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Effect of food intake on complete blood count of healthy dogs. Efeito da alimentação no hemograma de cães saudáveis.

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Abstract

The present determined the hematological alterations of healthy dogs at the peak of postprandial hypertriglyceridemia. Twenty-four clinically healthy dogs had blood samples collected to perform complete blood count on three consecutive days at the same time every day: first day after a 12-hour fast; second day three hours after feeding with commercial feed, during the peak of postprandial lipemia; and third day after a 12-hour fast. Feeding led to an increase in MCHC, hemoglobin and white blood cell count due to the increase in segmented neutrophil, monocyte and eosinophil concentrations. A significant increase in total plasma protein content was also observed. Postprandial condition at the peak of hypertriglyceridemia influences hematological parameters of healthy dogs, which is an important finding, however transient, when interpreting laboratory blood tests.

Keywords: Hemogram. Canine. Postprandial lipemia.

Resumo

O presente estudo determinou as alterações hematológicas de cães saudáveis no pico da hipertrigliceridemia pós-prandial. Vinte e quatro cães clinicamente saudáveis tiveram amostras de sangue colhidas para realização de hemograma completo em três dias consecutivos, no mesmo horário do dia: primeiro dia após um jejum de 12 horas; segundo dia, três horas após a alimentação com ração comercial, durante o pico da lipemia pós-prandial; e terceiro dia após jejum de 12 horas. A alimentação levou a um aumento do CHCM, teor de hemoglobina e contagem de leucócitos devido ao aumento de neutrófilos segmentados, monócitos e eosinófilos. Também foi observado aumento no teor de proteína plasmática total. A condição pós-prandial no pico da hipertrigliceridemia influencia os parâmetros hematológicos de cães saudáveis, o que é um achado importante, porém transitório, na interpretação dos exames laboratoriais sanguíneos.

Palavras-chave: Hemograma. Canino. Lipemia pós-prandial.

Introduction

The complete blood count (CBC) is one of the most requested tests laboratory analysis in the veterinary clinical routine due to its practicality and low cost combined with the information provided regarding the patient's physiological or pathological state at the time of collection. The CBC comprises the analysis of blood components, organized in erythrogram, leukogram and platelet count, with quantitative and qualitative results of these parameters (GROTTO, 2009, p.179).

Laboratory analysis, including CBC, involves a pre-analytical, an analytical and a post-analytical phase (LIPPI, FOSTINI; GUIDI 2008, p.285). The pre-analytical phase precedes the analysis of the material requested by the clinician, that is, preparation of the patient, collection and handling of the sample. The analytical phase comprises processing the material itself and the post-analytical phase includes transcription and interpretation of the results (CODAGNONE; GUEDES 2014, p. 33). Any interference in one of the stages could produce errors that affect the quality and reliability of the results (LIMA-OLIVEIRA et al., 2009, p. 442, LIPPI; FOSTINI; GUIDI 2008, p. 287, PLEBANI, 2007, p. 701).

Most laboratory errors occur in the pre-analytical phase, mainly due to poor sample handling, inadequate fasting and hemolysis (ALMEIDA et al., 2011, p. 13, LIMA-OLIVEIRA et al., 2009, p. 442, LIPPI et al., 2006, p. 1443). Insufficient fasting (less than 8 hours) induces blood sample lipemia due to accumulation of postprandial chylomicrons, producing turbidity in the serum or plasma, mainly when triglyceride levels exceed 300 mg/dL (BAUER, 2004, 672, THRALL et al., 2012). In healthy dogs fed with commercial feed, postprandial lipemia has been shown to occur from 2h to 5h, with maximum mean value at 3h (SILVA et al., 2019). However, little is known regarding the isolated effects of feeding on hematological parameters of dogs.

In human studies, Lippi et al. (2010, p. 96) observed an increase in neutrophil count and a decrease in the hematocrit, mean corpuscular volume (MCV), lymphocytes and monocytes after a light meal. Van Oostrom et al. (2003, p. 199), also in healthy human volunteers, detected an increase in total leukocyte count due to the increase in segmented neutrophils concentration 2 hours following a meal, whereas the lymphocyte count increased 8 hours after eating a diet based on glucose and retinol palmitate.

