DEVELOPMENT OF DOG FOOD BY UTILIZING MEAT, EGG AND DAIRY INDUSTRY BYPRODUCTS

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University
in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
LIVESTOCK PRODUCTS TECHNOLOGY
(Minor Subject: Veterinary Public Health and Epidemiology)

By

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(L-2018-V-27-M)

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CERTIFICATE-I

This is to certify that the thesis entitled, "**Development of dog food by utilizing meat, egg and dairy industry byproducts"** submitted for the degree of **M.V.Sc.** in the subject of **Livestock Products Technology** (Minor subject: **Veterinary Public Health and Epidemiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Dr. Tejinder Pal Singh (L-2018-V-27-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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ABSTRACT

The study was conducted for development of dog food by utilizing meat, egg and dairy industry byproducts such as chicken skin, chicken liver, gizzard, hatchery waste, lard and ghee residue. On the basis of proximate composition, physico-chemical quality and aflatoxin content, ghee residue and liver powder were selected for incorporation in dog food. Selected byproducts were incorporated at two different levels in the dog diet i.e. with 2.5% ghee residue, 5.0% ghee residue, 2.5% liver powder and 5.0% liver powder. Raw diets were subjected to different processing techniques viz. boiling and extrusion. Proximate composition and physicochemical parameters were evaluated for raw, boiled and extruded dog food. Boiling of dog food reduced the ether extract content of diets. Further, the diets were evaluated through *in- vitro* nutrient digestibility studies. Statistical analysis revealed that boiling of diets reduced the *in-vitro* digestibility of ether extract. Dry matter digestibility, crude protein digestibility and organic matter digestibility reduced significantly ($P \leq 0.05$) with inclusion of ghee residue and liver powder at 5% level, whereas as it was comparable to control diet at 2.5% level. Among different processing techniques, *in-vitro* nutrient digestibility was highest in case of extruded diets. Comparative evaluation of extruded dog food with all vegetarian dog food revealed that control dog food, dog food with 2.5% liver powder and 2.5% ghee residue have better nutritional digestibility than the all vegetarian dog food. The pH, FFA, PV and aflatoxin content of formulated dog foods were within permissible limits.

Keywords: Boiling, digestibility, dog food, extrusion, ghee residue, *in-vitro* analysis, liver powder.

Signature of Major Advisor **Signature of Student**

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ABBREVIATIONS USED

AA	:	Amino acid
AAFCO	:	Association of American Feed Control Officials
ACD	:	Apparent Colonic Digestibility
AIA	:	Acid insoluble ash
AID	:	Apparent Ileal Digestibility
ALT	:	Alanine transaminase
AOAC	:	Association of Official Analytical Chemists
AST	:	Aspartate transaminase
ATTD	:	Apparent Total Tract Digestibility
AV	:	Anisidine Value
BMBM	:	Beef meat and bone meals
CAGR	:	Compound Annual Growth Rate
CBPM	:	Chicken byproduct meals
CF	:	Crude fibre
CIAD	:	Coefficient for apparent ileal digestibility
CP	:	Crude protein
CPD	:	Crude protein Digestibility
CTTAD	:	Coefficient Of Total Tract Apparent Digestibility
CV	:	Coefficient Of Variation
DE	:	Digestible Energy
DM	:	Dry Matter
DMD	:	Dry Matter Digestibility
DORB	:	De oiled rice bran
EE	:	Ether extract
EED	:	Ether extract digestibility
FFA	:	Free fatty acid
GADVASU	:	Guru Angad Dev Veterinary and Animal Sciences University
GE	:	Gross energy
GIT	:	Gastrointestinal tract
GR		Ghee residue
HMD	:	Home-made diets
IIPTF	:	India International Pet Trade Fair\
LP	:	Liver powder
ME	:	Metabolizable energy

MJ/Kg	:	Metajoules/ kilogram
mmol/L	:	Millimoles Per Litre
NFE	:	Nitrogen-free extract
NIR	:	Near-infrared reflectance spectroscopy
NRC	:	National research council
OM	:	Organic Matter
OMD	:	Organic matter digestibility
PV	:	Peroxide value
R	:	Correlation coefficient
RH	:	Relative humidity
SCFA	:	Short-chain fatty acid
SHIME	:	Simulated human intestinal microbial ecosystem
SID	:	Standardized ileal digestibility
STZ	:	Streptozotocin
TA	:	total ash
TBA	:	Thio-barbituric acid
TBARS	:	Thio-barbituric acid reactive substances
USD	:	US dollar
VFA	:	Volatile Fatty Acids
w.r.t.	:	With Respect To
WHC	:	Water holding capacity

CHAPTER-I

INTRODUCTION

Rapid development of food processing industry yields higher abundance and concentration of byproducts (Helkar et al., 2016). The byproducts of meat, egg and dairy industry are rich in nutrients. Disposal of these byproducts as a waste material constitutes a major ecological burden because of very high biochemical oxygen demand (BOD). Efficient utilization of animal by-products has direct impact on the economy and environmental pollution of the country. Non-utilization or under-utilization of by-products not only leads to loss of potential revenues but also add increasing cost of disposal along with major aesthetic and serious health problems (Jayathilakan et al., 2012).

Meat by-products are produced by slaughter houses, meat processors, wholesalers and rendering plants. Slaughterhouse waste or animal by-products such as skin, head, liver, lung, kidney, brain, spleen and tripe have high nutritive value and these can be efficiently utilized for the production of pet foods as the animal proteins are the integral part of their diet. These can be used as inputs in feeds for the poultry, fish and pets like dogs and cats. Presently in India, pet food production is more of cereal based and less of animal protein (Gautam et al., 2018). Poultry skins are used to produce elastin, which is usually incorporated into the production of functional foods due to its antioxidant properties (Nadalian et al., 2013). Gelatin extracted from animal skins can be used in foods to improve elasticity, consistency and stability (Mariod and Fadul, 2014). Animal fats are very important byproduct from meat industry. These have been used for frying. However, their use is declining in the fast food industry due to consumer health concerns. Edible lard products are used in sausage or emulsified products (Toldra et al., 2012).

Waste products from the poultry and egg production industries must be efficiently dealt with as the growth of these industries depends largely on waste management. The main byproducts of egg processing include hatchery by-products consisting of egg shells, infertile eggs, unhatched eggs and dead as well as culled chicks. The common use of hatchery by-product is in the poultry feed upto 3–5% level. Chicken eggs are the concentrated source of high quality nutrients, available at low cost. Eggs have been described as “Nature’s original functional food” (Hasler,

2000) packed with thirteen important vitamins and minerals. Eggs are also considered to have highest quality protein, and as compared to other animal protein sources, eggs are the most inexpensive. Egg shell meal is also a rich source of calcium in diet. Infertile eggs, unhatched eggs and egg shell meal from the hatcheries may be utilized for the preparation of dog food. Different dairy industry by-products are produced during the manufacture of main product, which are rich in nutrients and of great economic value. Ghee residue is important by-product of dairy industry, generally it is utilized in preparation of candy, chocolate, edible paste for sandwich, dosa and samosa filling, burfi and bakery products.

As per 19th livestock census (2012) dog population in India was 1,16,72,617 (84, 44,251-males and 32, 28, 366-females). Whereas in Punjab dog population was 4,70,558 (3,63,708-males and 1,06,850 females). The field of dog nutrition is in cognition and still very young. The demand of pet food is increasing tremendously in India as well as other Asian countries. Therefore, the pet food market is developing very fast in these countries (Hand et al., 2010). In 2014, the indian pet food market was valued at around 198.6 million dollars and is expected to grow at a compound annual growth rate of 13.9 percent and expected to be worth 434.3 million dollars by 2020 (IPFM, 2015).

Modern pet owners, identified themselves as “pet parents,” and are focused on improving the health status, well-being, quality of life, and longevity of their pets. Concerns pertaining to the nutrition of companion animals, pet owners depend on the commercial pet foods, without considering the safety, quality, availability of ingredients and processing transparency which give rise to many diseased conditions in their pets. The feeding of pet dogs under Indian conditions is variable, due to its divergent social, economic and cultural factors. A large number of pet owners in India rely on homemade diets to raise their pet dogs. Pet owners follow unscientific feeding and keep their pets on home-made cooked food recipes which are nutritionally imbalanced (Sethi et al., 2019)

Pets especially the dogs are playing the role of companion in the modern society. For many people, their dog is a member of the family and people like to give their pets- a balanced and complete diet. This could be possible by providing a dog with a healthy diet made with good ingredients especially of animal origin. In India, majority of pet owners follow unscientific feeding and keep their pets on leftover homemade cooked food which are nutritionally unbalanced with respect to protein, energy and minerals especially calcium and phosphorus (Shakhar et al., 2010). Therefore, there is an urgent need to explore nutrient rich foods not competing with human and are economical which can be incorporated in dog foods.

Therefore, keeping in view the above points, utilization of byproducts from meat, egg and dairy industry to develop economic and balanced diet for dogs, a comprehensive study was conducted with the following objectives:

1. To evaluate the nutritional quality of meat, egg and dairy industry byproducts for their potential to be used in dog food.
2. Comparative *in-vitro* evaluation of best levels of feed with all vegetarian dog food.

CHAPTER-II

REVIEW OF LITERATURE

The literature is reviewed under the following heading:

2.1 Scenario of pet food industry in India

2.2 Feeding practices adopted by Indian pet owners

2.3 Utilization of meat, egg and dairy industry byproducts in dog food

2.4 *In-vitro* digestibility studies

2.5 *In-vivo* digestibility studies in dogs

2.6 Physicochemical properties and storage quality of dog food

2.7 Effect of dog food on growth, blood and biochemical parameters

2.8 Effect of extrusion on nutritional characteristics of dog food

2.9 Advanced technologies for evaluation of pet food

2.1 Scenario of pet food industry in India

In India, dogs are more popular pets than any other animals. Indian household dog population increasing by 26% every year and about 17% of the households has a pet dog (Sudarshan et al., 2006).

India pet food market was valued at USD 334.3 million and is expected to grow at a CAGR of 13.9% during the projected period (2019-2024). Rapid urbanization and nuclear family structure had led to increase in pet adoption rate. Thus, growth of the pet food market is largely determined by the pace of urbanization. People are now more concerned about pet health and nutrition, which is a major driving force to the growth of the pet food market.

Dog food segment is expected to continue its dominance in the coming period as the largest revenue contributor, followed by cat and fish food segments. Dry food is more popular in case of dog food segment due to the general liking of dogs towards dry food. Treat/snacks are also popular among dogs, however, due to high prices, most of the pet owners prefer buying dry food.

Pet Food Market in India is segmented by product into dry pet food, wet pet food, veterinary diet, treat/snack, liquid food and organic product; by pricing into economic, premium and super-premium segment: by animal type into dog, cat, bird and other animals; by ingredient type into animal-based, plant-based, cereals and cereal byproducts and other ingredient types and by sales channel into specialized pet shops, internet sales, hypermarket and other sales channel (IPFM 2015).

2.2 Feeding practices adopted by Indian pet owners

Out of total 1500 pet owners surveyed in punjab, 64.06% of pet dog owners kept their pets on home-made cooked food, while 11.35% of pet owners keep their pets solely on commercially available packed food and 24.59% of pet owners kept their pets on mix feeding i.e. both home-made cooked food and commercially available food. 39.86% of domesticated dogs receive vegetarian diet, whereas 55.32% of dog population are fed both veg and non-veg diet. Among various food items fed to dog chapati forms a major portion of pet diet irrespective of veg and non-veg along with commercial feed. 94.91% of dogs receive chapatti in their diets, 87.5% dogs receive milk and 55% curd. Amongst the non-veg diets, eggs were offered to 55.25% of dogs and 46% received meat (Sethi et al., 2019).

Selvakumar (2016) conducted a survey in the state of Tamil Nadu, found 12.90% of kanni dog owner fed their dogs vegetarian diet, while rest 87.10% kept them on non- vegetarian diet. Home-made dog food was being offered to 91.13% of pet population, while rest 8.87% were reared on commercially available dog food.

Shakhar et al. (2010) surveyed pet dog population covering 494 pet dogs to gather information on prevailing feeding practices along with collection of 251 blood samples to evaluate blood metabolic profile of dogs reared on homemade diets. Majority of the pet owners fed their dogs either twice (52.0%) or thrice (41.3%) a day, while only 4.9% pet owners choose to fed their pets four times a day. Home-made diets (HMD) were offered to 79.4% of dogs, 3.4% of dogs were exclusively reared on commercially available pet foods and rest 17.2% were offered both homemade diets and commercially available dog food. Further, they reported that use of vegetarian foods was restricted to 42.1% of the pet dogs, while non-vegetarian diets were limited to 57.9% of dog population. Most of the pet owners were feeding milk and bread-based diets to their dogs, either with no other supplement (23.1%) or along with vegetables (19%); egg or meat with vegetables (37.2%); egg/meat along with commercially available pet food (17.2%) and 3.4% kept their pets solely on pet foods with milk. Metabolic blood profiles revealed that 30.3% of the blood samples analyzed had high plasma cholesterol levels than the normal physiological range. 39.2 and 23.3% of the pet dogs had plasma calcium levels lower and higher than the normal range, respectively. 29.4% of animals had plasma phosphorous levels higher than the reference range together with 41.8% of dogs having ALP levels lower than normal physiological range, revealing unbalanced metabolism of these bone forming minerals. Also, 45.4% of the dogs surveyed exhibited blood urea nitrogen (BUN) levels lower than the normal physiological range.

Further an appraisal of plasma biochemical values revealed significantly lower ($P<0.05$) total protein, albumin, globulins and inorganic phosphorus in those reared on home-made diet as compared to those on commercially available food. The cholesterol level was significantly ($P<0.05$) lower in dogs fed on commercially available food than the dogs fed HMD which in turn was lower than those fed both HMD and commercially available food. The plasma glucose and ALP were significantly ($P<0.05$) higher on HMD feeding. Notably higher ($P<0.05$) levels of glucose, phosphorus and ALP were recorded in dogs fed vegetarian diets as compared to those kept on non-vegetarian diets, whereas a contrary trend was apparent ($P<0.05$) for total protein, albumin, globulin and calcium levels.

2.3 Utilization of meat, egg and dairy industry byproducts in dog food

Livestock sector plays an important role in Indian economy and it is an important sub-sector of Indian Agriculture. The overall growth rate in livestock sector is steady (4-5%) and has been achieved despite very low investment in this sector. Livestock contributed 16% to the income of small farm households as against an average of 14% for all rural households. Livestock provides livelihood to two-third of rural community. It also provides employment to about 8.8 % of the population in India. Livestock sector contributes 4.11% GDP and 25.6% of total Agriculture GDP (DAHD, 2019).This contribution would have been much greater had the animal by-products been also efficiently utilized.

Efficient utilization of by-products has direct impact on the economy and environmental pollution of the country. Non-utilization or underutilization of livestock sector by-products not only lead to loss of potential revenues but also increases the cost of disposal and may create major aesthetic and health problems.

Poultry is one of the fastest growing segments of the agricultural sector in India, rising at a rate of 8 to 10 percent per annum. It carts a pivotal position in current Indian economy and has evolved from subsistence farming to an extremely business oriented enterprise. India is the World's fifth largest in poultry population (851.81 million birds) producing 103.32 billion of eggs annually (DAHD 2019). Regardless of this topmost production, waste generation in poultry industry is a big concern. Besides pollution and hazard aspects, in many cases meat and poultry processing wastes like poultry skin, hatchery waste, eggshells, broken eggs etc. have a potential for recycling raw materials or for conversion into useful products of higher value.

Deveau-Greene and Larson (2018) developed pet treats by processing cattle ears using high pressure processing. The cattle ears are covered with a liquid, such as water, and are exposed to high hydrostatic pressures for about one minute. The high hydrostatic pressures remove hair and implants from the cattle ears while preserving the texture and consistency of the ears, which are appealing to dogs and other pets.

