B-4840
[1-6]

RESEARCH ARTICLE Indian Journal of Animal Research

# Clinical Evaluation of Weight Loss Diet on Adiposity Markers and Subcutaneous Fat in Obese Dogs

Zinnia Marcelo, Sujata Turkar, Amrit Pal Singh Sethi[1]

10.18805/IJAR.B-4840

## ABSTRACT

**Background:** This clinical study was conducted in thirteen apparently healthy overweight/obese dogs during the period 2018-19. The study involved nutritional interventions such as feeding of high protein and low fat diet and increased physical activity for these dogs, so as to assess effect of these interventions on adiposity markers and subcutaneous fat in obese dogs.

**Methods:** The prescribed weight loss diet consisted of high crude protein (%), crude fibre (%) and reduced fat (%) compared to the mean values of pre-weight loss diets. The study was conducted over a span of 12 weeks and various hemato-biochemical parameters, adiposity markers and subcutaneous fat thickness were measured on 0, 30, 60 and 90 days.

**Result:** Dogs included in this study showed reduction of an average of 9% of their body weight. Significant (P<0.05) difference was observed in mean values of Body Condition Score at day 60, from day 30 and this difference persisted until day 90, when compared with day 0 of treatment. Serum cholesterol and leptin concentrations were markedly reduced at the end of weight loss period. Decreased thickness of subcutaneous fat by real time ultrasonography was observed from day 0 to day 90 in five anatomical locations *i.e.* abdomen, mid abdomen, 9 Inter Costal Space, thigh and lumbar.

**Key words:** Adiposity, Canine, Subcutaneous fat, Weight loss diet.

## INTRODUCTION

Obesity is a common nutritional disorder in dogs, which is one of the greatest clinical challenges in contemporary Veterinary Medicine. This is increasing with a similar trend as observed in humans and is becoming a major health and welfare problem worldwide (Carreira *et al.* 2016). It is defined as an excess of adipose tissue that mainly develops as a result of a positive mismatch between energy intake and energy expenditure combined with poor lifestyle habits, particularly inactivity and is associated with decreased quality and longevity of life and increased incidence of secondary diseases, including metabolic diseases, respiratory distress, hypertension, cardiac disease, kidney diseases, neoplasia, orthopaedic and skin diseases (German 2006, Laflamme 2012).

Management of obesity involves nutritional as well as lifestyle modifications. Nutritional management as a weight reduction protocol is tailored towards an individual patient to overcome the problems due to obesity and it leads to weight loss. Lifestyle management which includes increasing physical activity is useful in combination with dietary therapy, as it promotes fat loss and may assist in lean tissue preservation and promotion of behaviour that aid in sustainable weight loss. It has also been observed that exercise helps prevent the rapid regain in weight after successful weight loss (Laflamme 2012, Brooks *et al.* 2014).

The majority of owners of obese dogs do not spontaneously seek veterinary advice to resolve the problem of excess weight. Generally, they are not fully aware of the condition of their animals and it is therefore up to the clinicians to make the diagnosis and convince the owner regarding seriousness of the condition and motivate them to undertake measures to control it.

Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.
[1]Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.

**Corresponding Author:** Sujata Turkar, Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India. Email: sujataturkar@gmail.com

**How to cite this article:** Marcelo, Z., Turkar, S. and Sethi, A.P.S. (2022). Clinical Evaluation of Weight Loss Diet on Adiposity Markers and Subcutaneous Fat in Obese Dogs. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4840.

**Submitted:** 13-12-2021 **Accepted:** 05-05-2022 **Online:** 28-06-2022

In view of paucity of available literature on weight management in obese dogs from India, this clinical study was planned to evaluate the effect of dietary intervention and lifestyle management for the treatment of canine obesity.

## MATERIALS AND METHODS

The present study was undertaken during the period 2018-19 in the Department of Veterinary Medicine at Multispecialty Veterinary Hospital, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana after taking due approval (GADVASU/2018/IAEC/47/10) from the Institutional Animal Ethics Committee (IAEC) of the University. This study was conducted in 13 apparently healthy overweight/obese Labrador retrievers dogs which were presented for general check-up or vaccination. They were screened for obesity on the 5-point scale of Body Condition Score (BCS) as designed by Laflamme (1997) and graded as 1: thin, 2: underweight, 3: ideal, 4: overweight, 5: obese. The dogs with BCS 4 and 5 were selected for weight management protocol after taking informed consent of the owners.