To date, studies that assess the isolated effect of feeding on postprandial hypertriglyceridemia peak in dogs remain scarce. Costa et al. (2020, p. 2224) observed an increase in red blood cells concentration (RBC), hemoglobin, hematocrit (HCT) and mean corpuscular volume (MCV) from as soon as 1 h following feeding in dogs, depending on the evaluated parameter. They also reported an increase in total leukocytes concentration due to increased segmented neutrophil count, in addition to decreased lymphocyte count at specific times following feeding with commercial ration. In addition, the concentration of total plasma protein (TPP) obtained through refractometry also decreased after feeding. Considering that in this study animals were removed from their homes and handled for 12 consecutive hours in an atypical environment, such laboratory changes cannot be exclusively ascribed to the postprandial state, as there was also an influence of agitation (ROVIRA; MUNOZ; BENITO, 2008, p. 334) and perhaps stress (HANSEN, 1997, p. 552) on these parameters. In addition, the fact that the samples were taken on the same day at different times suggests that the results could also be influenced by circadian cycle (SANTOS et al., 1995, p.69).

Based on these aspects, the present study aimed to assess postprandial hematological changes in healthy dogs at peak postprandial hypertriglyceridemia, in order to detect isolated pre-analytical errors caused by postprandial lipemia on hematological parameters of dogs, without there being any interference with the animal's routine habits.

Materials and methods

Approval by the Ethics Committee

All procedures were approved by the University Center of the Integrated Faculties of Ourinhos (Unifio) Animal Usage Ethics Committee (protocol No. 023/2016). Owners were asked to sign an informed consent form allowing inclusion of dogs in the study.

Animal selection and sampling

Twenty-four healthy dogs with no clinical, hematological and biochemical changes (Tables 1 and 2), aged 2-6 years and weighing 8-20 kg with a body condition score between 4 and 5 (LAFLAMME, 1997) were enrolled in the study.

Table 1 – Hematological parameters (mean \pm standard deviation) of the selected dogs (n=24).

Parameter	Results	Reference
Hematocrit (%)	51.4 \pm 4.3	37 – 55
Red blood cells ($\times 10^{12}/L$)	7.4 \pm 0.7	5.5 – 8.5
Hemoglobin (g/dL)	18.1 \pm 2.0	12 – 18
MCV (fL)	69.6 \pm 3.8	60 – 77
MCHC (%)	34.5 \pm 2.0	32 – 36
RDW (%)	14.6 \pm 0.8	14 – 17
White blood cells ($\times 10^9/L$)	8.3 \pm 3.0	6 – 17
Band neutrophils ($\times 10^9/L$)	0.128 \pm 0.35	0 – 0.3
Segmented neutrophils ($\times 10^9/L$)	4.6 \pm 1.5	3 – 11.5
Lymphocytes ($\times 10^9/L$)	2.1 \pm 1.0	1.0 – 4.8
Monocytes ($\times 10^9/L$)	0.27 \pm 0.98	0.15 – 1.35
Eosinophils ($\times 10^9/L$)	0.89 \pm 0.43	0.15 – 1.25
TPP (g/dL)	7.0 \pm 0.6	6.0 – 8.0
Platelets ($10^9/L$)	276.1 \pm 72.3	160 – 430

Reference values for the canine species according to Rizzi et al. (2010). Abbreviations: MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; RDW: red cell distribution width; TPP: total plasma protein.

Table 2 – Serum biochemical parameters (mean \pm standard deviation) of the selected dogs (n=24).

