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HEMATOLOGY, RENAL AND HEPATIC FUNCTION IN OVERFED GREAT DANE PUPPIES

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ABSTRACT

An adequate diet is important and nutritional errors may lead to irrevocable consequences. Nowadays, obesity is the most common nutritional disease in dogs, with a prevalence of 16%. In dogs, the level of physical activity, diet composition, food taste, and lifestyle are the most important factors which contribute to obesity. This study was carried out in order to assess the effects of overfeeding on clinical biochemistry and hematology of healthy puppies. Fourteen male Great Dane dogs at 10 weeks of age were studied during 27 weeks. The experimental design was completely randomized, with two treatments, seven replications, and six months of duration. Treatments were made up of high quality diet given ad libitum (GI – treatment 1) and restrictedly (GII – treatment 2). The evaluation of blood and urinary biochemistry as well as the determination of hematological parameters were carried out. It was concluded that overfed puppies revealed seric elevations of alpha1-globulin, and lower values of beta-globulin, gamma-globulin, ALT, ALP, and creatinine. In blood evaluation, higher values of lymphocytes and lower values in the erythrogram were observed. The urinary protein:urinary creatinine value did not reveal any difference between treatments.

KEYWORDS: Ad libitum feed; biochemical parameters; canine animal; hematological parameters.

RESUMO

PARÂMETROS HEMATOLÓGICOS, FUNÇÃO RENAL E HEPÁTICA DE CÃES DA RAÇA DOGUE ALEMÃO EM CRESCIMENTO SUPERALIMENTADOS

Uma dieta apropriada é importante e os erros nutricionais podem ter consequências irreparáveis. Atualmente, a obesidade é a doença nutricional mais comum em cães, com prevalência de 16%. Em cães, o nível de atividade física, composição dietética, sabor do alimento e estilo de vida são os fatores mais importantes que contribuem para a obesidade. Este trabalho foi desenvolvido no sentido de avaliar os efeitos da superalimentação na bioquímica clínica e hematologia de filhotes saudáveis. Foram estudados 14 cães da raça Dogue Alemão, machos, 10 semanas de idade, durante 27 semanas. O delineamento experimental foi inteiramente casualizado, com dois

tratamentos, sete repetições e seis meses de duração. Os tratamentos constituíram-se de dieta super prêmio ofertada à vontade (GI) (tratamento 1) e com quantidade restrita (GII) (tratamento 2). Procedeu-se a avaliação da bioquímica sanguínea e urinária e a determinação dos parâmetros hematológicos. Constatou-se que filhotes superalimentados apresentaram elevações séricas de alfa1 globulina e menores valores de beta globulina, gama globulina, ALT, ALP e creatinina. No hemograma observaram-se maiores valores de linfócitos e menores valores no eritrograma. O índice proteína urinária: creatinina urinária não apresentou diferença entre os tratamentos.

PALAVRAS-CHAVE: Alimentação à vontade; canino; hematologia; parâmetros bioquímicos.

INTRODUCTION

The proximity of pets to humans, the access to high energy diets, snacks and sweets and the reduction of physical activity are predisposing the animals to obesity (EDNEY & SMITH, 1986; ARMSTRONG & LUND, 1996; JERICÓ & SCHEFFER, 2002). Obesity is the most common nutritional disease in humans, dogs and cats living in developed societies. National data expresses a prevalence of 16% of obesity in dogs (JERICÓ & SCHEFFER, 2002), lower numbers than those described in other countries, in which values ranging between 24% and 30% of overweight dogs are reported (EDNEY & SMITH, 1986; LEWIS et al., 1994).

Weight excess can lead to several deleterious effects on animal health. Among them, the most important are the disturbances of the locomotor system, hypertension, damages to immune response, increased incidence of type II mellitus diabetes, dermatopathies, neoplasias, heat intolerance, lower reproductive efficiency and decrease in liver function (HAND et al., 1989, LEWIS et al., 1994, JERICÓ et al., 2006).

The assessment of blood biochemistry is fundamental in the clinical monitoring of obesity. The determination of serum proteins has become a useful procedure for the understanding of the pathophysiological processes and it is used in both healthy and sick animals (ECKERSALL, 2000). Hematological analysis provides a wide variety of information which complements the biochemical findings, even when most of the hematological findings are normal and are used to exclude various differential diagnoses (BUSH, 1999).

Studies of the physiological changes resulting from nutritional effects at different ages are increasingly necessary with the increase in life expectancy of pets, partly attributed to the improvement in diet quality and the access to veterinary care. By identifying hematological changes and serum biochemical profile related to liver and kidneys during the animal life, researchers can develop food formulations and methods which are more appropriate for each stage of life.

The present work aimed to verify, by laboratory tests – clinical biochemistry and hemogram – the healthiness of overfed Great Dane puppies.

MATERIAL AND METHODS

The experiment was conducted at the Veterinary Hospital (VH) of the School of Veterinary and Animal Science (EVZ), Universidade Federal de Goiás (UFG). A total of 14 male Great Dane puppies, with mean initial weight of 8Kg, at 10 weeks of age were used. The animals came from six different litters and were obtained from private kennels of the states of Goias, Minas Gerais, Sao Paulo and Brasilia. The selection of the dogs was performed after systematic investigation, in which clinical and orthopedic conditions were evaluated by routine semiological maneuvers.

Before starting the experiment, the puppies underwent an acclimation period of seven days and received the same commercial extruded dry feed (Ossobuco large size puppies, super premium - Nutron Alimentos, Campinas, SP) used in the experimental phase. At this stage, the animals also received ecto- and endoparasiticides and were primo-immunized with polyvalent vaccine (Galaxy DA2PPvl + CV, Fort Dodge Animal Health-USA) against parvovirus, leptospirosis, canine distemper, corona virus, adenovirus, parainfluenza and viral hepatitis viral, receiving three doses of reinforcement at 21 days intervals.

The experimental design was completely

randomized with two treatments and seven replicates. There were two groups of seven animals each, being the first group (GI) with dogs treated with ad libitum diet and the second group (GII) with dogs treated with diet restriction. The 14 puppies were tattooed with numbers on the right ear and distributed in each group so that each litter was equally represented in both treatments.

The experiment comprised a period of 27 weeks. All animals received water ad libitum and remained in the same accommodations under the same fixed feeding schedule. The composition of the extruded dry feed supplied to the dogs in the experiment is described in Table 1.