Abdolghafour et al. (2014) studied the effect of incorporation of buffalo meat by- products namely tripe meal and rice flour on development, quality evaluation and storage stability of pet foods under ambient condition. The quality of pet food was based on physicochemical characteristics namely moisture, ash, fat, protein content and pH. There was moisture content (8.211%), ash content (3.571%), protein (17.85%), pH (6.688) and fat in the range of 14.323%.

Nadalian et al. (2013) stated that poultry skins can be used to produce elastin, which is usually incorporated into the production of functional foods due to its antioxidant properties. Extracted elastin from chicken skin contains glycine as the main amino acid (19–20%), followed by glutamic acid. Mariod and Fadul (2013) reported that gelatin extracted from animal skins is used in foods to improve elasticity, consistency and stability. Gelatin is widely added to jelly and aloe to form the main ingredient, the main purpose of which is to produce gel desserts because of its "mouth-melting" nature. Jamilah and Harvinder (2002) reported that gelatin is also added to a range of meat products, especially meat pies.

Mahender et al. (2013) conducted an experiment on a 4 x 4 LSD trial using adult Labrador dogs to find out the effect of incorporation of different levels of poultry slaughter waste (PSW) in dog biscuits, on dry matter (DM) intake, palatability and digestibility of nutrients. DM intake, DM intake as percent body weight and DM intake per kg metabolic body weight were significantly higher for the PSW (5 % level) group than other two PSW (10 and 15 % level) and control groups. The adult dogs consumed all test diets with equal preference to the control diet indicating, palatability of the PSW based dog diet biscuits was good and extrusion cooking of the diets had positive impact on the intake of the diets, while shape of the biscuit had no effect on the palatability.

As per the NRC (2006), for practical foods made from cereals and various animal by-products, the crude protein level needed for maximum nitrogen retention appears to be about 25 per cent dry matter for newly weaned puppies, whereas for puppies over 14 weeks of age, it is 20 per cent dry matter. Calcium level in a pet food for early growth should be at least 1g/100g DM. During late growth, it is recommended that large breed and giant breed puppies continue to be fed a pet food containing at least 1% of calcium until about 6 months of age.

Fahey (2004) reported that a wide range of protein sources including meat and bone meals, poultry meals, poultry by-products meals and soybean meal are traditionally used by the pet food industry. Krestel-Rickert (2001) developed the pet food from soft tissues (striated muscle, viscera and other organ tissue) of spent hens. The soft tissue of spent hen can be used by the pet food industry for the following reasons as it provides palatable material at low cost which is both a proven winner with pets and also provides good protein nutrition. Pet food industry provides an environmentally friendly disposal method of light spent hens and it provides a solution to the spent hen disposal problem in the table egg industry.

Karthikeyan et al. (2002) studied the nutritional quality and palatability of pet food from poultry by-product meal. The studies were conducted to formulate 3 different foods containing 15% poultry by-products, meal in combination with leaker eggs, bakery waste, cereal and cereal by-products, edible oil permitted food additives, vitamin and mineral mixtures. The finished pet foods had high pepsin digestibility (69.7-71.4%) and were a good source of crude protein (22.9-23.7%), calcium (0.74-0.77%), phosphorus (0.67-0.70%) and available lysine (0.80-0.82%), Methionine (0.47-0.51 %) and ME (4.17-4.24 kcal/kg). Feeding 100g of these foods to adult pet dogs (10 kg) could meet 50 to 65% of their daily maintenance requirements for ME, CP, Ca, P, available lysine and methionine as prescribed by AAFCO (1993).

The dairy industry processes raw milk into many valuable products such as curd, butter, cheese, cream, yogurt, ghee, condensed milk, dried milk, ice cream, paneer etc. and produces various by-products including buttermilk, whey, ghee and skim milk. These dairy by-products have high nutritive value and have found applications in many food industries as well as nonfood applications . A number of by-products like whey, buttermilk, skim milk and ghee and derived by-products like caseins, caseinates, lactose, whey proteins etc. are produced by the dairy industry. Attempts have been made globally to utilize these by-products because of their high nutritive value. Dairy plants in India are still confronted with the problem of by-product utilization because of lack of adequate technology and high cost of new technologies.

Whey is the major by-product of the dairy industry. It is a useful resource of nutrients containing about 50% of the solids of milk (Fox et al., 2017). Whey production is steadily growing, and its high organic content is an important environmental and health issue. Therefore, suitable management of this by-product is required. Like milk, whey may have different origins (e.g., goat, sheep and buffalo), but the most relevant in terms of production volume and economical value is that obtained from cow milk processing. Skim milk is a by-product obtained from cream manufacture. It is rich in SNF content and has high nutritional value and has been utilized in the manufacture of a number of dairy products or in powder form. Buttermilk, a by-product of butter manufacture, has been used as such or in dried form. Ghee residue from ghee manufacture has also found applications in many food products.

Ghee is an important constituent of Indian meal prepared using different methods. It is clarified milk fat with incomparable organoleptic properties, which make it an important ingredient in a wide variety of food applications (Pawar et al., 2014). About 30–35% of the milk produced in India is converted into ghee. A blackish brown residue mainly the SNF part of cream was coagulated out during ghee preparation as a by-product when cream is heated is known as ghee residue. It is obtained as moist brownish sediment after molten ghee has been strained out (Dairy India, 1981). The amount of ghee residue was found to depend upon the method of preparation of ghee. This was due to the variation of non-fatty serum constituents of the different raw materials used for the preparation of ghee. Ghee yield was higher from creamery butter method in comparison to direct cream method, whereas ghee residue content was higher in direct cream method in comparison to creamery butter method. The average yield of ghee residue was maximum (12%) in direct creamery (DC) method followed by almost the same yield in creamery butter (CB) and desi butter (DB) methods, that is, 3.7%. Ripening of cream prior to clarification reduces the yield of ghee residue (Santha and Narayanan, 1978). It is one of the largest by-products of the dairy industry and consists mainly of milk proteins and small quantity of lactose and minerals.

The ghee residue has been used in food industries for making sweets, bakery products, and as a flavor enhancer. An appreciable amount of GR is produced in the country which is a nutritionally rich source of proteins and nitrogenous compounds. Ghee residue has been utilized for preparing burfi by mixing it with skim milk powder (SMP), khoa, chocolate and sugar. It can be utilized for preparing coconut burfi, candies, toffees, pinni etc. after mixing with other ingredients. The nutritious by-product should be utilized as a food supplement in a variety of foods, food spreads, soups etc. (Galhotra and Wadhwa, 1993). The utilization of this by-product in the preparation of some type of candies, toffees, edible pastes etc. was suggested.

Ramesh et al. (2018) revealed the moisture, crude protein, crude fibre, ether extract, nitrogen free extract and total ash contents of ghee residue are 12.10, 19.86, 3.49, 47.12, 25.63 and 3.90 per cent respectively. Fatty acid profile of ghee residue revealed that the palmitic acid registered the highest percentage (38.88) among saturated fatty acids and the oleic acid accounted for the highest percentage (25.15) among unsaturated fatty acids. Linoleic, linolenic, eicosapentaenoic and decosahexaenoic acid content of ghee residue are 2.02, 0.79, 0.36 and 0.25 per cent respectively. Amino acid profile of ghee residue revealed that the lysine and methionine, content were 0.99 and 0.61 per cent, respectively. Threonine and arginine levels are found to be at 1.44 and 0.76 per cent, respectively. The glutamic acid recorded the highest percentage (5.26), while cystine registered the lowest percentage (0.35) among amino acids in ghee residue.

Arumugam et al. (1989) reported that ghee residue, a by-product of the dairy industries is good source of protein and energy, containing in dry matter 25.8% crude protein, 50.8% fat, 12.3% lactose, 14.4 MJ of apparent ME g−1, 16.5 MJ of true ME kg−1, 43.7 gross protein value, 8.98% ash, 0.88% calcium, 0.50% phosphorus, 14.4% nitrogen free extract and no crude fibre. They incorporated ghee residue in chick starter diet at different levels and reported that ghee residue appeared to be a potential high protein and high energy feed ingredient for poultry and could be incorporated in chick starter diets at up to 35.4% without any adverse effect, if supplemented with lysine and methionine.

Singh et al. (2017) conducted 96-day feeding trial in four ponds of 0.04 ha each to evaluate the efficacy of ghee residue (GR) based diet against control diet on growth performance of rohu fingerlings (Av. wt. 10.81±0.91). The feed was prepared with 20% ghee residue (GR20) along with other feed ingredients. The net weight gain, protein efficiency ratio and daily growth co-efficient during the 96 days trial were significantly ($P<0.05$) higher in ghee residue fed group (GR20). The FCR also improved significantly ($P>0.05$) in GR20 (1.75±0.05) as compared to the GR0 (2.23±0.08) group. The specific growth rate and specific feeding rate did not differ significantly between the treatments. The carcass composition revealed that the crude fat content was significantly ($P<0.05$) higher in group (GR20). The fatty acid profile of the fish fillet revealed that both mono-unsaturated and poly-unsaturated fatty acid composition was significantly ($P<0.05$) higher in the group GR20 compared to GR0.

2.4 *In-vitro* digestibility studies

Biagi et al. (2016) developed a simple and quick procedure to determine digestibility of commercial diets for dogs. In-vitro trials with different concentrations of pancreatin, bile salts and different durations of gastric phase were conducted to validate the method and results were compared with data obtained from literature. In-vitro method developed had two incubation phases including gastric phase of 2h in the presence of pepsin (2g/L), gastric lipase (1g/L), and HCl (0.075N) and a second phase (intestinal phase) of 4h incubation in buffer solution in the presence of pancreatin (10g/L) and bile salts (25g/L). Incubation was done at 39^{o}C in shaking water bath. Further, to compare in vitro digestibility with in-vivo digestibility, 16 extruded diets for dogs were evaluated both *in-vitro* and *in-vivo* with adult dogs. A close linear relationship was found between *in-vivo* total tract and *in-vitro* dry matter digestibility (DMD) ($r^2=0.81$). However, accuracy of crude protein digestibility using the *in-vitro* method was less ((r^2=0.5) which may be attributed to endogenous protein losses. The digestibility results obtained with the *in-vitro* method for ether extract and starch were 95.3 and 98.7%, respectively which were very close to those obtained the *in-vivo* trial i.e. 94.8 and 99.1%, respectively.

Swanson et al. (2001) studied chemical composition and fermentation characteristics of different fruit pomaces using a canine *in vitro* model. Total dietary fiber (TDF) concentration varied among the different pomace sources; apple pomace had the highest TDF concentration (79%), whereas grape pomace had the lowest (55%), while tomato pomace and fruit blend (mixture of peach, almond, nectarine and plum) had intermediate values, 57% and 65%, respectively. Tomato and grape pomaces had a higher ratio of insoluble to soluble fiber (13:1 and 11:1, respectively) as compared to apple pomace, which had the lowest ratio, 6:1. Organic matter disappearance in case of apple pomace increased from 0h to 24h from 10.2 to 34.9%, while in case of tomato pomace it increased from 24.4 to 35% at 24h. Generally, fruit fibers with a greater insoluble to soluble fiber ratio have lower gas production and short chain fatty acid production after 12 h or 24h of *in-vitro* fermentation. Apple pomace had a greater total Short chain fatty acid concentration after 24h of fermentation, (2.1mmol/g) in contrast to grape pomace (0.83 mmol/g), which had the lowest concentration.

Boisen (1991) reviewed several types of *in vitro* models, including batch cultures, chemostat simulators and computer-controlled systems. They differ widely in complexity and competency. Some of the *in-vitro* models are capable of simulating either hydrolytic or fermentative digestion, while others can simulate both. Through monitoring of digestive events at particular time points, kinetic outcomes, such as rate of nutrient digestion, maximal rate of short-chain fatty acid production, and time to achieve maximal rate of short-chain fatty acid production, can be determined along with extent of nutrient or dry matter digestibility. Author laid emphasis on advantages of *in-vitro* models that simulate gastric and small intestinal digestion or fermentative digestion in the large intestine. These are relatively inexpensive and rapid means of simulating events happening throughout the gastrointestinal tract of the dog.

Sunvold et al. (1995) evaluated *in vitro* fermentation characteristics of different dietary fiber sources. Fibre sources viz. citrus pulp and citrus pectin were incubated for

6, 12, 24 and 48 hours with faeces from dogs, cats, pigs, horses, humans and ruminal fluid from cattle. Data analysis revealed that across all species, citrus fibers had the greatest organic matter disappearance (OMD) (>80%) and total short chain fatty acids (SCFA) production (>5.5 mmol/g substrate OM). The cat had the highest total short chain fatty acids production (3.38 mmol/g of OM). These data contradicted the concept that a strict carnivore like cats is unable to utilize and benefit from dietary fibers.

Minekus et al. (1995) designed a complex digestion model, TNO Intestinal Model (TIM-1). This computer-controlled system was being capable of modeling various characteristics such as meal size, meal duration, peristalsis, pH, gastric and intestinal secretions, gastrointestinal transit time, and absorption of water and nutrients. It had four consecutive compartments mimicking the parts of gastrointestinal tract in sequence (stomach, duodenum, jejunum, and ileum). TIM-1 is developed to simulate physiological characteristics like gastrointestinal transit rate and gastric and intestinal secretions) based on *in vivo* data from the species of interest (humans).

Molly et al. (1993) designed SHIME (Simulated Human Intestinal Microbial Ecosystem), a chemostatic system that consider the complete digestive process from stomach to colon in one closed environment. Hydrolytic and fermentative processes occurring in each region of the intestinal tract are predicted through the use of different vessels. Main limitation is that digested nutrients are not removed despite the consistent movement of media through the vessels, all of which enter the simulated colon vessels, causing error in results as additional hydrolytic end-products are fermented at this site.

2.5 *In-vivo* digestibility studies in dogs

Beynen et al. (2017) conducted a cross-over digestibility trial on six adult dogs (3 Beagles and 3 Schnauzers) which were fed an extruded dry food containing beet pulp at the inclusion level of 5 or 10%, being substituted at the expense of a grain mixture. Apparent digestibility of DM, CP, EE and carbohydrates at 5% inclusion level was 79.9, 7.2, 92.1 and 89.9%, respectively and at 10 % inclusion level it was 79.9, 77.4, 92.1 and 89.9%, respectively. Thus 10% versus 5% had no effect on the percentage of apparent total digestive tract digestibility of protein, while fecal output of dry matter and water were raised by 6 and 19%. It was concluded that supplementation of beet pulp from 5 to 10% in a dry food is practicable if it involves an exchange with grains, and provided that the associated increase in fecal volume is acceptable.

Shakhar et al. (2007) studied the effect of vegetable supplementation of rice-meat based homemade diet using four female Great Dane pups (3 months; 16 kg) in a crossover design. The pups were fed two diets, rice-meat based and rice-meat- vegetables (containing potato, tomato and cabbage in equal proportions) based diets in the ratio of 20:80 and 16:68:16, respectively, on as fed basis. The experimental protocol, consisted of two subsequent periods of 14 days each, with 3 days of digestion trial during 12-14 d, followed by blood collection on day 15. It was found that inclusion of vegetables in diet markedly reduced ($P<0.001$) the palatability as well as food intake leading consequently to a reduction in mean daily consumption of protein, energy (ME), calcium, phosphorus, iron, copper and zinc. Digestibility of DM, OM, protein, fat and fibre for rice-meat based diets was 91.1, 92.8, 96.8, 94.8 and 75.5, respectively and for rice-meat- vegetable diet was 73.7, 79.2, 93.4, 92.6 and 56.7%, respectively. Thus, digestibility of DM and OM decreased ($P<0.001$) on feeding the vegetables supplemented diet with a parallel trend for that of protein ($P=0.077$) and fibre ($P=0.099$). The faecal characteristics like volume, moisture, pH and excretion of dry and wet faeces per 100g DM intake showed an increasing trend in the vegetables supplemented pups with no effects on short chain fatty acids (SCFA) and lactate concentrations. Serum metabolic profiles of the two groups were similar except for higher ($P<0.05$) values of urea and uric acid in the vegetables-fed pups. The antioxidant status was also similar between the two groups except for the total and protein-bound thiols, which were higher ($P<0.01$) in the vegetables fed pups. It was concluded that irrational supplementation of vegetables in the diet may adversely affect the nutritional status of the pups.