Parameter	Results	Reference
Albumin (g/dL)	3.8 \pm 0.5	2.6 – 3.3
ALP (IU/L)	52 \pm 31.5	20 – 156
ALT (IU/L)	42 \pm 23.7	21 – 102
AST (IU/L)	30 \pm 9.8	23 – 66
Calcium (mg/dL)	10.3 \pm 0.7	9.0 – 11.3
Cholesterol (mg/dL)	234 \pm 74.4	135 – 270
Creatinine (mg/dL)	1.2 \pm 0.2	0.5 – 1.5
Glucose (mg/dL)	93 \pm 15.7	68 – 118
Globulins (g/dL)	3.8 \pm 0.9	2.7 – 4.4
Phosphorus (mg/dL)	5.7 \pm 0.6	2.6 – 6.2
Total protein (g/dL)	7.6 \pm 0.8	5.4 – 7.1
Triglycerides (mg/dL)	87 \pm 34.3	20 – 112
Urea (mg/dL)	40.6 \pm 7.8	10 – 50

Reference values for the canine species according to Kaneko et al. (2008). Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Feeding

Only dogs fed exclusively commercial feed were included and each one was fed according to the manufacturer's instructions twice daily. Prior to the start of the study, dogs received one portion of the feed and ingested it within 10 minutes. The content of the feed comprised maximum moisture content of 9%, minimum crude protein of 26%, ether extract of at least 15%, maximum fibrous matter of 2%, and maximum mineral matter of 7.5% with metabolizable energy of 3,810 kcal/kg (CIBAU Adult Medium Breeds, Farmina Pet Foods, São Paulo, Brazil). Each dog received 7.0 g of feed/kg body weight, according to manufacturer's recommendations for dogs with moderate physical activity.

Experimental design

To determine the effect of postprandial lipemia, dogs had blood samples drawn on three consecutive days, always at the same time of day (11:30 am) at their respective homes, according to the following groups:

Non-lipemic 1 (NL1): after a 12-hour fasting, a blood sample was collected at 11:30 am; no routine changes were made.

Lipemic (L): each dog received feed three hours prior (08:30 am) to blood sampling. Peak hypertriglyceridemia was observed three hours following feeding in dogs that received this type of food in a previous study (SILVA et al., 2019, p 255).

Non-lipemic 2 (NL2): harvest identical to NL1.

The effect of lipemia was not verified in a single day due to possible variations of the analytes induced by the circadian cycle (CHIKAZAWA et al., 2013, p. 1616, OHMORI et al., 2012, p. 403),

and thus analyses were performed at different days at the same time. Considering that within a 24-hour interval the animal can be affected by a pathological disorder, samples were obtained one day before (NL1) and one day after (NL2) induction of postprandial lipemia (L). Samples from NL1 and NL2 were compared statistically, and since there was no statistically significant difference in any analyzed variable, the mean of these times was compared to the L time.

Laboratory analysis

To perform CBC, determinations of RBC count, white blood cells count (WBC), platelet count, red blood cell distribution width (RDW), hematocrit (HCT), mean platelet volume (MPV), MCV, mean corpuscular hemoglobin concentration (MCHC) and hemoglobin concentration were performed in an automated veterinary hematology analyzer (ABX Micros ESV 60, Paris, France). The HCT was also determined using the Strumia's microcapillary method (11,400 rpm for 5 minutes) and the differential leukocyte count was performed on a blood smear stained with commercial hematological dye (Instant-Prov, Newprov, Pinhais, PR, Brazil), following previous recommendations (JAIN, 1986). The TPP was determined using a portable clinical refractometer (ATAGO, Mod. Master-SUR-NM, Tokyo, Japan). Hematimetric indices MCHC and MCV were also calculated from the HCT obtained using the microcapillary method as previously described (JAIN, 1986).

Biochemical analysis was performed as part of a health screening for inclusion in this study, according to previously published methodology (COSTA et al., 2020, p. 2222, SILVA, et al., 2019, p.255).

Statistical analysis

Data were first tested for normality using the Shapiro-Wilk test and non-lipemic times were compared statistically. Since there was no significant difference, the mean of those times was compared to the lipemic time using paired t test or Wilcoxon test. Analyses were performed using a computer software (GraphPad Prism, v.6.00 for Windows, GraphPad Software, La Jolla, CA, USA, www.graphpad.com) and considered significant when $p < 0.05$.

