ISSN: 1220-3173; E-ISSN: 2457-7618

LEPTIN – A USEFUL ADIPOKINE IN PREVENTING AND MANAGING OBESITY IN DOGS

LEPTINA – O ADIPOKINĂ UTILĂ ÎN PREVENIREA ȘI GESTIONAREA OBEZITĂȚII LA CÂINI

Violeta-Elena SIMION[1)], Adriana AMFIM[1)], Monica PÂRVU[1)], Mădălina BELOUS[1)], L. TUDOR[2)], Liliana TUDOREANU[2)], Elena MITRĂNESCU[2)]

ABSTRACT

Anthropomorphizing of dogs has drastically changed their environment, social, and food behavior with serious health consequences, obesity being at the top of the list. This article highlights the potential role of leptin as a biomarker associated with obesity in dogs. Leptin analysis (ng/mL) was performed on venous blood by the enzyme-linked immunosorbent assay (ELISA). All data on plasma concentration were recorded and classified based on animal profile: age, gender, race, BCS, weight, nutrition, and primary disease. Statistical analysis matched pairs analysis, and multiple regression models of leptin concentrations were employed to analyze the relationship between serum leptin levels and some independent variables. The data from dogs is not normally distributed, and 50% of the animals had leptin concentration between 3 ng/mL and 6 ng/mL in year 1 (2010), between 3 ng/mL and 9 ng/mL in year 2 (2011) and between 2 ng/mL and 4 ng/mL in year 3 (2015). Monitoring animals since they were born, nutrition counseling, including adipokine monitoring, are essential for preventing obesity. Active maintenance of the animal is also important. The multiple regression models generated for leptin concentrations are a useful tool to estimate the animal's potential for developing obesity.

Keywords: monitoring leptin, obesity, dogs

REZUMAT

Antropomofizarea câinilor le-a schimbat drastic mediul, comportamentul social și alimentar, cu consecințe grave asupra sănătății, în timp ce obezitatea este prima pe lista de afecțiuni. Acest articol evidențiază rolul potențial al leptinei ca biomarker asociat obezității la câine. Analiza leptinei (ng/mL) a fost efectuată pe sânge venos, prin tehnica imunoenzimatică (ELISA). Toate datele privind concentrația plasmatică au fost inregistrate și clasificate pe baza profilului animalelor: vârsta, sexul, rasa, Scorul Corporal de Greutate (BCS), greutatea, tipul de alimentație și afecțiunea primară. Analiza statistică a asocierilor și modelele de regresie multiplă a concentrațiilor de leptină au fost folosite pentru a analiza relația dintre nivelele leptinei serice și unele variabile independente. Datele obținute de la câini nu sunt distribuite în mod normal și 50% dintre animale au avut concentrația de leptină între 3 ng/mL și 6 ng/mL în anul 1 (2010), între 3 ng/mL și 9 ng/mL în anul 2 (2011) și între 2 ng/mL și 4 ng/mL în anul 3 (2015). Monitorizarea animalelor începând de la naștere, consilierea nutrițională, inclusiv monitorizarea adipokinelor, sunt esențiale pentru prevenirea obezității. Menținerea activă a animalului este de asemenea importantă. Modelele de regresie multiplă generate pentru concentrațiile de leptină reprezintă un instrument util pentru estimarea potențialului animalului de a dezvolta obezitatea.

Cuvinte cheie: monitorizarea leptinei, obezitate, câini

Obesity is one of the most important health problems of the animal population, especially those raised as pets, at the top of the list being cats and dogs. Approximately 55% of dogs and cats in the United States were considered overweight and obese in 2013 (1), while in 2017 it is estimated that the proportion reached 60% of cats and 56% of overweight or obese dogs (2, 3). Domestication, even the anthropomorphism of these pets has drastically changed their environment and with very serious consequences on social and food behavior. A recent study in Europe on the investigation of dog owner's perceptions about their own obesity and their dogs' obesity has demonstrated that among 3185 respondents from ten countries, between 19.1% and 48.8% of the owners and their dogs were overweight/obese.

The predisposing factors identified were the age,

1) "Spiru Haret" University, Faculty of Veterinary Medicine, Bucharest, Romania
2) University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania
*) *Corresponding author: mitranescuelena@gmail.com*

gender and owners' attitude towards diet and physical activity (4). These statistics are alarming, and obesity is not just a build-up of adipose tissue, it leads to the production of important metabolic and hormonal changes in the body. The high incidence of overweight and obese pets results in high morbidity and mortality and affects the quality of life, longevity and wellbeing of dogs and cats (5).

