumin by the colorimetric method with bromocresol green, creatinine by the colorimetric method of the alkaline picrate − Jaffé, calcium by the colorimetric method (cresolphthalein), ALP by the Bowers and McComb modified kinetic method, phosphate by the colorimetric method, total proteins by the biuret colorimetric method and urea by UV enzymatic methodology. Globulin levels were obtained by subtracting albumin from total protein.

OS parameters were also determined from serum by a semiautomated photocolorimeter (BIO 2000, BioPlus, São Paulo, Brazil). Total antioxidant capacity (CAT) was determined by reduction of ABTS cation (TAC-ABTS) described by Erel (2004), by reduction of the ABTS cation associated with peroxidase (TAC-ABTS+HRP) described by Rubio et al. (2016a), by ferric reducing ability (TAC-FRAP) described by Benzie and Strain (1996), and by cupric reducing antioxidant capacity (TAC-CUPRAC) described by Rubio et al. (2016b). Determination of total oxidant capacity (TOC) was also performed by the colorimetric method of xylenol orange described by Erel (2005) and lipid peroxidation was determined by thiobarbituric acid reactive substances (TBARS), as described by Hunter et al. (1985).

Statistical analysis

Data were first tested for normality using the Shapiro-Wilk test and mean comparisons were performed using paired-t or Wilcoxon tests. Analyses were performed

by a computer software (GraphPad Prism, v.6.00 for Windows, GraphPad Software, La Jolla, CA, USA, www.graphpad.com) and significance was considered when $P<0.05$.

RESULTS

Blood samples obtained after ODF showed lower concentration of RBC (Figure 1A), hemoglobin (Figure 1B), HCT by both methodologies (Figures 1C and D) and MCV measured by the cell counter (Figure 1E) compared with samples obtained after ONF, with no differences in other variables from the erythrogram.

Leukogram results only showed lower lymphocyte count in ODF samples compared with those obtained after ONF (Figure 1L). There was no difference in total leukocyte (Figure 1J), segmented neutrophils (Figure 1K), monocytes (Figure 1M), eosinophils (Figure 1N) and basophils (data not shown) counts.

There was no change in platelet counts obtained by the cell counter (Figure 1O) and by estimation on blood smear (Figure 1Q) in both groups. However, after ODF, a significant reduction in MPV (Figure 1P) and TPP (Figure 1R) was observed compared with ONF.

Regarding biochemical parameters, ODF caused a significant reduction in ALP (Figure 2C), total cholesterol (Figure 2G), phosphate (Figure 2L) and triglycerides (Figure 2N) compared with ONF. No differences were found in albumin (Figure 2A), ALT (Figure 2B), AST (Figure 2D), calcium (Figure 2E), HDL cholesterol (Figure 2F), creatinine (Figure 2H), GGT (Figure 2I), globulin (Figure 2J), glucose (Figure 2K), total protein (Figure 2M) and urea (Figure 2O).

Oxidative stress results showed a decrease of TOC (Figure 3E) and an increase of lipid peroxidation assessed by the TBARS method (Figure 3F) in ODF compared with ONF. There was no change in TAC-ABTS (Figure 3A), TAC-ABTS+HRP (Figure 3B), TAC-CUPRAC (Figure 3C), TAC-FRAP (Figure 3D) and uric acid (Figure 3G).