TABLE 1 - Nutritional composition of the commercial extruded dry feed (Ossobuco large size puppies) used in the experiment

Component	Values in Dry Matter
Dry Matter (%)	100.0
Humidity (%)	12.0
Crude protein (%)	34.0
Ether extract (%)	16.0
Calcium (%)	1.5
Phosphorus (%)	1.0
Fibrous matter (%)	3.0
Mineral matter (%)	9.0
Metabolizable energy (Kcal/kg)	3,400.0
Aflatoxin (ppb)	20.0
Salmonella	Absence in 25g

Source: Nutron Alimentos, Campinas, SP

Feed supply varied according to the treatments used in this experiment. The supply was free and individual from 8 a.m. to 6 p.m. for dogs from GI. The amount of feed and remains at the end of the day were weighed so as to allow the calculation of individual daily intake for each animal. For GII, the feed was offered following the quantities recommended by the manufacturer, and calculated according to the age and body weight. As the dogs were weighed every seven days, the adjustment of the amount of feed was done weekly. Three individual meals were provided daily at 7 a.m. 12:30 p.m. and 5 p.m. The maximum time of 30 minutes for each meal was established. At the time of the feeding, each puppy was placed alone in a cubicle, where they remained until the end of the established time or until the intake of all the feed

The dogs of GI were housed in individual stalls of 1.96m x 2.92m, with rough cement floor, brick walls and natural lighting and ventilation through openings in the side walls. The dogs of GII, except during the feeding period, were housed in two collective stalls of 3.92m x 5.84m, rough cement floor, brick walls and natural lighting and ventilation through openings in the side walls.

Dogs of the two groups were taken to the solarium for physical exercises twice a day, from 7 a.m. to 8a.m. and from 4 p.m. to 5 p.m. On evaluation days, the dogs were placed in the solarium at 8:30 a.m., where they remained until 11:30 a.m.

The dogs of the two groups were observed three times a day, at the feeding time of the restricted group and on the evaluation days. A detailed clinical examination (body temperature, heart rate, respiratory rate and pulse, skin and mucosa evaluation, lymph nodes and joints palpation) was performed whenever an animal showed any behavioral changes on their general condition.

The study comprised 27 blood and urine samples at regular intervals of seven days with the animals under food fasting of at least 12 hours, for the performance of the following laboratory tests: blood biochemistry profile (total protein, urea, creatinine, alanine aminotransferase and alkaline phosphatase) and urinary biochemistry (protein and creatinine). For the hemogram and serum protein electrophoretic profile, 14 blood samples were collected every two weeks. Blood samples were obtained by jugular vein puncture with the animals kept in lateral recumbency and urine samples were collected by urethra catheterization, with animals kept in station.

All laboratory assessments were performed at the Laboratory of Clinical Pathology of the VH / EVZ / UFG. For each metabolite analysis, standardized commercial reagents (Labtest ®, Labtest Diagnostica SA, Lagoa Santa, MG) were used. The reactions occurred at 37°C and the reading was carried out in a manual spectrophotometer (Micronal B 342, Sao Paulo, SP).

For urine biochemical tests, 20 ml samples were centrifuged after collection, divided into 1.5 mL polypropylene microtubes (Eppendorf ®,

Germany) and kept refrigerated until the assessment.

For the hemogram, 5mL of blood were collected using glass vacuum tubes (Vacutainer®, Becton Dickinson Ind. Cirúrgicas Ltda, Brazil) and with EDTA anticoagulant (ethylenediamine tetra-acetic acid, disodium salt). The examination was performed within a maximum period of six hours, using an automated hematology analyzer (ABX Micros 60 Haematologic Analyzer, France). The leukocyte count was done on blood smears.

To perform serum biochemistry, 10 ml blood were collected in disposable vacutainer® tubes without anticoagulant. After clot retraction and serum obtainment, the tubes were centrifuged. Then the serum was separated by aspiration, divided into aliquots and kept refrigerated for not more than six hours, and the biochemical tests were carried out within 12 hours.

The total serum protein was determined by colorimetric method, by biuret reaction and reading at 550 nm wavelength. The protein fractions were separated by agarose gel electrophoresis, using cold (2º to 8ºC) Tris buffer pH 9.5 and stained with black starch (starch black 10-B - art.1810 - CELM) at 0.1% in acetic acid 5%. The reading of the film was carried out by densitometry in 520 nm, using the system SE-250, CELM, according to the methodology described by the manufacturer.

Urea was determined by enzymatic-colorimetric method, by urease reaction, and the reading was carried out at 600nm wavelength. Serum creatinine was determined by colorimetric method, by alkaline picrate reaction and reading at 520 nm wavelength.

Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined by modified Frankel Reitman and Roy, respectively. The reading was performed in a spectrophotometer with 505 nm wavelength for ALT and 590 nm for ALP (Labetest).

The determination of urinary protein concentration was performed using a colorimetric method, by coomassie brilliant blue reaction at 610 nm absorbance. Urinary creatinine was determined by colorimetric method, by alkaline picrate reaction, and reading at 520 nm wavelength.

The urinary protein: urinary creatinine ratio was calculated as mentioned by FINCO (1995), by dividing the values of urinary protein by urinary creatinine, aimed at detecting renal tubular injury.

Descriptive statistical analysis was performed to verify mean values and standard deviation. The comparison of both treatments was carried out for each variable analyzed in the study, considering overall data and data from each evaluation period

As blood and urinary variables as well as the calculations originated from them (urinary protein / urinary creatinine relation) did not show normality and homogeneity of variance simultaneously, the nonparametric Wilcoxon test at 5% significance (SAMPAIO, 1998) was used. Statistical tests were performed by the software SAEG (UFV, 2003).

RESULTS AND DISCUSSION

The mean and standard deviation of red blood cells, hemoglobin, hematocrit, total leukocytes, neutrophils and lymphocytes in the blood of dogs of this study and the reference values used are described in Table 2.

TABLE 2 – Hemogram of dogs fed a high quality diet in GI and GII, with mean values, standard deviation and coefficient of variation, Goiânia, 2006

Variables	GI Mean ± Sandard Deviation	GII Mean ± Sandard Deviation	Reference Values*
Red blood cells (x10^{6}µL)	5.37^B ± 0.68	5.68^A ±0.66	5.5 – 8.5
Hemoglobin (g/dL)	11.70^B ±1.35	12.22^A ±1.30	12 – 18
Hematocrit (%)	35.93^B ±3.84	37.23^A ±4.22	37% - 55%
Leukocytes (x10^3 µL)	14,761.24^A ±5,541.33	14,498.02^A ±6,446.83	6,000 -1,7000
Neutrophils (x10^3 µL)	8,329 27^A ± 3,728.91	8,806.16^A ± 4,963.44	3,000 -11.500
Lymphocytes (x10^3 µL)	4,200.45^A ± 2,072.48	3,522.01^B ± 2,073.20	1,000 -4,800

^{AB} Means followed by different letters in the same line differ statistically by the Wilcoxon test ($p < 0.05$).
*BUSH (1999)

Mean red blood cell of G1 was higher ($p < 0.05$) compared to GII. However, the values of both groups remained within normal ranges (BUSH, 1999). The individual analysis of the collections during the experimental period showed no significant difference between the groups.

The mean hemoglobin values showed a statistical difference, being higher ($p < 0.05$) in GI (12.22 g/dL) than in GII (11.70 g/dL). These values are within the reference ranges (BUSH, 1999). Throughout the experimental period, there was a difference only in the fifth week, being higher in GII (11.24 g/dL) than in GI (10.67 g/dL).