Castrillo et al. (2001) analyzed the apparent digestibility of nutrients and energy of 38 dry commercial extruded dog foods using six adult (2 to 3 year-old) female Beagles. Mean apparent digestibility (%) of CP, EE, NFE, total organic matter and gross energy was 85.1, 90.3, 87.2, 85.4 and 85.8 %, respectively. Apparent energy digestibility ranged from 77.3 to 91.6%, and was closely related to CF content ($r = -0.85$), yielding the resultant equation: gross energy digestibility (GED) (%) = 94.00- 4.04×CF (% DM). It was concluded that the estimation of digestible energy content of foods from digestibility coefficients calculated from the above equation and gross energy measured or estimated from the Weende fractions, provided a more precise prediction of experimental values than the Atwater approach followed by NRC and AAFCO.

Nyachoti et al. (1996) concluded that endogenous nutrient losses interfere with the additivity of nutrient digestibility in mixtures of different ingredients present in complete foods, and with metabolic losses associated with the utilization of absorbed nutrients for production. To predict endogenous losses, ileal digestibility must be evaluated. This allows nutrients to be measured before they reach the large intestine and get modified by the substantial microbiota population present at this site. In this technique, animals are cannulated at the terminal ileum, and digesta is collected from this site at specific times throughout an experimental period. To rule out the passage of nutrients to this site, inert digestion markers are used. Ileal digestibility coefficients should be, however, considered "apparent" rather than "true," as endogenous secretions include ileal chyme. However, these values are much more precise and reflective of nutrient digestion events than total tract digestibility measurements.

Shields (1993) listed four major factors that can affect digestibility values. These were food processing effects viz. ingredient particle size and modifications to the pre-conditioner, pellet mill, extruder, retort apparatus, or drying oven; feeding management practices like previous diet fed, amount of food offered, animal factors including breed, age, gender, activity level, physiological state; and housing and environmental factors like metabolism cages versus covered kennels, effective environmental temperature, caretaker-animal relationship, photoperiod.

2.6 Effect of dog food on growth, blood and biochemical parameters

De Godoy et al. (2013) studied the effects of blends of soluble corn fibers with pullulan and sorbitol, both slowly digestible carbohydrate sources, and fructose, a non insulinemic sugar. Soluble corn fiber had an in-vitro hydrolytic digestion of approximately 50%. Similar monosaccharide digestibility values were obtained when soluble corn fiber was blended with a low concentration (5% or 15%) of fructose. However, mixing soluble corn fiber with 30% or 50% fructose, sorbitol or pullulan led to higher digestibility values, up to 91%. Soluble corn fiber and its blends had lower glycemic and insulinemic responses than maltodextrin. The lowest glycemic response was recorded for blends with fructose or sorbitol, resulting in an average relative glycemic response of 4.8% as compared to maltodextrin (100%).

Brambillasca et al. (2010) studied the digestibility, fecal characteristics, and plasma glucose and urea levels in eight healthy adult cocker spaniel dogs receiving 2 different dog foods once or thrice daily. The dogs were divided randomly to feed in a double 4×4 Latin square design. Dog food A was 5 times more expensive than dog food B with 292 and 186 g/kg protein respectively. No difference was observed in DM, OM, and CP apparent digestibilities when comparing feeding frequencies or the interaction between food and frequency. Apparent digestibility of DM, OM, CP and CF for food A was 81.8, 85.4, 86.8 and 19.0%, respectively when fed once and 84.9, 88, 88.3 and 44.3%, respectively when fed thrice. Apparent digestibility of DM, OM, CP and CF for food B was 71, 75.5, 81.5 and 36.7%, respectively when fed once and 70.1, 74.9, 81.6 and 28%, respectively when fed thrice. Thus, food A had higher digestibility of DM, OM, and CP (18.1, 15.3, and 7.4%, respectively) than food B ($P<0.001$) for both feeding frequencies (once or thrice). Neither feeding frequency nor type of food affected the digestibility of CF. The dogs had higher wet fecal output with lower fecal consistency scores when they were given food B. Fecal DM content was not different between feed sequences. Fecal pH was lower when dogs ate food A. Blood samples were collected from 30 minutes before to 60 minutes after a meal and it was found that the basal plasma glucose and urea levels for all feed sequences were similar before the meal time. Both plasma glucose and urea increased after the meal, and only food × time interaction was significant for glucose. The maximum increases in glucose w. r. t. the basal values were 0.78, 0.88, and 0.54 mmol/L for food A fed once and thrice and food B fed once respectively, at 60 min, and 1.05 mmol/L at 45 min after the meal for food B fed thrice. Furthermore, plasma urea concentrations tended to increase throughout the measurement period and higher values were recorded 60 min after the meal for all feed sequences. It was concluded that digestibility of DM, OM, and CP, and fecal consistency were higher, and daily fecal excretion and fecal pH were lower when dogs were fed food A ($P <0.001$). The feeding schedule had no influence on plasma glucose and urea level. Neither feeding frequency nor food × frequency interactions were significant for the parameters analyzed.

2.7 Physico-chemical properties and storage quality of dog food

Virk et al. (2018) determined the appropriate incorporation level of poultry by-products namely liver and gizzard along with calcium fortification for the development of dog biscuits. Poultry liver and gizzard were minced and air dried for 15 - 16 hrs at 60 °C after that these were converted into powder. Three inclusion levels of poultry liver and gizzard viz., 10%, 20% and 30% were used, replacing the refined wheat flour in standardized dog biscuits formulation. All the treatment products were appraised for sensory evaluation, dog acceptability test, texture profile and instrumental color profile. Amalgamation of liver powder at 30% level was found most appropriate for the preparation of dog biscuits. The developed product was evaluated for physico-chemical properties (viz. pH and cooking yield), proximate composition (moisture, protein, fat and ash), mineral content (Ca, P and Se) and texture profile analysis. The dicalcium phosphate was amalgamated at 2% level in the 30% liver powder incorporated dog biscuits with acceptable physico-chemical and sensory attributes. The final product was packed under aerobic and stored at ambient temperature (25°C) for 80 days. The samples were drawn and analyzed at 20 days intervals. The storage quality was assessed on the basis of different physico-chemical (pH, water activity, moisture, TBARS, PV, and FFA) microbiological (TPC, PC, coliforms count, yeast and mold counts), water activity and sensory analysis (5- point descriptive scale). The dog biscuits could be stored for 80 days without any marked loss in physico-chemical, microbiological and sensory attributes.

Gray et al. (2015) studied the effect of gradually oxidized protein meals on the keeping quality of extruded pet foods. Chicken byproduct meals (CBPM) and beef meat and bone meals (BMBM) divided in two treatment groups as unpreserved (U) or preserved with either mixed tocopherols (T) or ethoxyquin (E) which were kept at normal atmosphere conditions (25°C, 51% RH) and monitored for their peroxide value (PV) and anisidine value (AV) until peak values were obtained (i.e. at 41 and 63 days for CBPM and BMBM, respectively). Each “aged” meal was then amalgamated into a model extruded cat food. Samples of kibble for each treatment were stored at higher temperature and humidity (40°C, 70% RH) for 18 weeks and at normal atmospheric conditions (~22°C, 45% RH) for 12 months. The oxidized meal led to a shorter shelf life ($P < 0.05$) of the finished food as revealed by PV analysis. However, sensory analysis by quick evaluation did not completely validate these results. It was therefore concluded that peroxide value doesn’t fully substantiate rendered protein meal stability or have a direct effect on keeping quality for consumers, but may have a negative impact on pets due to oxidized lipid consumption.

Akhtar et al. (2015) analyzed the quality of extruded pet food by using buffalo meat by-products such as liver, trims and agro processing waste viz. sorghum, oats and corn flour. The quality of the extruded pet food was assessed on the basis of quality characteristics such as pH, TBA number, protein, fat and ash content. The pH values of the agro processing waste used extruded dog food were in the neutral range (6.28- 6.18). The pH of extruded pet food decreased considerably. The ash content, TBA value and pH content in fresh condition were 2.43%, 0.605 mg/kg and 6.295 respectively. During ambient storage, pH values decreased constantly. Protein content was also decreased significantly. Increasing storage period and increasing trend in TBA values indicated that natural antioxidant α-tocopherol individual or in combination of potassium sorbet was less stable in atmosphere packaging. It was concluded that after 120 days of storage, there was no quality deterioration observed in any sample, however, the rancidity value of meat by-products amalgamated in extrudates increased with increase in storage period which later will not be suitable for the dog health.

Goswami et al. (2015) developed the carabeef cookies by substituting refined wheat flour with carabeef powder. The carabeef cookies were sensory appraised and baked at three different time–temperature combinations namely 150-160° C for 35-40 minutes, 170-180°C for 25-30 minutes and 190-200°C for 15-20 minutes. Physicochemical properties like color values, proximate analysis and instrumental texture parameters were assessed. Parameters like moisture, protein, fat, diameter, pH, and ash percentage had no significant difference at higher baking temperature. Textural parameters such as shear force and hardness, and adhesiveness values increased considerably. While, mean cooking yield and thickness values exhibited decreasing trend, however spread ratio values presented increasing trend. Mean yellowness values had no notable difference, whereas mean redness, hue angle and chroma values increased significantly with a higher baking temperature and lower time combinations. All sensory attributes scores decreased at a higher baking temperature. Carabeef cookies with 50 % carabeef powder baked at 150-160°C for 35- 40 minutes were selected as best treatment.

Devatkal et al. (2004) studied the functional, physicochemical and microbiological quality of buffalo liver and stated that buffalo liver is an important edible meat by- product. Though, in developing countries including India, it is underutilized and has a low commercial value. Buffalo liver had 71.92% moisture, 18.44% protein, 5.60% fat, 2.72% carbohydrate, 1.32% total ash and total energy of 135 kcal. Mineral concentrations (mg%) in liver was: Na–60.04, K–274, Ca–5.60, Mg–6.20, Fe–20.86 and Cu–5.60. Mean glycogen (mg/g), total liver pigments(mg/g) and cholesterol (mg%) were 7.07, 8.49 and 283.88 respectively. The mean pH, WHC and cooking yield values of buffalo liver were 6.42, 38ml per100g and 73.15% respectively. It was concluded that liver contain higher amounts of water-soluble proteins than salt soluble proteins.

2.8 Effect of extrusion on nutritional characteristics of dog food

Tran et al. (2008) stated that extrusion process is usually used to produce dry pet foods. It is the process involving heat treatment. Extrusion cooking can have both favorable and unfavorable effects on the nutritional attributes of the product. Favorable effects of extrusion include increase in palatability, destruction of anti-nutritional factors and improvement in digestibility and utilization of proteins and starch. Detrimental effects of extrusion include decrease of protein quality (due to e.g. the Maillard reaction), reduced palatability and loss of heat labile vitamins.

Lankhorst et al. (2007) investigated the effect of different extrusion conditions and product parameters on the nutritional quality as measured by a number of *in-vitro* experiments (e.g. reactive lysine, and starch gelatinization degree) as well as physical quality of the kibble (durability and hardness) of a dog diet in 3×2×2 factorial trial. The parameters considered were mass temperature (110, 130 or 150°C), moisture content (200 or 300 g/kg) of the diets earlier to extrusion and number of times (once or twice) extruded. The extrusion conditions employed had no effect on total lysine and other amino acids. Extrusion conditions had significant effect on the reactive lysine content with the ratio of reactive to total lysine increasing from 0.71 to 0.80 and higher due to the effect of extrusion and temperature. Ratio decreased from 1 to 0.9 after second extrusion. Initial moisture levels influenced lysine reactivity. Protein digestibility as measured *in-vitro* remained unaffected by different extruding conditions. There were no apparent differences in protein dispersibility index (PDI) of all the extrudates. *In-vitro* glucose digestibility coefficients and starch gelatinization degree (SGD) exhibited an ability to increase with an increase in each individual parameter tested. The rise in temperature from 110°C to 150°C as well as extrusion for a second time decreased kibble durability. However, increasing moisture content increased durability. Optimization of extrusion conditions during commercial pet food production should include measurement of the reactive to total lysine ratio.

Svihus et al. (2005) found that when extrusion is done at low moisture levels, starch granules through the application of heat (loss of crystalline structure) and shear (granular fragmentation), are partially transformed, leading to formation of a homogeneous phase known as starch melt or gelatinisation. High moisture content and high-temperature extrusion resulted in complete gelatinisation and a significant increase in *in vitro* (Dust et al., 2004, Murray et al., 2001) and *in- vivo* (Svihus et al., 2005) starch availability. The modification of a dietary ingredients after extrusion to contain more retrograded starch/soluble dietary fibre, can enhance the production of short-chain fatty acids (especially butyric acid), compounds known to increase the colonic health of dogs (Dust et al., 2004, Murray et al., 2001).

Alonso et al. (2000) found that heat treatment during extrusion cooking can inactivate protein-based anti- nutritional factors by destroying the integrity of their structure, thus preventing their activity. Bednar et al. (2000) studied the influence of processing of different vegetable and animal protein sources on the ileal nutrient digestibility in dogs and found that extruded and pelleted diets including various vegetable protein sources provided adequate levels of highly digestible protein and amino acids (AA). Harper (1979) studied extrusion cooking, being a high temperature short time process, causes minimum nutritional losses and yields a better product. In addition, extrusion cooking produces a product with improved safety as the process destroys heat labile anti nutritional factors and enzymes that cause problems during storage and deteriorate product quality

2.9 Advanced technologies for evaluation of pet food

De Godoy et al. (2016) laid emphasis on the important use of quick and more accurate analytical methodology and new technologies to enhance pet food safety and quality through detailed evaluation of chemical composition of ingredients than that provided by proximate analysis. *In-vitro* and *in-vivo* studies continue to be exploited as screening tools to analyze nutrient quality and competence of novel ingredients before their use in animal diets. Molecular and high-throughput technologies allow implementation of non-invasive studies in dogs to study the effect of dietary interventions through systems biology approaches.

Use of Near-infrared reflectance spectroscopy (NIRS) to evaluate available nutrient concentrations has been limited by the lack of sufficient databases of *in-vivo* nutrient availabilities in food ingredients. Though, over the past few decades, NIRS has been used to estimate the metabolizable energy content of feed ingredients and diets for poultry (Valdes and Leeson, 1992, Valdes and Leeson, 1994), the digestible energy content of feeds and feed ingredients in case of swine (Van Barneveld et al., 1999), the ileal digestible protein and amino acid concentrations in feed ingredients for pigs and poultry (Van Kempen and Bodin, 1998) and the gross energy (GE) and metabolizable energy (ME) concentrations of cereal food products (Kays et al., 2002).

Hervera et al. (2012) analyzed 71 commercial extruded dog foods and found NIRS estimations accurate as estimated through application of the recommended equations from NRC (2006) for prediction of GE and DE in pet food. Alomar et al (2006) analyzed 59 extruded commercial dog foods, drawing calibration equations for gross energy (GE) of nutrients and metabolizable energy (ME) as estimated by National Research Council (NRC 1985) in addition to some amino acids and trace minerals. The results obtained for GE and for estimated ME were precise. A less accurate estimation of available energy was expected compared with GE, and subsequently DE and ME, because they vary with food characteristics as well as animal response to feeding.

CHAPTER-III

MATERIALS AND METHODS

Nutritional composition and physico-chemical quality evaluation of different poultry and dairy industry byproducts such as chicken skin, liver powder, gizzard powder, hatchery waste, lard and ghee residue was evaluated to explore the potential for the inclusion in the dog food. Individual ingredients were selected on the basis of proximate composition and physico-chemical quality. Later, diets were formulated using selected ingredients and were evaluated through *in- vitro* digestibility studies. The diets incorporated with best levels of byproducts were compared with all vegetarian dog food. The experiment was conducted as part of canine research centre under the subheading entitled "Nutritional and processing interventions for developing pet foods" under the ageis of Department of Biotechnology, Govt. of India. The detailed methodology of experiments conducted is mentioned as below:

3.1 SOURCE OF MATERIALS

3.1.1 Source of meat industry by-products

Poultry byproducts i.e. chicken liver, chicken skin and gizzard required for the experiments were collected from the Instructional poultry Processing Plant of Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. After slaughtering of poultry birds, poultry liver and gizzard were collected, cleaned and packed in LDPE bags and stored in freezer. Lard was purchased from the butchers shop in the local market of Ludhiana.

