5.1. Chemical composition of diets

The chemical composition of the experimental diets and the different ingredients used in the diet are presented in Table 4.1 and 4.2 respectively. All the four diets varied in their chemical composition. The percentage of moisture in HV and HNV was higher than the CV and CNV diets, since the HV and HNV foods were cooked and served freshly. The dry matter content of the HV diet was lower (51.27%) than the HNV diet (65.06%) since the milk was not included in the HNV diet. The CV and CNV food were dried and extruded, wherein the moisture content ranged from six to seven per cent. The energy content of the diets varied from 3.679 to 4.182 kcal/g (gross energy) while the protein content of the diets ranged from 11.03 to 23.70 per cent. The crude protein content in homemade diets were lower than the AFFCO (2007) specification of minimum of 18 per cent for adult maintenance diets. Nevertheless, the diets were individually fed to the dogs as a single meal daily at such quantities to meet the energy and protein requirement. The daily allowance of the different diet for individual dogs were calculated and offered at the rates to meet the ME requirement of 130 kcal per kg MBW, minimum crude protein requirement of 2.62 g per kg MBW and recommended protein allowance of 3.28 g per kg MBW (NRC, 2006). Intake of energy and crude protein by different treatment groups (Table 4.10) indicated that the requirement of these nutrients was met according to the stipulations of the NRC (2006). Since the daily quantities of the diets offered were calculated based on the energy density of the diets, the protein intake by the dogs fed CV and CNV diets far exceeded the requirement. The ME content (declared by the manufacturer) of CV and CNV was 3,500 and 3,400 kcal per kg respectively. The
crude fat contents of all the diets met the AFFCO (2007) specification of minimum five per cent crude fat although the Crude fat content of HNV was marginally lower (4.436 per cent). The crude fibre content of all the experimental diets was lesser than the permissible level of four percent. The experimental diets fed to the dogs in the study therefore provided adequate nutrients as per the requirements of NRC (2006).

5.2. In-vitro studies

5.2.1. In-vitro digestibility of diets

The in-vitro digestibility of nutrients in the experimental diets are presented in Table 4.3. The in-vitro digestibility of various ingredients used for preparing HV and HNV diets are presented in Table 4.9. Comparing the digestibility of different diets used in the experiment, the digestibility of DM or OM was highest in the HNV diet. In the HNV diet, chicken and egg were included as ingredients to replace the pulses (greengram, Bengalgram, greenpeas and fieldbean) used in the HV diet. Therefore, higher digestibility of HNV diet compared to the HV diet could be explained by the chicken and egg content of the HNV diet since the in-vitro digestibility of chicken and egg was higher (more than 75%) than the pulses (46 to 72%) (Table 4.9). Similarly, comparing the HV and CV diets, the digestibilities of DM and OM were higher in CV than HV diet. Higher digestibility of CV diet was perhaps due to the extrusion process involved in preparing the commercial dog foods, which results in higher digestibility compared to steam cooked foods (Singh, 1992). The overall DM digestibility of diets exceeded 80 per cent, which reflects the quality of diets used in the study since DM digestibility of 80 per cent is usual for good quality dog food (Tonglet et al., 2001).
The crude protein digestibility of HNV was higher than HV diet. This is in accordance with reports of Meyer et al. (1981) and Kendall and Holme (1982) who opined that animal proteins generally have a higher digestibility than do plant proteins. With lowered digestibility of HV, one would expect more amount of undigested protein passing to hindgut since the legumes contain naturally occurring physical barriers, such as cell walls which may reduce the bioavailability of legume proteins (Melito and Tovar, 1995). The potential nutritional value of legume seeds is limited by a number of factors, which include structural features of some of their protein fractions and the presence of antinutrients, such as trypsin inhibitors, lectins and polyphenols which may affect protein digestion (Liener, 1994). Besides, the physical form in which foods are consumed has proven to be an important factor governing the bioavailability of nutrients (Bjorck et al., 1994). Further, Tovar et al. (1990; 1991 and 1992) observed that the starch fraction of legumes, with both the cotyledon tissue structure and a thick cell walls represented a physical barrier for starch digestion in in vitro. Since the seed storage proteins are also located intracellularly, it is likely that cell structural factors affecting starch hydrolysis would exert similar effects on the protein digestibility.