The mean hematocrit values were 35.93% and 37.23% for the dogs of GI and GII, respectively; thus, there was no difference between the groups, despite being higher in GII. These values were within the reference range (BUSH, 1999). During the experiment there was no statistical difference.

According to NELSON & COUTO (2001), when evaluating the erythroid series, the clinician does not need to analyze all hemogram values, because they provide identical information. In this study, the hematocrit value reflected similar behavior as the red blood cells and hemoglobin, which agrees with these authors (Figure 1).

NAP et al. (1991) studied the effects of three diets with different protein levels (14.6%, 23.1% and 31.6%) and did not verify differences between hemoglobin and hematocrit values. However, FERREIRA (2006) found differences between the groups receiving diets with varying levels of protein (12%, 22% and 32%), and higher hematocrit in the group fed diet with the highest protein content. The results of this study differed from these two studies, since the highest values were detected in dogs fed restricted diet, i.e., the ones which received less protein.

According to LIPPERT (1992) and AGAR (2001), as red blood cells present high metabolism, they need readily usable energy for normal activity maintenance, and the reduction of protein and energy reserves in the organism can result in anemia. Contrary to the expected, in this current study, dogs in food restriction showed higher erythrocytes, hemoglobin and hematocrit values.

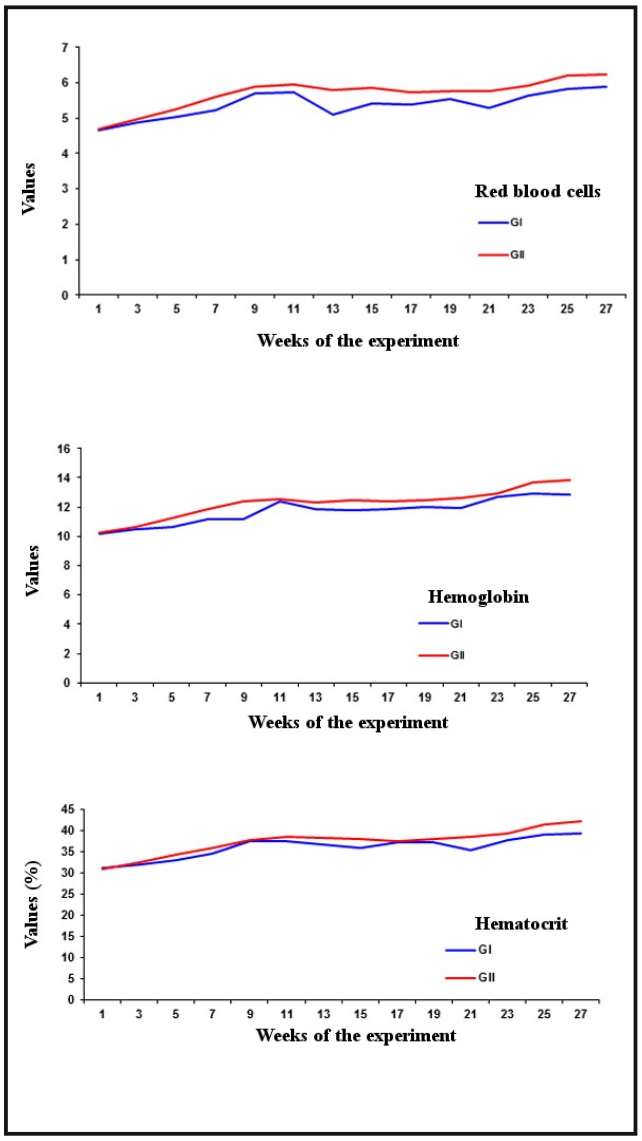

FIGURE 1 - Values of hematocrit, red blood cells and hemoglobin of dogs of GI and GII, throughout the evaluation period.

The mean absolute neutrophil values were within the reference ranges and there was no significant difference ($p > 0.05$). During the experimental period, significant differences ($p < 0.05$) were observed only in the third week of the experiment. The dogs of GII were more stressed than dogs of GI, probably due to severe food restriction during the experimental period, which may have contributed to higher values of neutrophils in this group.

The mean absolute lymphocyte values presented significant difference ($p < 0.05$), being higher in GI than in GII. These values are within the reference ranges, and there was no statistical difference throughout the experimental period.

The mean values and standard deviation of the metabolites determined in the serum of dogs of this study and the reference values used are described in Table 3.

The mean albumin values were 2.30 g/dL and 2.23 g/dL, respectively, for GI and GII, with no statistical difference between them. According to NAOUM (1999), the quantitative analysis of this protein has important clinical significance, because its decrease can be related to a defect in their synthesis in the liver or to renal losses. These values were within the reference ranges (HARRUS et al. 1996). During the studied periods, variations between GI and GII were identified only in the 11th week (2.21 g/dL vs. 2.04 g/dL), as shown in Figure 2.

TABLE 3 – Blood biochemistry of dogs fed a high quality diet in GI and GII, with mean values and standard deviation, Goiânia, 2006

Blood Biochemistry	GI - Mean ± Standard Deviation	GII - Mean ± Standard Deviation	Reference Values
Albumin (g/dL)	2.30^{A}±0.37	2.23 ^{A}±0.38	2.51 - 3.07***
Alpha1-globulin (g/dL)	0.70 ^{A}±0.13	0.67 ^{A}±0.12	0.58 - 0.80***
Alpha2-globulin (g/dL)	0.64 ^{A}±0.15	0.64^{A}±0.19	0.57 - 0.80***
Beta-globulin (g/dL)	1.18 ^{B}±0.26	1.28 ^{A}±0.25	1.41***
Gamma-globulin (g/dL)	0.65 ^{B}±0.25	0.70 ^{A}±0.22	0.37 - 0.59***
Urea (mg/dL)	33.32 ^{B}±10.82	32.74 ^{A}±10.03	15 - 40**
Creatinine (mg/dL)	0.80 ^{B}±0.31	0.86 ^{A}±0.32	0.5 - 1.5*
ALT (UI/L)	15.80^{B} ±6.85	19.11^{A}±8.50	21 - 102*
ALP (UI/L)	35.75 ^{B}±17.35	40.52 ^{A}±17.83	20 - 156*

^{AB} Means followed by different letters in the same line differ statistically by the Wilcoxon test ($p < 0.05$).
*KANEKO (1989)
**BUSH (1999)
***HARRUS et al.(1996)