Results

The induction of postprandial lipemia was effective, significantly increasing ($p < 0.0001$) the concentration of triglycerides in L (207.9 ± 90.92 mg/dL) when compared to NL1 (69.86 ± 17.61 mg/dL) and NL2 (78.55 ± 34.00 mg/dL) moments.

Regarding the erythrogram, an increase in hemoglobin levels (Figure 1B) and, consequently, MCHC by both methodologies (Figure 1G and H) was observed after feeding. There was no change

in RBC (Figure 1A), automated and microcapillary HCT (Figure 1C and D), automated and calculated MCV (Figure 1E and F), and RDW (Figure 1I).

Regarding the leukogram, feeding led to an increase in concentrations of WBC (Figure 2A) due to increased segmented neutrophils (Figure 2C), monocytes (Figure 2E) and eosinophils (Figure 2F) counts. No changes were observed on concentration of band neutrophils (Figure 2B), lymphocytes (Figure 2D) and basophils (Figure 2G).

Platelet count (Figure 3A), MPV (Figure 3B) and platelets obtained through field under optical microscopy (Figure 3C) were not influenced by feeding. There was a significant increase in TPP obtained through refractometry (Figure 3D).

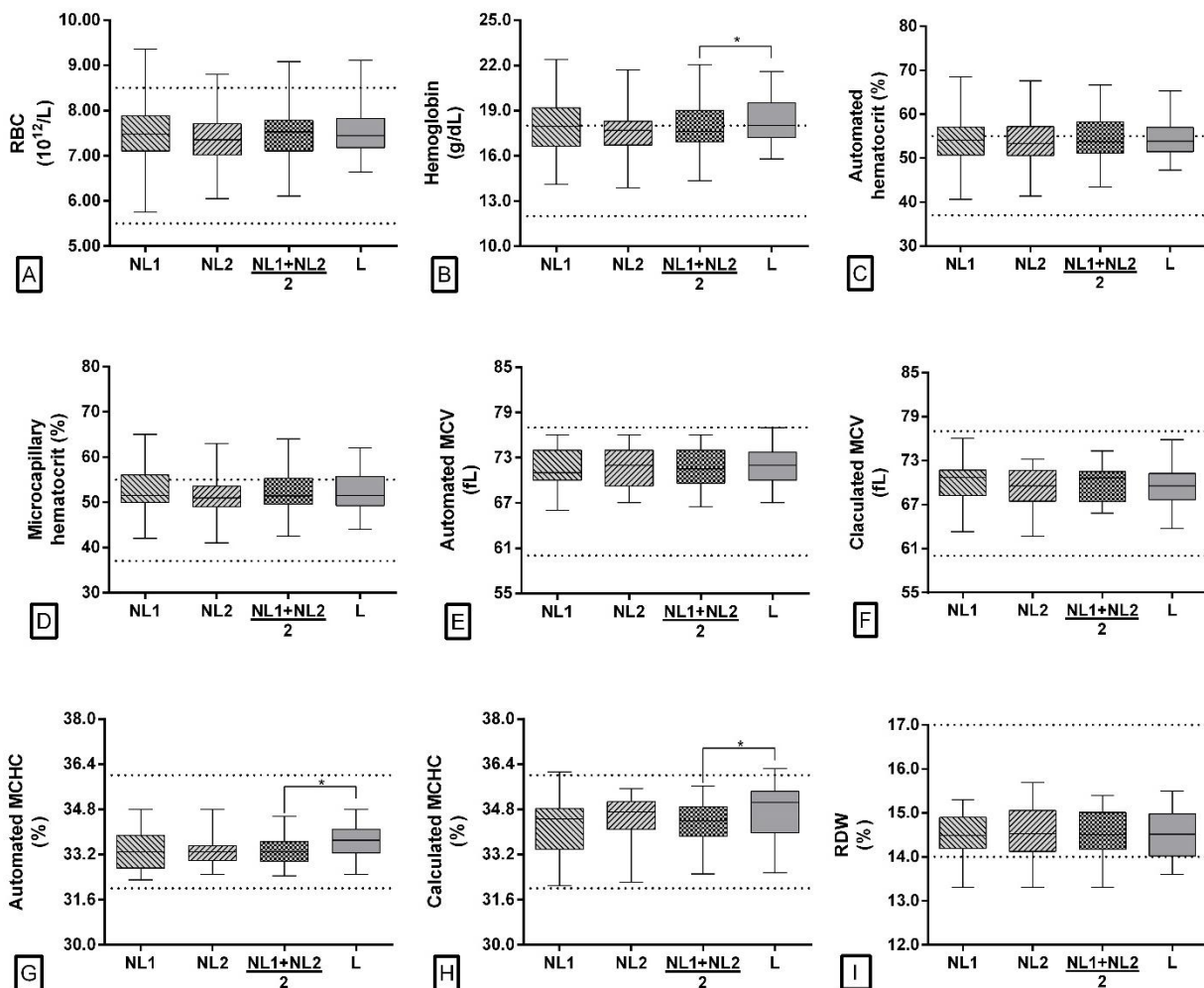


Figure 1 – Red blood cells (RBC, A), hemoglobin (B), automated (C) and microcapillary hematocrit (D), automated (E) and calculated (F) mean corpuscular volumes (MCV), automated (G) and calculated (H) mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW, F) of healthy dogs (n = 24) at two non-lipemic times, one day before (NL1) and one day after (NL2) the lipemic moment (L) induced by feeding with commercial feed. 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).