The crude fibre digestibility was lowest in HV diet (13.65%). This could be due to the lowered degradation of intact tissue/cell structure, viscous soluble dietary fibre components containing higher levels of amylose present in pulses and vegetables (Rehman and Shah, 2004). Further, the crystalline fibre affect the enzymatic digestibility by imposing physical barrier to the accessibility of the enzymes during hydrolysis. Such existence of physically inaccessible reserve biopolymers (starch and protein) is a
common feature of mild processed legume seeds, such as those prepared according to homemade food (Tovar et al., 1992).

The crude fat (EE) digestibility (%) was higher in HV (82.81) and HNV (92.71) diets when compared with CV (65.29) or CNV (36.84) which indicated that the quality of fat (sunflower oil) used to prepare home made diets was superior than the type of fat used in commercial diet.

5.2.2. *In-vivo* digestion trial

The data on the intake and digestibility of nutrients in the *in vivo* digestion trial are presented in Table 4.5. The intake of DM, OM, CP and EE were similar in HV and HNV diets. Similarly, there was no difference in the intake of these nutrients between CV and CNV diets. However, the intake of crude protein in the dogs fed commercial foods were two to three times higher than those fed home made diets. The protein intake of 86 to 114 g by the dogs fed commercial diets was 2 to 3 times than the actual requirement (of 30 to 40 g) recommended by NRC (2006). The digestibility of DM, OM, CP and EE were similar to those reported by Madhusudhan *et al.* (2009) who observed that the digestibility of DM, OM, CP and EE of 87, 91, 84 and 95 per cent respectively when the dogs were fed commercial chicken based diets.

The overall digestibilities of DM and OM observed in the *in vivo* trials were higher than those estimated in the *in vitro* experiment (p < 0.05) (Table 4.6). Drocher and Meyer (1991) observed that the diets are digested to about eight percent in the large intestines and therefore, if these values are discounted from the *in vivo* digestibility values, the *in vitro* estimates could be similar to the *in vivo* studies. Harvera (2009)
compared various in vitro methods with in vivo trials and concluded that the digestibility estimates predicted by in vitro experiments were similar to those obtained by in vivo trials. There was no difference between in-vitro and in-vivo values of CP and CF digestibility. Therefore, it could be concluded that the in vitro procedures used in the presented study could be used to predict the digestibility of dog foods when the values are corrected to the digestibility in large intestines.

5.3. Fermentation and Gas production

There is a complex inter-relationship between microbial community residing in the hindgut, nutrients available as a substrate and the fermentation that occurs. For better understanding the results from the present study are discussed here under by including all these factors.

The total gas production values obtained for different diets are presented in Table 4.7. Among the experimental diets, HV resulted in highest fermentation and gas production (37.70 mL/g DM of food), followed by HNV (31.29), CV (12.37) and CNV (5.79). Higher gas production by the HV diets could be attributed to the inclusion of pulses and vegetables in these diets. The amounts of fermentation gases depend on the dietary polysaccharides (Marthinsen and Fleming, 1982.). According to Borggreve et al. (1975) and Murray et al. (2001), wheat flour, rice, peas and potato are rich in starch. These ingredients contained the pectin, lignin and fermentable carbohydrates which are undegradable by gastric and small intestinal enzymes and these residues will be potentially available for fermentation by microorganisms habitating mainly in colon.
Starch exists in granules which have a crystallographic pattern is found in cereals (rice and wheat), potato and pulses are generally open to attack by amylase. (Katz, 1934). When starch is cooked it undergoes a process of gelatinization in which the crystal structure is broken down and the molecules become more accessible to amylase. However, during food processing of commercial diets, changes in starch structure occur, known collectively as retrogradation, renders the starch resistant to pancreatic amylase. These latter effects are very much concerned in case of commercial diets wherein the fermentation is very less than homemade diets (Fuwa et al., 1980). Although homemade diets were subjected to cooking, the food was served freshly without freezing or drying. Therefore, resistant starch was more in HV and HNV which clearly has a major role in determining how much dietary starch becomes available for fermentation. Ring et al. (1988) also explained that the legumes (peas) have high amount of resistant starch.

