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The Impact of Nutrition on Dog Blood Serum Malondialdehyde Levels as One of the Oxidative Stress Indicators

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Abstract: *The present work compares oxidative stress in two groups of dogs fed two different diets. Sixteen castrated bitches were divided into two groups. The first group was fed commercially balanced feed, and the second group of dogs was fed home-prepared feed without information on the nutritional composition. After two months of feeding, blood was taken and then the serum was examined for the amount of thiobarbituric acid reactive substances (TBARs). A higher amount of TBARs was recorded in the group of dogs fed home-prepared diets than in the group of dogs fed commercial diets ($p<0.01$). These findings indicate the importance of correct nutritional balancing in dog food, especially with regard to the amount of fat and its composition of fatty acids. Last but not least, it is necessary to mention the addition of antioxidants to feed, which help reduce this oxidative stress.*

I. INTRODUCTION

Oxidative stress is defined as an excess of reactive oxygen species (ROS) due to an imbalance between the production and removal of ROS. The excess may be due to overproduction of ROS, reduced antioxidant capacity, which may be caused by ROS, or both [1]. ROS can seriously alter the structure of molecules, e.g., proteins, lipids, and deoxyribonucleic acid (DNA) [2]. These changes can lead to cell degeneration and death-causing aging [3]. They play an important role in the pathogenesis of many diseases, for example, cardiovascular diseases, neuropathies, inflammatory diseases, suffering from immune deficiency syndrome (AIDS), diabetes mellitus, diseases, and cancer [4-14]. Oxidative stress triggers different mechanisms, including biomarkers such as lipid peroxidation products and endogenous substances with antioxidant properties. These have been identified by stress and used to assess the oxidants in mammals [1]. Malondialdehyde (MDA), one of the end products of lipid peroxidation, is widely distributed. It is used as a biomarker of oxidative stress. MDA

can be measured using a variety of assays, but the most common method is the thiobarbiturate reactive mixtures (TBARs) assay [15].

The antioxidant system contains enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include glutathione peroxidase (GPX), catalase, and superoxide dismutase (SOD). Non-enzymatic antioxidants are also present in time, including α-tocopherol (vitamin E), β-carotene, ascorbate, and glutathione [16-17]. Vitamin E, the most powerful antioxidant, is concentrated in cell membranes [18]. The feed itself and the composition of the feed affect the antioxidant activity and antioxidant capacity. Rezapour-Firouzi et al. [19] showed that n-6/n-3 polyunsaturated fatty acids (PUFA; polyunsaturated fatty acids) in a 3:1 or 6:1 diet improves the level of major ratios, act preventively against oxidative effects and can remove lipid peroxides. Singer et al. [20] also suggest that PUFA boosts immunity and hemp oil increases the body's levels of superoxide dismutase and glutathione peroxidase and decreases malondialdehyde levels. Recently, these opinions have diverged and, on the contrary, pointed out that it is PUFA that increases oxidative stress. The aim of our study is to show how unbalanced dog food can affect oxidative stress.

II. Material and Methods

2.1 Sampling

Totally 16 healthy dogs were divided into two groups, eight in each group. The first group consisted of individuals fed commercially nutritionally balanced feed, and in the second group, individuals were fed a homemade diet that was not nutritionally balanced in any way. All sixteen individuals were castrated bitches, whose ages varied between 3-6 years. These individuals were dewormed. All castrated bitches were crossbreds of medium height (10.3-23.6 kg). Commercial feed (Table 1) was given twice a day, according to the NRC [21], in a daily dose of 130 kcal/kg0.75. Basic feed ingredients: chicken meat (28%), rice (28%), corn (26%), chicken fat (7%), proteins from dried fish, dried beet pulp (4%), fish oil (2%), sodium chloride, and dried brewer's yeast (0.3%). Water was given ad libitum. The dogs were fed for 2 months and after this period, their blood was taken in the veterinary clinic and then processed for examination.