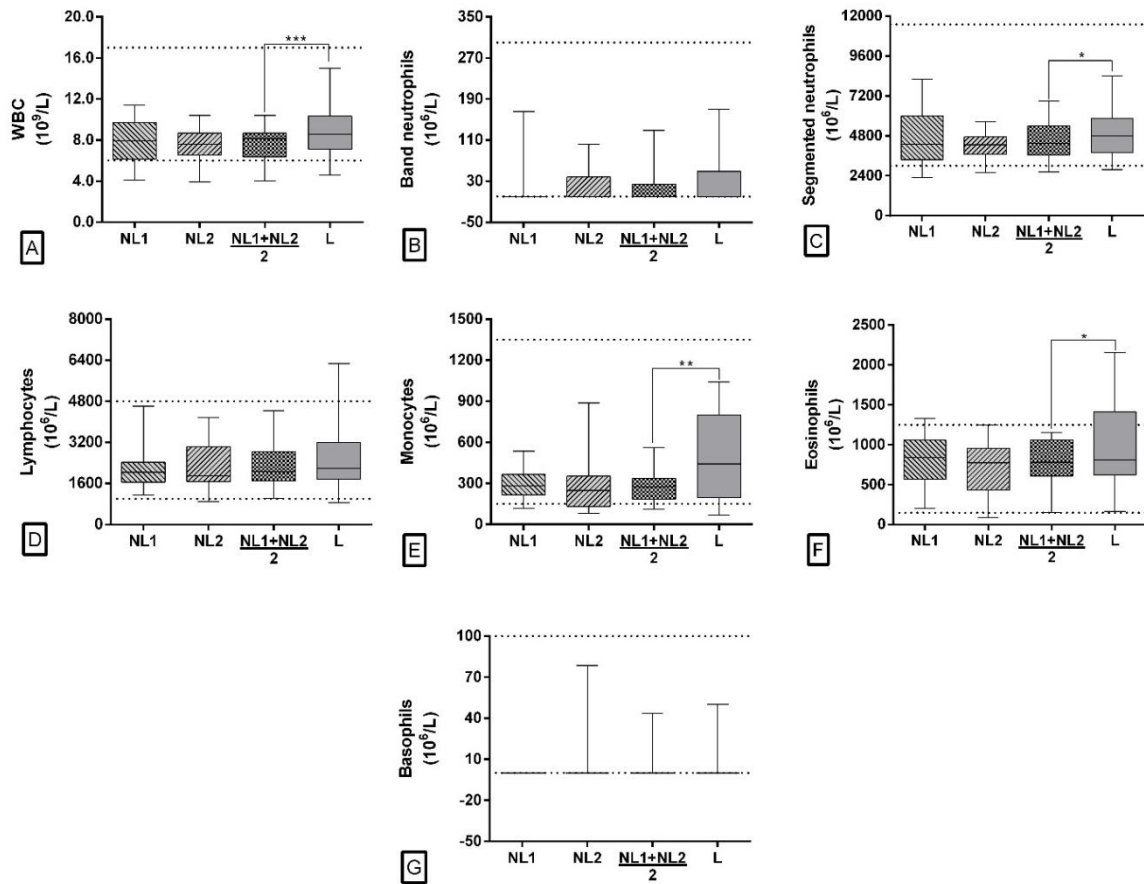


Figure 2 – White blood cells (WBC, **A**), band neutrophils (**B**), segmented neutrophils (**C**), lymphocytes (**D**), monocytes (**E**), eosinophils (**F**) and basophils (**G**) of healthy dogs (n = 24) at two non-lipemic times, one day before (NL1) and one day after (NL2) the lipemic moment (L) induced by feeding with commercial feed. 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).

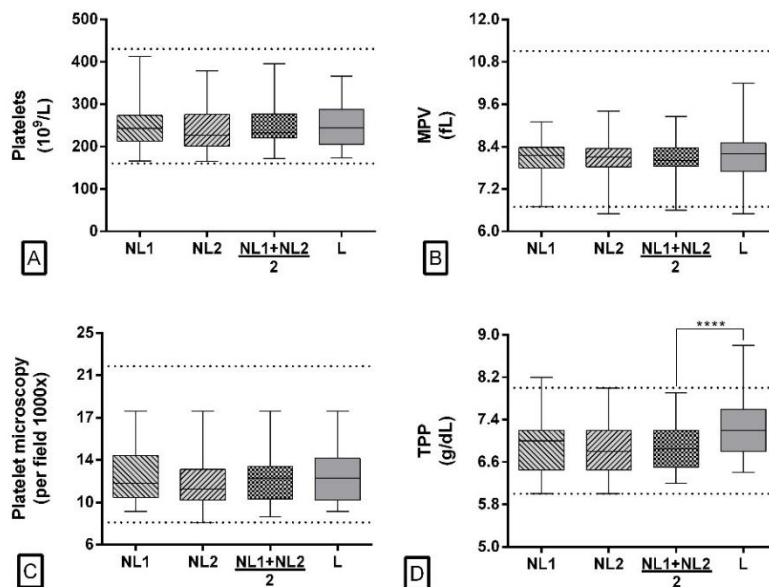


Figure 3 – Platelets (**A**), mean platelet volume (MPV, **B**), platelet estimation on 1000x magnification optical microscopy (**C**) and total plasma protein (TPP, **D**) of healthy dogs (n = 24) at two non-lipemic times, one day before (NL1) and one day after (NL2) the lipemic moment (L) induced by commercial feed. 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).

Discussion

The hematological and biochemical parameters of the dogs included in this study remained within the reference limits established by Rizzi et al. (2010) and Kaneko et al. (2008), respectively. As far as we know, the isolated effect of food intake during peak postprandial lipemia on canine hematological parameters has not been adequately addressed previously. We observed increased concentration of hemoglobin, MCHC, WBC, segmented neutrophils, monocytes, eosinophils and TPP, with no changes on RBC concentration and platelet parameters after feeding.