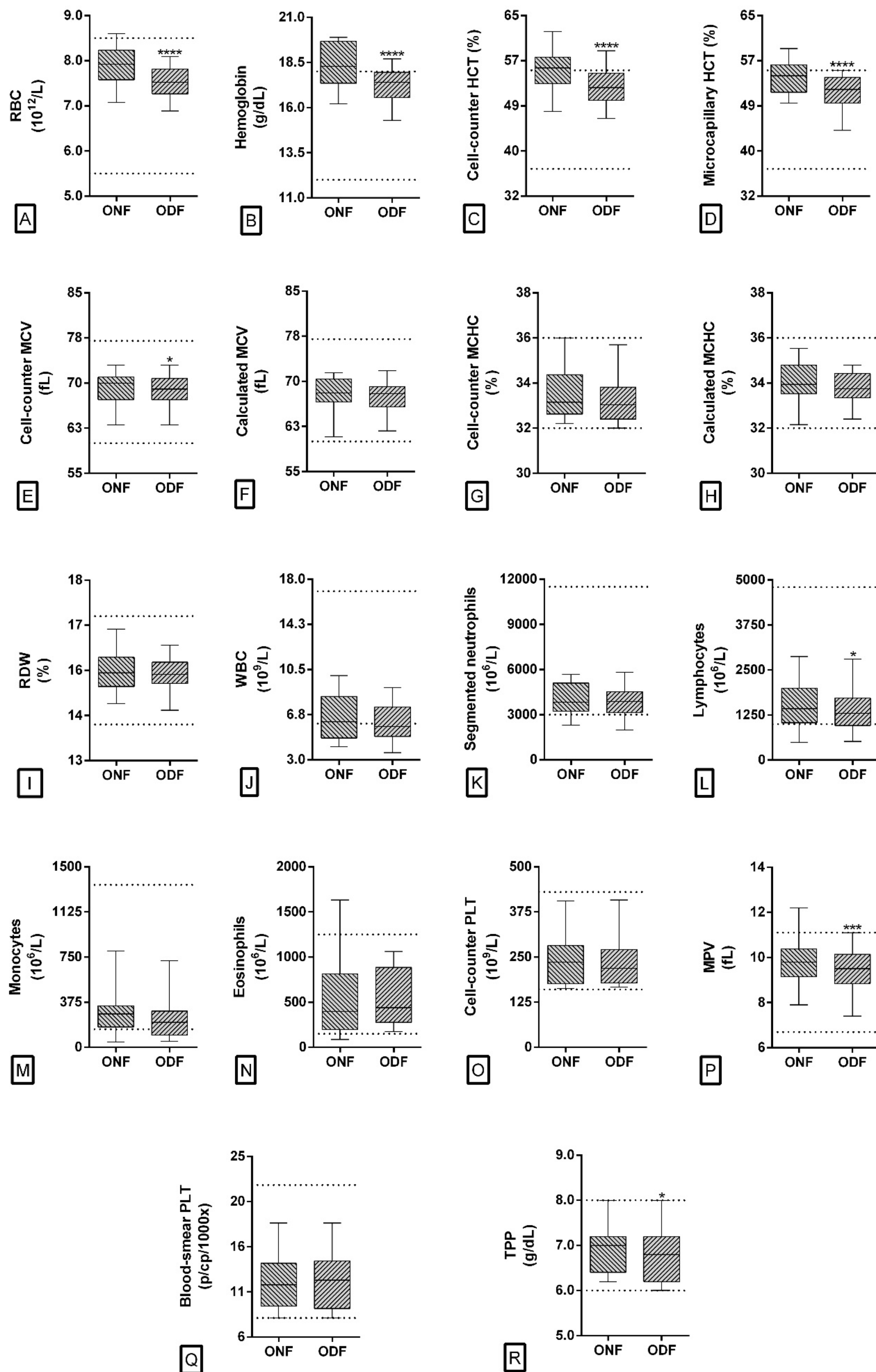
RBC (10^{12}/L)
Hemoglobin (g/dL)
Cell-counter HCT (%)
Microcapillary HCT (%)
Cell-counter MCV (fL)
Calculated MCV (fL)
Cell-counter MCHC (%)
Calculated MCHC (%)
RDW (%)
WBC (10^9/L)
Segmented neutrophils (10^6/L)
Lymphocytes (10^6/L)
Monocytes (10^6/L)
Eosinophils (10^6/L)
Cell-counter PLT (10^9/L)
MPV (fL)
Blood-smear PLT (p/cp/1000x)
TPP (g/dL)
ONF
ODF
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R