Detailed information regarding feeding habits and exercise status of 13 healthy overweight/obese dogs was obtained after applying unstructured questionnaires. Patient specific data *i.e*. age, breed, sex, weight and reproductive status were suitably recorded. The overweight/obese dogs were thoroughly evaluated physically at the beginning of the study and various clinical parameters such as rectal temperature, heart rate, respiration rate, body condition score, colour of mucous membrane were also recorded.

### Haemato-biochemical analysis

Blood was aseptically collected from the cephalic or the recurrent tarsal vein after proper restraining of the animals. Two millilitres (ml) of blood were collected in sodium ethylene diamine tetra acetic acid (EDTA) vials for complete blood count. Five ml of blood were collected in serum collection tubes, which was centrifuged to extract the serum and collected serum was refrigerated at -20°C for biochemical estimations. Whole blood sample collected in sodium EDTA coated vials was used for determination of following parameters by Fully Automatic Laser Based Hematology Analyser (ADVIA ® 2120 Hematology system, Siemens Healthcare diagnostics Inc, USA): Haemoglobin (g/dl), Total Erythrocyte Count (TEC, $\times 10^6$ mL$^{-1}$), Packed Cell Volume (PCV) (%), Platelet Count ($\times 10^3$ mL$^{-1}$), Total leukocyte count (TLC, mL$^{-1}$). Differential leukocyte count (DLC) was done using Giemsa stain using the method described by Jain (1986).

Serum biochemical analysis was performed with Vitros DT 350 Chemistry system (Ortho Clinical Diagnostics, Johnson and Johnson Company) for estimation of following parameters: Renal Markers: BUN (mg/dl) and Creatinine (mg/dl), hepatic markers: alkaline phosphatase (ALKP, IU/L), alanine aminotransferase (ALT, IU/L), total bilirubin (mg/dl), total protein (g/dl) and albumin (g/dl), minerals: calcium (Ca, mg/dl) and phosphorous (P, mg/dl), diabetic marker: glucose (mg/dl); lipid profile: cholesterol (mg/dl), triglycerides (mg/dl), high density lipoprotein cholesterol (HDL-C mg/dl), low density lipoprotein cholesterol (LDL-C, mg/dl) and very low-density lipoprotein cholesterol (VLDL-C mg/dl) was calculated by Friedewald formula, *i.e*.

$$\text{VLDL-C} = \text{Triglyceride} / 5$$

$$\text{LDL-C} = \text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C})$$

Adiposity markers, *i.e*. Adiponectin and Leptin estimations were done using canine specific sandwich ELISA kit of Bioassay Technology Laboratory. Ultrasonography of abdominal and thoracic region was done to obtain real time ultrasound (RTU) images of subcutaneous fat thickness using General Electric ultrasound scanner Logiq P5, which was equipped with a multifrequency linear transducer without any sedation or anaesthesia. RTU image acquisition was performed in right lateral recumbency. Subcutaneous fat thickness (SCT) was measured at 6 anatomical locations: lumber, abdomen, mid abdomen, chest, thigh and 9$^{th}$ intercostal space (flank).

Proximate analysis of thirteen pre-weight loss diets and prescribed diet was performed by standard methods for estimation of crude protein (CP), ether extracts (EE), total ash (TA), crude fibre and nitrogenous free extracts. The dogs were fed prescribed diet (66.08% nitrogen-free extract, 2.2% ether extract, 21.43% crude protein, 3.92% total ash and 6.35% crude fiber on dry matter basis) with restricted amounts, corresponding to 60% of their maintenance energy requirement (NRC, 1985) calculated for their target weight, standardized as the present weight minus 15%. Vitamins, minerals and Omega-3 fatty acids were supplemented in the diet of the animal during the entire course of weight loss protocol. Prescribed weight loss diet was fed for a period of three months. The therapeutic protocol also recommended with no treats or other foods to be given during the treatment and increased physical activity in the form of daily exercise/walk (45 min/day). Haemato-biochemical parameters and SCT were evaluated at monthly intervals for 3 months following nutritional management along with increased physical activity.

