Identifying drivers of palatability in beef and lamb ingredients used in commercial pet food

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Abstract

Palatability is an important criterion in pet food research and development and involves examining a pet's liking for certain foods by assessing intake. In a sense, palatability is very much a human concept that has been applied to pet food research to assess the success or failure of a product. Whether a pet eats a food or not can alter an owner's future repurchasing habits of pet foods.

Within pet care, pet food makes up the largest proportion of the sector. In cats, domestication has placed great importance on pet food, as compared to their carnivorous ancestors, domestic cats obtain most (if not all) their daily nutrition from commercial pet food. However, as a species, cats have retained much of their ancestral traits particularly in terms of their nutritional requirements and are still known as obligate carnivores.

Cats preferentially choose diets high in protein and palatability of food is known to be positively associated with the amount of protein, particularly if ingredients of animal origin are included. Nevertheless, there are few studies on the palatability of meat and its by-products as individual components. Additionally, reasons for differences in palatability between by-products have yet to be determined in detail. Research is particularly limited in new and emerging food formats such as air-dried and freeze-dried, which are of great interest to pet owners as providing nutrition to pets that has undergone minimal processing. With this being considered, this thesis examined the palatability of air-dried by-products (lung, heart, kidney, tripe, mechanically deboned meat (MDM), liver and spleen) from ovine and bovine sources and then examined potential nutrient drivers responsible for observed differences in palatability between the by-products. Finally, it aimed to understand whether inclusion of low- or highly- palatable ingredients would affect macronutrient selection in the cat.

Firstly, Chapter 2 details the methodology used to manufacture air-dried by-products for testing in Chapters 3 and 4. Following the method development, three experimental trials using a designated panel of eight cats were conducted to make up Chapters 3, 4 and 5, respectively.

Chapter 3 examined the within- and between-species differences in palatability between air-dried beef and lamb by-products. This was carried out to compare the same series of by-products that I investigated previously in their raw state to an air-dried state, to determine the influence of heat-treatment on palatability. The results reflected similar

findings to previous research of raw by-products where greater amounts of organ meats were consumed over MDM and lamb was preferentially consumed over the equivalent beef by-products. In terms of the between species finding, lamb was also preferred over the same beef by-product. Furthermore, when compared on a dry matter basis, heat-treatment via air-drying had a negative impact on by-product palatbility compared to raw.

Chapter 4 examined within species effects on palatability of by-products from young and old animals from ovine and bovine sources. Cats showed preferential consumption of organ meats from calves over beef and a general preference for lamb over mutton (except for mutton lung and tripe which were preferred from older animals), and no differences were observed between the liver of different ages. Metabolomic analysis of the by-products was also used as a tool to understand their metabolite composition and revealed that key compounds were associated with palatability in cats, particularly amino acids relating to umami taste, specifically glutamic acid, and kokumi dipeptides showed greater levels in the more palatable by-products. Whereas, dipeptides associated with lean meat, carnosine and anserine, were higher in the least palatable by-product MDM. These results highlight that although cats have a biological need for animal proteins, the type of by-product selected has a great influence on palatability.

From here, I developed a series of high and low palatability diets varying in protein fat and carbohydrate content with limited ingredients, formulated with lamb kidney as the ingredient of high palatability and sheep heart as the ingredient of low palatability. These were used to examine the macronutrient selection by cats when presented in a geometric framework study. The highly palatable series did show greater intake, but both sets of diets were highly palatable with cats consuming greater than 100% of the maintenance energy requirements throughout the two phases. When the intake patterns were compared, the high palatability series saw cats consume similar metabolisable energy percentages from fat to the low palatability series but more protein and less carbohydrate. Whereas cats seemed to maximise their carbohydrate intake to reach the carbohydrate ceiling on the low palatability diet and consumed less protein.

From this research, I have identified potential compounds of interest from Chapter 4 to undergo more controlled experimental protocols. Glutamic acid and the two γ-glutamyl dipeptides could be investigated further to determine the levels for inclusion in palatability enhancers and/or digests or in pet food formulations. Additionally, I have also discussed how further work could be done to reformulate the diets from the geometric framework study in Chapter 5 to achieve more variable protein, fat, and carbohydrate targets. The differences between the predicted and measured values for the series of diets with the consistent high fat contents of the diets across the study, need to be modified by using different or varying levels of meat ingredients to those selected. The study clearly demonstrated the variability of meat by-products and the challenges of using them in a controlled study.

With the high utilisation of meat and by-products in pet food both locally in New Zealand and worldwide, the work carried out in this thesis highlights the importance of these ingredients at a fundamental level. This thesis also explores the known drivers of palatability in cats and their links to individual ingredients and begins to determine reasons for differences between by-products that have not be reported in the literature to date.

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Abbreviations

AAFCO	American Association of Feed Control Officials
AOAC	Association of Official Analytical Chemists
Ca	Calcium
CaSR	Calcium sensing receptor
CE	Collision energy
CHO	Carbohydrate
CKD	Chronic kidney disease
DHA	Docosahexaenoic acid
DIA	Data independent acquisition
DM	Dry matter
EPA	Eicosapentaenoic acid
GSH	Glutathione
HC	High carbohydrate
HF	High fat
HILIC	Hydrophilic interaction liquid chromatography
HP	High protein
HPLC	High-performance liquid chromatography
IQR	Interquartile range
LPK	Less palatable kibble
LSM	Least squares means
MDM	Mechanically deboned meat
ME	Metabolisable energy
MPI	Ministry for Primary Industries
NAD	Nicotinamide adenine dinucleotide
NAPD	Nicotinamide adenine dinucleotide phosphate
NFE	Nitrogen-free extract
NRC	National Research Council
P	Phosphorus
PC	Principal component
PCA	Principal component analysis
QC	Quality control
RSD	Relative standard deviation

SEM	Standard error of the mean
TBARS	Thiobarbituric acid reactive substances
TVP	Textured vegetable protein
UHPLC	Ultra high-performance liquid chromatography
VPK	Very palatable kibble

Units of Measurement

a_w	Water activity
cm	Centimetre
F_0	Lethality value
g	Grams
kcal	Kilocalories
kg	Kilograms
kJ	Kilojoules
mm	Millimetres
IU	International units
°C	Degrees Celsius
µ	Micrograms

General Introduction

Palatability is a key criterion in pet food research and development. Palatability is described as the physical and chemical properties of the diet, which are linked with the promotion or suppression of feeding behaviour during the pre-absorptive period (National Research Council, 2006; Aldrich and Koppel, 2015). Rather than being related to an appetite or craving that indicates a want or need, palatability relates to taste pleasure, liking or happiness (Stasiak, 2002). Palatability is very much a human term that has been applied to pet food research and development with the assumption that food that is readily accepted by an animal is generally indicative of a food that is palatable (Stasiak, 2002; Tobie et al., 2015; Aldrich & Koppel, 2015).

Whilst important for both cats and dogs, cats are notably more selective about the foods they do and do not eat. Therefore, this thesis firstly details the literature available on the differences between the feeding behaviour and nutritional requirement of both cats and dogs, before undertaking more focused palatability studies using cats as the test subjects.

Although the importance of palatability has been recognised in the industry. Knowledge on the palatability and drivers of palatability for ingredients included in pet foods, particularly meat and its by-products, is lacking and requires further research. In response to this, the aims of this thesis were split into two parts. The initial aim was to develop and carry out pilot-scale production of air-dried by-products. In doing this, examining the effect of heat treatment (specifically via air-drying) on the palatability of beef and lamb by-products could be conducted.

