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Evaluation of *in vitro* digestibility of high protein and fibre diet in obese dogs

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Abstract
Four different therapeutic diets *viz.*, T_1 (control - 15.7% CP and 4.09% CF) T_2, T_3 and T_4 of high protein (21.90% CP) high fibre (11.28% CF), high protein (21.73% CP) medium fibre (8.23% CF), and control diet supplemented with choline chloride (15.72% CP and 3.99% CF) were prepared and subjected to *in vitro* enzymatic hydrolysis and *in vitro* fermentation characteristics, in which *in vitro* dry matter digestibility and gas production were estimated. The dry matter digestibility was significantly higher (p<0.05) in T_1 (84.30 ± 0.97) and T_4 (84.80 ± 0.41). Gas production were significantly higher in T_2 (16.82 ± 0.88 ml/g DM) followed by T_3 (10.76 ± 0.64) was observed. The overall DM digestibility of all the diets was more than 75%, which showed overall higher digestibility of all nutrients in the prepared therapeutic diets.

Keywords: Canine obesity, *in vitro* digestibility, *in vitro* fermentation

Introduction
Obesity in dogs is a clinical state of impaired general health and efforts are made to increase the longevity and improve the quality of life. Dietary modification is one the safest and easiest way to treat obesity in dogs. Evaluation of digestibility of modified diets often meets with technical difficulties. Therefore, to mimic gastrointestinal digestion and fermentation processes, *In vitro* models are attempted as an alternative to the *In vivo* studies (Peeters *et al*., 1998) [1].

Babinszky *et al.* (1990) [2] imitated digestive processes using commercially available enzymes to predict the content digestible crude protein in feedstuffs and diets. The *in vitro* digestibility was correlated with the *in vivo* results. The difference in the digestibility was noted and that was due to the contribution of endogenous and microbial protein in the feces, lowering the *in vivo* measurements. In simple-stomached animals, fecal samples were used as an inoculum for the gas production technique rather than cecal or colonic contents. Williams *et al.* (1997) [3] believed that fecal microbial inocula were indicative of colonic microflora. Becker *et al.* (2003) [4] studied the fermentation characteristics and synthesis of short chain fatty acids (SCFA) of fibrous whole plant products and industrial by products, after pre-treatment with digestive enzymes. A significant gas production was observed due to the presence of enzymatically resistant but microbially readily degradable compounds in the non-digestible residues.

Biagi *et al.* (2016) [5] developed a quick and simple procedure for predicting the digestibility of commercial diets for dogs. They found a close linear relationship between *in vivo* total tract and *in vitro* dry matter digestibility and concluded that following those methods would significantly reduce the need of *in vivo* digestion trials (r^2 = 0.81) with dogs.

Materials and Methods
The evaluation of *In vitro* digestibility in obese dogs was studied by preparing therapeutic diets and conducting a feeding trial in obese dogs. The feeding trial had four treatment groups. T_1 was kept as control (15.7% CP and 4.09% CF) and fed with normal diet (AAFCO, 2014 [6] recommendation for adult dog maintenance); T_2, T_3 and T_4 were fed with high protein (21.90% CP) high fibre (11.28% CF), high protein (21.73% CP) medium fibre (8.23% CF), and control diet supplemented with choline chloride (15.72% CP and 3.99% CF), respectively.

The prepared therapeutic diets were subjected to *in vitro* enzymatic hydrolysis and *in vitro* fermentation characteristics, in which *in vitro* dry matter digestibility and gas production of four different therapeutic pet foods were estimated as per Madhusudhan *et al.* (2010) [7]. The procedure was slightly modified that in the present study, 10 g DM of food samples were taken

instead of 20 g and incubated for digestion due to the difficulty during filtration of final residues of the enzymatic treatments. Therefore, volumes of solutions and reagents were added to the respective sample quantity.

10 g DM of samples were incubated in 250 ml 0.1 M HCl with 5 g/l pepsin (P-10000) for 1.5 hours. After 1.5 hours, pH was neutralised with 50 ml of 0.5 M NaHCO3, followed by another 1.5 hours incubation with 250 ml of 0.165 M phosphate buffer (pH=6.8) containing 2 g/l pancreatin (P-7545, Sigma-Aldrich Chemicals Co., USA) and 1% amylase (A-8220, Sigma-Aldrich Chemicals Co., USA). After incubation, the contents were filtered through nylon gauze and the residue obtained was dried at 70 ˚C and analysed for its proximate principles prior to incubation for fermentation study.

The final residues obtained after enzymatic digestion were incubated to measure the gas production by adopting the modified procedures of B ecker *et al.* (2003) [4] and Cone *et al.* (2005) [8].

The fecal samples were used as inoculums for gas production technique and they were collected from dogs after an adaptation period of 90 days. Fresh feces within an hour after defecation from all the dogs were brought in a sterile sample container. The fecal samples were mixed with buffer to obtain a 2 per cent fecal solution. 0.5 g DM of the residue was incubated anaerobically in 60 ml of fecal buffer solution in 100 ml glass syringe. The gas productions were compared with a blank incubation with no added sample. Fermentation of samples was continuously measured by registering gas production in the gas production technique (GPT) after 24 hours according to Biagi *et al*. (2016) [5].