Figure 1 – Red blood cells (RBC, **A**), hemoglobin (**B**), hematocrit (HCT) obtained by the cell counter (**C**) and by the microcapillary method (**D**), mean corpuscular volume (MCV) measured by the cell counter (**E**) and calculated (**F**), mean corpuscular hemoglobin concentration (MCHC) obtained by the cell counter (**G**) and calculated (**H**), RBC distribution width (RDW, **I**), white blood cells (WBC, **J**), segmented neutrophils (**K**), lymphocytes (**L**), monocytes (**M**), eosinophils (**N**), platelets obtained by the cell counter (**O**), mean platelet volume (MPV, **P**), platelet estimation on blood smear (**Q**) and total plasma protein (TPP, **R**) from healthy dogs (n=20) following overnight fasting (ONF) and over–day fasting (ODF) of 12 hours. Bars indicate minimum and maximum values and boxes represent the first and third quartiles. Statistically significant difference is indicated by * (P<0.05), ** (P<0.01), *** (P<0.001) or **** (P<0.0001). Dashed lines indicate reference values for the canine species according to Rizzi et al. (2010).

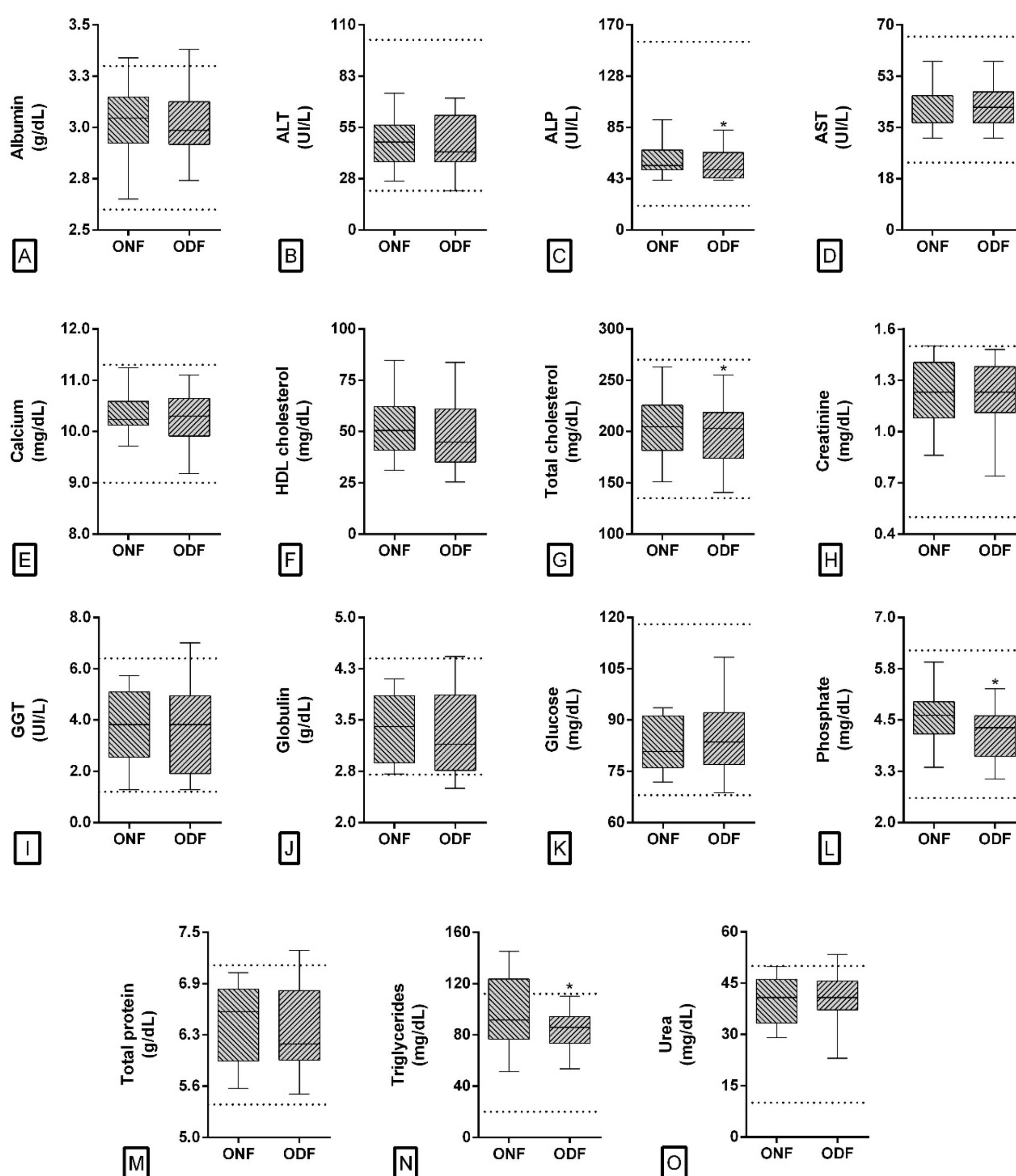