Following this, an assessment of the palatability of by-products from young and old ovine and bovine was carried out to determine whether cats show preference for by-products of different ages. This was accompanied using metabolomics as an analytical method to help identify compounds and/or nutrients of interest.

Lastly, to bring all the fundamental ingredient level work together, a final palatability and geometric nutrition study was conducted. The aim of this study was to look at the effect on macronutrient selection by cats when presented with air-dried diets that were formulated with limited ingredients and varied in palatability through the inclusion of high palatability and lower palatability by-products.

Chapter 1 Literature Review

1.1 Introduction

The pet food industry has shown strong growth both globally and more locally in New Zealand over the last decade. Global pet food sales reached US$96 billion in 2019 compared to $67.4 billion in 2009 and were projected to surpass $111 billion by 2022 (Euromonitor International, 2020). However, global pet food sales from 2021 have already exceeded US$110.2 billion, highlighting the rapid growth in the industry (Euromonitor International, 2022). Within the industry, cat and dog foods have the greatest market share, accounting for US$106.2 billion of the US$110.2 billion pet food sales made globally in 2021 (Euromonitor International, 2020; Tobie et al., 2015). Sales are now projected to reach US$156.9 billion globally by 2026, with cat and dog food contributing approximately US$152 billion (Euromonitor International, 2022). Growth in the New Zealand pet food industry has also been increasing exponentially with sales reaching NZ$499 million in 2009, growing to $650.4 million in 2019, and projected to reach $720.4 million by 2026 (Euromonitor International, 2022).

The dominance of cat and dog food is likely due to these species being the most common household pets (Legrand-Defretin, 1994). In New Zealand, the pet ownership rate was one of the highest in the world in 2020, with 64% of households being home to at least one companion animal (Companion Animals New Zealand, 2020). When comparing species of pets owned by New Zealanders, cats were identified as the most popular companion animals, with 41% of New Zealanders owning at least one cat, followed by dogs at 34% (Companion Animals New Zealand, 2020). Growth in the industry to date can be attributed to market trends resulting in a major increase in the number of new and innovative products that are available to pet owners (Aldrich & Koppel, 2015).

While pet food is primarily formulated to deliver complete and balanced nutrition, palatability has been identified as a crucial factor to consider in pet food products. Palatability plays a vital role in the evaluation of pet food, often determining the success or failure of a product in the market and is among the top determinants by owners when selecting food for their pets (Dodd et al., 2019; Knight and Satchell, 2021; Schleicher et al., 2019; Zaghini & Biagi, 2005).

Therefore, this review will consider the feeding behaviour and nutritional requirements of both dogs and cats, before focusing on the diet selection pattern of cats. The different types of pet food available and the ingredients commonly included in pet foods will also be assessed and from there, the methods used for assessing pet food palatability will be reviewed, along with a discussion of the known drivers of palatability.

1.2 Nutrient Requirements of Cats and Dogs

Cats and dogs are both members of the order Carnivora. While the name implies that both are specialised meat-eaters, each species originated from different branches, with the domestic cat (*Felis catus*) being part of the Felidae family, and the domestic dog (*Canis familiaris*) part of the Canidae family. However, the nutritional requirements, feeding behaviour and food selection choices vary considerably between the two species.

1.2.1 Domestication and Feeding Behaviours

Dogs were likely the first animals to be domesticated (Udell and Wynne, 2008). They share a long history of co-existence with humans, with the dog's direct wolf ancestor (*Canis lupus*), thought to be utilised as guards and hunters alongside human hunter-gathers (Driscoll et al., 2009b). It is believed that divergence from their carnivorous wolf ancestors took place between 13,000 and 17,000 years ago, when the increased availability of human food waste associated with the move to an agricultural existence created a new ecological niche (Bosch et al., 2015). Wolves that took advantage of this new niche and became more accustomed to human contact. Over time, humans became experienced at selecting for specific tameness traits in dogs and established control over proto-dog mating, ultimately resulting in the evolution of the domestic dog (Driscoll et al., 2009b).

Compared to their carnivorous wolf ancestors, domestic dogs can consume foods of both animal and non-animal origin and are therefore classified as facultative carnivores (Bosch et al., 2015; Knight & Leitsberger, 2016). They are often described as opportunistic eaters that spend a short period of time consuming large amounts of food (Aldrich & Koppel, 2015; Bradshaw 2006). As a result, food is normally eaten in a gluttonous manner with minimal chewing taking place, as dogs are likely to regurgitate and re-consume it later when away from other members of the pack (Aldrich & Koppel, 2015). In the wild, dogs eat a wide variety of food including insects, berries and grass, animal faeces as well as carrion and chew on bones, hides, and other animal parts (Aldrich & Koppel, 2015). The ability to eat a wide variety of food stems from their wolf ancestors having to adapt during times of feast and famine to cope with variable nutrient availability and allowed the change from a predominantly carnivorous to a more omnivorous diet during domestication (Bosch et al., 2015).

In contrast to dogs, cats were domesticated approximately 9,000 to 10,000 years ago from the African wildcat (*Felis silvestris*), making them one of most recently domesticated mammal species (Plantinga et al., 2011, Driscoll et al., 2009b). Rather than being actively sought as household pets by human, cats likely became associated with people to take advantage of food scraps and mice found in their settlements and are believed to have naturally diverged from their wildcat ancestors (Driscoll et al., 2009b, Driscoll et al., 2009a).

Cats, formally termed obligate carnivores, are described as prey-driven animals, and are solitary hunters that will wait for their prey to show themselves before making their kill (Aldrich & Koppel, 2015). Once caught, food is eaten quickly, as cats prefer freshly killed carcasses at body temperature as opposed to carrion at ambient temperature (Becques et al., 2014; Eyre et al., 2022). Small prey such as rodents are often consumed as a single unit but for larger prey, the flesh will be ripped off and whole sections consumed (Aldrich & Koppel, 2015). For example, Eurasian lynx ate all muscle, body fat and internal organs, except the digestive tract, of 359 prey species in the wild (made up of predominately roe deer at 69%) between the months of March and May 1988 (Jobin et al., 2000). Of these kills, the meat (muscle) and organ meats (lung, heart, kidney, liver, and spleen) were completely consumed in 90% of analysed cases (Jobin et al., 2000).

Cats are also classified as intermittent feeders, consuming multiple, small meals throughout the day and night (Bradshaw et al., 1996; Peachey & Harper, 2002; Pickering, 2009). In contrast to dogs, cats are selective about the food they consume and can detect small differences in the composition of food they are offered (Bradshaw et al., 1996; Aldrich & Koppel, 2015). Cats are obligate carnivores in their methods of ingesting, digesting, and metabolising such nutrients (Bradshaw et al., 1996). Without animal derived protein, severe nutritional deficiencies can occur in cats.

Both cats and dogs tend to display neophilic behaviour, defined as the preference for a food that has never been encountered before, as opposed to neophobia, the avoidance of new food (Péron & Tobie, 2018; Bourgeois et al., 2006). In extreme cases, some cats may also exhibit metaphilia, which is defined as a clear preference for change or variation from a familiar food (Péron & Tobie, 2018). Overtime, the feeding experiences become less variable for cats as a dynamic equilibrium between the purchasing habits of owners and the foods cat will and will not eat is reached (Bradshaw, 2006).