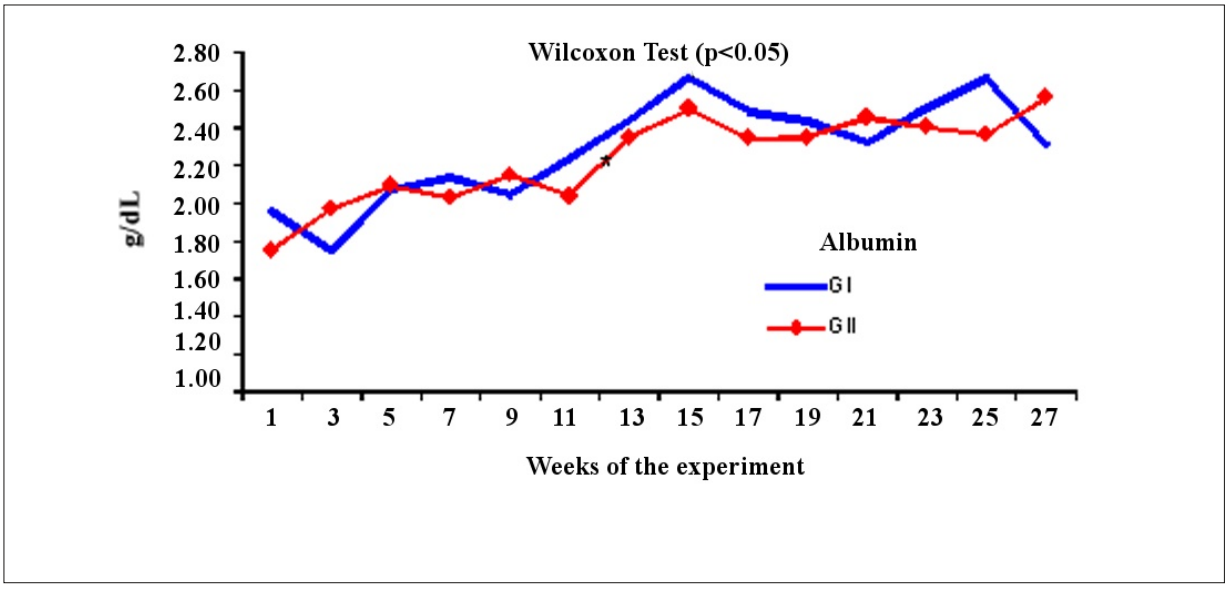

FIGURE 2 – Mean values of albumin of dogs of GI and GII, throughout the evaluation period..

The alpha1-globulin was higher ($p < 0.05$) in GI than in GII. However, the values of both groups remained within the reference range (HARRUS et al., 1996). During the weeks of the experiment, there was a statistical difference in the 17th week (Figure 3). The highest values of alpha1-globulin combined with the increased number of lymphocytes detected in GI suggest the stimulation of some components of the inflammatory process.

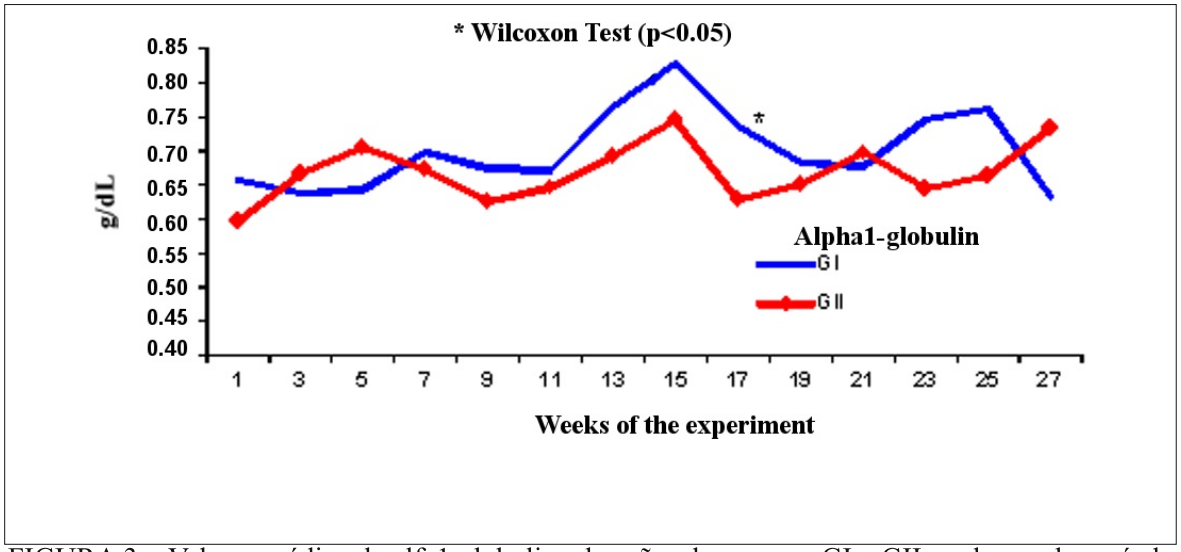

FIGURA 3 – Valores médios da alfa1 globulina dos cães dos grupos GI e GII, ao longo do período de avaliação.

The mean alpha2-globulin values were the same for dogs of both groups (GI and GII) and remained within the reference range (HARRUS et al., 1996). During the experimental period there was no difference ($p > 0.05$) between groups (Figure 4).

According to TRAYHURN & WOOD (2005), human obesity induces a state of chronic inflammation represented by the increase of haptoglobin. In this study there was no difference ($p > 0.05$) between the two groups in the alpha2-globulin fraction, which contains haptoglobin, suggesting that the dogs of the GI treatment, which were overweight or fat, did not present an increase of haptoglobin.

The average beta-globulin was higher ($p < 0.05$) in dogs of GII compared with dogs of GI. The values of both groups remained within the reference values (HARRUS et al., 1996). During the weeks of the experiment, the dogs of GII showed higher values than those of GI in the following weeks: 9th (1.09 vs. 1.27) and 21st (1.13 vs. 1.46) for goups GI and GII, respectively (Figure 5).

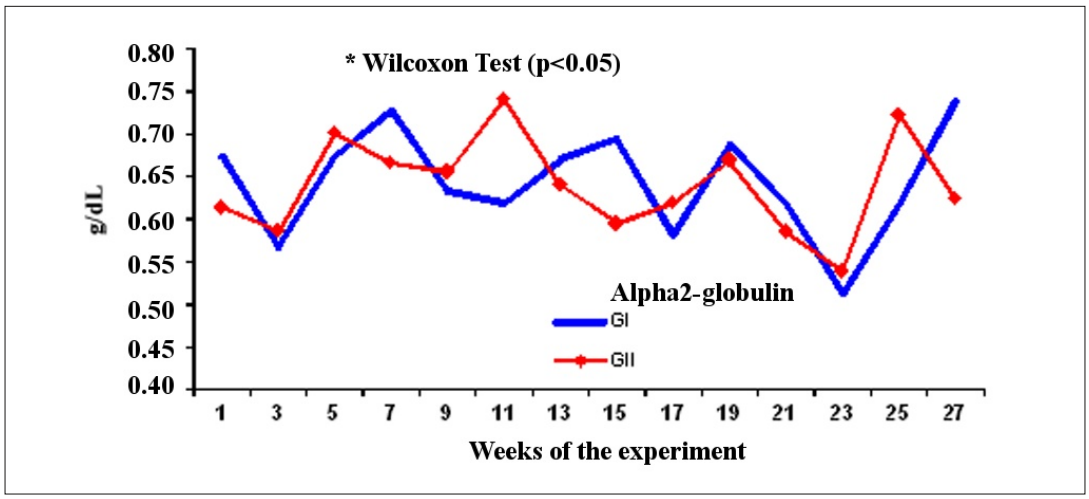

FIGURE 4 – Mean values of alpha2-globulin of dogs of GI and GII, throughout the evaluation period.