Postprandial lipemia caused a significant increase in hemoglobin levels, as reported by Hansen et al. (1997, p. 551). In our study, 45.8% of the animals (11/24) exceeded the reference value of this parameter. Determination of hemoglobin in automated cell counters is performed through spectrophotometry and since lipemia increases the absorption of light, values can be overestimated, as reported by Nikolac (2014, p.59). Since MCHC is calculated from the hemoglobin value, the error is repeated in this parameter, although this increase was clinically relevant only in 2 animals, with values exceeding the reference range and leading to a false hyperchromia.

Red blood cells concentration and therefore HCT were not influenced by feeding. Previous studies in dogs, with blood samples obtained during a 12 h period after feeding reported a decrease in these parameters and hemoglobin since 1 h following the meal (COSTA et al., 2020, p. 2224). In humans with samples also obtained at the same day before and after the meal, the decrease seen on these parameters was attributed to fluid intake during the meal (LIPPI et al., 2010, p. 98). As pointed out by Costa et al. (2020, p. 2226), the reduction of the RBC series concentration in dogs following feeding could also be related to water intake. In the present study, the analysis was carried out in a timely manner at the same time of the day, with no interference from water intake by successive collections at the same day or the same effect of the circadian cycle, which therefore explains the discrepancies.

MCV and RDW were unaffected by the postprandial condition in our study 3 h following feeding. Costa et al. (2020, p. 2227), observed a decrease in MCV 4 h following feeding in dogs. Korbonits et al. (2007, p. 162) explain that feeding leads to an increase in plasma osmotic pressure, causing RBC to lose fluid and thus reducing their size, leading to a decrease in MCV values. Being this a longer process, such changes were not observed at peak postprandial hypertriglyceridemia in dogs.

Regarding the leukogram, the postprandial condition was accompanied by an increase in WBC concentration due to increased segmented neutrophils, monocytes and eosinophils counts. Only eosinophil counts showed clinical relevance, as 29% of the animals (7/24), exceeded the reference values (data not shown). Previous studies that assessed the effect of postprandial lipemia on the hematological parameters of human volunteers reported an immune response at the systemic level that led to changes in the leukogram such as increased neutrophil counts (CHENG et al. 2010, p. 1421, HANSEN et al., 1997, p. 551, KLOP et al., 2013, p. 4, LIPPI et al. 2010, p. 96, VAN OOSTROM et al., 2003a p.199, 2004b p.180) and could also be extrapolated to eosinophil count. This increase in WBC concentration due to increased segmented neutrophils count has also been reported in dogs (COSTA et al., 2020, p. 2227). There is evidence that postprandial hypertriglyceridemia triggers the activation of endothelial cells and initiates the production of inflammatory cytokines, contributing to the increase on postprandial leukocytes (CHENG et al. 2010, p. 1421, HENNIG; TOBOREK; MCCLEIN, 2001, p. 99, NORATA et al., 2007, p. 321). Interleukins

(IL) such as IL-6 and IL-8 are responsible for mediating leukocyte migration to the vascular endothelium (WILLERSON; RIDKER, 2004, p. II-2).

Lymphocyte count was not influenced by the postprandial state in dogs, but in humans there are reports of increased lymphocyte counts (KLOP et al., 2013, p. 4, VAN OOSTROM et al., 2003a, p. 199, 2004b, p. 177). The IL-6 is the main stimulus for lymphocyte recruitment, which originate in adipose tissue and endothelial cells (PAPANICOLAOU; VGONTZAS, 2000, p. 1331). Costa et al. (2020, p. 2224) observed a decrease in lymphocyte count 2 and 6 h following feeding, but values were unchanged during lipemic peak (3 h), which corroborates our findings. Other human studies have also reported a decrease in lymphocyte count after feeding (HANSEN et al., 1997, p. 551, LIPPI et al., 2010, p. 96), probably due to migration of these cells from the blood to the tissues, given that food intake leads to a high exposure to intestinal antigens that requires action by the immune system (COSTA et al., 2020, p. 2227, HANSEN et al., 1997, p. 552). This effect on lymphocytes was not seen during peak postprandial hypertriglyceridemia in dogs.