## RESULTS AND DISCUSSION

History with reference to feeding and exercise status in overweight/obese dogs showed that majority of the dogs were fed only home-cooked food (n=07, 54%) followed by a mixture of both home-cooked and commercial diet (n=06, 46%). Out of thirteen dogs, four dogs (31%) had access to table scraps and leftover food, whereas nine dogs (69%) did not eat table scraps. Snacks and treats were provided by majority of owners *i.e*. 12 (92%) regularly, whereas one dog (8%) did not get any treats. Nine dogs (69%) were fed meals twice a day and four dogs (31%) were fed several times a day. Nutritional supplements were provided to two dogs (15%), whereas eleven dogs (85%) were not provided with any nutritional supplements in this study. All dogs were provided with walking as the form of exercise. Seven (54%) dogs were taken for walk and six dogs (86%) were taken for exercise for less than 0.5 hour and one dog (14%) was taken for walk for 0.5-1 hour.

Feeding is the most handy and agreeable mode of communication and interaction of owners with their pets which ultimately results in obesity (Kienzle *et al.* 1998). Feeding snacks and treats, table scraps which are high in calorie content proved to be risk factors for obesity (Courcier *et al*. 2010, Heuberger and Wakshlag 2011) which was also seen in present study. There is 2.06-fold greater risk of obesity development in dogs that are fed table scraps or homemade foods as compared with dogs not given these items (Sallander *et al*. 2010). Similar results were also observed by Mao *et al*. (2013) and Preet (2018), who found that several feeding times were associated with higher rates of obesity in dogs and also reported that dogs were at increased risk of obesity, when fed home cooked food exclusively as compared to those fed either commercial diet only or a combination of commercial and home cooked food.

Results of proximate analysis of pre-weight loss diets and the prescribed weight loss diet are shown in Table 1.

The mean values of body weight, BCS and Muscle Condition Score (MCS) are shown in Table 2. Significant (P<0.05) difference was observed in mean values of BCS at day 60, from day 30 and that difference persisted until day 90, when compared with day 0 of treatment. There was non-significant decrease in body weight at day 30, 60 and 90, when compared to day 0. No significant differences were found in MCS on day 30, 60 and 90 after nutritional and physical activity management.

The results of effects of weight loss on haemato-biochemical parameters are depicted in Table 3 and 4, respectively. There was no significant (p<0.05) difference in the haematological parameters observed on day 0, 30, 60 and 90 (Table 3). No significant differences were found among the biochemical values at the end of day 30, day 60 and day 90 (Table 4) of weight loss period. However, there was non-significant decrease in adiposity markers (serum cholesterol and leptin) concentrations on day 30, 60 and 90, when compared to day 0.

The mean values of subcutaneous fat thickness (SCT) from six anatomical locations *i.e.* abdomen, mid abdomen, flank (9ICS), thigh, lumbar and chest have been depicted in Table 5. An insignificant decrease in SCT was observed from day 0 to day 90 in five locations (abdomen, mid abdomen, 9ICS, thigh and lumbar). These results might be due to the small sample size and/or due to the short duration of study.

A low-calorie diet with an increased nutrient-to-calorie ratio should be considered for body weight (BW) loss which promotes fat loss while minimizing loss of lean body mass (LBM). Consumption of low-calorie diets with increased protein significantly increases fat loss and reduces the loss of LBM in dogs (Bierer and Bui, 2004). Protein has a significant diet-induced thermogenesis effect *i.e.* postprandial metabolic energy expenditure is increased more when protein is consumed, compared with carbohydrates or fats (Laflamme, 2012). The thermic effect provided by a high-protein diet could help offset the reduction in resting energy expenditure, which can slow BW loss and might be contributed to BW rebound (Wei *et al.*, 2011). A higher protein diet can help reduce the oxidative stress and chronic inflammation associated with obesity. Dietary fiber is another important consideration for BW loss diets. When dietary fiber replaces fat or digestible carbohydrates, the caloric density of the food is reduced and it also provides a satiety effect that might be of value in BW management (Laflamme 2012). Yamka *et al.* (2007) conducted a study comparing 3 diets in the weight loss programme and reported that dogs with higher protein and low-fat diet lost more weight and maintained greater lean muscle mass. This

**Table 1:** Proximate analysis of pre-weight loss diets and prescribed weight loss diet.

| | Crude protein (%) | Crude fibre (%) | Ether extract (%) | Total ash (%) | N.F.E. (%) |
|---|---|---|---|---|---|
| Pre-weight loss diet (n=13) (Mean±S.E.) | 8.91±1.28[a] | 2.04±0.18[a] | 10.23±1.24[b] | 3.36±0.56 | 33.36±5.40[a] |
| Prescribed weight loss diet | 21.4375 | 6.35 | 2.2 | 3.925 | 66.0875 |

Values with superscript letter "a" is lower than prescribed weight loss diet.