Genetics plays an important role in the predisposition to obesity in dogs, like for example at dog breeds such as Terrier Cairn, Dachshund, Scottish Terrier, Beagle, Cocker Spaniel, Basset Hound, Labrador, Golden Retriever, Rottweiler, Newfoundland, Saint Bernard, Bernese Mountain Dog (6). Also, another predisposing factor for obesity is age. Like the elderly, the dogs, but also the cats are the most exposed to the risk of becoming overweight, although younger animals may find themselves in this condition. Gender is another predisposing factor, females, like most species, are at increased risk for obesity because physiologically during the gestation and lactation period the animal accumulates energy reserves in the form of fat deposits and nutrients and greatly restrains the movement, remaining around the nest to defend and nourish the offspring. Also, sterilization of females and males is another predisposing factor of obesity, sex hormones influencing basal metabolism but ensuring daily moderate movement after sterilization and a balanced diet can prevent weight gain. However, studies have shown that early sterilization in females at about 5-6 months seems to reduce the risk of weight gain, although hormonal changes have a direct effect on nerve centers responsible for satiety and metabolism (hypothalamus, for example), and indirectly affect cell metabolism and the activity of hormones that regulate food consumption (ghrelin, leptin etc.). Estrogen is a hormone with inhibitory activity of lipogenesis and is also known to be a determinant in the production of adipocyte count (5). Miller (2011) shows in a study the association between obesity and castration in dogs and cats. It is essential to educate the owners about how to feed animals and adapt the post-neutering diet (7). In addition to genetic or predisposing factors, oral or injectable contraceptives, endocrine diseases (diabetes or hyperadrenocorticism), hypothalamus lesions, drugs such as glucocorticosteroids (dexamethasone, for example) can indirectly induce polyphagia and then increase in weight (5). In obesity, metabolic, hereditary, nervous, endocrine and nutritional factors are incriminated (8).

The rate of obesity in dogs and cats (and not only in these species) is increasing and the study of adipokines as biomarkers is essential in assessing the risks for the development of obesity-related complications (9). Adipose tissue is a group of several endocrine glands that act differently and produce over 50 bioactive adipokines involved in metabolism with influence on the immune system and inflammatory effects (1, 11). The most well-known and studied are: leptin, which regulates the functions of the immune and reproductive system and modulates insulin resistance; resistin that increases the production of cytokines with pro-inflammatory effect and increase inflammatory markers (TNF α, Il-1, IR-6, Protein C reactive); adiponectin, also with inflammatory effects and increasing insulin sensitivity, apelin, an adipokine involved in increasing insulin sensitivity as a visfatin, otherwise participating in the absorption of glucose by the muscle and adipocyte cells.

With the discovery of leptin in 1994, studies on this satiety hormone, as it was called, began to develop, its role in energy homeostasis and the maintenance of body mass is extremely important (10). The circulating concentrations of leptin correlate with the fat mass in both dogs and cats. Thus, increased fat mass, either from experimentally induced obesity or from pets with BCS (Body Corporal Score) over ideal (BCS= 3), results in predictable and measurable increases in leptin. On the contrary, the reduction in fat mass also leads to a decrease in leptin concentrations in both species (5). This study presents the results of 30 analyzed samples from 10 subjects that were monitored over a period of about 5 years between November 2010 and April 2015. The animals were at least once each year registered in a veterinary clinic in Bucharest, regarding various routine treatments, vaccinations, spayed/neutering or other necessary treatments. Detailed information: name, race, gender, physiological status, number of meals/days, type of food used in the animal's diet, and medical history of each individual patient.

MATERIALS AND METHODS

All data on plasma leptin concentration were recorded and classified based on animal profile, for ten dogs: age (months, gender, race, assessment of BCS (with values from 1 to 5), weight monitoring periodically (kg), type of diet, spaying/neutering or not, and primary disease. The monitoring was done between 2012 -2015, and the leptin concentration analysis was performed at the beginning of the study (2010), one year after the start (2011), and the end of the study (2015).