Figure 2 – Albumin (**A**), ALT (**B**), ALP (**C**), AST (**D**), calcium (**E**), HDL cholesterol (**F**), total cholesterol (**G**), creatinine (**H**), GGT (**I**), globulin (**J**), glucose (**K**), phosphate (**L**), total protein (**M**), triglycerides (**N**) and urea (**O**) from healthy dogs (n=20) following overnight fasting (ONF) and over–day (ODF) of 12 hours. Bars indicate minimum and maximum values and boxes represent the first and third quartiles. Statistically significant difference is indicated by * (P<0.05), ** (P<0.01), *** (P<0.001) or **** (P<0.0001). Dashed lines indicate reference values for the canine species according to Kaneko et al. (2008).

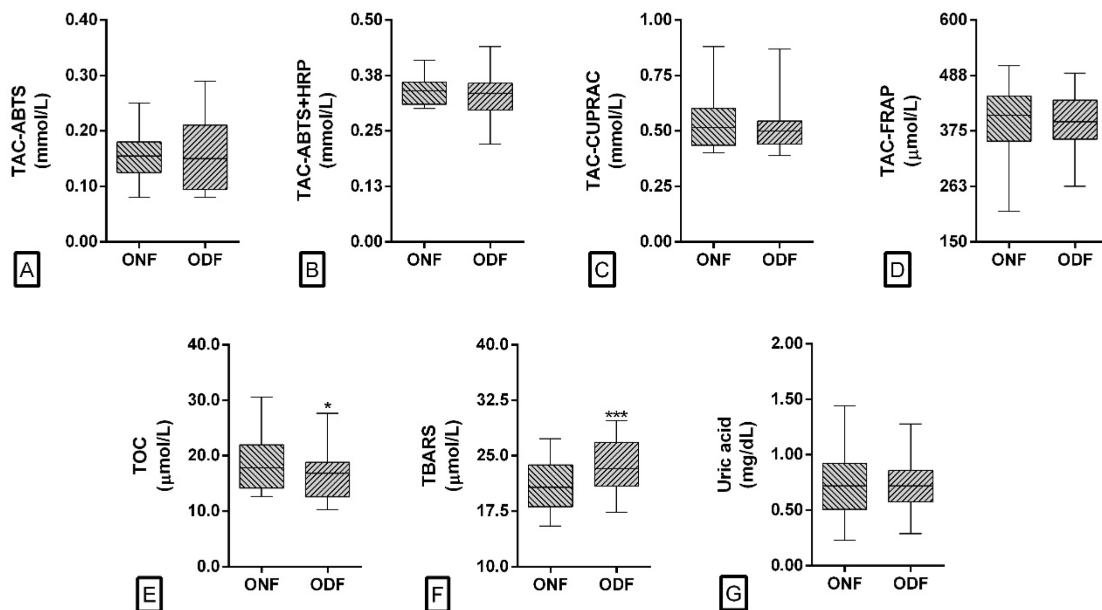

Figure 3 – TAC-ABTS (**A**), TAC-ABTS+HRP (**B**), TAC-CUPRAC (**C**), TAC-FRAP (**D**), TOC (**E**), lipid peroxidation determined by thiobarbituric acid reactive substances (TBARS, **F**) and uric acid (**G**) from healthy dogs (n=20) following overnight fasting (ONF) and over–day fasting (ODF) of 12 hours. Bars indicate minimum and maximum values and boxes represent the first and third quartiles. Statistically significant difference is indicated by * (P<0.05), ** (P<0.01), *** (P<0.001) or **** (P<0.0001).