2.2 Laboratory analyses

Lipid peroxidation product concentrations (MDA levels) in the serum were measured as thiobarbituric acid reactive substances (TBARS) according to the spectrophotometric method described by Costa et al. [22] with slight modifications. A 400 µl serum sample was mixed with 4 ml of a solution containing equal volumes of 15% trichloroacetic acid, 0.38% thiobarbituric acid, and 0.25 N hydrochloric acid. In addition, 40 µl of 0.2% butylated hydroxytoluene was used to prevent lipid peroxidation during heating. The reaction mixture was vortexed and then heated in a boiling water bath for 30 minutes. After cooling in ice water for 10 minutes, the reaction mixture was centrifuged at 3000 rpm for 15 minutes. The same procedure was repeated for MDA standards and a blank test (distilled water). MDA standards were prepared by acid hydrolysis of 1, 1, 3, 3-tetramethoxypropane (malondialdehyde-bis [dimethyl acetal]) and were diluted in the range of 0 to 2 nmol/ml. The absorbance of the supernatant was determined at 535 nm against a blank test. TBARS concentration was read from a calibration curve prepared according to MDA standards. TBARS values in the serum were expressed in nmol /ml.

2.3 Statistical analyses

The obtained results were evaluated statistically using the T-test. The statistical program GraphPad Prism 5 was used to calculate the unpaired Student`s t-test. The owners of the dogs agreed to blood sampling and their subsequent inclusion in the experiment. The dogs were in their home environment throughout the experiment. The relevant animal welfare legislative provisions were met while handling the experimental animals.

Table 1: Nutritional value of the commercial feed (% in DM)

Items	Composition
Dry matter %	92.68
Crude protein %	25.0
Fat %	12.0
Crude fiber %	2.0
ASH %	6.5
Calcium %	1.2
Phosphorus %	0.9
Metabolizable energy	3540 (kcal/kg)

III. Results and Discussion

The results of measuring TBARs in serum between the two groups of dogs showed significant differences (Table 2). Higher values of TBARs were measured in the group of dogs that were fed home-prepared feeds ($p<0.01$) than in the group of dogs that were fed commercially balanced feed. Oxidative stress values are influenced by several factors, such as the amount of antioxidants present in the feed and, on the other hand, the composition of the feed itself. The amount of fat, the composition of fatty acids, and especially the presence of fatty acids in the feed, which contain double bonds, can be quite risky for dogs from the point of view of a higher susceptibility to oxidation. PUFAs are mainly sensitive to such susceptibility [23].

As mentioned in the introduction, the presence and ratio of n-6/n-3 PUFA in the diet 3:1 or 6:1 improves the level of serum immune antibodies, the activity of antioxidant enzymes, the ability to remove lipid peroxides and the level of MDA [19; 20]. Previous studies have investigated numerous effects of n-3 fatty acid supplementation on the health of dogs and cats, and the observed results are conflicting. Although some studies suggest that n-3 supplementation is beneficial for animal health [24-26], its excess has been shown to cause adverse effects [27; 28].

This discrepancy between results is partially justified by the lack of data on the effective level of health maintenance and the maximum level of possible supplementation for companion animals. Diets rich in PUFAs such as n-6 and n-3 are known to have the potential to increase cell and tissue peroxidation [29]. Due to the number of double bonds, PUFA incorporated into cell membranes and organelles such as mitochondria, endoplasmic reticulum and peroxisomes are particularly sensitive to free radical attack. According to NRC and FEDIAF [21; 30], supplementation with exogenous antioxidants is necessary when the amount of PUFA in the diet is high. Likely other factors and mechanisms that have not been well studied, such as changes in gene expression, act on the body's oxidative balance when high amounts of PUFA are consumed, and therefore further studies are needed.