Table 1
Summary statistics leptin concentration (ng/mL) in dogs - year 1 (2010)

Mean	Std Dev	Std Err Mean	Upper 95% Mean	Lower 95% Mean	N
7.64	4.9947083	1.5794655	11.212999	4.0670009	10

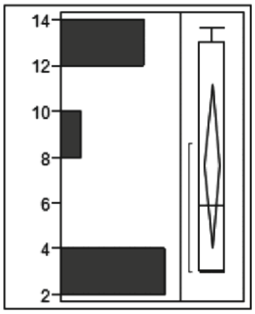

Fig. 1. Distribution of leptin concentration (ng/mL) in dogs – year 1 (2010)

Table 2
Summary statistics leptin concentration (ng/mL) in dogs - year 2 (2011)

Mean	Std Dev	Std Err Mean	Upper 95% Mean	Lower 95% Mean	N
7.9	5.2008546	1.6446546	11.620467	4.1795327	10

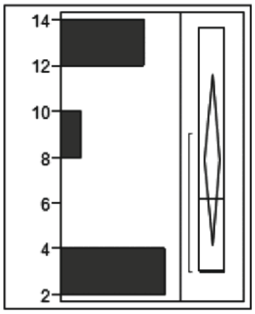

Fig. 2. Distribution of leptin concentration (ng/mL) in dogs – year 2 (2011)

Table 3
Summary statistics leptin concentration (ng/mL) in dogs - year 4 (2015)

Mean	Std Dev	Std Err Mean	Upper 95% Mean	Lower 95% Mean	N
7.57	5.7986684	1.8337	11.718118	3.4218825	10

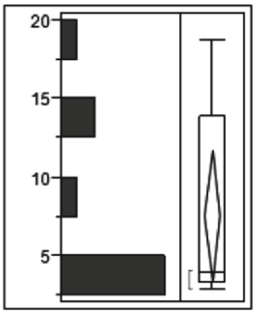

Fig. 3. Distribution of leptin concentration (ng/mL) in dogs – year 4 (2015)

Leptin analysis (ng/mL) was done on venous blood, at least 2 mL of serum, collected in vacutainer without anticoagulant; the serum samples were separated by centrifugation and analyzed for leptin dosing by the enzyme-linked immunosorbent assay (ELISA) using an automated immunodiagnostic device with Leptin Sandwich ELISA kit.

The blood plasma sample was from all subjects investigated, during the monitoring period. Thus, blood was collected at least 10 hours after the last meal or at least 10 hours after the last dose of insulin. For the statistical analysis, the data were processed using the Matched pairs platform and the Fit model platform of the JMP10 software.

The linear model that has been tested is a multiple regression.

The independent variables considered were: *age* (numerical continuous), *weight* (numerical continuous), *gender* (binary variable), *BCS* (ordinal variable).

RESULTS AND DISCUSSION

The summary statistics are shown in Tables 1-3 and Figures 1-3. The data are not normally distributed. The summary statistics suggest that a larger group of animals should be observed covering all variables that are indicated in the literature to influence the level of leptin. Figures 1-3 show that *50% of the animals under observation had leptin concentration* between 3 ng/mL and 6 ng/mL in year 1 (2010), between 3 ng/mL and 9 ng/mL in year 2 (2011), and between 2 ng/mL and 4 ng/mL in year 3 (2015).

Appetite, food intake, body weight, and BCS

It is well known that, as has been proven by many studies, the main role of leptin is to induce satiety and to regulate food intake. All ten dogs were monitored between 2010 and 2015, and the leptin concentration was determined in 2010, then at one year (2011), and at the end of the monitoring period (2015). Among the dogs, one received diet (Intestinal Low Fat), three received home-made food, one received a mix of commercial food and food scraps, and five have received commercial food premium/super premium.

A study on the influence of food on the microbiome showed that a ''high-protein, low-carbohydrate'' diet caused the development of proteolytic bacteria while ''moderate-protein, moderate-carbohydrate'' diet has led to the development of saccharolytic bacteria and the growth of bifidobacterial and ghrelin in the blood which stimulates appetite and lactobacilli, whose value was correlated with the increase in total blood cholesterol and leptin (11).

These statements were also valid in this study. Dogs who received home-made diet and commercial food economic range with a moderate-low nutritional value, BCS was in two dogs 5, and in one, 3; in all dogs, the plasma leptin concentration increased during the monitoring period. Findings on the role of intestinal microbiota in obesity have been mentioned by Ostro and Lutz (2015) (13). The same results were also obtained and presented by Vester et al.(2012) (14).