3.1.2 Source of ghee residue

Dairy industry byproducts i.e. ghee residue was procured from dairy processing plant, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.

3.1.3 Source of hatchery waste

Source of egg industry byproducts i.e. hatchery waste was procured from hatcheries, Department of Animal Genetics and Breeding.

3.1.4 Preparation of liver and gizzard powder

Poultry liver and gizzard were collected from the healthy birds after their slaughter and thorough postmortem examination. These were minced in the meat mincer (Mado Eskimo Mew-714, Mado, Germany). Minced poultry liver and gizzard were air dried at 60 °C for 15-16 hr. in industrial tray dryer. After drying, the by-products were converted into powder form with help of grinder separately. These were stored in PET jar for the subsequent use.

3.1.5 Chemicals and standards

Analytical grade chemicals and high purity standards required for analysis were procured from standard firms like SRL, Fisher Scientific, Loba Chemie, Himedia, etc.

3.1.6 Food ingredients

Rice, De-oiled rice polish (DORP), Bengal gram, Soya bean meal (SBM), Skim milk powder(SMP), Meat cum bone meal(MBM), Rice gluten meal(RGM) are procured from the local market of Ludhiana.

3.2 Experimental details

3.2.1 Experiment 1: Nutritional evaluation of meat, egg and dairy industry Byproducts

Chicken liver powder, chicken skin, gizzard powder, ghee residue and hatchery waste were analysed for proximate composition i.e Dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), Ash, acid insoluble ash (AIA) and mineral estimation i.e phosphorus estimation as per AOAC (2005) and calcium estimation as per Talapatra et al. (1940). These byproducts are also analysed for physio-chemical properties (pH, free fatty acid, peroxide value) and aflatoxin estimation.

3.2.1.1 Formulation of dog food

The different food ingredients i.e. rice, de-oiled rice polish (DORP), bengal gram, soya bean meal (SBM), skim milk powder (SMP), meat cum bone meal (MBM), rice gluten meal (RGM) were analysed for proximate composition and the proportion of the ingredients were calculated using wet chemistry analysis in MS Excel using two selected ingredients at different levels. Diets were formulated for adult Labrador dogs according to the nutritional requirements as given by AAFCO (2014) containing 18 % CP, 5.5% EE, 0.5 % Ca and 0.4% P. Energy density of diet was kept 3500 kcal ME/kg in accordance with Indian climatic conditions as described by ICAR (2013). The formulation of control diet is computed as follows:

Table 1: Formulation of Control Dog food

Ingredient	Quantity (%)	CP%	ME	EE	CF	Ca%	T.P %
Rice	**49**	7.44	3630	0.78	0.78	0.04	0.06
De-oiled Rice Polish	**5**	16	2100	1.5	23.15	0.07	1.33
Bengal gram	**20**	24.08	3120	4.95	4.4	0.08	0.13
Soyabean meal	**12**	45	2870	1.5	9.1	0.32	0.28
Skim milk powder	**4**	37	3590	0.285	0.22	1.2	0.993
Rice gluten meal	**2**	55.12	3150	3.9	5.02	0.62	0.78
Oil	**5**	-	8500	98	-	0	0
Di-calcium phosphate	**0.5**	-	0	-	-	23	18
Meat cum bone meal	**2.5**	41.87	2500	5.9	5.944	13.86	6.78
Total	**100**	18.290	3546.2	6.7641	3.7695	0.599	0.470

(CP-crude protein, ME- metabolizable energy, EE-ether extract, CF-crude fibre, Ca-calcium, T.P-total phosphorus)

According to the proximate composition, physico-chemical quality and aflatoxin estimation, chicken liver powder and ghee residues were selected for incorporation in the dog diet. Chicken liver powder and ghee residues were incorporated at the level of 2.5% and 5.0% in the control dog diet after replacing the rice gluten and soyabean meal.

Table 2: Formulation of dog diet with ghee residue

Ingredient	2.5% Ghee residue	5.0% Ghee residue
Rice	49	49
Deoiled rice polish	6	6
Bengal gram	20	20
Soyabean meal	12	10.5
Skim milk powder	4	4
Ghee residue	2.5	5
Oil	3.5	2.5
Meat bone meal	3	3

Table 3: Formulation of dog diet with liver powder

Ingredient	2.5% liver powder	5.0% liver powder
Rice	50	52
Deoiled rice polish	6.5	7
Bengal gram	19.7	17.7
Soyabean meal	10	7.5
Skim milk powder	4.5	4
Liver powder	2.5	5
Oil	4	4
Di calcium phosphate	0.2	0.2
Lime stone powder	0.1	0.1
Meat cum bone meal	2.5	2.5

3.2.2 Preparation of dog foods

The ingredients were weighed as per the standardized formulation and three types of dog foods were prepared i.e. raw, boiled and extruded. These were analyzed for proximate composition and physico-chemical attributes. Method of preparation of dog foods is described below:

3.2.2.1 Raw dried diets

Raw dried Diets were prepared using dried ingredients that were kept overnight in hot air oven at 100°C.

3.2.2.2 Boiled diets

100 g dried diets were boiled with 300 mL of water in pan for 15 minutes. Later on boiled feed was transferred into trays and kept overnight at 100°C in hot air oven for drying. After drying, the boiled feed was grinded.

3.2.2.3 Extruded Diets

The extruded diets were prepared in twin Screw extruder in the Department of Livestock Products Technology. All the ingredients were grinded in the mill and converted into the flour. All the ingredients were weighed as per the formulation and properly mixed in the mixer. The mixture was kept in vats. The die of desired product shape (star, bone, triangle and round) was fitted at the front portion of the extruder. The twin screw extruder was switched on. The main motor was switched on and the speed of the barrel was set to 35 Hz. The heating temperature in all three heaters was set as Heater 1: 80°C, Heater 2: 120°C, Heater 3: 160°C respectively. After reaching at set barrel temperature, the barrel of the extruder was cleaned with hot water. The mixture of ingredients (powder form) was filled in the feeding chamber. The feeding machine was switched on and the speed was fixed at 15 Hz. The ingredients mixture comes in the barrel and the finished product comes out of the machine. Initial product was discarded till a good quality extruded product came out. Then the cutter motor was switched on at the speed of 10 Hz. Extruded dog biscuits were collected in large vessels. The product was cooled for about 30 minutes.

3.2.3 *In vitro* nutrient digestibility analysis of dog foods

Dog diets i.e. control, 2.5% level of ghee residues, 5.0% level of ghee residues, 2.5% level of chicken liver powder and 5.0% level of chicken liver powder were analysed for in- vitro digestibility trials.

2.5% level of ghee residues and 2.5% level of chicken liver powder was found most suitable for the preparation of dog food.

3.2.4 Experiment 2: Comparative *in-vitro* evaluation of best levels of feed with all vegetarian dog food

The dog diets selected from the experiment no. 1 i.e. dog diets incorporated with 2.5% level of ghee residues and 2.5% chicken liver powder were compared with the all vegetarian diet (all the ingredients are from the vegetable sources). The formulation of all vegetarian diet is presented in table 4

Table 4: Formulation of all vegetarian dog diet

Ingredient	Quantity (%)	CP%	ME	EE	CF	Ca%	T. P%
Rice	50	7.44	3630	0.78	0.78	0.04	0.06
De-oiled rice polish	0	16	2100	1.5	23.15	0.07	1.33
Bengal gram	17	24.08	3120	4.95	4.4	0.08	0.13
Soya bean meal	10.5	45	2870	1.5	9.1	0.32	0.28
Skim milk powder	4.1	37	3590	0.285	0.22	1.2	0.993
Rice gluten meal	6	55.12	3150	3.9	5.02	0.62	0.78
Oil	4.8	-	8500	98	-	0	0
Di-calcium phosphate	0.9	-	0	-	-	23	18
Lime stone powder	0.5	-	0	-	-	35	0
Wheat bran	6.2	13.5	2100	2.1	17.3	0.12	1.38
Total	100	18.19	3521.14	6.7641	3.7695	0.543	0.416

(CP-crude protein, ME- metabolizable energy, EE-ether extract, CF-Crude fibre, Ca-calcium, T.P-total phosphorus)

3.3 Analytical Procedures

3.3.1 Proximate analysis and mineral estimation

The procured samples were analyzed for proximate composition viz. dry matter (DM), crude protein (CP), ether extract (EE), total ash (TA), acid insoluble ash (AIA), crude fiber (CF) and phosphorus as per AOAC (2005) and calcium (Talapatra et al., 1940).

3.3.1.1 Dry matter

About 100 g of food samples in duplicate were weighed in aluminum cups of known weight and dried in the oven at 100°C for overnight. The dried samples were weighed after cooling and DM was calculated as follows:

$$\text{DM\%} = \frac{\text{Weight of cups with oven dried sample} - \text{wt. of empty cup}}{\text{Wt. of sample before drying}} \times 100$$

3.3.1.2 Crude protein

The crude protein content was estimated as per method described in AOAC (2005) with suitable modifications using automatic digestion and distillation unit (Kel Plus-KES 12L, Pelican Industries, Chennai). Pre-weighed moisture free sample of approximately 0.2-0.3 g was digested in a Kjeldahl's digestion tubes after adding 10 ml of concentrated sulphuric acid and a pinch of digestion mixture (potassium sulphate and copper sulphate in 5:1 ratio) at 420ºC in the digestion unit. The appearance of clear green coloured liquid indicated the completion of digestion. The sample was cooled and then diluted with distilled water (10-20 ml). Exactly 40 ml of 40 percent sodium hydroxide was added to the aliquot to make it alkaline. Distillation was carried automatically in the distillation unit. The ammonia liberated during the process gets collected in boric acid containing indicator (Toshiro's indicator) placed at the receiver end of the distillation unit. The distillate obtained was titrated against standard N/10 hydrochloric acid to light pink end point. The percentage crude protein was calculated using the following formula. A parallel blank was run to eliminate the error.

$$\text{Nitrogen (\%)} = \frac{14.01 \times 0.1\ \text{N} \times (\text{TV} - \text{BV})}{\text{W} \times 1000} \times 100$$

$$\text{Crude protein (\%)} = \%\ \text{Nitrogen} \times 6.25$$

Where: 14.01 = Molecular weight of ammonia

0.1N = Titration solution’s normality

TV = Titre value

BV = Blank value

W = Sample weight in g

3.3.1.3 Ether Extract

The fat content of dog food samples was estimated by solvent extraction method as per AOAC (2005) using Socs Plus (SCS-6-AS, Pelican Industries, and Chennai). Two gram of dried, ground sample was taken in an extraction thimble (Whatsman No. 1 filter paper) fitted in a specially designed beaker. The initial weight of the empty beakers was noted (W_1).The thimbles with the samples were placed in the beakers containing around 80 ml of solvent (petroleum ether). The extraction was carried out automatically using 5 segments programme. After the process was over the beakers containing the fat residue were placed in hot air oven (100ºC) for 20-30 minutes. Than the beakers removed and cooled in desiccators. The final weight of the beakers was recorded as W_2. The fat percentage in the sample was calculated using the following formula:

$$\text{Fat (\%)} = \frac{\text{Final weight of beaker } (W_2) - \text{Initial weight of beaker}}{(W_1) \text{ Weight of sample in g}} \times 100$$

3.3.1.4 Crude Fibre

Weigh 1 g sample accurately and note down the weights. Transfer the weighed samples into oven dried crucibles. Place the crucibles into the metal adapters of Fibra Plus hot extract unit and ensure proper sealing of crucibles against the adapter rubber. In acid wash, pour 150 ml of 1.25% sulphuric acid into the extractors from the top for each sample. Set the initial temperature to 400°C and when the boiling starts reduce the temperature to 300°C. Allow the samples to boil for 45 minutes in acid. After 45 minutes boiling, drain the acid and wash the samples twice or thrice with distilled water. In alkali wash, pour 150 ml of 1.25% NaOH into the extractors from the top for each sample. Set the initial temperature to 400°C and after boiling reduce it to 300°C. Allow the samples to boil for 45 minutes in alkali. After 45 minutes boiling, drain the alkali and wash the samples twice or thrice with distilled water. During draining ensure that the knob is in vacuum mode and if draining is not effective due to clogging of sample in the crucible, then keep the knob in pressure mode, press the pressure button twice or thrice and immediately turn the knob to vacuum mode.

3.3.1.5 Ash content

The ash content was estimated as per AOAC (2005) method using muffle furnace. Around 2 g of moisture free sample was taken in pre-weighed moisture free crucibles. The crucibles were then placed on a hot plate for charring. After charring, the crucibles were transferred to muffle furnace set at 550°C for around 6 hrs. After cooling of the furnace the crucibles were taken out in desiccators and final weight is recorded. The ash content was calculated using the formula:

$$\text{Ash\%} = \frac{\text{(Final weight of the crucible + ash)} - \text{(Initial weight of the crucible)}}{\text{Weight of the sample}} \times 100$$

3.3.1.6 Organic matter

Organic matter was calculated by estimating total ash of weighed amount of dried sample.

3.3.1.7 Acid Insoluble Ash

The ash was transferred into 100 ml beaker with about 25 ml of (1:1) HCl and heated over hot plate to reduce the volume to half. Again 5 ml of concentrated HCl was added to the beaker and heated to complete dryness. Then added another 10 ml of (1:1) HCl into the beaker and heated for a few minutes. The content of the beaker was filtered through whatman No. 1 filter paper. The residue was quantitatively transferred from beaker to the filter paper and washed with distilled water till it became acid free. The filtrate was collected in 250 ml volumetric flask. The volume was made up to the mark with distilled water. The residue along with filter paper was transferred to the previously used silica crucible. Then it was charred over heater till it became smokeless. Ashing was completed in the muffle furnace at 700°C for 6 hours . Then it was cooled in the desiccator and weighed. The percent of acid insoluble ash was calculated as follows:

$$\text{AIA\%} = \frac{\text{Wt. of crucible with acid insoluble ash} - \text{Wt. of empty crucible}}{\text{Weight of the sample}} \times 100$$

3.3.1.8 Calcium

50 ml of aliquot (ash solution) was pipetted out into 250 ml beaker in duplicate. To this 10 ml of saturated ammonium oxalate solution was added and heated the solution to boiling. Then red litmus paper was added to the solution and mixed properly, which

was gradually neutralized with concentrated ammonia adding drop wise from pipette. Then the contents were boiled until white precipitates were coarsely crystalline. The pH of the liquid was adjusted with dilute (1:4) HCl until faint pink color of litmus paper appeared and it was then left for overnight. On the next day, it was filtered through Whatman No. 42 filter paper, washed the precipitate repeatedly by using hot distilled water till it became oxalate free (test with silver nitrate). Carefully removed the filter paper along with the precipitate and then transferred to the same beaker. 50 ml of 10 % sulphuric acid was poured on the filter paper and the precipitates were dissolved. The solution was warmed to about 70-80°C and was titrated with 0.1 N KMNO, to a faint pink end point. The filter paper was mashed, if the pink colour disappeared, then titrated again. Calcium percentage was calculated as follows:

$$\text{Ca\%} = \frac{\text{(ml of 0.1N } KMNO_4 \text{ (0.1ml of 0.1 N } KMNO_4 \text{ used for blank) x 0.002 x 250)}}{\text{25 x weight of sample}} \times 100$$

3.3.1.9 Available Phosphorus

Pipetted out 25 ml of the aliquots of acid insoluble ash into 250 ml of beakers in duplicate and added 10 ml of concentrated $HNO3$ and a piece of red litmus paper. Ammonia was added drop wise by using pipette with continuous stirring till blue colour appearance of litmus paper. Then few drops of dilute (1:10) HNO_3 was added to make solution slightly acidic. After that, 25 ml of ammonium molybdate solution was added and the precipitates were left for overnight. Next day, the precipitates were washed with distilled water till it became acid free and then filtered using Whatman filter paper No. 42. The filter paper along with the precipitates was transferred into the same beaker. Measured excess of 0.1 N NaOH was added to dissolve the precipitates and titrated against 0.1 N H_2SO_4 using phenolphthalein as indicator to colorless end point. The available phosphorus was calculated by using following formula:

$$\text{Av. P\%} = \frac{\text{(ml of 0.1N NaOH- ml of 0.1 N } H_2SO_4\text{- blank) x 0.067 x 250}}{\text{25 x weight of sample}} \times 100$$

3.3.2 Physiochemical properties

Physical properties like pH, Free Fatty acid (FFA), peroxide were recorded.