Overall, cats and dogs continue to be the most used animal models in assessing pet food palatability. While dogs show greater acceptance of a wide variety of foods, their opportunistic feeding behaviour and tendency to consume the first food chosen may prove challenging when looking to identify the fundamental components that drive food intake. In comparison, cats show greater selectivity and can detect small changes in food composition. Therefore, greater focus on the nutrient requirements of cats will be examined in this review, with reference to dogs provided for comparative purposes.

1.2.2 Protein

Despite being classed as carnivores, cats and dogs have specific dietary nutrient requirements, with cats notably having more specialised nutrient needs than dogs. For example, cats have a higher minimum requirement for dietary protein than dogs at 26% versus 18% on a dry matter (DM) basis, with protein requirements increasing to 30% and 22.5%, respectively, in growing or lactating animals (National Research Council, 2006). Within these protein requirements, essential amino acids must also be present at specified levels to deliver a complete and balanced diet (AAFCO, 2020; National Research Council, 2006). This is likely due to their limited ability to regulate catabolic enzymes of amino acid metabolism (MacDonald et al., 1985). Table 1.1shows the higher minimum dietary levels of essential amino acids required by cats compared to dogs (Knopf et al., 1978; National Research Council., 2006).

Table 1.1. Protein and amino acid requirements in the cat and dog for maintenance

Requirement on dry matter (DM) basis (%)	**Adult Maintenance**			
	Cat		**Dog**	
	Minimum	Maximum	Minimum	Maximum
Crude Protein	26	-	18	-
Essential amino acids[1]				
Taurine (canned/extruded)	0.2/0.1	-	No requirement	-
Arginine	1.04	-	0.51	-
Histidine	0.31	-	0.19	-
Isoleucine	0.52	-	0.38	-
Leucine	1.24	-	0.68	-
Lysine	0.83	-	0.63	-
Methionine	0.20	1.5	0.33	-
Phenylalanine	0.42	-	0.45	-
Threonine	0.73	-	0.48	-
Tryptophan	0.16	1.7	0.16	-
Valine	0.62	-	0.49	-

[1]The 11 essential amino acids listed for cats and 10 essential amino acids listed for dogs (AAFCO, 2020)

Taurine is the only amino acid able to conjugate bile acids in cats, so they are unable to use glycine as an alternative like other mammals (Zaghini, & Biagi, 2005). Cats also require taurine to maintain retinal function and structure, and it has roles in cardiac function, sight, and reproduction. However, cats, and kittens, are unable to synthesise or recycle enough taurine to meet their needs and hence it must be provided in their diet.

Arginine is another essential amino acid required for growth and in the detoxification and excretion of ammonia as urea (Morris & Rogers, 1978). It is of great importance to cats, as severe and rapid ammonia intoxication can result if they are fed diets lacking in arginine (Anderson et al., 1979). Unlike other mammals that can synthesise arginine from ornithine and citrulline in the intestine, cats have lost this ability due to their lack of pyrroline-5-carboxylate synthase and ornithine aminotransferase, the two enzymes needed in the urea cycle pathway (Hamper, 2016). Cats require arginine in their diets to replenish their liver levels for urea cycle function.

1.2.3 Vitamins and Minerals

Cats and dogs also have requirements for vitamins and minerals as outlined in Table 1.2. Briefly, the twelve essential vitamins required for adult cat maintenance are vitamins A, D and E (fat-soluble), along with thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, vitamin B12, biotin and choline (water-soluble). In terms of minerals, the macro-minerals which are essential for cats are calcium, phosphorus, magnesium, sodium, potassium, and chloride. The microminerals essential for cats are iron, copper, zinc, manganese, selenium, and iodine (National Research Council, 2006). In this discussion, focus will be placed on vitamin A and niacin, as these two follow unique synthesis pathways in the cat. Calcium and phosphorus will also be examined as the key microminerals required in the highest amounts for adult cat maintenance.

Vitamin A is an essential fat-soluble vitamin for cats but is known to cause toxicity when fed in excess. While the main source of dietary vitamin A for most species is in the non-toxic plant pigment form of β-carotene, cats lack the dioxygenase enzyme required to start the conversion of carotenoids to retinal, and therefore require a dietary source of pre-formed vitamin A (Hayes, 1982; Legrand-Defretin, 1994; Schweigert et al., 2002; Zaghini & Biagi, 2005). In contrast to β-carotene, preformed vitamin A can be extremely toxic if consumed in large amounts (Hayes, 1982). As a fat-soluble vitamin, excess vitamin A is not excreted through urine when consumed in excess. Instead, appreciable amounts are stored in the liver as well as fatty tissues throughout the body (Hayes, 1982; Kantorosinski and Morrison, 1987). Whilst vitamin A is important for vision, bone and tooth growth and reproduction and maintenance of skin and mucous membranes, toxicity in cats can result in muscle soreness, tenderness of joints and hyperesthesia, particularly along the neck and forelimbs of cats due to the development of bony exostoses (Green and Fascetti, 2016; Hayes, 1982; Kantorosinski and Morrison, 1987). As carnivores, most of the vitamin A is consumed as preformed retinyl palmitate stored in tissue and is particularly abundant in liver of their prey (Hayes, 1982). In commercial diets, a safe upper limit for vitamin A levels for cats has been set at 333,300 IU/kg which is equivalent to 99.99µg/g as retinol (AAFCO, 2022). Beef liver has been shown to have vitamin A levels of 283.19µg/g (Purchas & Wilkinson, 2013), which limits daily intake to 35 g assuming no other ingredients supply any vitamin A.

In contrast to the synthesis pathways of taurine and arginine which are characterised by low enzymatic activity at different points, cats possess all the enzymes and pathways required for niacin synthesis (Morris, 2002). Niacin is a water-soluble vitamin essential for energy metabolism and can be metabolised one of two ways using tryptophan, one results in the production of acetyl CoA and CO_2 and the other to nicotinamide adenine dinucleotide (NAD) (Morris, 2002). In cats, the activity of picolinic carboxylase, the enzyme catalysing the first step of the degradative pathway to acetyl CoA and CO_2, is upregulated resulting in niacin being broken down faster than it is produced (Ikeda et al., 1965). Cats, however, are supplied with enough NAD and nicotinamide adenine dinucleotide phosphate (NADP) coenzymes through the dietary consumption of meat, meaning they have no need to produce niacin from tryptophan (Morris, 2002).