During the first year of monitoring, 2010-2011, dogs were medically evaluated on the basis of variables (nutrition, gender, spayed / neutered, weight, BCS, and leptin concentration). The level of leptin is a good indicator of the body's fat content in humans, dogs, and rodents. Shibata et al. (2003) have shown that ELISA-determined leptin concentration may be useful in assessing obesity (13). In the monitored dogs, the leptin concentration increased in 2010-2011 in 6 dogs (predominantly those with BCS 5 and 4); these dogs generally received 2-3 meals/day.

The results presented in Tables 1-2 and Figures 1-2, show an increase in leptin concentration during this year. Dogs' leptin concentrations are increased after a fat meal or after a high energy consumption. In dogs, this effect may increase by 2 to 3 times leptin concentrations, for 8 hours. A study by Yamka (2007) on nutrients in experimentally administered food for weight loss of the dog showed that formulas with high content of amino acids and proteins and increased content in soluble fiber compared to commercially available diets in commerce for obesity prevention, with increased content in total dietary fiber, have been effective in reducing weight and loss of body fat (14).

The animals monitored in this study spend their time in the house and go out controlled, limited periods of time. Owners have been advised to adopt a controlled feeding of animals so as to prevent the installation of obesity. During the monitored period (2010-2015) no food was changed but strict control of the administered amount was recommended, and motion was also recommended. Adult animals which live in a stress-free environment and who receive healthy diets from a nutritional point of view, balance the expenses with long-term energy consumption (1). At the end of the monitoring period, the results presented in Table 3 and Figure 3 in dogs show a variation in leptin concentration for the three periods in the investigated animals.

The effectiveness of an obesity prevention program is even more efficient as animals are growing and evaluation and monitoring is done from birth. An adequate diet during the key development periods (growth, pregnancy, lactation, weaning) both in dogs and cats is essential for their health and reproduction (16). Surely, diet is essential in a weight loss program along with an effective program of movement.

Sunvold (2008) shows that weight loss is influenced by changes in the carbohydrate metabolism by the use of nutrients that influence blood glucose (e.g., starch source), some mineral substances (e.g., chromium) and fermentable fibers; changes in lipid metabolism by carnitine supplementation, for example as a longchain fatty acid carrier via the mitochondrial membrane or supplementation with some vitamins (e.g., vitamin A) as well as vitamin D (17). Generally, proper protein intake, the use of low-glycemic carbohydrate sources (e.g. cereals such as barley, sorghum or buckwheat) as well as mineral and vitamin supplements are useful in preventing obesity equally in humans and animals (dogs, cats).

Regarding race influence on BCS, fat mass and adipokine concentrations, studies are fairly low. Ishioka et al. (2007) shows that the race may still influence the leptin concentrations for the dog while this does not apply to the cat. Thus, comparative analysis of BCS and leptin concentration in Shetland Sheepdog breeds was higher compared to the results obtained in dogs from the Miniature Dachshund and Shih Tzu breeds, for example (18). In this study, 6 mixed-breed dogs and 4 breed dogs were investigated (Bullmastiff, 84 months, BCS 5; Golden Retriever, 120 months, BCS 3; Cocker, 132 month, BCS 3; Bichon, 84 month, BCS 4) in which no BCS variation was observed during the monitoring period, except for the Bichon for which BCS was reduced to 3.

Plasma leptin concentration is a good indicator of obesity. A study of male Beagle dogs that received a high calorie diet for 3 months showed that they all became obese, and the plasma leptin concentration increased significantly from 2.4+/-1.2 to 4.9+/-0.9 ng/mL, correlating positively with the body fat content estimated by the deuterium oxide dilution method (r=0.87) (19).

In this study, in the 10 dogs monitored, plasma leptin concentration based BCS values were: for BCS 3, the leptin concentration was of 2.9-4.0 ng/mL; for BCS 4, the leptin concentration was of 8.2-13.5 ng/mL, and for BCS 5, the leptin concentration was of 13.0-18.7 ng/mL. The results are almost similar to those from other studies. Also, Ishioka et al. (2002) shows that the leptin concentrations of plasma samples collected from 59 dogs in veterinary clinics were compared with BCS. Plasma leptin concentrations in obese dogs were 9.7+/-, and 12.3+/- ng/mL at BCS 4, and respectively, BCS = 5, which were significantly higher than those of optimal dogs (BCS = 3) 2.7+/-0.3 ng/mL. There was no significant effect of gender and race. We found a poor positive correlation (r=0.37) between the plasma and age leptin concentration, probably due to the lower visceral fat content at puppies younger than 1 year. These results indicate that leptin from plasma is a good index of adiposity in dogs, irrespective of race, age, and gender, and can be useful for quantitative assessment of obesity in small animals (19).