3.3.2.1 pH

The pH of dog food samples were determined as per the method described by Trout et al. (1992) with digital pH meter (FE-20-1-KIT, Mettler-Toledo India Pvt. Ltd., Mumbai) equipped with a combined glass electrode. Ten gram of sample was homogenized with 50 ml of distilled water for 1 min using pestle and mortar. The electrode was dipped into the suspension and the pH value of the sample was recorded.

3.3.2.2 Free fatty acid (FFA)

The FFA was estimated by the method of Koniecko (1979). 5 g of the sample was blended with 30 ml of chloroform in the presence of anhydrous sodium sulphate. The filtrate (Whatman filter paper No. 1) was added with 2 drops of 0.2 percent phenolphthalein indicator and titrated against 0.1N alcoholic KOH to get the end point (pink colour). The quantity of KOH consumed during the titration was recorded. Percent FFA content was calculated as,

FFA (%) = [(0.1 × ml of 0.1N alc. KOH × 0.282) / sample weight (g)] × 100

3.3.2.3 Peroxide value (PV)

The PV was determined by the method of Koniecko (1979). 5 g of RPC sample blended with 30 ml chloroform in the presence of anhydrous sodium sulphate. The filtrate (Whatman filter paper No. 1) was added with 30 ml of glacial acetic acid and 2 ml of saturated KI solution and 100 ml of distilled water and 2 ml of fresh 1 percent starch solution were added. The content was titrated against 0.1N sodium thiosulphate to get the end point (non-aqueous layer turned to colorless). It was calculated as,

PV (meq/kg sample) = [(0.1 × ml of 0.1N sodium thiosulphate)/sample weight (g)] × 1000

3.3.3 Estimation of *In-vitro* nutrient digestibility

In-vitro digestibility protocol as developed by Biagi et al. (2016) was used with some modifications. At the end of two digestion phases the undigested samples was filtered using nylon bag and washed with cold water instead of centrifugation.

3.3.3.1 Preparation of solutions

1 N HCl, 0.075N HCl, 1N NaOH and phosphate buffer solutions were prepared well in advance. Enzymes were added on the spot. Preparation of each solution is given as follows.

- **1 N HCl**

Molar mass = 36.46;

Volume= mass/density =36.46/1.18= 30.89 Concentration =35.4%

30.89 mL of HCl = (30.89)*(100/35.4)=87.28 mL

Hence, to prepare 1 N HCl add 87.28 Ml of HCl in 1000mL of distilled water (DW). Add 8.728 in 100Ml of DW

- **0.075 N HCl**

  Add 6.54 in 1000mL of distilled water. Add 13.08 in 2000 mL of DW

- **1N NaOH**

  Add 4g of NaOH crystals to 100 mL of distilled water

- **Phosphate buffer**

  Na_2HPO_4 = 3.72 g
  $NaHCO_3$ = 3.92 g
  NaCl = 0.19g
  KCl = 0.23g
  $MgCl_2$ = 0.12g
  $CaCl_2$ = 0.08 g in 1 L of distilled water

Above contents were mixed with magnetic stirrer and the pH of the phosphate buffer was adjusted to 7.5 with pH meter by adding HCl (1N) drop by drop.

Enzymatic solutions

- **Pepsin-HCl solution:** 2 g of pepsin and 1 g of lipase was weighed and added to 1L of 0.075N HCl solution and was mixed on magnetic stirrer.
- **Pancreatin-phosphate buffer solution:** 10 g of pancreatin was weighed and added to 1L of previously prepared buffer solution.

Above two enzymatic solutions were prepared fresh before each incubation phase.

3.3.3.2 Sample preparation:

One day before weighed amount of samples were kept in hot air oven at 100℃ until constant weight was obtained. It was finely ground after drying (<1mm particle size). Nylon bags were properly washed and kept in hot air oven at 65℃.

3.3.3.3 Gastric digestion simulation:

10g of food samples was weighed and put in to respective 1 L bottles and labeled. Sample and 400 mL of a pepsin-HCl solution (HCl 0.075N; pepsin 2 g/L) containing gastric lipase (1 g/L) were incubated in a 1 L bottle in a shaking water bath at 39°C for 2 h.

3.3.3.4 Small intestine digestion simulation

First, the pH level was adjusted to 7.5 with NaOH (1 N). Then, 400mL of a pancreatin solution was added to each bottle. Immediately prior to the addition of the pancreatin solution, bile salts (Cholic acid-Deoxycholic acid sodium salt mixture) were added to each bottle at a final concentration of 25 g/L. Finally, the bottles were placed in a shaking water bath at 39°C for 4 h.

3.3.3.5 Collection of the undigested fraction:

Nylon Bags were first labeled by tying knots and weighed empty after cooling. The undigested residue was filtered through nylon bags and washings were given with cold water and then nylon bags were tied securely and kept in hot air oven at 65℃ until constant weight. After weight of nylon bags was recorded and later analyzed for the determination of crude protein, ether extract and total ash according to AOAC standard methods (AOAC, 2005).

3.3.3.6 Calculation and data analysis:-

In order to determine the dry matter digestibility of the food samples, the residue obtained from each bottle after the *in-vitro* digestion was weighed and digestibility was calculated with the following equation:

Dry matter digestibility = (100- ([residue weight×100]/sample weight)

The undigested fraction was analyzed for crude protein, ether extract, and ash according to AOAC standard methods (AOAC, 2005). Nutrient digestibility was calculated with the following equation:

100-{[nutrient% in residue× (100-diet digestibility)]/ nutrient % in diet}

3.3.4 Aflatoxin estimation

3.3.4.1 Calibration settings

Mycotoxin calibration standards:

Instrument	Green	Red	Yellow
VICAM series 4 and 4EX	-2	110	54±5
Tor Bex Model FX-100 series 3	-2	120	59±5

Range : **0-500 ppb** Limit of detection : **2 ppb**

3.3.4.2 Preparation of solutions

Preparation of methanol: water solution: Add 80 mL of methanol to 20mL of water.

Afla test developer solution: Add 1mL to 9mL of water.

3.3.4.3 Sample extraction:

50g of ground sample was weighed and 5g of salt was added and was taken in blender jar. 100mLof methanol water solution was added to it and blended at high speed for 1 minute. The extract was filtered through fluted filter paper and collected in clean vessel.

3.3.4.4 Extract dilution

10mL of filtered extract was taken in clean vessel and was diluted with 40mL purified water and was mixed properly. Diluted extract was filtered through 1.5µm glass microfiber into clean vessel or directly into glass syringe barrel using marking on barrel to measure 2ml (2mL =0.2g sample equivalent).

3.3.4.5 Column Chromatography

2mL of filtered extract was passed through Afla Test column at the rate of about 1drop/second until air comes out through column. 5mL of purified water was passed through column at the rate of about 1-2drop/second twice. Cuvette (VICAM part #34000) was placed under column and 1 mL of HPLC grade methanol was added into glass syringe barrel. It was eluted at the rate of 1drop/second and collected in cuvette placed below. 1 mL of Afla test developer solution was added to cuvette and mixed properly. Cuvette was placed in calibrated fluorometer and readings were taken.

3.3.5 Statistical analysis

The data was analyzed using software package for social science (SPSS version 21.0) and SAS software. Duplicate samples was drawn for each parameter and the whole set of experiment will be repeated three times to have total number of observations. The average values was reported along with standard error. The statistical significance will be estimated at 5% level ($p<0.05$) and will be evaluated with Duncan’s Multiple Range Test (DMRT).

Plate 1: Extruded dog food and twin screw extruder

Plate 2: Bench top orbital shaker

Plate 3: Afla test column and Fluorometer

CHAPTER-IV

RESULT AND DISCUSSIONS

Under the present study entitled "Development of dog food by utilizing meat, egg and dairy industry byproducts", nutritional composition and physico-chemical quality of different poultry and dairy industry byproducts such as chicken skin, liver powder, gizzard powder, lard, hatchery waste and ghee residue was evaluated to explore the potential for the inclusion in the dog food. Individual ingredients were selected on the basis of proximate composition and physico-chemical quality. Selected food ingredients were incorporated at two different levels in the dog diet. Three different cooking methods were used. Later, diets were formulated using selected ingredients and were evaluated through *in- vitro* digestibility studies. The diets incorporated with best levels of byproducts were compared with all vegetarian dog food. These objectives were achieved by conducting two different experiments. The critical analysis of the results with suitable support of available literature to draw the inference, have also been attempted in the present chapter. The results are presented in the statistical analysed tables **(5-21).**

4.1 Experiment No. 1: Nutritional evaluation of meat, egg and dairy industry by-products

This experiment details the results obtained from the experiment carried out during our investigation in accordance with the above mentioned objectives. The results are presented in the statistically analyzed tables 5 to 16. These are critically discussed with support of suitable available literature to draw the inference.

4.1.1 Selection of meat, egg and dairy industry byproducts

Byproducts of meat industry (chicken skin, liver, gizzard) were collected from the Instructional Poultry Processing Plant, Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. Lard was collected from the butcher shops in local market of Ludhiana. Dairy industry byproduct (ghee residue) was collected from Dairy Processing Plant, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences. Egg industry byproduct (hatchery waste) was procured from hatchery of Department of Animal Genetics and Breeding. Poultry liver and gizzard were minced in the meat mincer (Mado Eskimo Mew-714, Mado, Germany) separately. Minced poultry liver and gizzard were air dried at 60℃ for 15 -16 hr in industrial tray dryer. After drying, the by-products were converted into powder form with help of grinder separately. These were stored in PET jar for the subsequent use. All the byproducts were analyzed for proximate composition and the results are presented in Table 5.

Among the meat and egg industry byproducts, liver powder has 60% crude protein, 18% EE, 0.6% crude fibre and 7.52% total ash. Gizzard powder was analyzed for its proximate composition and was found to have 58.6% crude protein, 17.45% EE, 0.75% crude fibre and 7.44% total ash. Chicken skin have 12.94% crude protein, 20.13% EE, 2.6% crude fibre and 3.50 % total ash. Hatchery waste was also analyzed for its proximate composition and have 22.73% crude protein, 4.06% EE, 10.8% crude fibre and 23.6% total ash. Ether extract content of lard was 99.45% and other nutrients were present in traces. Among dairy industry byproducts, ghee residue has 25.37% crude protein, 43% ether extract and crude fibre 0.4%.

The crude protein content in chicken liver powder and gizzard powder was significant ($P<0.05$) higher than the other byproducts, whereas the ether extract content of the ghee residue was highest among all the byproducts. Crude fibre content was highest in the hatchery waste, whereas the lowest was reported in the ghee residue. Ash content was reported highest for the hatchery waste as it includes the broken out egg shells. Calcium content was significant ($P<0.05$) higher than the other byproducts in hatchery waste, whereas the lowest value was reported for the chicken skin. Phosphorus content was highest in the gizzard powder.

Devatkal et al. (2004) studied the physicochemical, functional and microbiological quality of buffalo liver and reported that buffalo liver is an important edible meat byproduct. However, in developing countries including India, it has a low commercial value and is underutilized. Proximate composition was: moisture – 71.92%, protein – 18.44%, fat – 5.60%, carbohydrate – 2.72%, total ash – 1.32% and total energy – 135 kcal. Mineral concentrations (mg%) in liver were: Na – 60.04, K – 274, Ca – 5.60, Mg – 6.20, Fe – 20.86 and Cu – 5.60.

Table 5: Proximate and Mineral estimations of Meat, egg and Dairy Industry Byproducts (DMB)

Parameters	Meat, Egg and Dairy Industry Byproducts				
	Ghee Residue	**Liver Powder**	**Gizzard Powder**	**Chicken Skin**	**Hatchery Waste**
Dry matter	88.8±1.62^{b}	94.65±1.85^{a}	94.62±2.51^{a}	30.7±2.38^{d}	53.84±2.62^{c}
Crude protein	25.37±0.83^{b}	60±2.53^{a}	58.6±1.88^{a}	12.94±0.89^{d}	22.73±1.23^{c}
Ether extract	43±1.21^{a}	18±0.13^{c}	17.45±0.25^{c}	20.13±1.68^{b}	4.06±0.92^{d}
Crude fiber	0.41±0.03^{d}	0.62±0.04^{c}	0.75±0.08^{c}	2.61±0.32^{b}	10.8±1.62^{a}
Ash	3.98±1.42^{c}	7.52±0.68^{b}	7.44±0.72^{b}	3.50±0.21^{c}	23.6±1.23^{a}
Acid insoluble ash (AIA)	0.08±0.01^{d}	0.10±0.02^{d}	0.30±0.06^{c}	0.96±.05^{b}	3.20±0.62^{a}
Calcium	1.50±0.07^{b}	0.40±0.04^{c}	0.43±0.03^{c}	0.11±0.02^{d}	11.6±1.22^{a}
T. Phosphorus	0.31±0.04^{c}	0.52±0.03^{b}	0.70±0.06^{a}	0.16±0.03^{d}	0.46±0.02^{b}

N=6; The values are given on dry matter basis (DMB).

*Mean ± S.E. with different superscripts row wise (small alphabets) differ significantly ($P<0.05$).

4.1.1.1 Physicochemical Properties of different byproducts

Different byproducts viz. chicken skin, liver powder, gizzard powder, lard, ghee residue and hatchery waste were analysed for physiochemical properties (pH, free fatty acid and peroxide value). The results are presented in statistically analysed Table 6. pH value of all ingredients was found to be in acidic range. The pH values of Gizzard powder was significantly ($P<0.05$) lower than all other byproducts. Peroxide value and free fatty acid content was determined to check the rancidity of fat content in dog food ingredients. Free fatty acid content of all byproducts except lard was less than the critical limits ranging from 0.46 to 1.11 % which indicate that ingredient were of good quality. Peroxide value of ghee residue, liver powder, gizzard powder, chicken skin and hatchery waste was 2, 2, 4 , 6 and 6 (mEq/kg) respectively. Peroxide value and free fatty acid content of lard was significantly ($P<0.05$) higher than the other byproducts. Osawa et al. (2008) reported the value of FFA and PV of pet food in the range of 4.6±0.1 to 28.0±0.6% oleic acid and 1.4±0.1 to 6.8±0.3 meq O_2/kg respectively. Pearson (1968) stated that minced beef had FFA content in the range of

0.38 to 1.74% and had a maximum acceptability limit of 1.8% FFA in view of their progressive increase during storage.

Table 6: Physicochemical Properties of different byproducts:

Ingredients	pH	FFA (% oleic acid)	PV (mEq/kg)
Ghee Residue	6.1±0.06^{a}	0.75±0.05^{b}	2.0±0.32^{d}
Liver Powder	5.7±0.05^{b}	0.46±0.07^{d}	2.0±0.85^{d}
Gizzard Powder	3.6±0.03^{d}	0.67±0.02^{c}	4.0±0.61^{c}
Chicken Skin	4.8±0.03^{c}	0.91±0.08^{b}	6.0±0.26^{b}
Hatchery waste	6.7±0.05^{a}	1.11±0.08^{b}	6.0±0.52^{b}
Lard	6.4±0.05^{a}	11.15±0.18^{a}	16.0±0.93^{a}

N=6; *Mean±S.E. with different superscripts column wise (small alphabets) differ significantly ($P<0.05$).