Values with superscript letter "b" is higher than prescribed weight loss diet.

**Table 2:** Effect of weight loss diet along with increased physical activity on body weight, BCS and MCS of overweight/obese dogs.

| | Day 0 (Mean±SD) | Day 30 (Mean±SD) | Day 60 (Mean±SD) | Day 90 (Mean±SD) | P value (α=0.05) |
|---|---|---|---|---|---|
| Weight (kg) | 49.15±10.04 | 47.12±9.97 | 45.69±9.73 | 44.69±9.89 | 0.687 |
| BCS | 4.76±0.43 | 4.69*±0.48 | 4.23*±0.59 | 4.15* ±0.68 | 0.011 |
| MCS | 2.00±0.81 | 2.00±0.81 | 2.07±0.86 | 2.15±0.89 | 0.960 |

Values with superscript "asterisk (*)" differ (Pd"0.05) from day 0 of treatment.

**Table 3:** Effect of weight loss diet along with increased physical activity on haematological parameters in overweight/obese dogs.

| Parameter | Day 0 (Mean±SD) | Day 30 (Mean±SD) | Day 60 (Mean±SD) | Day 90 (Mean±SD) | P value (α=0.05) |
|---|---|---|---|---|---|
| Hb (g%) | 14.7±1.98 | 12.25±3.36 | 12.25±2.63 | 13.42±2.63 | 0.076 |
| TLC ($\mu L^{-1}$) | 12894.62±1596.66 | 12089.23±2983.57 | 11779±2071.16 | 11854.31±2417.33 | 0.599 |
| N (%) | 85.92±6.27 | 82.15±5.58 | 81.46±10.33 | 84.15±8.46 | 0.472 |
| L (%) | 12.38±5.88 | 15.23±4.06 | 16.07±9.98 | 13.69±7.43 | 0.570 |
| E (%) | 2.07±2.05 | 2.92±2.69 | 1.92±2.95 | 2.46±2.96 | 0.784 |
| TEC ($\times 10^6 \mu L^{-1}$) | 7.20±0.97 | 6.21±1.29 | 6.08±1.05 | 6.71±1.31 | 0.071 |
| PCV (%) | 42.90±6.10 | 38.96±7.30 | 37.63±6.02 | 40.03±8.59 | 0.285 |
| Platelets ($\times 10^3 \mu L^{-1}$) | 335.31±196.39 | 317.46±110.81 | 271.77±66.87 | 290.31±156.90 | 0.672 |

**Table 4:** Effect of weight loss diet along with increased physical activity on biochemical parameters in overweight/obese dogs.