Table 2: Average values of TBARs

Items	Commercial feed	Homemade diet
TBARs	0.73	1.13
SEM	0.053	0.103
P	0.0042	

TBARs - thiobarbituric acid reactive substances; P – significant differences if $P<0.01$ among the means by t-test; SEM – standard error of the mean

IV. Conclusion

Because a nutritional analysis of the home-prepared diets and an analysis of the fatty acids in both administered diets were not performed, one must be careful when drawing strict conclusions. However, it is necessary to focus on the amount of fat in feed, the choice of components in the production of feed, and the composition of fatty acids in dog feed. Last but not least, it is also necessary to pay attention to antioxidants that reduce oxidative stress and their inclusion in the ration for dogs.

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References

[1] M. A. McMichael, Oxidative stress, antioxidants, and assessment of oxidative stress in dogs and cats, *Journal of the American Veterinary Medical Association*, *231(5)*, 2007, 714–720.

[2] S. Khanna, D. Pande, R. Negi, K. Karki, R. S. Khanna, H. D. Khanna, Oxidative stress induced damage in benign and malignant breast diseases: histopathological and biochemical aspects. *The Journal of Stress Physiology and Biochemistry*, *(8)*, 2012, 210–214.

[3] N. Hermans, P. Cos, L. Maes, T. De Bruyne, D. Vanden Berghe, A. J. Vlietinck, L. Pieters, Challenges and pitfalls in antioxidant research. *Current Medicinal Chemistry*, *14(4)*, 2007, 417–430.

[4] J. F. O'Connell, A. J. Klein-Szanto, D. M. DiGiovanni, J. W. Fries, T. J. Slaga, Enhanced malignant progression of mouse skin tumors by the free-radical generator benzoyl peroxide, *Cancer Research*, *46(6)*, 1986, 2863–2865.

[5] R. G. Cutler, Human longevity and aging: possible role of reactive oxygen species, *Annals of the New York Academy of Sciences*, *(621)*, 1991, 1–28.

[6] K. Frenkel, Carcinogen-mediated oxidant formation and oxidative DNA damage. *Pharmacology and Therapeutics*, *53(1)*, 1992, 127–166.

[7] B. Halliwell, Antioxidants and human disease: a general introduction. *Nutrition Reviews*, *(55)*, 1997, 44–49.

[8] J. E. Klaunig, Y. Xu, J. S. Isenberg, S. Bachowski, K. L. Kolaja, J. Jiang, D. E. Stevenson, E. F. Walborg Jr, The role of oxidative stress in chemical carcinogenesis. *Environmental Health Perspectives*, *106(1)*, 1998, 289–295.

[9] D. Hristozov, V. Gadjeva, T. Vlaykova, G. Dimitrov, Evaluation of oxidative stress in patients with cancer. *Archives of Physiology and Biochemistry*, *109(4)*, 2001, 331–336.

[10] Y. Sanchez-Perez, C. Carrasco-Legleu, C. Garcia-Cuellar, J. Perez-Carreon, S. Hernandez-Garcia, M. Salcido-Neyoy, L. Aleman-Lazarini, S. Villa-Trevino, Oxidative stress in carcinogenesis. Correlation between lipid peroxidation and induction of preneoplastic lesions in rat hepatocarcinogenesis. *Cancer Letters*, *217(1)*, 2005, 25–32.

[11] M. Valko, C. J. Rhodes, J. Moncol, M. Izakovic, M. Mazur, Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, *160(1)*, 2006, 1–40.

[12] B. Halliwell, Oxidative stress and cancer: have we moved forward? *Biochemical Journal*, *401(1)*, 2007 1–11.

[13] G. Mauldin, *Nutritional management of the cancer patient*. 4th edition. Missouri: Saunders, 2007.

[14] N. Sharifi, Commentary: Antioxidants for cancer: new tricks for an old dog? *Oncologist*, *14(3)*, 2009, 213–215.

[15] R. Lee, M. Margaritis, K. M. Channon, C. Antoniades, Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations. *Current Medicinal Chemistry*, *19(16)*, 2012, 2504–2520.