The postprandial condition also caused an increase in monocyte counts in dogs. Klop et al. (2013, p. 4) also observed an increase in monocytes concentration close to peak lipemia, while Hansen et al. (1997, p. 551) and Lippi et al. (2010, p. 96) did not observe that change in humans. Hansen et al. (1997, p. 551) reported that the postprandial state can induce release of cortisol in humans, which results in a stress leukogram and could favor the increase in neutrophil counts associated with a decrease in lymphocyte and monocyte counts. In dogs, however, cortisol can lead to monocytosis (THRALL et al., 2012). In addition, postprandial hypertriglyceridemia can cause activation of monocytes (CHENG et al., 2010, p. 1421, KLOP et al., 2013 p. 7) and neutrophils (KLOP et al., 2013, p. 7, VAN OOSTROM et al., 2003, p. 200). Klop et al. (2013, p. 6) highlighted that such leukocyte changes are transient and unlikely to represent clinically significant changes in humans, similarly to previous reports in dogs (COSTA et al., 2020, p. 2228) and to our study.

Platelet counts and MPV were unaffected by the postprandial condition in dogs. Hansen et al. (1997, p. 551) observed an increase in platelet count after food intake, which, according to the authors, was due to the release of epinephrine. Pavlishchuk, Petrik, Nikol'skaya (2004, p. 350) reported that following feeding, plasma fibrinogen can increase with a consequent increase in platelet aggregation in humans. Furthermore, Krüger et al. (2015, p. 334) state that this response is likely due to the endothelial dysfunction that follows feeding. Such findings do not appear to occur in dogs or are mild enough not to significantly influence platelet count.

We observed an increase in TPP during the postprandial state, with 12.5% of the animals (3/24) exceeding canine reference values (data not shown). Considering that lipoprotein molecules that cause plasma turbidity are solutes and, therefore, are measured by clinical refractometry, such an increase could be attributed to lipemia. Similarly, Legendre et al. (2017 p. 4) observed a significant increase in TPP concentration obtained through refractometry in dogs with azotemia, mainly due to increased urea levels. Thus, refractometry, as well as hemoglobin determination and MCHC seem to be significantly affected by postprandial lipemia, enhancing the importance of knowing sources of pre-analytical variation for adequate interpretation of hematological parameters in these conditions.

It is also worth mentioning that, in the present study, the effect of feeding was assessed in isolation, with samples taken at different days so that possible circadian variations or even fluid intake could not interfere with the results. A previous study carried out with dogs by our research group (COSTA et al., 2020, p. 2224) also showed significant changes in CBC of dogs fed with commercial feed. However, such changes may have been influenced not only by the postprandial condition, but by other factors such as circadian cycle and the excitatory state of the animal that was removed from

its routine environment. The organism undergoes several physiological changes during the circadian cycle. Studies with dogs show that over the period of 24 h there is a change in glucocorticoid level (OHMORI et al., 2012, p. 404), body temperature (REFINETTI, PICCIONE 2003, p. 936), blood pressure and heart rate (PICCIONE, CAOLA, REFINETTI, 2005, p. 378), intraocular pressure and tear production (GIANNETTO, PICCIONE, GIUDICE, 2009, p. 304) and values of neutrophils, lymphocytes, eosinophils, monocytes, platelets and fibrinogen undergo changes influenced by sunlight (SANTOS, 1995, p. 69), so there may be many other unknown changes. In addition, as a response to adrenaline during arousal, physiological leukocytosis can occur due to recruitment of neutrophils and lymphocytes to the blood circulating compartment, thereby increasing the count of these cells as well as monocytes and eosinophils (HANSEN et al., 1997, p. 551). Our study is pioneer in determining the isolated effect of the postprandial condition and peak hypertriglyceridemia on hematological parameters of healthy dogs.

Conclusions

Postprandial condition, at peak hypertriglyceridemia, significantly changes hematological parameters of healthy dogs, making it important to know such changes, even if transient, during interpretation of laboratory tests in these species. Changes can be avoided, whenever possible, through correct preparation of the patient for blood sampling.

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