| | Day 0 (Mean±SD) | Day 30 (Mean±SD) | Day 60 (Mean±SD) | Day 90 (Mean±SD) | P value |
|---|---|---|---|---|---|
| Total Bilirubin (mg/dl) | 0.30±0.18 | 0.33±0.13 | 0.28±0.08 | 0.24±0.15 | 0.412 |
| ALT (IU/L) | 42.76±23.21 | 44.46±18.12 | 43.69±14.08 | 36.30±16.50 | 0.656 |
| ALKP (IU/L) | 75.61±36.61 | 95.92±35.11 | 63.34±30.21 | 81.46±34.37 | 0.121 |
| Total Protein (g/dl) | 6.30±2.10 | 6.58±1.94 | 6.77±1.78 | 7.29±1.86 | 0.609 |
| Albumin (g/dl) | 2.60±0.50 | 2.65±0.48 | 3.00±0.52 | 2.69±0.58 | 0.229 |
| BUN (mg/dl) | 5.53±1.80 | 5.38±1.26 | 4.58±1.53 | 5.23±1.16 | 0.371 |
| Creatinine (mg/dl) | 0.92±0.27 | 0.86±0.18 | 0.87±0.26 | 0.83±0.19 | 0.788 |
| Calcium (mg/dl) | 9.46±1.40 | 9.37±1.03 | 8.38±2.30 | 9.52±0.86 | 0.189 |
| Phosphorous (mg/dl) | 3.96±1.16 | 4.25±1.62 | 4.97±1.23 | 5.01±2.14 | 0.251 |
| Glucose (mg/dl) | 77.76±44.44 | 80.69±32.69 | 101.15±34.52 | 77.53±33.55 | 0.303 |
| Cholesterol (mg/dl) | 234.84±97.18 | 171.07±62.05 | 191.38±72.24 | 153.00±79.34 | 0.063 |
| Triglycerides (mg/dl) | 137.15±134.23 | 98.76±26.87 | 107.15±24.79 | 100.92±59.50 | 0.545 |
| LDL (mg/dl) | 134.87±103.46 | 85.01±61.13 | 107.95±64.23 | 93.04±67.78 | 0.366 |
| VLDL (mg/dl) | 27.43±26.84 | 19.75±5.37 | 21.43±4.95 | 20.18±11.90 | 0.545 |
| HDL (mg/dl) | 72.53±35.42 | 66.30±45.99 | 62.00±39.20 | 39.76±45.75 | 0.223 |
| Adiponectin (pg/ml) | 11.51±5.26 | 12.51±7.43 | 16.29±12.78 | 16.57±7.00 | 0.327 |
| Leptin (ng/ml) | 40.56±33.40 | 31.99±19.62 | 25.71±9.85 | 18.61±12.20 | 0.063 |

**Table 5:** Effect of weight loss diet along with increased physical activity on subcutaneous fat thickness in overweight/obese dogs. (Mean±SD).

| | Day 0 | Day 30 | Day 60 | Day 90 | P Value(α=0.05) |
|---|---|---|---|---|---|
| Abdomen | 0.71±0.38 | 0.74±0.31 | 0.61±0.21 | 0.62±0.15 | 0.547 |
| Mid Abdomen | 0.78±0.39 | 0.75±0.32 | 0.64±0.19 | 0.62±0.16 | 0.410 |
| 9$^{th}$ ICS | 0.80±0.32 | 0.61±0.24 | 0.60±0.16 | 0.67±0.26 | 0.178 |
| Thigh | 0.37±0.21 | 0.44±0.21 | 0.41±0.16 | 0.33±0.19 | 0.528 |
| Lumbar | 2.75±1.11 | 2.63±1.07 | 2.35±0.93 | 2.37±0.70 | 0.665 |
| Chest | 1.51±0.87 | 1.32±0.81 | 1.37±0.90 | 1.78±1.18 | 0.608 |

study suggested that increasing the protein and fiber content and decreasing fat content of the food helped to maintain lean muscle mass while enhancing fat mobilization for energy in obese dogs.

The present study was conducted over a span of 12 weeks (3 months) and dogs included in this study reduced an average of 9% of their body weight and 0.75% per week. A weight loss rate of 1-2% per week is usually the target for weight loss based on AAHA weight management guidelines (Brook *et al.* 2014). The possible reason for not getting the recommended target rate of weight loss per week as per AAHA weight management guidelines might be due to the fact that this was an uncontrolled study, with small sample size and poor compliance of diet protocol by the owners. Carciofi *et al.* (2005) compared the effectiveness of the same hypocaloric diet in an experimental setting and a home setting and reported an average weight loss of 1.39% per week in the experimental group and an average weight loss of 0.75% in home setting. This study suggested that owner compliance is critical for the success of a weight loss protocol. Labrador retrievers were shown to need greater energy restrictions to achieve weight loss (Bissot *et al.* 2006). This might be one possible reason for not getting the desired weight loss in the present study.

Saker and Remillard (2005) reported that the BCS decreased in a 3-month weight loss programme. They also reported increased activity and improved social interactions in dogs having lost as little as 1% of their body weight per week. The starting BCS can provide an approximate initial target weight in obese dogs, but that the actual weight that a dog must lose can vary quite dramatically and therefore, safe and successful weight loss is most likely to be achieved when dogs are closely monitored during weight loss and changes made to the plan as required (German *et al.* 2009, German *et al.* 2012).