Crude fibre digestibility was lower in HV diet compared to other diets (Table 4.3). Therefore fibre available for hindgut fermentation was more in case of HV compared to HNV or CV. Crude fibre includes cellulose, lignin and some hemicelluloses like hexosans, pectin, pentosans and polyuronoides (Hungate, 1966). Except some of them, e.g. pectin are fermentable rapidly, these fibre get digested rather slowly than the starch and soluble carbohydrates, so they support a more sustained fermentation. Figure 3 supports the above statement that the HV and HNV showed a continuous and sustained increase in the gas production compared CV and CNV.

Higher fermentation leading to the higher volume of gas and associated end products in the hindgut can be considered as negative indices from the point of
evaluation of dog foods since the nutrients and end products released from hindgut fermentation are of little nutritional significance and are not absorbed and utilized by the body (Clinton et al., 1988). Monogastric mammals like rabbits and hare derive much of the nutritional benefit from the microbial cells in the hindgut flora through coprophagy. Dogs being non-coprophagic animals, derive little benefit unless molecules produced by or released from lysing microbial cells are absorbed into the blood in the cecum and colon. The contribution of energy generated from hindgut fermentation to the maintenance energy requirement of the dog is also limited (accounted for only two percent) whereas it could contribute 10-31 percent of the maintenance energy requirement in other species like pigs (Stevens and Hume, 1998).

Some of the potential toxic end products released because of fermentation also have a toxic effect to the animal and detrimental to their performance (Clinton et al., 1988). Detrimental effects of the enteric flora include competition for calories and essential nutrients, particularly by bacteria located in the small intestine and damage to the mucosa causing or contributing to inflammatory bowel disease. Compounds produced by the microflora from exogenous and endogenous material may induce cancer or other diseases in host (Stevens and Hume, 1998).

The fermentation processes in hindgut (leading to normal levels of gas) however are vital from the point of providing nutrition and maintenance of intestinal health in dogs (Stevens and Hume, 1998). Significance of fermentation processes therefore cannot be down played since some of the soluble carbohydrate fractions during fermentation stimulates and increase the growth or metabolism of specific bacterial species which are
potentially beneficial for health. Such fermentation leads to the production of mainly Short Chain Fatty Acids (SCFA) *i.e.* lactic, acetic, propionic and butyric acids. These SCFA are beneficial for health since they lower luminal pH (Blaut, 2002) that can have a negative effect on some potentially pathogenic and non desirable bacteria. Among the experimental diets CNV diet showed lowest gas production, which may be due to less substrate available for fermentation in the large intestine. Nevertheless, fermentation (and lowered gas production) levels in CNV diet, could also be considered as normal and optimal from the beneficial point of view. Such conclusions however should be drawn with caution as there are no literature available on the optimal levels of fermentation indicated by the normal levels of gas produced in the hindgut of dogs. Considering these facts, it could be concluded that the feeding of home made diets (HV or HNV) which resulted in significantly higher volume of gas (causing irritation, flatulence problems and frequent defaecations) as undesirable from the point of evaluation of canine diets.

5.4. Microbiota of hindgut

Knowledge on the fecal microbiota gives an insight to host, microbe and nutrient interactions in the hindgut. The bacterial counts in feces of dogs fed different experimental diets are presented in Table 4.8. The fecal bacterial counts in all the experimental diets were within the range of reports of Murray *et al.* (2000), Swanson *et al.* (2002) and Mentula *et al.* (2005).