The relationship between spaying or neutering, and leptin concentrations

According to Godoy (2018) there are changes between gonadectomy, hormonal changes (especially those of ghrelin, leptin, adiponectin, glucagon-like peptide-1) and appetite regulation. Therefore, a therapeutic approach to obesity has to start from the investigation of physiological hormones to a dietary management adapted to satiety control and hunger, the more so as the composition of food in nutrients greatly influences the phylogeny and the functional capacity of the microbiota (5). Of the 10 monitored dogs, half of them were neutered; in 4 of these, the leptin concentration increased during the monitoring period, average values found between 9.12-9.72 ng/mL. In non-neutered dogs, both females and males, mean values of leptin concentration during the monitoring period were between 6.28–5.42 ng/mL.

Kawauchi et al. (2017) shows in a study that a diet with 94.0 g protein/1000 kcal ME seems to be a beneficial nutritional strategy to maintain the dog's body condition after neutering (22). It is worth noting that after sterilization in cats, the corporal weight increases significantly from the moment the animal is neutered. In general, for animals with age under one year, the concentration of plasmatic leptin was lower, as fat tissue is reduced (23).

Leptin and its role in the development of diabetes

Wakshlag et al. (2011), show that in a dog, following a weight loss process, a series of chronic inflammations (e.g. serum C-reactive protein, monocyte chemoattractant protein-1, total adiponectin, HMW adiponectin, resistin, leptin) have remained unchanged, except for leptin that recorded a significant reduction after the weight reduction program which suggests that insulin resistance and type 2 diabetes is less prevalent in dogs (20).

Although the consequences of obesity are very similar to those of humans, there are differences between the two species: diabetes in dogs is not necessarily associated with obesity, they develop a type 1 diabetes while in cats; this pathology is similar to human type 2 diabetes in terms of pathophysiology, risk factors and treatment (12, 21).

CONCLUSIONS

Obesity is on the rise in pets – dogs and cats, and this is evidenced by a series of studies around the world. The list of diseases caused by obesity is long, and prevention is the best solution in this case.

Adipokines are currently being analyzed as potential biomarkers for risk assessment in the development of obesity-related complications.

In this study, in the dogs monitored, plasma leptin concentration based BCS values were: for BCS 3, the leptin concentration was of 2.9-4.0 ng/mL; for BCS 4, the leptin concentration was of 8.2 -13.5 ng/mL, and for BCS 5, the leptin concentration was of 13.0-18.7 ng/mL. There was no significant effect of gender and race, and the results are almost similar to those from other studies. The approach to therapy has to go from investigating physiological hormones to dietary management adapted to satiety control and hunger, and last but not least, the change in physical activity as an important factor in weight maintenance.

Acknowledgments:

The authors thank DVM Iacob Roxana for the technical support given in serum leptin concentration analysis and for sorting and monitoring cases.