Table 1.2. Vitamin and mineral requirements in the cat and dog for maintenance

Nutrient	Units DM Basis	Cat Minimum	Cat Maximum	Dog Minimum	Dog Maximum
Minerals					
Calcium	%	0.6		0.5	2.5
Phosphorus	%	0.5		0.4	1.6
Ca:P ratio				1:1	1:2
Potassium	%	0.6		0.6	
Sodium	%	0.2		0.08	
Chloride	%	0.3		0.12	
Magnesium	%	0.04		0.06	
Iron	mg/kg	80		40	
Copper (extruded)	mg/kg	5		7.3	
Copper (canned)	mg/kg	5			
Manganese	mg/kg	7.6		5.0	
Zinc	mg/kg	75		80	
Iodine	mg/kg	0.6	9.0	1.0	11
Selenium	mg/kg	0.3		0.35	2
Vitamins and others					
Vitamin A	IU/kg	3332	333,300	5000	250,000
Vitamin D	IU/kg	280	30,080	500	3000
Vitamin E	IU/kg	40		50	
Vitamin K	mg/kg	0.1		-	
Thiamine	mg/kg	5.6		2.25	
Riboflavin	mg/kg	4.0		5.2	
Pantothenic acid	mg/kg	5.75		12	
Niacin	mg/kg	60		13.6	
Pyridoxine	mg/kg	4.0		1.5	
Folic acid	mg/kg	0.8		0.216	
Biotin	mg/kg	0.07		-	
Vitamin B12	mg/kg	0.020		0.028	
Choline	mg/kg	2400		1360	

Calcium and phosphorus are the two most abundant minerals in the body and have important structural and functional roles. Both are vital for growth and maintenance of bones and teeth, with calcium also being involved in blood coagulation and nerve impulse transmission, and phosphorus playing a key part in energy metabolism as a component of adenosine triphosphate (Stockman et al., 2021). Raw meaty bones and fish provide a good amount of calcium to pet foods. Phosphorus is also provided by meat and vegetables, particularly cereal, however, in grains phosphorus is presented in a less bioavailable form known as phytate (Baker and Czarnecki-Maulden, 1991; Stockman et al., 2021). In addition to these organic forms of calcium and phosphorus, inorganic sources (additives) are also used in the industry but have a significant difference in bioavailability, with inorganic sources providing higher bioavailability than organic sources (Dobenecker et al., 2018). For example, phosphate salts are highly soluble compared to raw bony ingredients resulting in increased absorption and postprandial serum levels. This can have a negative impact on phosphorus homeostasis and contribute to renal damage (Stockman et al., 2021).

In addition to bioavailability, dietary levels of phosphorus and calcium can also have varying adverse effects on feline health. Regarding phosphorus, low dietary levels are associated with increased risk of hypercalcemia (Stockman et al., 2021). High dietary phosphorus levels greater than 3.0 or 3.6 g/1000 kcal may lead to kidney damage or dysfunction and chronic kidney disease (CKD) in healthy cats, particularly when provided in a highly available form of soluble inorganic salts (Dobenecker et al., 2018; Stockman et al., 2021). High levels of phosphorus also severely disrupt the hormonal regulation of phosphate, calcium, and vitamin D (Böswald et al., 2018; Dobenecker et al., 2018). In terms of calcium, plasma calcium levels are generally well-regulated, however, low levels can have immediate detrimental effects including cardiac arrhythmias, which can be fatal to cats (Stockman et al., 2021). In contrast, a sudden increase in dietary calcium may increase the risk of calcium oxalate urolithiasis, as well as lead to soft tissue mineralisation and possibly to kidney injury and impaired function (Stockman et al., 2021).

The availability of calcium and phosphorus is also impacted by their relative proportion to each other (Stockman et al., 2021). Many cases of hyperparathyroidism have resulted from a Ca:P imbalance which can result from feeding high meat products which are sufficient in phosphorus but low in calcium (Baker and Czarnecki-Maulden, 1991; Kantorosinski and Morrison, 1987; Stockman et al., 2021). Conversely a high calcium-to-phosphorus ratio may result in increased calcium absorption (Stockman et al., 2021).

As a result, a Ca:P ratio of 1:1 to 2:1 is thought to be acceptable to reduce the likelihood of the disease taking place (Kantorosinski and Morrison, 1987).

1.2.4 Fat

Cat foods must also contain a minimum of 9% crude fat on a dry matter basis for maintenance compared to 5.5% in dog foods (National Research Council, 2006). Within the fat requirements, arachidonic acid is essential for cats and must be present at 0.2% on a dry matter basis in cat food (AAFCO, 2020). While there are no additional fatty acid requirements for adult cats and dogs, kittens and puppies have the additional requirements for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA is important for supporting the body's natural anti-inflammatory response and DHA play a vital role in neurological and retinal development (Bauer, 2017; Debraekeleer, 2005; Hemmings, 2016)

Along with these essential nutrients described, cats require additional nutrients in their diets, some of which are found abundantly in animal tissue, notably preformed vitamin A (Aldrich & Koppel, 2015; Stasiak, 2002; Zaghini & Biagi, 2005; Corbin, 1992).

1.3 Diet Selection (Macronutrient Selection)

While the specific minimum nutrient requirements of cats and dogs have been established, research has shown that cats and dogs are able to select for a 'target intake' of protein, fat, and carbohydrates to achieve nutritional adequacy when given the choice between diets with varying levels of macronutrients (Hewson-Hughes et al., 2011, Hewson-Hughes et al., 2013a).

An extensive study by Hewson-Hughes et al. (2013b) evaluated the geometric analysis of macronutrient selection in dogs when presented with six dry-format (extruded) diets and six wet-format (retorted) diets to five different dog breeds; papillon, miniature schnauzer, cocker spaniel, Labrador retriever, and St Bernard. It was found that after initially selecting a diet significantly lower in fat, dogs were able to regulate their dietary macronutrient level based on the metabolisable energy compositions of 30% protein, 63% fat and 7% carbohydrate, with values showing similarities across the different breeds.

Prior to the work in dogs, Hewson-Hughes et al. (2011) conducted the same study with cats and found that they select dietary macronutrient based on the metabolisable energy compositions of 52% protein, 36% fat and 12% carbohydrate. As well as the optimal levels, the study revealed that cats displayed a ceiling for carbohydrate intake of approximately 300kJ/day. It is believed that the subsequent low intake of carbohydrates in a cat's diet is due to many sensory and metabolic adaptations, including their inability to detect sweetness due to their lack of sweet taste receptors (Hewson-Hughes et al., 2011).

Similar macronutrient limits were also displayed by Salaun et al. (2016), when wet diets of varying protein and carbohydrate contents were fed to cats. With fat levels being stabilised at 36% of total metabolisable energy provided by the diets, cats were able to regulate their macronutrient intake to obtain 53% of metabolisable energy from protein and 11% from carbohydrate. Both studies also reflect similar levels of protein-fat-carbohydrate levels of prey consumed by free-roaming cats of 52:46:2% (Plantinga et al., 2011). All three geometric nutrition studies illustrate that in terms of macronutrient selection, cats are driven to foods with a high protein and fat content and avoid carbohydrate rich foods.

As the diets by Hewson-Hughes et al. (2011) were manufactured to be complete and balanced based on commercial formulations, and the study focused solely on the macronutrients selection by cats and did not explore the impact of diet palatability on food intake. The study by Salaun et al. (2016) did assess the effect of adding a palatability enhancer to diets and found that cats showed increased food intake but no change in protein or carbohydrate intake patterns. A follow up study by Hewson-Hughes et al. (2016) also examined the effect of adding fish (positive), rabbit (neutral) and orange (negative) flavours to diets varying in protein:fat energy ratios of 10:90, 40:60 and 70:30. Cats were able to distinguish between flavours added to the foods with fish preferred over rabbit and no addition or flavour, and orange flavour being the least preferred in the short-term. However, in the long term, cats selected similar protein and fat intake regardless of flavour combination, suggesting that macronutrient balancing is key driver for longer-term food selection and intake in the domestic cat (Hewson-Hughes et al., 2016).