The importance of the microbiota to the nutrition and homeostasis of the body is obvious since the imbalance of the microbiota may cause several different disturbances of the gastrointestinal functions such as diarrhoea and impairs the health status of an
individual. Microorganisms colonise all mucosal surfaces forming a complex and dynamic entities. The intestine harbours a vast majority of the microbes of the body and therefore the microbes also have prominent impact on health (McCartney et al., 1996; Franks et al., 1998). The gut microbiota can be described to be in a state of unstability with reference to the relatively stable composition of the host specific main bacterial groups and their proportions within an individual, and to the simultaneous constant flow of new bacterial clones co-existing and replacing the previous ones in a balanced and seemingly homologous living system (Zoetendal et al., 1998; Falk et al., 1998). Gut microbiota can be regarded as a metabolically active organ of the body, beneficial to both microbes and the host.

In the present study, the increased fermentation and gas production didn’t affect the Lactobacilli and Bifidobacterial counts. The reason for this is unknown but the decreased Clostridial counts in HV compared to CV were in accordance with Blaut (2002). Contrary to the results of Clostridium counts observed by Zentek et al. (2004) that animals fed with commercial diet showed the least counts compared to canned and dry diets prepared for experiment purpose, in the present study the Clostridium counts were higher in commercial diet. Clostridium counts were found to increase substantially in dogs that are fed large quantities of low- quality protein diet. In the present study, the higher counts of Clostridium counts in CV and CNV can also be correlated with Zentek et al. (2004) such that in the present study the quality of protein in commercial diets may be low. This might have lead to increased counts of Clostridium bacteria. A limited species composition and thus possibly pronounced effects of single species, like clostridia
should not be overlooked, since specific microbial features of different niches may have clinical relevance in various disorders and diseases of the gastrointestinal tract.

The bacteria compete for nutrient availability in ecological niches and maintain their collective habitat by administering and consuming all resources. The host actively provides a nutrient that the bacterium needs and the bacterium actively indicates how much it needs to the host. This symbiotic relationship prevents unwanted overproduction of the nutrient, which would favour intrusion of microbial competitors with potential pathogenicity for the host. Finally, bacteria can inhibit the growth of their competitors by producing antimicrobial substances called bacteriocins. The ability to synthesise bacteriocins is widely distributed among microbiota of gastrointestinal tract. The host can control production of such substances since most of them are protein compounds degradable by digestive proteases. Thus, the role of bacteriocins is mainly restricted to localised niches.

In the present study, there was no difference in *coliform* counts among the different treatment groups, lower counts of coliforms in vegetable based diets was not expected as these organisms are known to ferment lactose. In HV diet 96.22 per cent of milk was digested during the pre-digestion step *i.e.* gastric and small intestinal simulation (Table 4.9). Therefore, counts of Coliforms was numerically lesser in HV. This observation may also be due to the fact that these bacteria depend on the starch content of the diet *i.e.* Coliforms decreases with increase in the starch content of the diet (Hartman *et al.*, 1966). The pH of fecal samples obtained from the dogs fed with HV was lowest among other diets. This could possibly lower the fecal coliforms.
Streptococci count was lowest in HV and highest in CNV. This could be due to the fact that streptococci counts decreases in the hindgut when animals are fed with more of lactose (Wilber et al., 1960). The in-vitro protein digestibility was very low in HV compared to HNV and CV. Among chicken based diets, HNV showed less in-vitro crude protein digestibility than CNV, so protein availability for microbial fermentation was more in case of HV and HNV compared to other diets. End products of protein fermentation are branched chain SCFA, ammonia, phenols, indoles and amines which have potentially negative effect on host health (Cummings et al., 1989).

Dragteadt et al. (1922) reported that diet rich in protein of animal origin leads to increase in proteolytic putrefactive organisms. Therefore HNV diets resulted in higher counts of total anaerobes and Clostridial organisms compared to HV. This trend was not seen in CNV diet fed dogs which may be due to good quality meat used in HNV rather than chicken byproducts used in CNV. Chicken byproducts are not much easily degradable by microbes. They take long duration to get degraded in the large intestine but breast muscle used in HNV serves as readily available and good substrate for putrefactive proteolytic microbes.

Among the enormous anaerobic bacteria population, Bacteroides are the dominant which accounts up to 30 percent of the total anaerobes residing in the hindgut (Stevens and Hume, 1998). In this perspective, the counts of Bacteroides in the present study were observed to be more in the treatment group which had higher fermentation. So, higher population of Bacteroides may influence the significant gas production. Bifidobacterium
are also high among the bacterial population (up to 25 percent) but not dominant (Stevens and hume, 1998).