DISCUSSION

Considering that the effects of overnight or over–day fasting on canine laboratory determinations are not elucidated, the present study is the first to detect significant laboratory changes in hematological, biochemical and oxidative stress parameters of healthy dogs following overnight and over–day fasting.

After ODF, there was significant reduction of RBC, hemoglobin, HCT, cell-counter MCV, MPV and TPP compared with ONF. In humans, Lippi et al. (2010) reported decreased RBC, hemoglobin and HCT 4 hours after a meal. The authors attributed these variations to postprandial hemodilution resulting from fluid intake. A similar situation was observed in dogs by Costa et al. (2020), in which reductions in erythrogram parameters and TPP were observed from 2 hours after feeding throughout the day. In the present study, the decrease in HCT and TPP following ODF is likely a result of higher water intake

during the day. As a consequence, upon waking up from ONF, the HCT and TPP are higher due to hemoconcentration, similar to what was observed by Costa et al. (2020) in samples obtained during the early hours of the day. Korbonits et al. (2007) reported a considerable decrease in MCV 4 hours after feeding without any change in RDW, as well as a decrease in RBC, hemoglobin, HCT and proteins. According to the author, the increase in postprandial sodium concentrations would attract intracellular and tissue fluid, thus contributing to increased vascular volume and decreased cell volume. In the present study, the lowest volume of RBC (MCV) and PLT (MPV) was recorded after ODF, which likely demonstrates such diurnal changes in sodium metabolism that are responsible for attracting fluid from RBC and PLT, thereby reducing their volume. To date, studies evaluating the effect of fasting time during different periods of day on canine blood counts are scarce and the variations that may occur are poorly described.

Daytime fasting caused a significant reduction of lymphocyte concentration compared with ONF, with 6 dogs (30%) showing lymphocyte counts below the reference interval for the species. Costa et al. (2020) also reported a decrease in lymphocytes 2 and 6 hours after feeding at daytime, which was attributed to feeding. Studies in humans have observed decreased lymphocytes between 2 and 5 hours following a meal (Hansen et al., 1997; Lippi et al., 2010). Postprandial lymphocyte reduction could be justified by greater migration of these cells to intestinal tissue, where they will act in the immune defense (Hansen et al., 1997). However, in the present study, both samples were obtained 12 h after feeding, which makes it possible to suggest that hormonal influences such as diurnal cortisol levels may have contributed to these results. Cortisol levels are known to increase during the day and decrease at night (Bernardi et al., 2009). When released during stressful situations, cortisol induces lysis of lymphocytes, thus decreasing its blood counts (Jain, 1993).

Regarding biochemical parameters, samples obtained after ODF showed lower levels of total cholesterol, phosphate, triglycerides and activity of ALP compared with samples obtained after ONF. When compared to the reference interval previously established for dogs (Kaneko et al., 2008), 8 dogs (40%) were hypertriglyceridemic following ONF with normalization of levels following ODF. GGT activity exceeded reference intervals in eight dogs (40%) following ODF. Albumin, calcium, HDL cholesterol, creatinine, glucose, globulin, total protein and urea levels and ALT, AST and ALP activities failed to show clinically relevant changes. Piccione et al. (2008), while studying dogs undergoing 12–hour fasting during light and dark cycles, have observed changes only on serum triglyceride levels with decreased levels during the day. The authors then suggest

that the circadian light–dragging oscillator possibly influenced the liver, one of the main organs responsible for triglyceride biosynthesis. However, there are no studies that report changes in other biochemical constituents during different periods of fasting in dogs.