Canine obesity research suggested that adipose is not an inert tissue, but rather it releases a variety of adipokines that drive the chronic inflammatory response in peripheral tissues, thereby exacerbating many disease processes (German *et al.* 2010). Leptin and adiponectin are two important adipokines produced by the white adipose tissue and these adipokines have been shown to be valuable quantitative markers of adiposity in dogs. Leptin positively correlates with body condition score (BCS) in dogs and adiponectin negatively correlates with body fat mass and is therefore more abundant in lean animals (Ricci and Bevilacqua 2012). Weight loss in obese subjects has been shown to reverse this low-grade inflammatory state and to improve insulin sensitivity. Markers of chronic inflammation and adipokines have been assessed in canine obesity and weight loss, with evidence showing a decrease in pro-inflammatory adipokines, such as leptin and an increase in the adiponectin (Ishioka *et al.* 2006, Jeusette *et al.* 2007. Similar findings were also observed in the current study.

The main serum biochemical finding of canine obesity is hyperlipidemia, which is characterized by hypercholesterolemia and/or hypertriglycemia. Hyperlipidemia could be caused by a quantitative increase in circulating lipoproteins (LP) or by a higher lipid concentration in the various LP classes. When adipose accumulates in humans, the lipoprotein composition is altered with LDL% tending to extend while HDL% generally decreases. In contrast to humans, whose LDL are the main lipoprotein, the dog may be a species with predominant HDL and few VLDL. Hence, in dogs, HDL is the main plasma carriers of cholesterol, with 2 HDL sub-fractions being identifiable (Mori *et al.* 2011). Similar findings were also found in the present study. Pema *et al*. 2014 reported a significant decrease in cholesterol and triglycerides levels after weight loss with a specific diet. In our study, the obese dogs presented with hyperlipidemia whereas at the end of the study, we observed statistically non-significant further decrease of triglycerides and cholesterol levels in obese dogs treated with weight loss diet, which could be attributed to high protein and less fat diet content.

Carreira *et al.* (2016) reported a highest fat deposition in abdomen followed by lumbar, thigh and chest using RTU. Preet (2018) in a study on subcutaneous fat thickness estimation in overweight and obese dogs concluded that maximum fat deposition was found in lumbar region followed by chest, abdomen, flank, thigh and mid abdomen. No literature reports could be traced for the comparison of the effect of weight loss on subcutaneous fat thickness measurement by ultrasonography in overweight/ obese dogs.

## CONCLUSION

A high protein and low-fat diet along with increased physical activity helps in weight management which is reflected by marked reduction in adiposity markers and subcutaneous fat in overweight/obese dogs, but this may require a longer time and better owner compliance to achieve target weight in an uncontrolled clinical study.

**Conflict of interest:** None.

## REFERENCES


Bierer, T.L. and Bui, L. M. (2004). High-protein low-carbohydrate diets enhance weight loss in dogs. Journal of Nutrition. 134: 2087-89.

Bissot, T., Servet, E. and Biourge, V. (2006). Energy Allowance to Induce Weight Loss is Affected by Breed and Sex but Not Diet. In: 10th Congress of the European Society of Veterinary and Comparative Nutrition.

Brooks, D., Churchill, J., Fein, K., Linder, D., Michel, K.E., Tudor, K., Ward, E. and Witzel, A. (2014). AAHA weight management guidelines for dogs and cats. Journal of the American Animal Hospital Association. 50: 1-11.

Carciofi, A.C., Goncalves, K.N.V., Vasconcellos, R.S., Bazolli, R.S., Brunetto, M.A. and Prada, F. A.(2005). Weight loss protocol and owners participation in the treatment of canine obesity. Cienc Rural. 35: 1331-38.

Carreira, P.R., Martins, L., Miranda, S., Oliverio, P. and Silva, S.R. (2016). *In vivo* assessment of subcutaneous fat in dogs by real time ultrasonography and image analysis. Acta Veterinaria Scandinavica. 58: 11-18.

Courcier, E.A., Thomson, R.M., Mellor, D.J. and Yam, P.S. (2010). An epidemiological study of environmental factors associated with canine obesity. Journal of Small Animal Practice. 51: 362-67.

German, A.J., Hervera, M., Hunter, L., Holden, S.L., Morris, P.J., Biourge, V. and Trayhurn, P. (2009). Improvement in insulin resistance and reduction in plasma inflammatory adipokines after weight loss in obese dogs. Domestic Animal Endocrinology. 37: 214-26.