The chemical composition of the buffer solution (g/l)

Chemical	Quantity (g/l)
NaHCO3	10.03
Na_2HPO_4	1.43
KH_2PO_4	1.55
$MgSO_4.7H_2O$	0.15
Na_2S	0.52
$CaCl_2.2H_2O$	0.017
$MnCl_2.4H_2O$	0.015
$CoCl_3.6H_2O$	0.002
$FeCl_3.6H_2O$	0.012
Resazurin (mg/l)	0.125

Digestibility of Nutrients

The digestibility of dry matter and various nutrients were calculated as the difference of sample and residue of any nutrient and expressed in percentage.

$$\text{Digestibility (\%)} = \frac{\text{Nutrient in sample} - \text{Nutrient in residue}}{\text{Nutrient in sample}} \times 100$$

The amount of gas produced (ml) per g (DM) of residue of *in vitro* enzymatic digestion was found and the amount of gas produced (ml) per g of DM of food was estimated.

Results

In vitro dry matter digestibility and gas production of four different therapeutic pet foods were estimated and the digestibility results obtained are presented in Table 1.

The dry matter digestibility was significantly higher ($p<0.05$) in T_1 (84.30 ± 0.97) and T_4 (84.80 ± 0.41). Significantly ($p<0.05$) lower digestibility was observed in high fibre supplemented groups viz., T_2 (76.44 ± 0.93) than T_3 (80.46 ± 0.76). Protein digestibility was comparable in all the treatment groups. Though there was no significant ($p>0.05$) difference in crude fibre digestibility among the treatments, T_2 had lower digestibility (64.21 ± 2.10).

Gas production were significantly higher in T_2 (16.82 ± 0.88 ml/g DM) followed by T_3 (10.76 ± 0.64) and no significant difference in the gas production of control and choline supplemented diet (5.73 ± 0.35 *Vs* 5.32 ± 0.34 ml/g DM) was observed.

The overall DM digestibility of all the diets was more than 75%, which reflected the quality of diets used in this study. However, increasing the fibre level in the diet found to decrease the digestibility of other nutrients.

Table 1: *In vitro* digestibility of the nutrients (%) and gas production (ml/g of DM) of the different therapeutic pet foods (Mean ± SE)

Parameters	T_1	T_2	T_3	T_4
***In vitro* digestibility of the nutrients (%)**				
Dry matter	84.30^{c}±0.97	76.44^{a}±0.93	80.46^{b}±0.76	84.80^{c}±0.41
Crude protein^{NS}	81.72±0.23	81.25±0.61	82.71±0.78	81.75±0.72
Crude fibre^{NS}	74.49±4.22	64.21±2.10	69.38±0.89	74.51±1.83
Ether extract	84.08^{c}±0.40	68.52^{a}±1.52	73.87^{b}±0.55	83.40^{c}±1.24
Organic matter^{NS}	82.94±1.11	83.83±1.35	82.23±0.50	82.66±0.49
Gas production (ml/g of DM)				
Gas production	5.73^{a}±0.35	16.82^{c}±0.88	10.76^{b}±0.64	5.32^{a}±0.34

Each value is a mean of six observations

Mean bearing different superscripts within a row differs significantly ($p<0.05$)

Discussion

The dry matter digestibility was found to be lower in high protein high fibre diet, when compared with other diets. The overall DM digestibility was more than 75%, which reflected the quality of diets used in this study. The significantly lowered ($p<0.05$) digestibility observed in protein and fat in T_2 diet could be the reason for the overall decrease in DM digestibility.

Pasupathi *et al.* (2002) [9] observed that the digestibility of nutrients was significantly reduced in dogs on high fibre diet (8.2% CF). Biagi *et al.* (2016) [5] determined *in vitro* digestibility of dry extruded diets for dogs and observed the DM digestibility in the range of 65.7 to 73.2%, CP digestibility of 79.3 to 84.7% and EE digestibility in between 28.9 to 45.3%. The increased protein digestibility observed in this study is in accordance with Biagi *et al.* (2016) [5].

The present study showed overall higher digestibility of all the nutrients and that was perhaps due to the extrusion process which increased the digestibility (Singh, 1992) [10].

The higher gas production in T_2 diet compared to other diets could be due to high fibre in the diet, which probably increased the availability of substrates for fermentation by microorganisms habituating mainly in colon. This is in agreement with Marthinsen and Fleming (1982) [11] who reported that the quantity of gas produced by fermentation is directly dependent on the level of dietary polysaccharides.

Conclusion

The current study has demonstrated the *in vitro* digestibility of different therapeutic diets for obese dogs and showed overall higher digestibility of all nutrients in the prepared therapeutic diets.

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