4.1.1.2 Aflatoxin content of different byproducts:

Aflatoxin content of meat, egg and dairy industry byproducts (hatchery waste, ghee residue, liver powder, gizzard powder and chicken skin) is depicted in table 7. Aflatoxin content in liver powder was 21 ppb. High amount of aflatoxin content was present in Hatchery waste i.e. 47 ppb. Aflatoxin content of chicken skin and gizzard powder was 41 ppb and 33 ppb respectively. Lowest aflatoxin content was present in ghee residue (7.1 ppb).

Table 7: Aflatoxin content of the different byproducts :

Ingredients	Aflatoxin (ppb)
Liver Powder	21±1.34^{c}
Ghee Residue	7.1±0.88^{d}
Hatchery waste	47±2.56^{a}
Gizzard Powder	33±1.72^{b}
Chicken Skin	41±2.47^{a}

N=6; *Mean±S.E. with different superscripts column wise (small alphabets) differ significantly ($P<0.05$).

On the basis of proximate analysis, physico-chemical properties and aflatoxin content in the different byproducts, liver powder and ghee residue were found most suitable for the incorporation in the formulation of dog food.

4.1.2 Formulation of control dog food

Common food ingredients i.e. Rice, De-oiled rice polish (DORP), Bengal gram, Soya bean meal (SBM), Skim milk powder (SMP), Meat cum bone meal (MBM), Rice gluten meal (RGM) were analysed for proximate composition and the proportion of the ingredients were calculated using wet chemistry analysis in MS Excel using two selected ingredients at different levels. Diets were formulated for adult Labrador dogs according to the nutritional requirements as given by AAFCO (2014) containing 18% CP, 5.5% EE, 0.5% Ca and 0.4% P. Energy density of diet was kept 3500 kcal ME/kg in accordance with Indian climatic conditions as described by ICAR (2013). The formulation of control diet is computed as follows: rice (49%), de-oiled rice polish (5%), bengal gram (20%), soya bean meal (12%), skim milk powder (4%), meat cum bone meal (2.5%), rice gluten meal (2%) and di-calcium phosphate (2.5%).

4.1.2.1 Proximate and mineral composition of the ingredients selected for preparation of different dog food formulation

The results for the proximate and mineral composition of the ingredients are presented in Table 8. Proximate analysis of rice showed that it contains 7.44 percent CP, 0.78 percent EE and 0.78 percent CF and ME was taken as 3630 kcal / kg as per Kumar et al. (2014). DORB evaluation showed that it had the highest amount of crude fibre i.e. 23.15 percent, it have 16% CP and 1.5% EE which is in close agreement with the findings of Bhanja et al. (2001). ME value of DORP was taken 2100 kcal/Kg. In Bengal gram CP, EE, CF, calcium and phosphorus were found to be 24.08, 4.95, 4.4, 0.08 and 0.13 % respectively. Dhaliwal (2015) also reported almost similar composition, except for ether extract and crude fibre.

Soybean meal used in this study had 45% crude protein with significant quantity of crude fibre, i.e. 6.8%. The meat cum bone meal was used as animal protein source and rich source of calcium and phosphorus. MBM contained 41.87% CP with 13.86% calcium and 6.78% phosphorus content, which was comparable with the standard composition in red meat handbook APEDA (2000). Skim milk powder had 37 % CP, 0.285 % EE, 1.2% Ca and 0.993% P content, which was consistent with the findings of Gopalan et al. (2004). Rice gluten meal selected for formulation of control dog food had high protein content (55.12%) and moderate amounts of EE

(3.9%) and crude fibre content 5.02%, 0.62% Ca and 0.78% P (Metwally and Farahat, 2015).

Table 8: Proximate composition and mineral content of the ingredients (on dry matter basis)

Ingredient	DM%	CP%	ME	EE%	CF%	Ca%	T. P%
Rice	89.38	7.44	3630[1]	0.78	0.78	0.04	0.06
De-oiled rice polish (DORP)	88.7	16	2100[2]	1.5	23.15	0.07	1.33
Bengal gram	89.38	24.08	3120[3]	4.95	4.4	0.08	0.13
Soya bean meal (SBM)	87.17	45	2870[3]	1.5	6.8	0.32	0.28
Skim milk powder (SMP)	95.8	37	3590[4]	0.285	0.22	1.2	0.993
Meat cum bone meal (MBM)	90.9	41.87	2500[5]	5.9	5.944	13.86	6.78
Rice gluten meal(RGM)	89.4	55.12	3150	3.9	5.02	0.62	0.78
Ghee Residue (GR)	88.8	25.37	5078	43	0.4	1.5	0.3
Liver Powder (LP)	86.2	60	3945	18	0.6	0.4	0.52

1Kumar et al. (2014); 2 Bhanja et al. (2000); 3 Dhaliwal. (2015); 4 Gopalan et al. (2004); 5 LPEDA)

Since the nutritional requirements of dog are given on dry matter basis, Nevertheless, the values reported on dog and cat food labels are often given on an "as is" or "as fed" (AF) basis. This inconsistency makes direct comparison between the values listed on a pet food label and the profile table values impossible without first correcting moisture level so that both are on an equal-moisture basis. Thus, moisture correction was done by dividing each AF value by the proportion of DM in the food [(100 - % moisture)/100]. Chicken liver powder and ghee residues were incorporated at the level of 2.5% and 5.0% in the control dog diet after replacing the rice gluten and soybean meal. The standardized formulation of dog diets incorporated with 2.5% Ghee residue, 5.0% ghee residue, 2.5% liver powder and 5.0% liver powder are presented in Table 2 and Table 3 under materials and methods section. Diets were formulated using dried ingredients and later on subjected to different processing techniques (boiling and extrusion). The ingredients were weighed as per the standardized formulation and three types of dog foods were prepared i.e. raw, boiled and extruded. The proximate composition of developed diets were analyzed and the results are presented in Table 9.

4.1.3 Proximate composition of raw dog food

The value of crude protein content of dog food containing 5% liver powder, 5% ghee residue and control diet was 19.25% . Similarly, the CP content of dog food containing 2.5 ghee residue and 2.5% liver powder were found to be uniformly 18.81%. The ether extract value was highest (5.475%) in dog food containing 5% LP and lowest (5.225%) in dog food containing 2.5% GR as depicted in (Table 9). Crude fiber was highest (6.15%) in dog food containing 5% GR and lowest (4.1%) in dog food containing 2.5% LP. Crude fiber content of dog foods containing 2.5% GR, 5% LP and control diet was 5.1, 4.7 and 5.7% respectively. Total Ash (TA) content in these five diets varied between 3.50 to 4.24% with maximum value in the diet having 5% LP and minimum in control diet.

4.1.4 Proximate composition of boiled dog food

The proximate composition of boiled food is presented in Table 9. The crude protein content of control diet and dog food containing 2.5% liver powder and 2.5% ghee residue was 18.81%. The crude protein content of dog food containing 5% Ghee Residue and 5% liver powder was 19.25% . Ether extract was highest (3.925%) in dog food containing 2.5% liver powder and lowest (3.5%) in control diet. Among boiled dog foods, dog food containing 2.5% liver powder had the lowest (4.15%) crude fiber content whereas boiled dog food containing 5% ghee residue had highest CF content i.e. 5.35%.

It was observed that on boiling both ether extract and crude fiber decreased in diets. High ash content of 4.6% was found in dog food containing 2.5% LP, hence had the lower organic matter content. Total ash content in boiled dog foods containing 2.5% GR, 5% GR, 5% LP and control diet was 4.56, 4.47, 4.44 and 4.08% respectively.

4.1.5 Proximate composition of extruded dog foods

The crude protein content of extruded control dog food, and dog foods containing 2.5% ghee residue and 2.5% liver powder was 19.25, 18.81 and 19.25% respectively. EE was lowest (4.85%) in extruded dog food containing 2.5% GR and highest (5.13%) in dog food containing 5% LP. Dog food having 5% GR had highest crude fibre content i.e. 5.54%. Extruded dog food containing 2.5% LP and control dog food has 4.08% and 5.32% of crude fibre content respectively. Total ash content of dog food containing 2.5% ghee residue, 5% ghee residue and 2.5% liver powder dog food was 4.03, 4.01 and 4.08 and highest TA content was observed in dog food incorporated with 5 % liver powder i.e. 4.11 (Table 9).

Table 9: Proximate composition of different types of dog foods

Control Dog Food			
Parameter	**Raw**	**Boiled**	**Extruded**
CP	19.25 ± 0.08^{a}	18.81 ± 0.09^{b}	19.25 ± 0.12^{a}
EE	5.375 ± 0.18^{a}	3.55 ± 0.12^{c}	4.98 ± 0.15^{b}
CF	5.70 ± 0.065^{a}	5.25 ± 0.042^{b}	5.32 ± 0.07^{b}
Total Ash	3.50 ± 0.81^{c}	4.08 ± 0.27^{a}	3.91 ± 0.43^{a}
OM	96.5 ± 0.28^{a}	96.92 ± 0.82^{a}	96.09 ± 0.62^{a}
2.5% Ghee residue			
CP	18.81 ± 0.08^{a}	18.38 ± 0.12^{b}	18.81 ± 0.25^{a}
EE	5.225 ± 0.62^{a}	3.50 ± 0.22^{c}	4.85 ± 0.51^{b}
CF	5.10 ± 0.07^{b}	4.80 ± 0.12^{b}	4.92 ± 0.15^{b}
Total Ash	4.24 ± 0.61^{a}	4.56 ± 0.94^{a}	4.03 ± 0.82^{b}
OM	95.76 ± 0.42^{a}	95.44 ± 0.18^{b}	95.97 ± 0.28^{a}
5% Ghee residue			
CP	19.25 ± 0.12^{a}	18.81 ± 0.25^{b}	19.25 ± 0.09^{a}
EE	5.325 ± 1.17^{a}	3.77 ± 0.66^{b}	4.92 ± 0.78^{b}
CF	6.15 ± 0.16^{a}	5.35 ± 0.11^{b}	5.54 ± 0.14^{b}
Total Ash	4.13 ± 0.91^{b}	4.47 ± 0.68^{a}	4.01 ± 0.82^{b}
OM	95.87 ± 0.10^{a}	95.53 ± 0.33^{a}	95.99 ± 0.29^{a}
2.5% Liver Powder			
CP	18.81 ± 0.12^{b}	18.81 ± 0.08^{b}	19.25 ± 0.15^{a}
EE	5.30 ± 0.60^{a}	3.925 ± 0.55^{b}	5.13 ± 0.46^{b}
CF	4.10 ± 0.23^{a}	4.15 ± 0.34^{a}	4.08 ± 0.43^{a}
Total Ash	4.17 ± 0.22^{b}	4.60 ± 0.16^{a}	4.14 ± 0.31^{b}
OM	95.83 ± 0.78^{a}	95.40 ± 0.84^{b}	95.86 ± 0.69^{a}
5% Liver powder			
CP	19.25 ± 0.12^{a}	18.38 ± 0.25^{b}	19.25 ± 0.18^{a}
EE	5.475 ± 0.15^{a}	3.60 ± 0.18^{b}	5.18 ± 0.19^{a}
CF	4.70 ± 0.12^{a}	4.35 ± 0.17^{b}	4.46 ± 0.09^{b}
Total Ash	4.24 ± 0.66^{a}	4.44 ± 0.52^{a}	4.11 ± 0.28^{a}
OM	95.76 ± 0.34^{a}	95.56 ± 0.48^{a}	95.89 ± 0.72^{a}

(DM-dry matter, CP-crude protein , EE-ether extract, OM-organic matter)
Figures with different superscripts in each row differ significantly ($P \leq 0.05$)

The perusal of data of proximate composition of dog foods subjected to different processing techniques revealed that ether extract and crude fiber contents reduced after boiling of dog food. Extrusion had almost no effect on crude protein content. However EE value slightly decreased after boiling in all the diets. Ash content of boiled diet was higher than extruded and raw diets. Shruthi et al. (2016) observed that fat content in the corn during extrusion reduces as the temperature increases. This may be due to the burning of fat that takes place during the extrusion process due to high temperature. Kajihausa et al (2014) observed the decrease in crude protein and crude fiber content of sprouted sesame flour after boiling. This may be due to leaching of soluble component into cooking water (Yagoub and Abdalla, 2007). Nsa et al. (2011) reported that crude fiber content of undecorticated castor oil seeds reduced after boiling. This may be due to softening of fiber fraction during boiling.

4.1.6 *In-vitro digestibility* analysis of different types of dog foods

Dog diets were prepared by incorporating 2.5% Ghee residue, 5.0% Ghee residue, 2.5% liver powder and 5.0% liver powder in the standardized formulation and subjected to different processing techniques (boiling and extrusion). All the diets were analysed by in-vitro digestibility trials. The results are presented in the statistical analysed tables (10-14).

4.1.6.1 *In-vitro* nutrient digestibility of control dog food prepared by different processing techniques

In-vitro nutrient digestibility of control group is illustrated in table 10**.** Dry matter digestibility (DMD) and organic matter digestibility (OMD) improved significantly ($P<0.05$) as the processing of raw dog food was done either using boiling or extrusion method. Extruded diets had highest ($P<0.05$) DMD, ether extract digestibility (EED) and organic matter digestibility (OMD) among different processing techniques. There was significant ($P<0.05$) difference in crude protein digestibility (CPD) among different processing techniques. Significant difference was observed between EED of raw and extruded dog food. EED (83.86%) reduced significantly ($P<0.05$) in case of boiled diets, fat could have liquefied into the boiling water during the boiling of diets, thereby causing a drop in fat content (Lola, 2009). DMD and OMD of boiled dog foods was significantly ($P<0.05$) higher than raw dog food but significantly ($P<0.05$) lower than that of extruded diets.

Table 10: *In-vitro* nutrient digestibility of control dog food prepared by different processing techniques

Parameters	In-vitro digestibility%			P-value
	Raw	Boiled	Extruded	
DM	77.11±0.14^{c}	80.18±0.24^{b}	86.08±0.12^{a}	<0.05
CP	90.63±0.11^{b}	91.24±0.08^{b}	92.72±0.06^{a}	<0.05
EE	94.69±0.08^{a}	83.86±0.07^{b}	96.05±0.04^{a}	<0.05
OM	83.51±0.24^{c}	87.32±0.32^{b}	91.63±0.36^{a}	<0.05

N=6, (DM-dry matter, CP-crude protein, EE-ether extract, OM-organic matter).
Figures with different superscripts in each row differ significantly ($P \leq 0.05$)

4.1.6.2 *In-vitro* nutrient digestibility of dog food with 2.5% ghee residue prepared by different processing techniques

In-vitro digestibility with respect to various nutrients in 2.5% ghee residue-based dog food subjected to different processing techniques (e.g. dried, boiled and extruded) is depicted in table 11. There was significant difference between DMD of extruded (84.45%), boiled dog food (80.85%) and raw diet (74.28%). The DMD of extruded dog food was significantly higher (P<0.05) than both boiled and raw diet. Crude protein digestibility of boiled dog food (90.64%) was significantly lower (P<0.05) than extruded dog food (91.65%) but it was significantly higher (P<0.05) than raw dog food (89.23%), El-Adawy (2002) reported that significant increase in *in- vitro* protein digestibility (IVPD) of unprocessed chickpea (*Cicer arietinum*) after cooking for 90 min. Park et al. (2010) also reported that apparent ileal digestibility of CP for extruded pea seeds were higher than unprocessed pea seeds, suggesting that the extrusion process improves protein digestibility. There was slight difference between EED of raw (94.43%) and extruded diet (95.09%). EED declined significantly (P<0.05) after boiling (83.85%). OMD of boiled dog food was significantly higher (P<0.05) than raw diet i.e. 87.48.There is also significant difference in OMD between boiled and extruded diet.