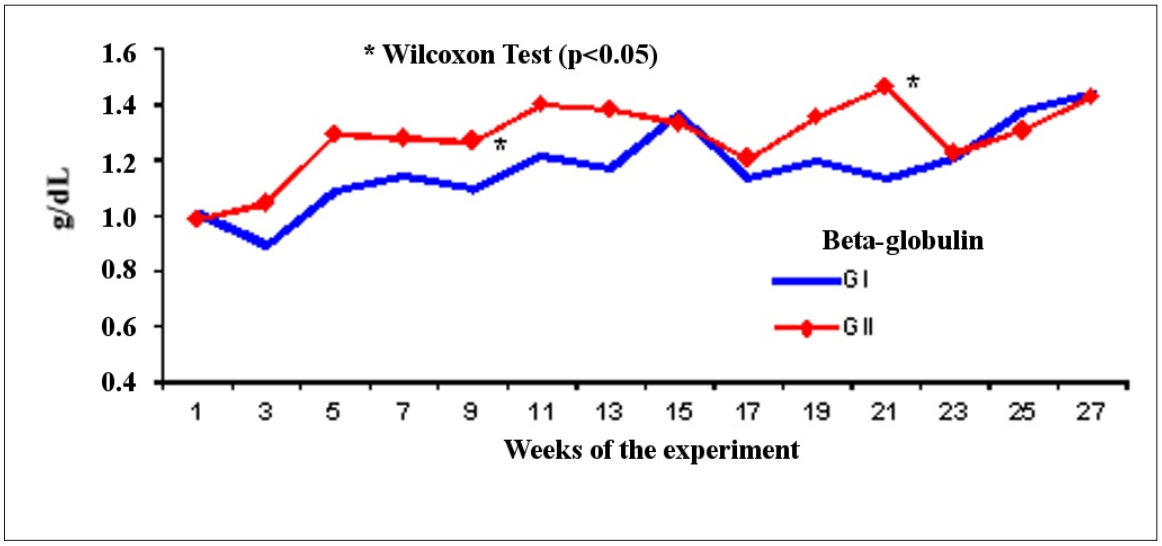

FIGURE 5 – Mean values of beta-globulin of dogs of GI and GII, throughout the evaluation period.

According to NAOUM (1999), it is not uncommon that the beta-globulin zone, being very close to the gamma zone, is covered by the monoclonal bands, which may justify the same behavior of the former fraction when compared to the response pattern of gamma-globulin.

The gamma-globulin showed higher average in GII and the values were above reference interval. During the weeks of the experiment there was statistical difference in the 13th week, it was higher in GII (0.77 g/dL) than in GI (0.56 g/dL), as shown in Figure 6.

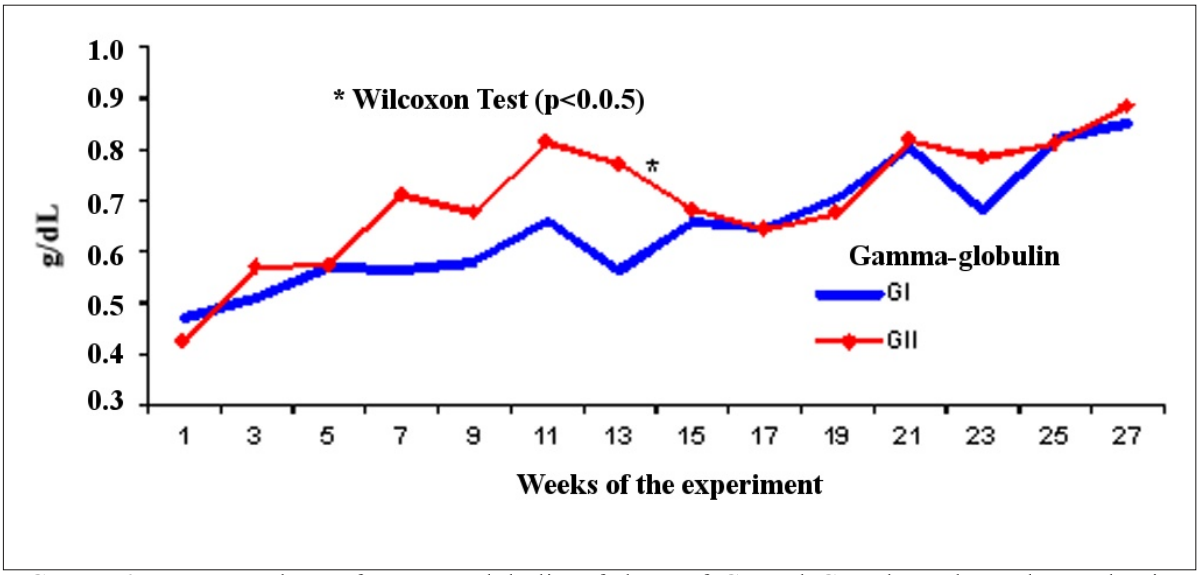

FIGURE 6 – Mean values of gamma-globulin of dogs of GI and GII, throughout the evaluation period.

The mean ALT value was higher ($p < 0.05$) in dogs of GII compared with GI. The averages of both groups were within the reference values. Difference was observed during the experimental period, being higher in dogs of GII compared to GI in nine weeks, as shown in Figure 7.

The results for this enzyme were different than those found by DIEZ et. al. (2004), who found no differences in serum ALT activity in dogs submitted to different diets, i.e., one with high protein level and other with high fiber level.

VÄHALA et al. (1991) did not observe differences in values related to age, unlike this work because, over the course of the experiment, in both treatments, these values increased, suggesting the effect of age on this enzyme. Similarly to this work,

SWANSON et al. (2004) observed the effect of age on the activity of serum ALT, which increased in older dogs.

The mean ALP values was higher ($p < 0.05$) in dogs of GII (40.52 IU) than in those of GI (35.75 IU); however, these values were within the reference range. During the experimental period there was no difference ($p < 0.05$), being higher within nine weeks (Figure 8).