With regard to OS markers, ODF caused a significant reduction in TOC and a significant increase in lipid peroxidation levels, without changing other markers. It was evident that during the night, there was greater production of oxidants, thus contributing to greater lipid peroxidation during the day without any change in TAC. Cellular concentrations of many antioxidants and small protective molecules are influenced by the circadian rhythm, as seen in animal and human models (Wilking et al., 2013). In rats, which are nocturnal animals, the activity of superoxide dismutase (SOD), an enzyme with antioxidant potential, increases during the night together with peak lipid peroxidation (Diaz-Munoz et al., 1985). These findings corroborate the findings of the present study, in which peak lipid peroxidation in dogs happened during the day, which is the most active period of this species. In humans, the peak of catalase, another antioxidant enzyme, occurred at the beginning of the light phase (Singh et al., 2005). If we were to extrapolate these findings to dog's antioxidant enzymes that were not assessed in the present study likely contributed to the reduction of TOC observed during the day, and consumption of precisely these enzymes contributed to greater lipid peroxidation. However, so far, the effect of several periods of fasting on oxidative stress biomarkers in dogs is not well understood and still warrants further investigation.

For a given pattern to be considered circadian, it must have five main attributes, including (1) the ability to become synchronized; (2) the persistence of the cycle after removing external stimuli; (3) the ability to change the phase of the cycle; (4) duration of about 24 h; and (5) maintenance of its periodicity regardless of temperature (Bell-Pedersen et al., 2001). The main limitation of the present study was that these characteristics were not assessed to prove that the observed changes were purely a result of circadian variations. However, when considering that the dogs were healthy and that the feeding times of the animals were standardized with variations only in the time for obtaining blood samples, it is plausible to assume that such variations are in fact circadian variations.

CONCLUSION

Overnight or over–day fasting significantly affects a few hematological, biochemical and oxidative stress parameters in healthy dogs. Although changes are mostly discrete and do not lead to significant clinical errors, they are especially important in scientific research, and therefore the time for obtaining blood samples should be standardized to minimize data variability.

REFERENCES

ALMEIDA, B.F.M.; NARCISO, L.G.; MELO, L.M. et al. Leishmaniasis causes oxidative stress and alteration of oxidative metabolism and viability of neutrophils in dogs. **The Veterinary Journal**, v.198, p.599-605, 2013.

ALMEIDA, B.F.M.; SILVA, K.L.O.; CHIKU, V.M. et al. The effects of increased heme oxygenase-1 on the lymphoproliferative response in dogs with visceral leishmaniasis. **Immunobiology**, v. 222, n. 5, p. 693-703, 2017.

ALMEIDA, B.F.M.; ZUCATTO, A.S.; VIEIRA, R.F.C. et al. Effect of hemolysis on canine, bovine and equine serum chemistry. **Medicina Veterinária**, v.5, p.12-17, 2011.

BARREIROS, A. L. B. S; DAVID, J. M; DAVID, J. P. D. L. Estresse oxidativo: relação entre geração de espécies reativas e defesa do organismo. **Química Nova**, v. 29, p.113-123, 2006.

BEIGH, S.A.; SOODAN, J.S.; BHAT, A.M. Sarcoptic mange in dogs: its effect on liver, oxidative stress, trace minerals and vitamins. **Veterinary Parasitology**, v.227, p.30-34, 2016.

BELL-PEDERSEN, D.; CROSTHWAITE, S.K.; LAKIN-THOMAS, P.L. et al. The Neurospora circadian clock: simple or complex? **Philosophical Transactions of the Royal Society B: Biological Sciences**, v.356, p.1697-1709, 2001.

BENZIE, I.F.F.; STRAIN, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. **Analytical Biochemistry**, v. 239, p.70-76, 1996.

BERNARDI, F.; HARB, A.B.C.; LEVANDOVSKI, R.M. et al. Transtornos alimentares e padrão circadiano alimentar: uma revisão. **Revista de Psiquiatria do Rio Grande do Sul**, v. 31, p.170-176, 2009.

BONATTO, N.C.M.; OLIVEIRA, P.L.; MANCEBO, A.M. et al. Postprandial lipemia causes oxidative stress in dogs. **Research in Veterinary Science**, v.136, p. 277-286, 2021.

BOSCO, A.M.; PEREIRA, P.P.; ALMEIDA, B.F.M. et al. Free p-cresol alters neutrophil function in dogs. **Artificial Organs**, v.40, p.480-488, 2016.