There are still limitations to the geometric work that has been published, with reference to the level of carbohydrate inclusion of commercially prepared pet foods. As stated by Watson (2011), wet foods contain generally lower amounts of carbohydrate (less than 15 per cent energy) compared with up to 40 per cent in dry foods. Hewson-Hughes et al. (2011) does acknowledge this in their study as well as in commercial dry pet foods in general, with the explanation that the high carbohydrate content of dry food is essential for the manufacture of extruded kibble. As such, this may have limited the full range of carbohydrate contents available to the cats and confounded the true macronutrient selection by domestic cats. There is the need to examine whether the defined macronutrient intake targets for cats would remain if the carbohydrate content in dry foods was reduced substantially in the high protein and high fat diet options presented.

1.4 Types of Pet Food

Today, domestic cats and dogs receive most, if not all, their nutrient requirements through commercially prepared pet foods. Although there are a variety of foods available, pet foods typically fall under one of three broad categories; dry, wet, and semi-moist foods, depending on their processing method, methods of preservation and moisture content (Case et al., 2010). Along with these three main diet types, foods can also be formulated to be complete and balanced or complementary. Complete and balanced foods deliver all nutrients at the correct levels to pets when fed as a single food source. In contrast, complementary foods, such as pet treats and mixers, generally lack some essential nutrients so can only form 10% of the daily intake and must be fed alongside another type of food to ensure the animals nutrient requirements are met.

1.4.1 Dry Food

Dry pet foods have a typical moisture content between 10 and 12% and rely on this low moisture content for preservation. Dry pet foods often include cereal grain and by-products, soybean products, animal by-products, fats, and oils, as well as the inclusion of vitamins and minerals, which are generally mixed to form a dough (Agar, 2001; National Research Council, 2006). The ingredients included in dry pet food are much the same for cats and dogs, although more emphasis is given on the inclusion of proteins and fat of animal origin into dry cat foods (Edney, 1982). Dry pet foods have the benefits of being a relatively cheap and useful source of energy compared to wet and semi-moist pet food. Dry foods are also very easy to store and dispense, however, they are often less palatable than the other food formats, particularly to cats (Edney, 1982). There are also many forms of dry foods including baked, air or freeze-dried and extruded products, with the latter accounting for most dry pet foods available on the market (Case et al., 2010; National Research Council, 2006).

Through use of extrusion processing, manufacturers can produce a range of products with different shapes, sizes, and colours. This often has little to do with nutritional adequacy for pets but provides visual variety to pet owners (Agar, 2001; National Research Council, 2006). The process involves the dry ingredients and water entering the extruder through different streams where they are mixed and cooked under pressure. The mixed material is then pushed through a die, which is located at the end of the extruder. The food is then dried to reduce moisture to ensure preservation and may be coated with natural flavours or fat before being cooled (National Research Council, 2006).

Baked kibble and biscuits are the least common types of dry pet food, which are made by mixing the ingredients to form a dough. To form a dough suitable for biscuits, a formulation with a high proportion of wheat is traditionally used. For biscuits, the dough is cut into shape before baking in an oven, whereas for kibble a large sheet is baked and then broken up to form a kibble (Agar, 2001; Case et al., 2010).

Air-dried and freeze-dried pet foods are also becoming increasingly popular types of dry food on the market. Compared to traditional dry food cooking methods, air-dried pet food typically adopts the use of low drying temperatures (usually below 100°C) with gentle airflow for a long drying time (Giri and Prasad, 2007; Sturm et al., 2013; Xu et al., 2019). Freeze-drying is another method used to produces a high-quality product, however, it is an expensive process (Giri and Prasad, 2007). Freeze-drying involves lowering the temperature of a food to below freezing, then applying a high-pressure vacuum to extract the water in the form of vapour, which turns back to ice and is removed (Cuddon Freeze Dry, 2022). Freeze-drying is beneficial in retaining the properties of the raw material better than the air-dried product (Michalczyk et al., 2009). However, both options provide end products that are minimally processed to help maintain the nutritional value of the raw material, which can be lost in traditional manufacturing processes.

1.4.2 Wet Food

Wet foods typically have a moisture content of 74 to 78% and exist in a variety of forms, with canned and pouch products being the most common (Agar, 2001; Case et al., 2010; National Research Council, 2006). Many of the same ingredients used in dry pet foods are also included in canned food at differing levels (National Research Council, 2006). These ingredients include muscle meat, poultry, or fish meats or by-products, cereal grains, textured vegetable protein, as well as vitamins and minerals (Case et al., 2010). In canned foods, there is a much higher inclusion of fresh or frozen meat, poultry or fish products and animal by-products, usually at levels between 25 to 75%, and cereal flour is used as gelling agents (National Research Council, 2006).

Figure 1.1. Canned wet food formats (left to right) loaf, chunks in gravy, and chunk in loaf

There are three general types of wet food: loaf, chunks or chunks in gravy, and a chunk in loaf combination, as shown in Figure 1.1. All three are preserved via heat treatment where cans are filled with the wet slurry of ingredients, sealed with a double seam lid, and retorted at a specified temperature/time profile to achieve a defined lethality value (F_0). The F_0 of a thermal process represents the time equivalent (minutes) of a heating process to destroy microorganisms at the reference temperature of 121.1°C to kill any food-borne pathogens. (Dainton et al., 2021; Hagen-Plantinga et al., 2017; Hendriks et al., 1999; van Rooijen et al., 2013). Cans are then cooled, dried, and labelled to deliver safe final products which have a long shelf life and no special storage considerations (Agar, 2001; National Research Council, 2006; Edney, 1982).

Usually there is considerable damage or loss of nutrients during heat processing and storage. A study conducted by a major vitamin supplier to the human and pet food industries summarises the losses of vitamins during processing and storage (Crane et al., 2010). During canning, ascorbic acid was unstable in the high-moisture environment of wet pet food. Heat- and moisture-liable vitamins such as thiamine, folic acid and β-carotene also showed losses. In contrast, fat-soluble vitamins used as protective coatings make them much more resistant to processing losses. Vitamins that are usually stable, such as riboflavin, niacin, pantothenic acid, choline, vitamin B12 and biotin have good processing resistance, except for biotin in wet dog food. Vitamin losses during storage were minimal compared to the losses during processing, due to the protective environment of the can. However, thiamine and vitamin B12 were the two main vitamins lost during storage. To combat these losses, manufacturers add compensatory amounts to formulations to ensure adequate levels are retained following heat treatment (Edney, 1982).

In addition to loss of nutrients, Maillard products formed via a chemical reaction between amino acids and reducing sugars in wet food during heating, result in the production of different flavours and a brown colour (Zaghini & Biagi, 2005; Tamanna & Mahmood, 2015). Compared to dry foods, canned foods are generally more palatable particularly when little or no cereal or carbohydrate source is included in formulations. (Agar, 2001; Case et al., 2010; Edney, 1982). However, the production of Maillard products is known to decrease the digestibility of proteins in a diet but may increase the palatability of a diet through mechanisms such as the loss of L-arginine, considered a bitter amino acid to cats, during Maillard reaction between free reactive amino groups of specific amino acids and reducing sugar (Hagen-Plantinga et al, 2017; Morris et al., 1994; van Rooijen et al., 2013).

1.4.3 Semi-Moist Food

Semi-moist products are relatively uncommon and exhibit a moisture content which can range from 25 to 35% and are stable at room temperature (Agar, 2001; National Research Council, 2006). To achieve its shelf-life stability of several months, the water activity (α_w), defined as the water which is available for bacterial and fungal growth in or on the surface of food, needs to be controlled (Edney, 1982). Manufacturers will include ingredients classified as humectants, such as salts, simple sugars, glycerol, and corn syrup, in formulations which control the water activity (National Research Council, 2006, Edney, 1982). To prevent growth of yeasts and moulds, preservatives such as potassium sorbate may also be added (Case et al., 2010).