German, A. J., Ryan, V. H., German, A. C., Wood, I. S. and Trayhurn, P. (2010). Obesity, its associated disorders and the role of inflammatory adipokines in companion animals. The Veterinary Journal. 185: 4-9.

German, A.J. (2006). The growing problem of obesity in dogs and cats. Journal of Nutrition. 136: 1940-46.

German, A.J., Holden, S.L., Morris, P.J. and Biourge, V. (2012). Long-term follow-up after weight management in obese dogs: the role of diet in preventing regain. Veterinary Journal. 192: 65-70.

Heuberger, R. and Wakshlag, J. (2011). The relationship of feeding patterns and obesity in dogs. Journal of Animal Physiology and Animal Nutrition. 95: 98-105.

Ishioka, K., Omachi, A., Sagawa, M., Shibata, H., Honjoh, T., Kimura, K. and Saito, M. (2006). Canine adiponectin: cDNA structure, mRNA expression in adipose tissues and reduced plasma levels in obesity. Research in Veterinary Science. 80: 127-32.

Jain, W.C. (1986). Schalm's Veterinary Haematology 4th edn. Lea and Febiger, Philadelphia, London.

Jeusette, I.C., Detilleux, J., Shibata, H., Saito, M., Honjoh, T., Delobei, A., Istrasse, L. and Diez, M. (2007). Effect of chronic obesity and weight loss on plasma ghrelin and leptin concentrations in dog. Research in Veterinary Science. 79: 169-75.

Kienzle, E., Bergler, R. and Mandernach, A.A. (1998). Comparison of the feeding behavior and the Human-Animal relationship in owners of normal and obese dogs. Journal of Nutrition. 128: 2779-82.

Laflamme, D.P. (1997). Development and validation of a body condition score system for dogs: A clinical tool. Canine Practice. 22: 10-15.

Laflamme, D.P. (2012). Companion animal's symposium: obesity in dogs and cats: what is wrong with being fat? Journal of Animal Science. 90: 1653-62.

Mao, J., Xia, Z., Chen, J. and Yu, J. (2013). Prevalence and risk factors for canine obesity surveyed in veterinary practices in Beijing, China. Preventive Veterinary Medicine. 112: 438-442.

Mori, N., Lee, P., Kondo, K., Kido, T., Saito, T. and Arai, T. (2011). Potential use of cholesterol lipoprotein profile to confirm obesity status in dogs. Veterinary Research Communications. 35(4): 223-35.

NRC. (1985). National Research Council - Nutrient Requirements of Dogs. Washington National Academy.

Pena, C., Suarez, L., Bautista-Castano, I., Juste, M.C., Carreton, E. and Montoya-Alonso, J.A. (2014). Effects of low-fat high-fibre diet and mitratapide on body weight reduction, blood pressure and metabolic parameters in obese dogs. Journal of Veterinary Medical Science. 76(9): 1305-08.

Preet, G. (2018). Risk factors and diagnosis of obesity in companion dogs. M.V. Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Ricci, R. and Bevilacqua, F. (2012). The potential role of leptin and adiponectin in obesity: A comparative review. The Veterinary Journal. 191(3): 292-8.

Saker, K. E. and Remillard, R. L. (2005). Performance of a canine weight-loss program in clinical practice. Veterinary Therapeutics. 6: 291-302.

Sallander, M., Hagberg, M., Hedhammar, A., Rundgren, M. and Lindberg, J.E. (2010). Energy-intake and activity risk factors for owner-perceived obesity in a defined population of Swedish dogs. Preventive Veterinary Medicine. 96: 132-41.

Wei, A., Fascetti, A.J., Liu, K.J., Villaverde, C., Green, A.S., Manzanilla, E.G., Havel, P.J. and Ramsey, J.J. (2011). Influence of a highprotein diet on energy balance in obese cats allowed ad libitum access to food. Journal of Animal Physiology and Animal Nutrition. 95: 359-367.

Yamka, R.M., Frantz, N.Z. and Friesen, K.G. (2007). Effects of 3 canine weight loss foods on body composition and obesity markers. International Journal of Applied Research in Veterinary Medicine. 5: 125-132.