BOTTARI, N.B.; MUNHOZ, T.D.; TORBITZ, V.D. et al. Oxidative stress in dogs with multicentric lymphoma: effect of chemotherapy on oxidative and antioxidant biomarkers. **Redox Report**, v.20, p.267-274, 2015.

COSTA, L.R.; SILVA, N.L.T.; OLIVEIRA, P.L. et al. Diurnal variations in canine hematological parameters after commercial feed feeding. **Semina Ciências Agrárias**, v.41, p.2219–2230, 2020.

CURTIS, A.O. **Oxidative stress parameters in dogs naturally infected with canine distemper virus.** 2013. 61 f. Dissertação (Mestrado em Medicina Veterinária) - Universidade Federal de Santa Maria, Santa Maria, 2013.

DIAZ-MUNOZ, M.; HERNANDEZ-MUNOZ, R.; SUAREZ, J. et al. Day-night cycle of lipid peroxidation in rat cerebral cortex and their relationship to the glutathione cycle and superoxide dismutase activity. **Neuroscience**, v.16, p.859-863, 1985.

EREL, O. A new automated colorimetric method for measuring total oxidant status. **Clinical Biochemistry**, v.38, p.1103-1111, 2005.

EREL, O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. **Clinical Biochemistry**, v.37, p.277-285, 2004.

FERREIRA, A.L.A.; MATSUBARA, L. Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. **Revista da Associação Médica Brasileira**, v.43, p.61-68, 1997.

HANSEN, K.; SICKELMANN, F.; PIETROWSKY, R. et al. Systemic immune changes following meal intake in humans. **American Journal of Physiology-Regulatory**, v.273, p.548-553, 1997.

HUNTER, M.I.; NLEMADIM, B.C.; DAVIDSON, D.L. Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluid from multiple sclerosis patients. **Neurochemical Research**, v.10, p.1645–1652, 1985.

JAIN, N.C. **Essentials of Veterinary Hematology**, 1st ed. Philadelphia: Wiley-Blackwell, 1993, 417p.

KANEKO, J.J.; HARVEY, J.W.; BRUSS, M.L. **Clinical Biochemistry of Domestic Animals**, 6th, Academic Press: London, 2008.

KANABROCKI, E.L.; MURRAY, D.; HERMIDA, R.C. et al. Circadian variation in oxidative stress markers in healthy and type II diabetic men. **Chronobiology International**, v.19, p.423-439, 2002.

KORBONITS, M.; BLAINE, D.; ELIA, M.; POWELL-TUCK, J. Metabolic and hormonal changes during the refeeding period of prolonged fasting. **European Journal of Endocrinology**, v.157, p.157-166, 2007.

LANANNA, B.V.; MUSIEK, E.S. The wrinkling of time: Aging, inflammation, oxidative stress, and the circadian clock in neurodegeneration. **Neurobiology of Disease**, v.139, e104832, 2020.

RADIN, M.J. Laboratory evaluation of lipids. In: THRALL, M.A.; WEISER, G.; ALLISON, R.W.; CAMPBELL, T.W. **Veterinary Hematology and Clinical Chemistry**, 2nd ed., John Wiley & Sons: Ames, p.480-496, 2012.

LIPPI, G.; LIMA-OLIVEIRA, G.; SALVAGNO, G. L. et al. Influence of a light meal on routine haematological tests. **Blood Transfusion**, v.8, p.94-99, 2010.

MACHADO, V.S.; CRIVELLENTI, L.Z.; BOTTARI, N.B. et al. Oxidative stress and inflammatory response biomarkers in dogs with mammary carcinoma. **Pathology - Research and Practice**, v.211, p.677-681, 2015.

MEYER, D.J; HARVEY, J.W. **Veterinary Laboratory Medicine: Interpretation and Diagnosis**. Saunders: St. Louis, 2004, 351p.

NELSON, R.W.; COUTO, C.E. **Medicina Interna de Pequenos Animais**. Guanabara Koogan: Rio de Janeiro, 2001, 1084p.

NILSEN, L.; KJELGAARD-HANSEN, M.; JENSEN, A.L. et al. Breed-specific variation of hematologic and biochemical analyses in healthy adult Bernese Mountain dogs. **Veterinary Clinical Pathology**, v.39, p.20-28, 2010.