Semi-moist foods use similar ingredients to dry and wet foods. They are prepared in a similar manner to dry foods, with the addition of meat or meat by-products prior to extrusion. The ratio of dry to wet ingredients can range from 4:1 to 1:1 in this type of food. Semi-moist products often come out in patties or roll-like form for dogs, or single serve packages of small-bite sized pieces for both cats and dogs (National Research Council, 2006). This type of food has a softer texture than dry food, which has a positive influence on food acceptance and palatability (Case et al., 2010).

1.4.4 Nutritional Comparison of Different Types of Pet Food

While the three main type of pet foods have different methods of processing, preservation techniques and moisture contents, products can be compared nutritionally on a dry matter basis (Table 1.3).

Table 1.3. Macronutrient contents of dry, semi-moist and canned dog foods (from Case et al., 2010)

	Dry		Semi-Moist		Wet	
	As Fed Basis	DM Basis	As Fed Basis	DM Basis	As Fed Basis	DM Basis
Moisture (%)	6-10	0	15-30	0	75	0
Fat (%)	7-20	8-22	7-10	8-14	5-8	20-32
Protein (%)	16-30	18-32	17-20	20-28	7-13	28-50
Carbohydrate (%)	41-70	46-74	40-60	58-72	4-13	18-57
ME ($kcal.kg^{-1}$)	2,800-4,050	3,000-4,500	2,550-2,880	3,000-4,000	875-1,250	3,500-5,000

1.4.5 Emergence of Vegetarian and Vegan Pet Food

Vegetarian and veganism have become increasingly popular dietary choices amongst the global human population (Wakefield et al., 2006). Vegetarians are defined as those who do not consume meat, poultry, or fish; with vegans being seen as a subset of vegetarians, who eschew consumption of all animal products (Key et al., 2006).

A study by Leahy and colleagues (2010), estimated that there are one and half billion vegetarians globally. Of these, 75 million are vegetarians by choice, with this figure predicted to rise with increasing affluence and education. The remaining are vegetarian by necessity, such as those in the developing world with a lack of choice of foods that they can consume. Adoption of a vegetarian lifestyle by individuals is largely due to ethical, ecological, religious, empathy for animals, and health reasons (Dodd et al., 2019; Kanakobu et al., 2015; Wakefield et al., 2006; Zafalon et al., 2020).

In terms of pet food, ethical concerns about commercial pet food appears to be the primary motive for owners feeding cats vegetarian diets (Wakefield et al., 2006). However, there have been several reports of nutritional inadequacy of vegetarian and vegan diets for dogs and in particular, cats due to their obligate carnivore status, as well as the associated health implications of such diets on the animals (Dodd et al., 2019; Gray et al., 2004; Kanakobu et al., 2015; Knight and Leitsberger, 2016; Knight and Light, 2021; Wakefield et al., 2006; Zafalon et al., 2020).

1.4.6 Raw (non-heat treated) Pet Food

Raw pet food, in the form of non-heat treated food made from raw meats, seafood, fruits, and vegetables, are also becoming increasingly among pet owners (Soffer et al., 2016). However, raw pet foods have been subject to disagreement among veterinarians and owners over many years, particularly regarding their nutritional benefits and risks to the health of animals and human (Finley et al., 2006). Raw foods do have the advantage of being high in palatability and nutrient digestibility. However, there is still concern over the safety of these diets, primarily in relation to the associated microbiological hazards, particularly the improper handling of these pet foods by owners and the human health hazards of raw feeding (Davies et al., 2019; Knight & Leitsberger, 2016; Marks et al, 2011).

1.4.7 Diet Formulation

In the pet food industry, diets are often formulated using diet formulation models and/or software which pulls across average values from ingredients tested by the manufacturers or those obtained from nutritional databases into a recipe (National Research Council, 2006). By selecting the desired amount of each ingredient, the macronutrient and micronutrient compositions can be determined. The addition of premixes, a selection of vitamins and minerals, are then added at appropriate levels deliver complete and balanced diets.

1.5 Ingredients in Pet Foods

Although a wide variety of pet foods exist, most utilise significant quantities of by-products globally and pet food production is tightly interlinked with livestock production and the human food system (Swanson et al., 2013). By making use of by-product streams, the pet food industry does not directly compete with the human food industry. Instead, it reduces the environmental load of the human food system by utilising inedible meat, poultry and fish by-products and by-products that would otherwise go to waste (Carrión & Thompson, 2014). As a result, the transformation of low-value animal by-products into value-added pet food has played a major role in the growth and expansion of the pet food industry (Corbin, 1992).

In addition to the utilisation of these by-product streams, cats impose a requirement for animal-sourced ingredients in their diets due to their obligate carnivore status (Case et al., 2010). Meat and meat by-products are highly palatable, digestible and contain high nutrient levels when included in a feline diet. However, little has been reported on the palatability of individual ingredients, particularly those of animal origin.

1.5.1 Meat

Meat is defined as the clean flesh derived from any species of slaughtered mammal and is made up of muscle tissue but may include intramuscular fat, connective tissue of the muscle sheaths and tendons, as well as blood vessels (Case et al., 2010; Edney, 1982). In this definition, mechanically deboned meat (MDM), the residual meat attached to the bones of a carcass, is included at a meat source. However, it generally contains high levels of calcium and phosphorus, which varies considerably depending on its method of collection (Ang & Hamm, 1982). The toughness and texture of meat depends on the relative proportion of muscle fibres, connective tissue, and fat. Lean meats lacking fat tend to have similar proportions of water and protein (75% and 25% respectively), whether from different parts of the same carcass or even from different animals such as cattle, lamb, pigs, or poultry (Edney, 1982; Table 2).

Meats are a good source of amino acids, fat, iron, and some B vitamins such as niacin, thiamine, riboflavin, and vitamin B12 (Edney, 1982). Compared to that for human consumption, meat for pet food is obtained by mechanically separating excess muscle meat from bones using a machine to deliver a final product that is finely ground and paste-like in texture (AAFCO, 2022).

1.5.2 Meat By-Products

By-products are classified as “a protein source consisting of organ meats, scrap meat, bone, blood, and fatty tissue from mammals, but do not include hair/hide, horns, hoofs or teeth, or intestinal contents” (LaFlamme et al., 2014). They are obtained fresh or frozen directly from the processing plant and while unappealing and largely unused for human consumption, by-products are often highly nutritious, highly digestible, and extremely palatable for pets and hence included as major ingredients in pet food (Corbin, 1992; Edney, 1982 Laflamme et al., 2014; Michel, 2006). In terms of live weight of beef, by-products make up nearly 50% of the animal and has substantial value as a protein source with high nutrient bioavailability (Aldrich, 2021). Additionally, the consumption of these ingredients often reflects the feeding behaviour of the larger wild cats who, when hunting prey, will often preferentially consume organ tissues as well as skin, bones, and viscera (Thompson, 2008, Laflamme et al., 2014). These components are also beneficial in providing essential nutrients such as vitamin A from liver, and calcium and phosphorus in the correct ratio from bone that may be lacking in lean meat (Laflamme et al., 2014).