Table 11: *In-vitro* nutrient digestibility of dog food with 2.5% ghee residue prepared by different processing techniques

Parameters	*In-vitro* digestibility (%)			P-value
	Raw	Boiled	Extruded	
DM	74.28±0.16^{c}	80.85±0.22^{b}	84.46±0.20^{a}	<0.05
CP	89.23±0.06^{b}	90.64±0.06^{a}	91.65±0.08^{a}	<0.05
EE	94.43±0.11^{a}	83.85±0.21^{b}	95.09±0.05^{a}	<0.05
OM	82.33±0.63^{c}	87.48±0.52^{b}	91.01±0.32^{a}	<0.05

N=6, (DM-dry matter, CP-crude protein , EE-ether extract, OM-organic matter).
Figures with different superscripts in each row differ significantly ($P \leq 0.05$)

4.1.6.3 *In-vitro* nutrient digestibility of dog food with 5% ghee residue prepared by different processing techniques

The *in-vitro* nutrient digestibility of dog food containing 5% ghee residue is presented in table 12. Significant difference ($P<0.05$) was recorded in DMD of boiled (79.28%) and extruded (83.01%) dog food. DMD (72.28%) was lowest ($P<0.05$) in case of raw diet. The CPD of extruded diet was significantly higher ($P<0.05$) than both boiled and raw diet. Lankhorst et al. (2007) also reported that extrusion of canine diets at temperatures of 110, 130 and 150ºC caused no reduction in digestibility of CP however it increased the digestibility of starch. There was no significant difference in EED of raw (95.84%) and extruded (93.08%) diet, however EED was significantly lowest ($P<0.05$) i.e. 83.09% in boiled diet. OMD of boiled (86.41%) and extruded dog food (90.08%) had significant difference, it was significantly lowest ($P<0.05$) in raw (81.04%) diet .

Table 12: *In-vitro* nutrient digestibility of dog food with 5% ghee residue prepared by different processing techniques

Parameters	*In-vitro* digestibility (%)			P- Value
	Raw	Boiled	Extruded	
DM	72.28±0.78^{c}	79.28±0.66^{b}	83.01±0.62^{a}	<0.05
CP	88.66±0.11^{b}	88.91±0.08^{b}	90.73±0.06^{a}	<0.05
EE	93.64±0.12^{a}	83.09±0.25^{b}	94.40±0.18^{a}	<0.05
OM	81.04±0.28^{c}	86.41±0.41^{b}	90.08±0.22^{a}	<.005

N=6, (DM-dry matter, CP-crude protein, EE-ether extract, OM-organic matter),
Figures with different superscripts in each row differ significantly ($P \leq 0.05$)

4.1.6.4 *In-vitro* nutrient digestibility of dog food with 2.5% Liver powder prepared by different processing techniques

Nutrient digestibility of dog food containing 2.5% liver powder is presented in table 13. The DMD of extruded dog food was significantly higher (P<0.05) than the raw and boiled dog diets. The DMD of raw diet (76.85%) was significantly (P<0.05) lower than the boiled dog food (80.48%). Significant difference (P<0.05) in the CPD was observed in dog food when subjected to different processing techniques viz. raw (89.77%), boiled (91.37%) and extruded (92.54%). EED was significantly (P<0.05) lower in boiled diets (84.13%) (fig.4).

Table 13: *In-vitro* nutrient digestibility of dog food with 2.5% Liver powder prepared by different processing techniques

Parameters	*In-vitro* digestibility%			P-value
	Raw	Boiled	Extruded	
DM	76.85 ± 0.72^{c}	80.48 ± 0.63^{b}	85.08 ± 0.52^{a}	<0.05
CP	89.77 ± 0.06^{b}	91.37 ± 0.07^{a}	92.54 ± 0.04^{a}	<0.05
EE	95.23 ± 0.21^{a}	84.13 ± 0.41^{b}	95.78 ± 0.18^{a}	<0.05
OM	83.76 ± 0.45^{c}	87.86 ± 0.56^{b}	91.78 ± 0.38^{a}	<.005

N=6, (DM-dry matter, CP-crude protein, EE-ether extract, OM-organic matter),
Figures with different superscripts in each row differ significantly ($P \leq 0.05$)

EED of raw and extruded diets had non-significant difference but these were significantly higher (P<0.05) than boiled diets. OMD was significantly highest (P<0.05) in extruded diets and the OMD of raw (80.61%) dog food was lowest among all the treatments.

4.1.6.4 *In-vitro* nutrient digestibility of dog food with 5% Liver powder prepared by different processing techniques

Nutrient digestibility of dog food containing 5% Liver powder is depicted in table 14. DMD of boiled diet containing 5% LP was significantly (P<0.05) higher (79.13%) than raw diet (75.19%) but it was significantly lower (P<0.05) than extruded diet (83.92%) (fig.5). There was significant difference in CPD of raw (88.72%), boiled (90.05%) and extruded diets (83.92%). EED was significantly lower in case of boiled diets (83.01%), however there was non-significant (P>0.05) difference in EED of raw (94.83%) and extruded (95.43%) diets. The OMD of boiled dog food (86.45%) was significantly lower (P<0.05) than extruded dog food (90.96%), however it was significantly higher (P<0.05) than raw diet (82.56%).

Table 14: *In-vitro* nutrient digestibility of dog food with 5% Liver powder prepared by different processing techniques

Parameters	*In-vitro* digestibility %			P-value
	Raw	Boiled	Extruded	
DM	75.19±0.22^{c}	79.13±0.32^{b}	83.92±0.51^{a}	<0.05
CP	88.72±0.04^{b}	90.05±0.06^{a}	91.59±0.09^{a}	<0.05
EE	94.83±0.15^{a}	83.01±0.67^{b}	95.43±0.11^{a}	<0.05
OM	82.56±0.26^{c}	86.45±0.16^{b}	90.96±0.22^{a}	<.005

(DM-dry matter, CP-crude protein, EE-ether extract, OM-organic matter) Figures with different superscripts in each row differ significantly ($P \leq 0.05$)

4.1.7 Effect of processing techniques and incorporation of liver powder and ghee residue (GR) on *in-vitro* digestibility (%) of nutrients in dog diets.

4.1.7.1 Effect of processing techniques and level of ghee residue (GR) on *in-vitro* digestibility (%) of nutrients in dog diets.

This study was conducted to assess the impact of the processing technique (i.e. raw, boiled and extruded) and the amount of ghee residue (0, 2.5 and 5%) in dog food on *in-vitro* nutrient digestibility. The analysis of these findings (Table 15) showed that the processing technique had a major effect on the percentage of dry matter digestibility (DMD) regardless of the ghee residue present on the diet. Significantly higher (P≤0.01) DMD was found in extruded diets (84.52%) regardless of the level of ghee residue. Significantly lowest (P≤0.01) DMD was detected in the raw diet (74.55%). The dry matter digestibility in boiled dog food was 80.10%, which was significantly higher (P≤0.01) than the raw dog food but significantly lower (P≤0.01) than the extruded dog food.

Crude protein digestibility (CPD) and organic matter digestibility (OMD) was effectively improved (P≤0.01) with type of processing techniques from raw to boiled and extrusion. Crude protein digestibility and organic matter digestibility were significantly lower (P≤0.01) for the raw diets as compared to boiled dog food CPD (90.26%), boiled dog food OMD (87.07%), extruded dog food CPD (91.70%) and extruded dog food OMD (90.91%). Hullar et al. (1998) and Overland et al. (2007)

revealed that the crude protein digestibility of cat and dog foods has not been impaired by extrusion process. Ether extract digestibility (EED) was not affected by extrusion. The raw dog food EED (94.25%) was comparable with extruded dog food EED (95.18%), but it was significantly ($P \leq 0.01$) higher than the boiled dog food EED (83.60 %). Cipollini (2008) found that the digestibility of the ether extract of dry dog food was greater than that of wet dog food (94.9 ± 3.69 vs 87.7 ± 0.82 percent).

In vitro digestibility trials carried out to find out the effect of incorporation of ghee residue in dog food, irrespective of the processing methods used (Table 15) showed that the use of ghee residue had a substantial effect on the digestibility of nutrients. Dry matter digestibility was observed significantly higher ($P \leq 0.01$) at 0% inclusion level (81.12%). The DMD (79.86%) significantly reduced ($P \leq 0.01$) when inclusion level was increased to 2.5%. and was further reduced at the inclusion level at 5% ghee residue. Non-significant ($P > 0.01$) difference was observed in CPD either when 2.5% ghee residue (90.51%), or 5% ghee residue (89.43%) was included in the dog food. CPD was significantly higher ($P \leq 0.01$) (91.53%) when 0% inclusion level of ghee residue was used.

Inclusion of ghee residue in the dog food showed non-significant ($P > 0.01$) difference with regards to ether extract digestibility when inclusion level of ghee residue was 0 and 2.5% and was significantly lower ($P \leq 0.01$) in 5% ghee residue inclusion level. Significantly higher ($P \leq 0.01$) organic matter digestibility (OMD) (87.48%) was seen when 0% ghee residue was added in the dog food. Significantly lower OMD (85.84%) was in the group where 5% GR was added. At 2.5% of GR inclusion, the OMD was significantly higher (86.94%) than the 5% GR supplemented group.

As *in-vitro* digestibility (%) of most of the nutrients in extruded dog food was significantly higher than the other processing technique (raw and boiled), hence the extrusion was selected as cooking method for the processing of dog food. In the dog diets incorporated with different levels of ghee residue (2.5 and 5%), in-vitro digestibility (%) of most of the nutrients was observed highest for the dog diets incorporated with 2.5% ghee residue, hence the level of incorporation at 2.5% was adjudged best for the preparation of dog food.

Table 15: Effect of processing technique used and level of ghee residue in the dog food on *in vitro* digestibility (%) of nutrients

Parameters	Processing technique[1] (P)			Level of ghee residue [2], % (L)			PSE	P-value		
	Raw	Boiled	Extruded	0	2.5	5.0		P	L	P×L
DM	74.55±0.62^{c}	80.10±0.44^{b}	84.52±0.32^{a}	81.12±0.22^{A}	79.86±0.78^{B}	78.19±0.96^{C}	1.18	<0.001	<0.001	<0.001
CP	89.51±0.18^{b}	90.26±0.09^{b}	91.70±0.10^{a}	91.53±0.14^{A}	90.51±0.44^{A}	89.43±0.72^{B}	0.394	<0.001	<0.001	<0.001
EE	94.25±0.26^{a}	83.60±0.38^{b}	95.18±0.12^{a}	91.53±0.18^{A}	91.12±0.25^{A}	90.38±0.06^{B}	1.276	<0.001	<0.001	<0.001
OM	82.29±0.35^{c}	87.07±0.62^{b}	90.91±0.45^{a}	87.48±0.14^{A}	86.94±0.22^{A}	85.84±0.26^{B}	0.577	<0.001	<0.001	<0.001

[1] Irrespective of level of ghee residue in the diet; [2] Irrespective of processing technique; Means with different superscripts^{a,b,c} for different processing techniques and superscripts^{A,B,C} for different levels of ghee residue with in a row differ significantly; PSE Pooled standard error

Table 16: Effect of processing technique used and level of Liver powder in the dog food on *in vitro* digestibility (%) of nutrients

Parameters	Processing technique[1] (P)			Level of liver powder [2], % (L)			PSE	P-value		
	Raw	**Boiled**	**Extruded**	**0**	**2.5**	**5.0**		**P**	**L**	**P×L**
DM	76.37±0.22^{a}	79.93±0.36^{b}	85.03±0.52^{c}	81.12±0.26^{A}	80.80±0.65^{A}	79.41±0.61^{B}	0.9605	<0.001	<0.001	<0.001
CP	89.71±0.76^{b}	90.89±0.16^{b}	92.28±0.12^{b}	91.53±0.12^{A}	91.23±0.16^{A}	90.12±0.22^{B}	0.394	<0.001	<0.001	<0.001
EE	94.92±0.19^{b}	83.67±0.11^{a}	95.75±0.13^{b}	91.53±0.15^{A}	91.71±0.19^{A}	91.09±0.11^{A}	0.378	<0.001	0.01	0.32
OM	83.28±0.32^{a}	87.21±0.61^{b}	91.46±0.18^{c}	87.48±0.25^{A}	87.80±0.16^{A}	86.65±0.12^{B}	0.979	<0.001	0.01	0.12

[1] Irrespective of level of liver powder in the diet; [2] Irrespective of processing technique; Means with different superscripts^{a,b,c} for different processing techniques and superscripts^{A,B,C} for different levels of liver powder with in a row differ significantly; PSE Pooled standard error

4.1.7.2 Effect of processing techniques and level of liver powder (LP) on *in-vitro* digestibility (%) of nutrients in dog diets.

Effect of processing technique (raw, boiled and extruded) and level of liver powder (0, 2.5 and 5 %) on *in-vitro* digestibility (%) of nutrients in dog food is presented in table 16. Analysis of data revealed that processing technique irrespective of the level of liver powder in dog diet significantly influence digestibility of all nutrients. DMD (irrespective the level of liver powder in diet) was highest ($P \leq 0.01$) in extruded diets (85.03%) and lowest ($P \leq 0.01$) in case of raw dog food (76.37%). DMD of boiled diet (79.93%) was significantly higher ($P \leq 0.01$) than raw diets but significantly lower than extruded diets.

Crude protein digestibility (CPD) significantly ($P \leq 0.01$) differ when diets were subjected to either boiling (90.89%) or extrusion (92.08%). CPD of raw diets (89.71%) was significantly lower ($P \leq 0.01$) than both boiled and extruded diets. Drulyte et al. (2019) and Park et al. (2010) also reported that *in-vitro* protein digestibility (IVPD) increases significantly after cooking. Alonso et al. *(*2000) also reported that extrusion increases *in-vitro* protein digestibility as compared to other processing method e.g. dehulling or soaking.

Ether extract digestibility (EED) remained unaffected after extrusion. EED ($P>0.01$) of raw and extruded dog food was 94.92 and 95.75% respectively. These findings are in covenant to the findings of Baigi et al. (2016) who reported the ether extract digestibility of extruded dog food between 93 and 99%. However, EE digestibility of boiled dog food (83.67%) was significantly ($P \leq 0.01$) lower than both raw and extruded dog food. Hur et al. (2014) reported that in *in-vitro* human digestion model, total lipid digestibility was lower in the boiled samples as compared to microwave and oven cooked samples.

Same trend was followed in organic matter digestibility (OMD) as in DMD of extruded dog foods i.e. 91.46% was highest ($P \leq 0.01$) for the extruded diets among different processing techniques and for raw dog food it was (83.28%), which was lowest ($P \leq 0.01$) among all the treatments. OMD of boiled dog food (87.21%) was significantly lower ($P \leq 0.01$) than the extruded diet but it was significantly higher ($P \leq 0.01$) than the raw dog food.

The effect of incorporation of liver powder, irrespective of processing methods on nutrient digestibility is depicted in Table 16.

Non-significant (P>0.01) difference was observed in DMD of diet at 2.5 % liver powder (LP) inclusion level (80.80%) than the 0% LP inclusion level (81.12%). DMD at 5 % LP level was significantly lower (P≤0.01) (79.41%) than both 2.5% LP supplementation group and group in which LP was not supplemented. Burrows et al. (1982) revealed that with the increase in fibre content in dog diet, decreases the intestinal transit time. Decreased intestinal transit time lead to reduced dry matter digestibility.

Crude protein digestibility (CPD) of dog food containing 2.5% LP (i.e. 91.23%) was comparable with that of 0% liver powder inclusion level (91.53%). While at 5% inclusion level, CPD (90.12%) was lowest (P≤0.01) among all the treatments. Burkhalter et al. *(*2001) also reported that protein digestibility decreases with increase in fibre content. Supplementation of liver powder in the dog food showed non-significant (P>0.01) difference w.r.t. to ether extract digestibility. Organic matter digestibility for 2.5% LP inclusion level (87.80%) was significantly higher (P≤0.01) than the dog food with 5% liver powder, but it was comparable with digestibility of organic matter at 0 % inclusion level i.e. control diet.

As in-vitro digestibility (%) of most of the nutrients in extruded dog food was significantly higher than the other processing technique (raw and boiled), hence the extrusion was selected as cooking method for the processing of dog food. In the dog diets incorporated with different levels of liver powder (2.5 and 5%), in-vitro digestibility (%) of most of the nutrients was observed highest for the dog diets incorporated with 2.5% liver powder, hence the level of incorporation at 2.5% was adjudged best for the preparation of dog food.