REFERENCES

1. *Backus R., Wara A.,* (2016), Development of obesity mechanisms and physiology. Vet Clin North Am Small Anim Pract, 46(5): 773-784
2. *Barbany M., Foz M.,* (2002), Obezity: concept, classification and diagnosis. An Sist Sanit Navar, 25 (Suppl 1):7-16
3. *de Godoy M.R.C.,* (2018), Pancosma comparative gut physiology symposium: all about appetite regulation: effects of diet and gonadal steroids on appetite regulation and food intake of companion animals. J Anim Sci, 96(8):3526-3536
4. *de Godoy M.R.C., Swanson K.S.,* (2013), Nutrigenomics: using gene expression and molecular biology data to understand pet obesity. J Anim Sci, 91(6):2949-2964
5. *Fontaine E.,* (2012), Food intake and nutrition during pregnancy, lactation and weaning in the dam and offspring. Reprod Domest Anim, 47(Suppl 6):326-330
6. *Hoenig M.,* (2002). Comparative aspects of *diabetes mellitus* in dogs and cats. Mol Cell Endocrinol, 2002, 197(1-2):221-229
7. *Hooda S., Vester Boler B.M., Kerr K.R., Dowd S.E., Swanson K.S,* (*2012*), The gut microbiome of kittens is affected by dietary protein: carbohydrate ratio and associated with blood metabolite and hormone concentrations*. Br J Nutr, 109(9):1637-1646*
8. *Ishioka K., Hosoya K., Kitagawa H., Shibata H., Honjoh T., Kimura K., Saito M.,* (2007), Plasma leptin concentration in dogs: effects of body condition score, age, gender, and breeds. Res Vet Sci, 82(1):11–15
9. *Ishioka K., Soliman M.M., Sagawa M., Nakadomo F., Shibata H., Honjoh T., Hashimoto A., Kitamura H., Kimura K., Saito M* , (2002), Experimental and clinical studies on plasma leptin in obese dogs, J Vet Med Sci, 64(4):349-353
10. *Kawauch I.M., Jeremias J.T., Takeara P., de Souza D.F., Balieiro J.C.D., Pfrimer K., Brunetto M.A., Pontieri C.F.F.,* (2017), Effect of dietary protein intake on the body composition and metabolic parameters of neutered dogs, J Nutr Sci, 6:e40
11. *Kuryszko J., Sławuta P., Sapikowski G.,* (2016), Secretory function of adipose tissue. Pol J Vet Sci, 19(2):441-446
12. *Lund E.M., Armstrong P.J., Kirk C.A., Klausner J.S.,* (2005), Prevalence and risk factors for obesity in adult cats from private US veterinary practices. Intern J Appl Res Vet Med, 3(2):88-96
13. *Lund E.M., Armstrong P.J., Kirk C.A., Klausner J.S.,* (2006), Prevalence and risk factors for obesity in adult dogs from private US veterinary practices. Int J Appl Res Vet Med, 4(2):177-186
14. *Miller D.,* (2011), The link between obesity and neutering in companion animals, Banfield Journal, 7(3):16-21
15. *Muñoz-Prieto A., Nielsen L.R., Dąbrowski R., Bjørnvad C.R., Söder J., Lamy E., Monkeviciene I., Ljubić B.B., Vasiu I., Savic S., Busato F., Yilmaz Z., Bravo-Cantero A.F., Öhlund M., Lucena S., Zelvyte R.,Aladrović J., Lopez-Jornet P., Caldin M., Lavrador C., Karveliene B., Mrljak V., Mazeikiene J., Tvarijonaviciute A.,* (2018), European dog owner perceptions of obesity and factors associated with human and canine obesity. Sci Rep, 8(1):13353
16. *Osto M., Lutz T.A.,* (2015), Translational value of animal models of obesity – Focus on dogs and cats. Eur J Pharmacol, 759:240-252
17. *Radin M.J., Sharkey L.C., Holycross B.J.,* (2009), Adipokines: a review of biological and analytical principles and an update in dogs, cats and horses. Vet Clin Path, 38(2):136-156
18. *Robić M., Belić M., Turk R.,* (2016), Pathophysiology of obesity in dogs and cats. Veterinarska Stanica, 47(1):73-78
19. *Shibata H., Sasaki N., Honjoh T., Ohishi I., Takiguchi M., Ishioka K., Mohamed Ahmed, Mohamed Soliman, Kimura, K., Saito, M.,* (2003), Feline leptin: immunogenic and biological activities of the recombinant protein and its measurement by ELISA. J Vet Med Sci, 65(11):1207-1211
20. *Simion V., Mitranescu E., Parvu M., Tudor L.,* (2016), Obesity at cats and the role of leptin as biomarker for its control, J Biotechnol, 231(Suppl): S45-S45
21. *Sunvold G.D.,* (2008), A nutritional innovation for weight control. Bollettino AIVPA, 2:25-33
22. *Wakshlag J.J., Struble A.M., Levine C.B., Bushey J.J., Laflamme D.P., Long G.M.,* (2011), The effects of weight loss on adipokines and markers of inflammation in dogs. B J Nutr, 106(Suppl 1):S11-S14
23. *Yamka R.M., Frantz N.Z., Friesen K.G.,* (2007), Effects of 3 canine weight loss foods on body composition and obesity markers. Int J Appl Res Vet M, 5(3):125-132.