Large differences in nutrient content are generally exhibited between different offal, particularly in terms of the fat and vitamin contents (Edney, 1982; Murray et al., 1997; Shariff & Mona, 2013). However, between species, the same offal tends to have a similar nutrient content (Edney, 1982). As ingredients, offal meats are a rich source of trace elements, with levels being much higher than that in muscular tissue (Biel, et al., 2019; Purchas & Wilkinson, 2013).

Table 1.4. Typical nutrient content of selected meat and meat by-products (from Edney, 1982)

	Water g/100g	Protein g/100g	Fat g/100g	Calcium g/100g	Phosphorus g/100g	Energy kcal/100g
Raw lean meats						
Pork	71.5	20.6	7.1	0.008	0.20	147
Beef	74.0	20.3	4.6	0.007	0.18	123
Veal	74.9	21.1	2.7	0.008	0.26	109
Lamb	70.1	20.8	8.8	0.007	0.19	162
Chicken	74.4	20.6	4.3	0.01	0.20	121
Average	**73.0**	**20.7**	**5.5**	**0.008**	**0.20**	**132**
Offals						
Fatty lungs	73.1	17.2	5.0	0.01	0.19	114
Heart	70.1	14.3	15.5	0.02	0.18	197
Heart (trimmed)	76.3	18.9	3.6	0.005	0.23	108
Liver (fresh)	68.6	21.1	7.8	0.001	0.36	163
Green tripe	76.2	12.3	11.6	0.01	0.10	154
Dressed tripe	88.0	9.0	3.0	0.08	0.04	63
Sheep lungs	76.0	16.9	3.2	0.01	0.20	96
Beef kidney	79.8	15.7	2.6	0.02	0.25	86
Average	**76.0**	**15.7**	**6.5**	**0.020**	**0.19**	**123**

Animal-sourced proteins are generally regarded to be of higher quality and superior in amino acid balance compared to other ingredients in pet food (Case et al., 2010; Donadelli et al., 2019). However, both muscle and offal meats have very low calcium contents and have unfavourable calcium to phosphorus ratios that can range from 1:15 to 1:26 (Table 1.4). Most meat and offal are also deficient in vitamin A and D. Liver and kidney are the exceptions and provide a good source of these vitamins, although vitamin A toxicity can be a problem with liver (Edney 1982).

Although ingredients are primarily used in a blend to provide specific nutrients in diets, examining the compositional variation of individual meat by-products may help determine what drives food selection and preference on a fundamental level in companion animals.

1.5.3 Textured Vegetable Protein (TVP)

Many canned and pouched pet foods contain considerable amounts of textured vegetable protein (TVP), an extruded soybean product typically made from defatted soy grits or flour used to mimic the look of meat (Davenport, 1994; Harper and Clark,1979). While the aim of TVP is for it to look like meat, it usually has a similar nutrient profile as soy flours (Hill and PAS, 2004). However, nutrient composition and protein quality of plant-based proteins used in pet food manufacturing have been reported by Donadelli and colleagues (2019) as having less complete amino acid profiles than animal-based proteins. Soy is the best of the plant-based sources protein, however in terms of amino acids, it is rich in lysine and limiting in sulfur amino acids, namely methionine and cysteine (Lusas et al., 1995; Peisker, 2001)

1.5.4 Carbohydrate Sources

Although carbohydrates are not considered essential for cats, as their natural diet contains little carbohydrate, commercial cat foods, particularly dry diets, can contain as much as 40% carbohydrates (Hilton, 1987; Thompson, 2008). Despite their obligate carnivore status, carbohydrates do provide a fibre source in the diet, which is important for gut health (Thompson, 2008). Additionally, cats can utilise starch as a glucose source to provide cellular energy (Thompson, 2008). This does provide a cheaper source of energy for pet food manufacturers; however, it also results in a protein-sparing effect and the long-term effect of this is not known in the cat (Laflamme, 2010). Typical sources of carbohydrate in pet foods include various grains, brown rice, oats, sorghum, potatoes, and legumes (Buff et al., 2014; Thompson, 2008; Verbrugghe and Hesta, 2017).

1.6 Palatability and Preference

With the increasing number of pet foods available on the market, palatability is the major criteria used to measure product performance. Although interpreted in many ways, palatability is defined as the physical and chemical properties of the diet, which are linked with the promotion or suppression of feeding behaviour during the pre-absorptive period (National Research Council, 2006; Aldrich and Koppel, 2015). Rather than being related to an appetite or craving that indicates a want or need, palatability relates to taste pleasure, liking or happiness (Stasiak, 2002). Food that is readily accepted by an animal is generally indicative of a food that is palatable (Stasiak, 2002; Tobie et al., 2015; Aldrich & Koppel, 2015).

1.6.1 Palatability Testing

Consumption testing (i.e., how much diet is consumed over time) is the most used technique for assessing the palatability of pet foods. During the product development stages, pet food manufacturers will often use palatability studies to test product acceptance and/or preference. Briefly, acceptance testing is used to determine a single product's intrinsic palatability (Tobie et al., 2015), while preference testing utilises the simultaneous presentation of different diets (generally two or three diets), to determine whether one is preferred over the other(s) based on intake (Tobie et al., 2015). Further distinction between testing methods is described further in this section.

1.6.2 One-Bowl Test

The one-bowl test is used to measure the acceptability of food when only one product is presented to an animal (Tobie et al., 2015). This method involves the use of multiple animals and is generally repeated over multiple days (typically five days), to eliminate environmental influences.

The benefits of this test are that it more closely reflects the home setting where animals are not given a choice of what to eat and any breed and size of animal can be used. Kennel or pet animals can be used, and no training is required for the animals to detect small differences in foods (Aldrich & Koppel, 2015). In addition, the cost of carrying out this test is relatively low and the use of between eight to ten animals is appropriate to detect a trend. It may also help to identify a product that is completely unacceptable due to off-flavours, aromas, or textures.

Although the one-bowl test is advantageous in many aspects, a few limitations also exist which have been identified by Aldrich and Koppel (2015). Firstly, this method of testing is only suitable for determining the acceptance of a single food and no information on the preference or degree of liking of the food by the animals can be obtained. In addition, using this method alone often does not provide enough information for a company to use such information to develop marketing claims or product improvements. Finally, the results from pet animals are likely to vary more than kennel animals due to the variation in prior feeding that pet animals receive. To overcome these variations, it is recommended that for in-home testing, animals undergo a period where they are fed a control diet for four to five days before being presented with the test diet, however this can be very time consuming.

1.6.3 Two-Bowl Test

The two-bowl test involves presenting two diets simultaneously to the subjects for a defined period (Tobie et al., 2015). This enables a graded choice for one product over the other to be assessed and hence a preference for one diet over the other to be determined, based on the quantities of food consumed (Aldrich & Koppel, 2015). It is the most common and reliable type of test used in expert panels for palatability assessment studies in both cats and dogs. Two-bowl testing can be used for both kennel and pet panels, although the inability to control the testing environment in the home can result in less precise findings.

In a two-bowl test, animals are put in individual testing booths to avoid social interaction and competition whilst they are given free access to food for a defined period (Tobie et al., 2015). These tests are normally repeated, and the bowl positions are switched to remove the effect of side preference and evaluate the consistency of results.