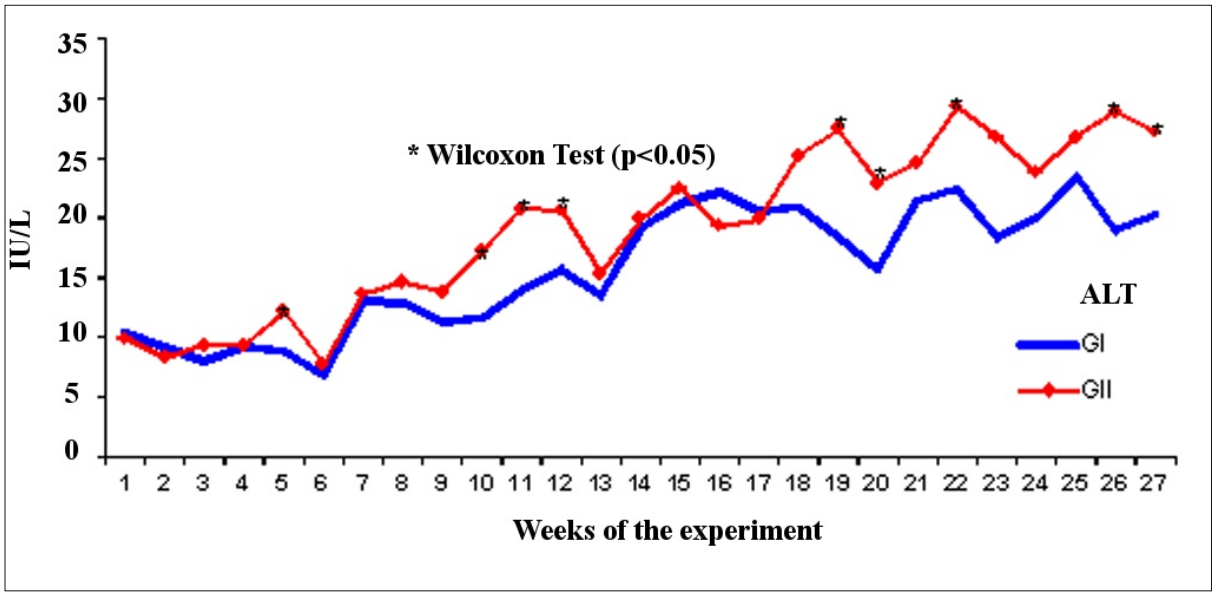

FIGURE 7 – Mean values of ALT of dogs of GI and GII, throughout the evaluation period.

Figure 8 shows the decline of serum ALP activity as dogs get older. This behavior is consistent with SWANSON et al. (2004) who studied the effects of age on weaned and old dogs and verified that ALP was higher in younger dogs. This fact explains the higher activity of bone alkaline phosphatase in the period of greatest growth, i.e., three to four months of age.

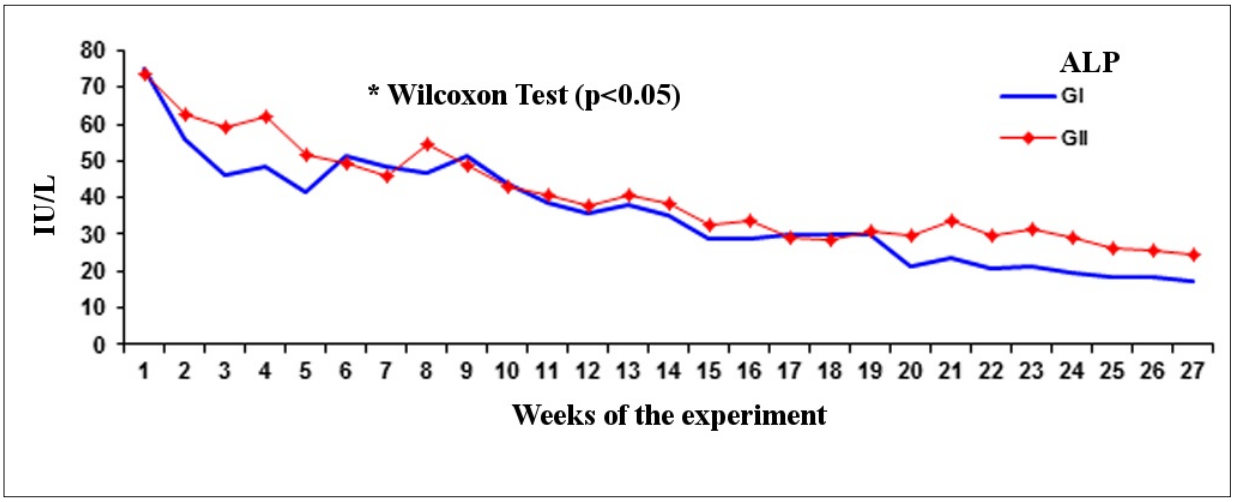

FIGURA 8 - Mean values of ALP of dogs of GI and GII, throughout the evaluation period.

The determination of serum urea and creatinine is a conventional method used for assessment of renal function (NELSON & COUTO, 2001). Over 90% of urea is excreted by the kidneys; however, urea, as an independent indicator of renal function, is limited by variability of its blood levels as a result of non-renal factors. According to CHEW & DIBARTOLA (1992) and WHELTON et

al. (1998), urea is the main nitrogenous metabolic product of the protein catabolism of the body, being synthesized in the liver via the ornithine cycle, using ammonia derived from catabolism of amino acids, which, in turn, are derived from the degradation of exogenous and endogenous proteins. Thus, while the urea is more directly involved in hepatic metabolism, the main clinical utility of determining serum urea, in the study developed here, is justified along with the determination of creatinine and discrimination of pre- and post-renal azotemia (CHEW & DIBARTOLA, 1992; VANDER, 1995 e WHELTON et al., 1998).

In this work, higher urea serum was expected in dogs of GI fed high protein diet. However, no significant difference ($p > 0.05$) was observed in mean serum levels of urea (Table 1), and the values were within the reference ranges (BUSH, 1999). In Figure 9, the behavior of serum urea in the weeks of the experiment between GI and GII dogs can be observed, showing difference ($p < 0.05$) at week 16 (35.42 mg dL vs. 43.28 mg dL).

Serum creatinine concentration was within reference values (KANEKO, 1989) in both groups throughout the experiment, but it was higher ($p < 0.05$) in dogs of the GII. There were differences between groups in four weeks (Figure 9).

Animals of both groups showed no clinical or laboratory evidence of renal disease in this experiment.

The urinary protein: urinary creatinine index (uP:uCr), according to WHITE et al. (1984), replaces with advantages the volume of 24 hours since creatinine is produced at constant rates and filtered freely, being neither secreted nor reabsorbed by the renal tubules. Thus, when applying the index, the effect of urine volume on the protein in a single sample is canceled. According to LULICH & OSBORNE (1990), healthy dogs have uP:uCr index lower than 0.5, being values between 0.5 and 1.0 questionable and the ones greater than 1.0 abnormal.