The Bacteroides counts in HV were higher than HNV and CV. The same trend was seen in HNV and CNV wherein HNV showed higher Bacteroides counts than CNV. This result also supports that Englysth and Cummings (1987a and 1987b), Salyers and Leedle (1983), that the polysaccharides that either have not or cannot be hydrolysed by host digestive enzymes in the small bowel were degraded mainly by the gram negative anaerobes belonging to the Genus Bacteroides and this observation can be correlated with the total gas production. The Bacteroides counts was highest in HV where the gas production was also highest. Therefore as the gas production decreased the Bacteroides counts also decreased. Bacteroides counts can also be correlated with residue of enzymatic digestion. As the residue of enzymatic digestion decreased the Bacteroides count was also decreased so both the factors were positively correlated. The high population of Bacteroides may also be due to the endogenous macromolecular polymers like pectin which passes to the large intestine and become available to the microflora (Bacteroides). These pectin get hydrolyzed and utilized as carbon and energy sources (Savage, 1986).

Among the commercial diets the fat digested in-vitro was very less in CNV (36.84%) compared to CV (65.29%). Homemade diets showed a significantly higher in-vitro fat digestibility than commercial diets. Therefore undigested fat that passed towards hindgut was more in case of CNV compared to other diets. When fat percentage is more in the residue passed to the hindgut (Table 4.3), other nutrients in the residue get entirely
covered by fat, which decreases the hydrophily of the surface. Since microbial digestive enzymes in the hindgut occur in an aqueous phase enzymatic attack is diminished (Hungate, 1966). Digestion and emulsification of the fat permit gradual entrance of the microbial enzymes but a longer time is required for complete digestion and the residue leave the large intestine before digestion proceeds to the extend found in substrate free of added fat. Therefore the higher percentage of fat in commercial diets resulted in decreased fermentation and accordingly the gas production was very lesser than homemade diets.

Marteau et al. (2001) reported facultative bacteria including Lactobacilli and *E. coli* species to be part of the dominant flora in the human feces. We found similar results though with lower counts of *E. coli*. in a study of beagle dogs (Buddington, 2003) and Labrador dogs (Greetham et al., 2002) where no Bifidobacterium but numerous Lactobacillus organisms were reported. However, these organisms were found in substantial numbers in the present study.

Findings of the present study have indicated that a change in the type of diet reflected in a change in microbiota of the hindgut. Generally, it can be expected that the total anaerobic bacterial count in the hindgut to be proportional to the total gas produced. Contrary to this, the total anaerobic bacterial count was not consistent with the volume of gas produced by feeding different diets. Nevertheless, the count of bacteroides species was concomitant with the total volume of gas produced in the respective diets. Bacteroides, being a dominant species in the hindgut, reflecting the anaerobic bacteria, it
could be concluded that the bacteroides count could be an index in the evaluation of canine diets to reflect the fermentation and gas production.

5.5. In-vivo studies

5.5.1. Daily intake of food and nutrients

Results on the data of daily intake of food, ME and CP are presented in Table 4.10. The entire quantity of diets offered was completely consumed by the dogs without any left over in any of the treatment groups. The home made diets were more palatable compared to commercial diets which was due to better flavor, high moisture content and oil used while preparing chapathi, salt added in cooking pulses, chicken and egg, boiled milk added in rice and most importantly the food was served fresh. Before starting the experiment, the HV and HNV dogs were on commercial diets and shifting them to a new diet also accounted for palatability. This observation supports the opinion of Mugford (1977) that well adjusted adult dogs will select new flavor rather than the one they were fed. Dogs consumed the home made food within three minutes of serving whereas the dogs on CV and CNV took more than 45 minutes to consume (Table 4.10). Commercial diets and HV were more bulky than HNV. Though the fat content was higher in commercial diets, the palatability was less in CV and CNV. This may also be due to dryness of the diet.