4.2 Experiment 2: Comparative *in-vitro* evaluation of best levels of feed with all vegetarian dog food.

The dog diets selected from the experiment no. 1 i.e. extruded dog diets incorporated with 2.5% level of ghee residue and 2.5% chicken liver powder were compared with the all vegetarian diet (all the ingredients are from the vegetable sources). The formulation of all vegetarian diet is presented in table 4 in the materials and methods section. The proximate composition of all vegetarian diet is presented in table 17.

Table 17: Proximate composition of extruded all vegetarian diet

Parameters	Composition (%)
CP	19.25±0.12
EE	5.11±0.21
CF	4.16±0.11
Total Ash	3.62±0.12
OM	96.38±0.88

(CP-crude protein, EE-ether extract, CF-crude fibre, OM-organic matter)

4.2.1 *In-vitro* digestibility of all vegetarian dog diet

In-vitro nutrient digestibility of all vegetarian diet is presented in table 18. Dry matter digestibility, crude protein digestibility, ether extract digestibility and organic matter digestibility of all vegetarian diet were 81.65, 90.82, 94.54 and 88.70 % respectively.

Table 18: *In-vitro* nutrient digestibility of vegetarian dog food

Parameters	*In-vitro* digestibility%
DM	81.65±0.27^{d}
CP	90.82±0.06^{b}
EE	94.54±0.16^{a}
OM	88.70±0.32^{c}

DM-dry matter, CP-crude protein , EE-ether extract, OM-organic matter) Figures with different superscripts in column differ significantly ($P \leq 0.05$)

4.2.2 Comparative evaluation of *In-vitro* nutrient digestibility of extruded all vegetarian diet, extruded control diet and extruded dog food incorporated with 2.5% ghee residue and 2.5% liver powder

The comparative evaluation of *in-vitro* nutrient digestibility of extruded all vegetarian diet, extruded control diet and extruded dog food incorporated with 2.5% ghee residue and 2.5% liver powder is depicted in table 19. DMD and CPD of control dog food was highest ($P \leq 0.05$). DMD of all vegetarian diet was lowest ($P \leq 0.05$). DMD of 2.5% LP incorporated extruded dog food was higher ($P \leq 0.05$)

than 2.5% GR incorporated dog diets but it was lower ($P \leq 0.05$) than control diet. CPD of control diet (92.72%) and LP incorporated diet (94.54%) were comparable among themselves but higher ($P \leq 0.05$) than that of all vegetarian diet (90.62%) and 2.5% GR (91.65%) containing dog food. Ether extract digestibility of control diet (96.05%), 2.5% LP incorporated dog diet (95.78%) were comparable among themselves but the values were significantly higher ($P \leq 0.05$) than 2.5% GR (95.09%) containing dog foods and all vegetarian dog diet (94.54%). Organic matter digestibility was highest ($P \leq 0.05$) in control dog food. Bednar et al. (2000) studied the effect of processing on ileal nutrient digestibility of several protein sources of plant and animal origin in dogs and stated that extruded and pelleted dog food with different vegetable protein sources provided adequate levels of highly digestible protein and amino acids. Kore et al. (2009) also reported that digestibility of DM, CP and EE of rice-based diet was 89.4, 96.1 and 95.2% respectively.

Table 19: *In-vitro* digestibility of extruded all vegetarian dog diet and its Comparative evaluation with control, 2.5% ghee residue and 2.5% liver powder containing dog diets

Parameters	***In-vitro nutrient* digestibility (%)**				**PSE**	**P-Value**
	All vegetarian diet	**Control diet**	**2.5% ghee residue**	**2.5% liver powder**		
DM	81.65 ± 0.27^{c}	86.08 ± 0.12^{a}	84.45 ± 0.20^{b}	85.08 ± 0.52^{a}	0.621	<0.05
CP	90.82 ± 0.06^{c}	92.72 ± 0.06^{a}	91.65 ± 0.08^{b}	92.54 ± 0.04^{a}	0.287	<0.05
EE	94.54 ± 0.10^{b}	96.05 ± 0.04^{a}	95.09 ± 0.05^{b}	95.78 ± 0.18^{a}	0.22	<0.05
OM	88.70 ± 0.32^{b}	91.63 ± 0.36^{a}	91.01 ± 0.32^{a}	91.78 ± 0.38^{a}	0.466	<0.05

DM-dry matter, CP-crude protein, EE-ether extract, OM-organic matter) Figures with different superscripts in each row differ significantly ($P \leq 0.05$)

4.2.3.1 Physico-chemical properties of extruded control dog diet, extruded dog diets incorporated with 2.5% level of ghee residues and 2.5% chicken liver powder and extruded all vegetarian dog diet

Physico-chemical properties of extruded control dog diet, extruded dog diets incorporated with 2.5% level of ghee residues and 2.5% chicken liver powder and extruded all vegetarian dog diet are depicted in table 20. Among the selected diets and all vegetarian diet, it was observed that all vegetarian diet (pH 4.87) was acidic and followed by control dog food (pH 5.13), 2.5% LP and 2.5% GR based diets. Free fatty acid content of extruded dog diets incorporated with 2.5% chicken liver powder were significantly lower than all other treatments. FFA content of control dog diet was 0.738%. Peroxide value of control and 2.5% GR incorporated dog diet were comparable but these were significantly lower than all vegetarian diet and 2.5% LP incorporated diet. Osawa et al. (2008) reported the value of FFA and PV of pet food in the range of 4.6 ±0.1 to 28.0±0.6% oleic acid and 1.4±0.1 to 6.8±0.3 meq O_2/kg respectively.

Table 20: Physico-chemical properties of extruded control dog diet, extruded dog diets incorporated with 2.5% level of ghee residues and 2.5% chicken liver powder and extruded all vegetarian dog diet

Dog foods	pH	FFA (% oleic acid)	PV (mEq/kg)
Vegetarian	4.87 ± 0.01^d	0.902 ± 0.02^a	6.00 ± 0.2^a
Control	5.13 ± 0.03^c	0.738 ± 0.05^c	4.00 ± 0.3^b
GR 2.5%	5.87 ± 0.02^a	0.812 ± 0.09^b	4.00 ± 0.7^b
LP 2.5%	5.29 ± 0.03^b	0.678 ± 0.04^d	6.00 ± 0.5^a

N=6, Figures with different superscripts in each column differ significantly ($P \leq 0.05$)

4.2.3.2 Aflatoxin estimation

Dogs are susceptible to the toxic effects of mycotoxins particularly aflatoxin B_1 in dog food (> 60 µg/kg of dog food) with an LD_{50} of 0.5 to 1.5 mg/kg of body weight (Rumbeiha, 2001). Dog foods selected in *in-vitro nutrient digestibility* study were analyzed for aflatoxin content estimation. The results are presented in Table 21. Among different types of dog foods, lowest Aflatoxin content was found in control dog food. Aflatoxin content in vegetarian dog food was higher (19 ppb) than control diet (5.7 ppb), 2.5% ghee residue (9 ppb) and 2.5% liver powder (17 ppb) incorporated dog diets. Gazzoti et al. (2015) reported the presence of deoxynivalenol, fumonisins and ochratoxin A in extruded commercial dog food with values higher than the limit of quantification (5µg/kg) in 100%, 88% and 81% of the samples analyzed, respectively. Aflatoxins and zearalenone were present in moderate amounts with 88% and 75% of the samples, respectively, showing concentrations less than the corresponding limit of quantification (5µg/kg for aflatoxins and 10µg/kg for zearalenone). According to Food and Drug Administration (2019), maximum permissible limit for aflatoxin in complete pet food of all ages should not be more than 20 ppb. Consequently all the dog foods formulated had aflatoxin content with-in permissible limits.

Table 21: Aflatoxin content of dog foods

Extruded dog foods	**Aflatoxin (ppb)**
Vegetarian	18.5 ± 1.32^{a}
Control	6.1 ± 1.24^{c}
GR 2.5%	9 ± 2.44^{b}
LP 2.5%	17 ± 1.88^{a}

N=6, Figures with different superscripts in column differ significantly ($P \leq 0.05$)

The appraisal of data of in-vitro nutrient digestibility, physicochemical properties and aflatoxin content revealed that extruded dog diets incorporated with 2.5% level of ghee residues and 2.5% chicken liver powder were significantly better than extruded all vegetarian dog diet, whereas these dog foods were comparable to extruded control dog diet.

CHAPTER-V

SUMMARY AND CONCLUSIONS

Under the present study entitled "Development of dog food by utilizing meat, egg and dairy industry byproducts", nutritional composition and physico-chemical quality evaluation of different poultry and dairy industry byproducts such as chicken skin, liver powder, gizzard powder, lard, hatchery waste and ghee residue was evaluated to explore the potential for the inclusion in the dog food. Liver powder was analyzed and was found to contain 60% CP, 18% EE and crude fiber 0.6%. Proximate analysis of gizzard powder revealed that it contained 58.6% crude protein, 17.45% EE, 0.75% crude fiber. Proximate analysis of chicken skin revealed that it contained 12.94 % crude protein, 20.13% EE, 2.6% crude fiber .Ghee residue was having 25.37% CP, 43% EE and 0.4% crude fibre whereas hatchery waste was having 22.73% crude protein, 4.06% EE, 10.8% crude fiber. As far as physical parameter are concerned, pH of all test ingredient was found to be acidic however, pH of ghee residue was found to be near to neutral. FFA and PV of all ingredients was less than critical limits except the lard. Among all the ingredients, aflatoxin level of hatchery waste (47) was highest and aflatoxin level of ghee residue, Chicken skin, liver and gizzard powder was 7.1, 41, 21 and 33 ppb respectively. Thus, on the basis of proximate analysis, physico-chemical parameters, aflatoxin content and availability of ingredients, ghee residue and liver powder were selected as test ingredients for incorporation in dog food. Control dog food was formulated on dry matter basis using rice, deoiled rice bran, bengal gram, soyabean meal, skim milk powder, meat cum bone meal, di-calcium phospahte, lime stone powder and mono sodium orthophosphate added in diet to meet the calcium and phosphorus (AAFCO 2014). Ghee residue and liver powder were incorporated at different levels (2.5% and 5.0%) using all above ingredients to formulate dog food meeting the standard dietary requirements. These diets were subjected to different processing techniques viz: boiling and extrusion. These processed diets were analyzed for their proximate composition before conducting *in vitro* trials. Ether extract in boiled feeds decreased significantly due to liquefaction of oil. Crude fibre content reduced non significantly after boiling of raw feeds.

In-vitro nutrient digestibility studies revealed that in control dog food, dry matter and organic matter digestibility were significantly higher ($P \leq 0.05$) in extruded dog food. However, there was no significant difference in protein digestibility among different processing techniques. Non-significant difference between ether extract digestibility of raw and extruded feed was observed but, ether extract digestibility reduced ($P \leq 0.05$) significantly (83.09%) in boiled diets.

In-vitro nutrient digestibility of ghee residue based diets at different levels revealed that dry matter digestibility (DMD) and organic matter digestibility (OMD) of boiled diet was significantly higher($P \leq 0.05$) than raw diet but lower ($P \leq 0.05$) than without ghee residue supplemented dog food. DMD and OMD of boiled and extruded feeds had significant difference at both (2.5 and 5% GR) level of inclusion. As far as crude protein digestibility (CPD) is concerned, Non-significant difference was observed among different processing techniques at 0 and 5% GR inclusion level. However, at 2.5% inclusion level CPD of boiled diet was significantly higher ($P \leq 0.05$) than raw diet and it was significantly lower than extruded diet. Non-significant difference was observed with respect to ether extract digestibility (EED) of raw and extruded diets. However, EED reduced ($P \leq 0.05$) significantly after boiling of diet. OMD was lowest ($P \leq 0.05$) in case of raw diets at 2.5 and 5% inclusion level of ghee residue, significant difference was observed w.r.t. OMD of boiled and extruded diets at both the levels.

Irrespective of inclusion level, DMD and OMD was lowest in case of raw diets, it improved after boiling. DMD and OMD of extruded food was best ($P \leq 0.05$). CPD was lowest ($P \leq 0.05$) in case of raw diets, however no significant difference was observed in CPD in case of boiled and extruded diets. Non-significant difference was observed between raw and extruded diets w.r.t EED while lowest ($P \leq 0.05$) EED was found in-case of boiling. This may be due to the fact that liquefaction of fat in boiling water, resulting in decrease in fat content in dog food.

Irrespective of processing techniques, Highest ($P \leq 0.05$) DMD and OMD was recorded in control diet. DMD and OMD was better at 2.5% ghee residue inclusion level and decreased with subsequent increase in the level of ghee residue. Highest CPD and EED was observed in diets where ghee residue was not added. Both levels of GR had non-significant difference w.r.t. CPD and EED.

Extruded feed having 2.5% liver powder had highest DMD and significant difference was observed between raw and boiled feed with inclusion of 2.5% liver powder. DMD of boiled diet containing 5% liver powder was significantly higher ($P \leq 0.05$) than raw diet but significantly lower ($P \leq 0.05$) than 5% liver powder supplemented extruded diets. Significant difference ($P \leq 0.05$) in the crude protein digestibility was observed at both levels of liver powder supplementation when subjected to different processing techniques. Ether extract digestibility was lowest ($P \leq 0.05$) in boiled diets at both the levels of liver powder inclusion. Organic matter digestibility of raw and boiled dog food with 2.5% liver powder had significant difference, however it was highest in extruded feed at 2.5% liver powder inclusion. Organic matter digestibilty of boiled dog foodd containing 5% liver powder was significantly lower ($P \leq 0.05$) than extruded but was significantly higher ($P \leq 0.05$) than raw diet containing 5% liver powder.

Data analysis revealed that irrespective of level of liver powder in dog food, dry matter digestibility and organic matter digestibility was highest ($P \leq 0.05$) in extruded dog food and lowest ($P \leq 0.05$) in raw dog food. Crude protein digestibilty of extruded dog food was best and was lowest in raw dog feed. Non- significant difference was observed between ether extract digestibility of raw and extruded dog food. Ether extract digestibility reduced ($p<0.05$) in case of boiled food. As far as the level of liver powder in diet is concerned, irrespective of processing technique, DMD and CPD of feed with 2.5% liver powder was lower ($P \leq 0.05$) than dog food without inclusion of liver powder however it was higher ($P \leq 0.05$) than the dog food with 5% liver powder. Highest ($P \leq 0.05$) CPD was observed at 2.5% liver powder supplementation level, increasing the liver powder at 5% inclusion level decreased the CPD. Organic matter digestibility was highest in dog food with 2.5% liver powder supplementation and decreased sequentially with every inclusion level of liver powder.

Comparative *in-vitro* evaluation of all vegetarian dog food , control dog food, 2.5% ghee residue and 2.5% liver powder inclusions in dog foods showed organic matter digestibility and crude protein digestibility of control diet was highest ($P \leq 0.05$). Crude protein digestibility had non-significant difference between control dog food and 2.5% liver powder containing dog food however, these diets had significantly higher ($P \leq 0.05$) crude protein digestibility than all vegetarian diet and 2.5% ghee residue supplemented diets. Ether extract digestibility had non-significant difference among 2.5% ghee residue and 2.5% liver powder supplemented diets and was better ($P \leq 0.05$) than all vegetarian diet. Persual of results showed that control dog food, 2.5% ghee residue and 2.5% liver powder supplemented dog foods were significantly better ($P \leq 0.05$) than all vegetarian dog food.

Dog foods prepared were analyzed for aflatoxin content and physicochemical properties. Aflatoxin content of all dog foods was with in permissible limit (less than 20ppb) . pH of all diets was acidic. FFA content and peroxide values of all the dog foods were less than the critical limits showing that diets were not rancid.

Conclusions

1. Among different byproducts, liver powder and ghee residue were found most suitable for the incorporation in dog food.
2. Extrusion was found to be most suitable processing technique for dog food.
3. Ghee residue can be incorporated at 2.5% level in the extruded dog foods without affecting the digestibility of the nutrients.
4. Liver powder can be incorporated at 2.5% level in the extruded dog foods without affecting the digestibility of the nutrients.

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