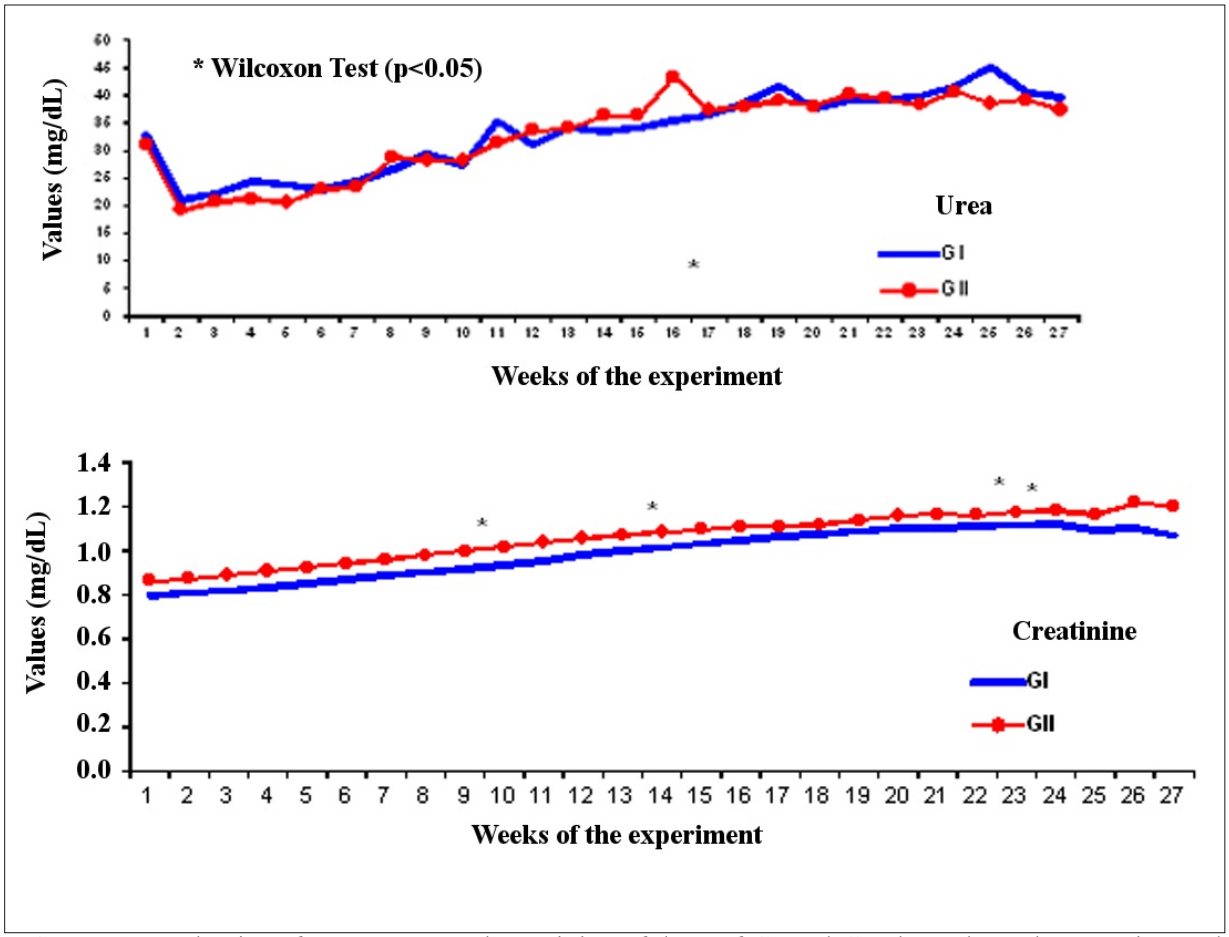

FIGURE 9 – Behavior of serum urea and creatinine of dogs of GI and GII throughout the experimental period.

The uP:uCr index showed mean and standard deviation of 0.27 (0.23) and 0.24 (0.13) for GI and GII, respectively, with no difference (p> 0.05) between them. There were differences (p <0.05) in the first week of the experiment, being higher in dogs of GII (Figure 10). In this study, in the second and 11th weeks, the dogs of GI had mean uP:uCr index greater than 0.5, which can be attributed to the excessive intake of animal protein by the animals of this group.

TOLEDO (2001) found values between 0.22 and 0.13, respectively, for males and females, and the values found for males were close to the findings of the study. Values higher than those observed here were described by ZARAGOZA et al. (2003), who found a mean of 0.5 in the calculation of this index in healthy dogs of various breeds and both sexes, being therefore within the limits of normality.

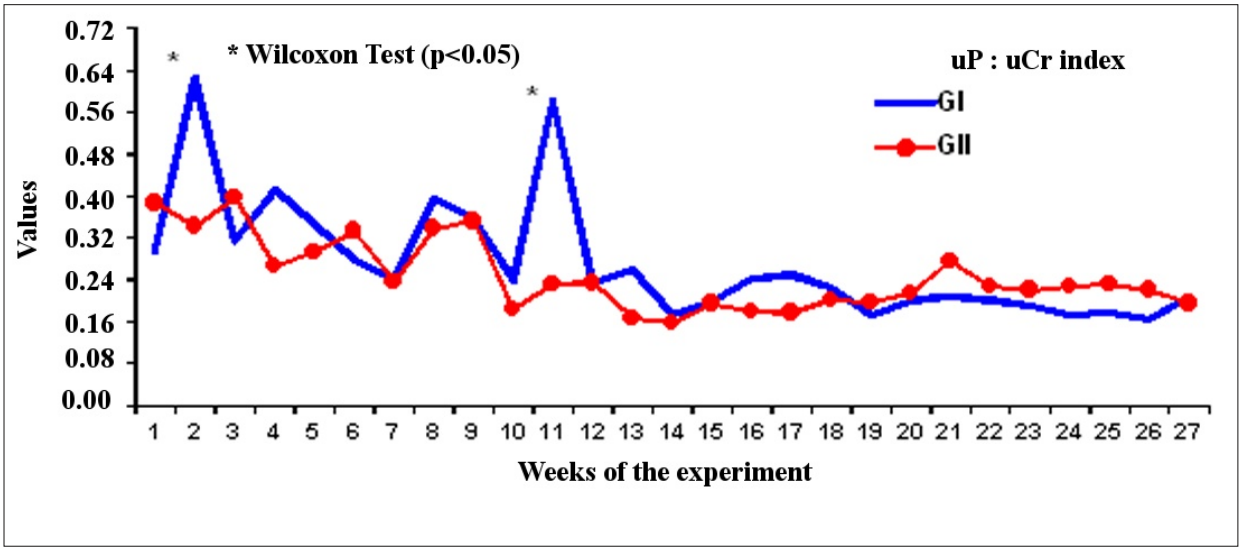

FIGURE 10 – Mean values of the urinary protein:urinary and creatinine indexes of dogs of GI and GII throughout the experimental period.

CONCLUSION

The high digestibility food offered ad libitum, with 34% CP and 16% lipid, is not related to hematological changes or liver and kidney function alterations in Grate Dane puppies.

SOURCE OF FUNDING – Nutron Alimentos

REFERENCES

AGAR, S. **Small animal nutrition**. Edinburgh: Butterworth-Heinemann, 2001. 187 p.

ARMSTRONG, P. J.; LUND, E. M. Changes in body composition and energy balance with aging. **Veterinary Clinical Nutrition**, Montreal, 1996. v.3, .n.3, p.83-87.