[16] J. M. Mates, C. Perez-Gomez, M. Blanca, Chemical and biological activity of free radical scavengers in allergic disease. *Clinica Chimica Acta (296)*, 2000 1.

[17] S. Briganti, M. Picardo, Antioxidant activity, lipid peroxidation and skin diseases. What's new. *Journal of the European Academy of Dermatology and Venerology*, *(17)*, 2003, 663–669.

[18] R. Brigelius-Flohe, M. G. Traber, Vitamin E: function and metabolism. *Federation of American Societies for Experimental Biology Journal*, *(13)*, 1999, 1145–1155.

[19] S. Rezapour-Firouzi, M. Mohammadian, M. Sadeghzadeh, M. Mazloomi, Effects of co-administration of rapamycin and eve-ning primrose/hemp seed oil supplement on immunolo-gic factors and cell membrane fatty acids in experimental autoimmune encephalomyelitis. *Gene*, *(759)*, 2020, 144987.

[20] P. Singer, E. Richter-Heinrich, Stress and fatty liver possible indications for dietary long-chain N-3 fatty acids. *Medical Hypotheses*. *(36)*, 1991, 90–94.

[21] NRC. *Nutrient requirement of dogs and cats*. The National Academies Press., Washington, DC, 2006

[22] C. M. Costa, R. C. C. Santos, E. S. Lima, A simple automated procedure for thiol measurement in human serum samples, *Jornal brasileiro de patologia e medicina laboratorial*, *42(5)*, 2006, 345-350.

[23] G. F. E. Pacheco, R. C. Bortolin, P. R. Chaves, J. C. F. Moreira, A. M. Kessler, L. Trevizan, Effects of the consumption of polyunsaturated fatty acids on the oxidative status of adult dogs. *Journal of Animal Science*, *96(11)*, 2018, 4590–4598.

[24] G. K. Ogilvie, M. J. Fettman, J. A. Mallinckrodt, R. A. Walton, R. A. Hansen, K. L. Davenport, K. L. Gross, K. Richardson, M. S. Hand, Effect of fish oil, arginine, and doxorubicin chemotherapy on remission and survival time for dogs with lymphoma: a double-blind, randomized placebo-controlled study. *Cancer*, *(88)*, 2000, 1916–1928.

[25] R. S. Mueller, K. V. Fieseler, M. J. Fettman, S. Zabel, R. A. W. Rosychuk, G. K. Ogilvie, T. L. Greenwalt, Effect of omega-3 fatty acids on canine atopic dermatitis. *Journal of Small Animal Practice*, *(45)*, 2004, 293–297.

[26] R. S. Mueller, M. J. Fettman, K. Richardson, R. A. Hansen, A. Miller, J. Magowitz, G. K. Ogilvie, Plasma and skin concentrations of polyunsaturated fatty acids before and after supplementation with n-3 fatty acids in dogs with atopic dermatitis. *The American Journal of Veterinary Research*, *(66)*, 2005, 868–873.

[27] R. C. Wander, J. A. Hall, J. L. Gradin, S. H. Du, D. E. Jewell, The ratio of dietary (n-6) to (n-3) fatty acids influences immune system function, eicosanoid metabolism, lipid peroxidation and vitamin E status in aged dogs. *The Journal of Nutrition*, *(127)*, 1997, 1198–1205.

[28] C. E. Lenox, J. E. Bauer, Potential adverse effects of omega-3 fatty acids in dogs and cats. *The Journal of Veterinary Internal Medicine, 27(2)*, 2013, 217-226.

[29] J. A. Hall, Potential adverse effects a long-term consumption of (n-3) fatty acids. *Compendium on Continuing Education for the Practising Veterinarian*, *18(8)*, 1996, 879–895.

[30] FEDIAF. *The European pet food industry, Nutritional Guidelines*. Bruxelles. 1–100, 2016.