The fast intake of food in HV and HNV might have influenced on poor fecal quality because rapid transit of certain dietary components may impact negatively on stool quality and contribute to lose feces in dogs with non specific dietary sensitivity as reported by Rolfe et al. (2002).
According to NRC (2006), normal dogs will maintain optimal health if fed only one meal a day. About 30-40 per cent of dogs will overeat and become overweight or even obese if allowed to eat as much as they want at all times during the day. Therefore in this study, for the convenience of feeding and for optimum health of animal, they were fed only one meal a day.

The ME intake was similar in all the treatment groups (Table 4.10). Feeding of dogs according to the NRC (2006) the MER recommendation has resulted in no statistical difference on average body weight gain between dietary groups. The average BW of CV and CNV was higher than HV and HNV respectively. This may be due to excess intake of protein in commercial dietary groups. The crude protein included in commercial diets was two to three times higher than the requirement of animals. The body condition score in all the animals were lesser than ideal score of 4. Dogs didn’t show obesity in any group. The dogs on commercial diets were able to maintain BW and BCS during the observation period compared to homemade diets wherein BW and BCS were reduced. However, the observation period of three weeks was not sufficient to judge these parameters and it requires minimum four to six months for absolute effect of diet on BCS and BW.

Among the homemade diets, the higher fecal output (as is basis) was observed in HV than HNV. This may be due to higher amount of fibre in HV compared to HNV which had the water holding capacity. Fibre in the diet helps in better fecal quality and avoids constipation problem.
Lower fibre in the HNV therefore, resulted in constipation problem i.e. the number of defecation was lesser than the HV. This observation supports the report of Clapper et al. (2001) that dogs fed the chicken based diet had lower fecal output than dogs fed soy protein concentrate diets.

The fecal DM of HNV, CV and CNV were higher than that reported by Zentek et al. (2004) wherein the DM content of feces was 26.8-29.7 percent and poor fecal consistency especially in large breed have been described after dogs were fed certain animal proteins. This was related to an impact on the intestinal microflora by favouring the condition for growth and toxins fermentation of Clostridium perfringens and other proteolytic bacteria by effects on water holding capacity of the intestinal content and by decreasing water absorption capacity of gut wall. In the present study also the feces of HNV did not have the ideal consistency but other treatment groups had the fecal score almost near to ideal score and Clostridium counts higher in all the groups except HV. The tendency for dry diets to result in better fecal quality was coincident with a higher fecal dry matter (Zentek et al., 2004), this was also true in case of CV and CNV wherein the fecal score was better and fecal dry matter was higher compared to homemade diets.

Number of defecations are comparatively more in case of homemade diets than commercial diets. This might influence the retention time of food in the gastro-intestinal tract in turn the exposure of food to fermentation will be more. According to Van Soest et al. (1982), diet and transit time are the major factors which influence the VFA availability, correspondingly, HV and HNV resulted in higher gas production whereas the gas production was lesser in commercial diets due to less retention time and less exposure
to microbial fermentation. From the owner preference point of view, lesser number of defecations is always convenient to dog care taker but high gas production or flatulence problem is undesirable, which creates discomfort to the animal as well as the dog owner. This may lead to bad odor in and around the place where the animal reside.

All the experimental dogs were closely monitored for their health status and treated for minor ailments if any, during the study period. Generally all the dogs were alert with pink colour of mucous membrane and shiny hair coat which reflected the good health condition of dogs during entire experimental period.

Hindgut fermentation and gas production offer a new concept in the nutritional evaluation of canine foods. While these parameters are important not only to provide nutrition and good health of dogs but also of concern by the dog owners in terms of frequency of defaecation and flatulence problems. Based on these criteria, evaluation of homemade or commercial diets can be viewed since feeding of homemade diets (HV or HNV), resulted in significantly higher gas production while the commercial diets (CV and CNV) resulted in the lowest gas production. Secondly, from the present study on microbiota of the hindgut, it was concluded that enumeration of total anaerobic bacteria may not reflect the fermentation characteristic of hindgut, while the enumeration of Bacteroides species can be the microbiological index to reflect the fermentation and hindgut health of dogs.