OLIVEIRA, P.L.; BONATTO, N.C.M.; BOSCULO, M.R.M. et al. Effect of post-prandial lipemia on canine biochemical parameters. **Comparative Clinical Pathology,** v.29, p.763-775, 2020a.

OLIVEIRA, P.L.; SOUZA, S.L.; BONATTO, N.C.M. et al. Effect of food intake on complete blood count of healthy dogs. **Revista Agrária Acadêmica**, v.3, p.105-116, 2020b.

PICCIONE, G.; BERTOLUCCI, C.; FAZIO, F. Daily rhythms of serum lipids in dogs: influences of lighting and fasting cycles. **American Association for Laboratory Animal Science**., v. 58, p. 485-489, 2008.

PITTENDRIGH, C.S. Circadian systems, general perspective. In: ASCHOFF, F. **Handbook of Behavioral Neurobiology: Biological Rhythms**, New York: Plenum Press, v. 4, p. 331-336, 1981.

REBAR, A.H. et al. **Guia de Hematologia para Cães e Gatos.** 1. ed. São Paulo: Roca, 2003, 304p.

RIZZI, T.E.; MEINKOTH, J.H.; CLINKENBEARD, K.D. Normal hematology of the dog. In: WEISS, D.J.; WARDROP, K.J. **Schalm´s Veterinary Hemathology**. 6th ed. Wiley Blackwell: Iowa, Cap 104, p.799-810, 2010.

ROTENBERG L.; MARQUES, N.; BARRETO, M. História e perspectivas da cronobiologia. In: MARQUES, N.; BARRETO, M. **Cronobiologia: princípios e aplicações**. EDUSP/FIOCRUZ, p. 31-53, 1997.

RUBIO, C.P.; HERNÁNDEZ-RUIZ, J.; MARTINEZ-SUBIELA, S. et al. Validation of three automated assays for total antioxidant capacity determination in canine serum samples. **Journal of Veterinary Diagnostic Investigation**, v.28, p.693-698, 2016a.

RUBIO, C.P.; MARTINEZ-SUBIELA, S.; HERNÁNDEZ-RUIZ, J. et al. Analytical validation of an automated assay for ferric-reducing ability of plasma in dog serum. **Journal of Veterinary Diagnostic Investigation**, v.29, p.574-578, 2016b.

RUBIO, C.P.; YILMAZ, Z.; MARTÍNEZ-SUBIELA, S. et al. Serum antioxidant capacity and oxidative damage in clinical and subclinical canine ehrlichiosis. **Research in Veterinary Science**, v.115, p.301-306, 2017a.

RUBIO, C.P.; MARTÍNEZ-SUBIELA, S.; HERNANDEZ-RUIZ, J. et al. Serum biomarkers of oxidative stress in dogs with idiopathic inflammatory bowel disease. **Veterinary Journal**, v.221, p.56-61, 2017b.

SILVA, A.C.R.A.; ALMEIDA, B.F.M.; SOEIRO, C.S. et al. Oxidative stress, superoxide production, and apoptosis of neutrophils in dogs with chronic kidney disease. **Canadian Journal of Veterinary Research**, v.77, p.136-141, 2013.

SILVA, N.L.T.; BONATTO, N.C.M.; OLIVEIRA, P.L. et al. Post-prandial lipemia and glycemia in dogs fed with industrialized pet food. **Comparative Clinical Pathology**, v.28, p.253-258, 2019.

SINGH, R.; SINGH, R.K.; TRIPATHI, A.K. et al. Chronomics of circulating plasma lipid peroxides and anti-oxidant enzymes and other related molecules in cirrhosis of liver. In the memory of late Shri Chetan Singh. **Biomedicine & Pharmacotherapy**, v.59, Suppl 1, p.229-235, 2005.

WILKING, M.; NDIAYE, M.; MUKHTAR, H. et al. Circadian rhythm connections to oxidative stress: implications for human health. **Antioxidants & Redox Signaling**, v.19, p.192-208, 2013.