BURKHOLDER, W. J.; TOLL, P. W. Controle da Obesidade. In: HAND, M.S., THATCHER, C.D.; REMILLARD, R. L.; ROUDEBUSH, P. **Small animal clinical nutrition**. 4 ed. Topeka: Mark Morris Institute, 2000. p.401-430.

BUSH, B. M. **Interpretación de los análisis de laboratorio para clínicos de pequeños animale**s. Harcourt. Espanha. 1999. 616p.

CHEW, D. J.; DIBARTOLA, S. P. Diagnóstico e fisiopatologia da moléstia renal. In: ETTINGER, S. J. (Ed.). **Tratado de medicina interna veterinária:** moléstias do cão e do gato. 3.ed. São Paulo: Manole, 1992. v.4, p.1975-2046.

DIEZ, M.; MICHAUX, C.; JEUSETTE, I.; BALDWIN, P., ISTASSE, L.; BIOURGE, V. Evolution of blood parameters during weight loss in experimental obese Beagle dogs. **Journal of Animal Physiology and Animal Nutrition**, Berlin, v.88, p.166-171, 2004.

ECKERSALL, P. D. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. **Revue de Médiccine Vétérinaire**, Toulouse, v.151, p.577-584, 2000.

EDNEY, A. T. B.; SMITH, P.M. Study of obesity in dogs visiting veterinary practices in the United Kingdom. **The Veterinary Record**, London, v.118, n.14, p.391-396, 1986.

FERREIRA, R. P. **Função renal de cães adultos sadios**

alimentados com diferentes teores de proteína bruta. 2006. 81f. Dissertação (Mestrado em Ciência Animal) – Escola de Veterinária da Universidade Federal de Goiás. disponível em http://www.ufg.br/this2/uploads/files/66/Dissertacao2006_Renata_Pereira.pdf

FINCO, D. R. Urinary protein loss. In: OSBORNE, C. A.; FINCO, D. R. (Ed.) **Canine and feline nephrology and urology**. Philadelphia: Williams & Wilkins, 1995. p.211-215.

HAND, M. S.; ARMSTRONG, J.; ALLEN, T. A. Obesity: Occurrence, treatment, and prevention. **The Veterinary Clinics of North America: Small Animal Practice**, Philadelphia, v.19, n.3, p.447-474, 1989.

HARRUS, S., WANER, T., AVIDAR, Y., BOGIN, E., PEH, H., BARK, H. Serum protein alterations in canine ehrlichiosis. **Veterinary Parasitology**, v.66, p.241-249. 1996.

JERICÓ, M. M.; SCHEFFER, K. C. Aspectos epidemiológicos dos cães obesos na cidade de São Paulo. **Clínica Veterinária**, São Paulo, v.7, n.37, p.25-29, 2002.

JERICÓ, M. M.; SILVA, M. B. F. P.; MACHADO, F. L. A. Avaliação cardiovascular em cães obesos: mensuração da pressão arterial e achados eletrocardiográficos. **Clinica Veterinária**, São Paulo, v.11, n.61, p.66-72, 2006.

KANECO, J. J. **Clinical biochemistry of domestic animals**. San Diego: Academic Press, 1989. 932p

LEWIS, D. L.; MORRIS, L. M.; HAND, S. M. **Small animal clinical nutrition III**. Topeka: Mark Morris Institute, 1994. 369 p.

LIPPERT, A. C. The metabolic response to injury: enteral and parenteral nutritional support. In: MURTAUGH, R.; KAPLAN, P. M. (Ed.). **Veterinary emergency and critical care**. Saint Louis: Mosby-Year Book, 1992. p.593-617.

LULICH, J. P.; OSBORNE, C. A. Interpretation of urine protein-creatinine ratios in dogs with glomerular and non-glomerular disorders. **The Compendium on Continuing Education for the Practicing Veterinarian**, Princeton. v. 12, n. 1 p. 59-72, 1990.

NAOUM, P. C. **Eletroforeses - técnicas e diagnósticos**. 2.ed. São Paulo: Santos, 1999. 154 p.

NAP, R. C.; HAZEWINKEL, H. A. W.; VOORHOUT, G.; BROM, W. E.; GOEDEGEBUURE, S. A.; KLOOSTER, A. T. V. Growth and skeletal development in Great Dane puppies fed different levels of protein intake. **Journal of Nutrition**, Philadelphia, v.121, n.11, p.107-113, 1991.

NELSON, R. W., COUTO, C. G. **Medicina interna de pequenos animais**. Guanabara Koogan, Rio de Janeiro, RJ. 2.ed., 2001. 1084 p.

SAMPAIO, I. B. M. **Estatística aplicada à experimentação animal**. Belo Horizonte: FEPMV,1998. 221p.

SWANSON, K. S.; KUZMUK, K. N.; SCHOOK, L. B.; FAHEY JR. G. C. Diet affects nutrient digestibility, hematology, and serum chemistry of senior and wealing dogs. **Journal of Animal Science**, Champaign, v.82, p.1713-1724, 2004.

TOLEDO, E. G. H. **Perfil eletroforético de proteínas séricas e urinárias de cães normais e de portadores de insuficiência renal crônica**. 2001. 52f. Dissertação (Mestrado em Medicina Veterinária) - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal.

TRAYHURN, P., WOOD, L. S. Signalling role of adipose tissue: adipokines and inflammation in obesity. **Biochemical Society Transactions**, v.33, part 5, p. 1078–1081, 2005

UFV, UNIVERSIDADE FEDERAL DE VIÇOSA. **Sistema de análises estatísticas e genéticas: Manual do usuário** (SAEG). Versão 8.1, Viçosa, 2003. 301 p.

VÄHALA, J., POSPISIL, J., POKORNY, R., KASE, F. Blood serum biochemical values of cape hunting dogs (*Lycaon pictus*): variations whit age and sex. **Acta Veterinaria**, Scandinavica, v.60, p.219-224,1991.

VANDER, A. J. **Renal physiology**. 5.ed. New York: McGraw-Hill, 1995. 238p.

WHELTON, A.; WATSON, A. J.; ROCK, R. C. Metabólitos nitrogenados e função renal. In: BURTIS, C. A.; ASHWOOD, E. R. (Ed.). **Fundamentos de química clínica**. 4.ed. Rio de Janeiro: Guanabara Koogan, 1998. p.552-574.

WHITE, V. OLIVIER, N.B., REIMANN, K., JOHNSON, C. Use of protein-to-creatinine in a single urine specimen for quantification of canine proteinuria. **Journal of the American Veterinary Medical Association**, v.185, n.8, p.882-885, 1984.

ZARAGOZA, C.; BARRERA, R.; CENTENO, F.; TAPIA, J. A.; MAÑÉ, M. C. Characterization of renal damage in canine leptospirosis by sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS - PAGE) and western blotting of the urinary proteins. **Journal of Comparative Pathology**, Edinburgh, v.129, p.169-178, 2003.

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