The number of subjects used in the two-bowl test is also an important consideration. Formally, the use of ten animals over five to six days was used to gain 50 to 60 observations as described by Aldrich and Koppel (2015). However, the use of a trained panel of eight cats for a two-hour period over five days has been frequently used (Anderson, 2017; Rutherford, 2004; Tarttelin, 1997). The two-hour testing period and subsequent 40 measurements over five days has proven to deliver sufficient power to consistently detect differences between diets, as well as consistently reliable results (Tarttelin, 1997). Other researchers have used 20 animals for two or four days of palatability testing (Aldrich & Koppel, 2015).

Conducting the same test for a greater number of days on a smaller number of subjects gives more repeated observations per animal for the same measure. In contrast, the use of a greater number of subjects over less days provides more true observations of the animals and reveals more quickly whether the animals preferred one food over the other (Aldrich & Koppel, 2015).

The important parameters that can be measured in the two-bowl test include (Aldrich & Koppel, 2015; Tarttelin, 1997; Tobie et al., 2015):

- the first choice and/or the first food product tasted (initial response to the food's aroma)
- the amount of food consumed
- the ratio of food consumed
- the percentage of food intake

the preference ratio (quantity of food A consumed over the total of food distributed)
This method of testing is beneficial for evaluating new flavour systems and product enhancements and is used for competitive analysis or new product development (Aldrich & Koppel, 2015). The main limitations of two-bowl testing include, only being able to rank between the two foods tested, so only paired comparisons can be evaluated. This method also does not tell us whether the pet likes the food, if both are disliked or if both foods are equally liked and it does not help identify the components or ingredients that are attractive in a food (Aldrich & Koppel, 2015; Tobie et al., 2015).

1.6.4 Behaviour as a Measure of Palatability

As cats are not able to verbalise their likes and dislikes, studies have evaluated the behavioural response of cats to various foods as an additional objective measure of palatability (Hanson et al., 2016). A study by Van den Bos et al. (2000) was able to identify certain physical responses which appeared to be related to liking or aversion to different foods. Licking and sniffing the feeding bowl, licking of the lips, and grooming of their face indicated a liking of the food. In contrast, licking and sniffing of the food and licking of the nose were associated with food aversion. These differences are quite subtle and the difficulty in distinguishing between licking of the lips and licking of the nose has been acknowledged by Becques et al. (2014) when using feeding behaviour to evaluate pet food palatability.

It is also suggested that the time cats spent sniffing food may be used to assess palatability (Tobie et al., 2015). In the study by Becques et al. (2014), two kibble diets of "very palatable kibble" (VPK) and "less palatable kibble" (LPK) were presented to cats for 20 hours a day. It was found that cats spent more time sniffing the LPK on day one and showed hesitation in consuming the diet. Furthermore, consumption of the LPK was lower than the VPK throughout the study, indicating preference for VPK over LPK.

In a survey conducted by Knight and Satchell (2021) which compared owner-perceived palatability behaviours in cats fed vegan versus meat-based pet foods, little difference was observed in the food-orientated behaviour of cats fed conventional, raw, and vegan diets. A limitation of this survey was that results were extremely subjective and based solely on owner-reported behaviours which is likely to show greater variability and bias compared to using a trained panel of cats that have been exposed to a wider variety of diets compared to the typical household cat.

Although useful in determining which food is tastier, both consumption-based and behavioural palatability assessments are still unable to identify the specific components that drive food intake. Work is therefore required to relate food intake results from palatability studies to the nutrient and textural properties that are driving or hindering product performance.

1.6.5 Factors to Consider for Palatability Testing

Although testing methods have been established, it is also important to select suitable animal subjects to test for the palatability of foods. It is known that cats, like humans, will likely exhibit individual variation when it comes to food acceptance and preference. However, some cats can display undesirable traits, particularly "side bias", which may severely impact palatability testing results. Side bias is common in cats and can be characterised when an animal prefers to eat from the left- or right-hand bowl regardless of what diet is presented (Rofe & Anderson, 1970). Animals that exhibit this behaviour can skew results, so it is important to screen out such individuals prior to testing.

In addition to side bias, the animals' level of hunger leading into testing can also impact the amount of food eaten. To combat this, animals are normally fed a reduced amount of their usual food and/or are fasted prior to testing. Seasonal effects can also result in variability, with cats eating less in the winter than in summer (Tobie et al., 2015; Péron & Tobie, 2018). Therefore, it is necessary to ensure a standardised testing protocol is in place and ensure that it is followed.

1.6.6 Palatants/Palatability Enhancers

Palatants are complex systems that incorporate many different macro- and micro-molecules including proteins, amino acids, carbohydrates, fatty acids, peptides, vitamins, and minerals (AFB International, 2020b). The aim of these ingredients is to enhance the sensory experience of the companion animal, particularly the taste receptors of cats. In the pet food industry, animal protein hydrolysates have been used to create palatability enhancers via a Maillard reaction scheme (Nagodawithana et al., 2008). Additionally, animal proteins, emulsified meats, amino acids, animal fats, and acids are identified as flavours that are highly palatable to cats (Thombre, 2004).

Palatants exist in both dry and liquid forms and are commonly added to kibbles following extrusion to enhance the flavour of food (Haines et al., 2015). In comparison, wet foods tend to be of higher palatability than dry foods due to their higher moisture content and the processing techniques (AFB International, 2020a). As a result, the inclusion levels of palatants in wet foods is generally lower than in dry pet foods.

1.7 Palatability Drivers

While the most commonly used methods of assessing pet food palatability have been described above, limited studies have taken place to identify the dietary components which drive food intake.

1.7.1 Biological Aspects

In addition to the differences in feeding behaviour and nutrient requirements already discussed, the main factors influencing food preference in cats and dogs also vary. In dogs, odour preference has been identified as the likely driver for palatability. Hall et al. (2017) presented dogs with two bowls containing different diets. Multiple tests were performed and bowl position for each diet was changed to remove any positional bias. It was found that in 89% of the tests the dogs consumed more of the diet they initially selected first. Similar results were observed by Roberts et al. (2018). It was concluded that dogs were able to select their preferred diet before tasting and it is possible that odour was a key factor in making this selection.

In kittens, preference for food is often strongly influenced by the food preferences exhibited by their mothers (Bradshaw, 2006), and exposure to foods during their mother's pregnancy via amniotic fluid and in early life can also affect a cat's feeding behaviour (Aldrich & Koppel, 2015; Bradshaw, 2006; Watson, 2011; Zaghini & Biagi, 2005). For example, cats raised from birth on a single diet of mackerel and rice showed neophobia when offered novel foods, which contrasted with cats that were raised on a variety of foods (Houpt & Zicker, 2003). With cats, limited exposure to different foods in early life can result in preference for that flavour, which is referred to as the primacy effect (Stasiak, 2002). However, the primacy effect may not be observed in practise, with some cats exhibiting neophilia (i.e., the preference for a new food rather than a pet's accustomed diet) when pet owners make a range of food experiences available (Bradshaw et al., 1996; Stasiak, 2002; Church et al., 1996). Furthermore, when cats are presented with two foods that are both familiar and abundant, they will eat a mixture of the two to obtain a wide range of nutrients to maximise the long-term nutritional benefits from what is made available to them (Church et al., 1996; Bradshaw et al., 1996).

Several factors play an important role in diet selection for cats. Cats use both smell and taste in the detection and selection of foods (Pickering, 2009; Alegría-Morán et al., 2019; Hullár et al., 2001). While not as developed as dogs, the olfactory senses are used by cats to recognise both novel and untrusted aromas (Aldrich & Koppel, 2015). These senses are also able to detect the freshness and safety of food and may also explain why cats display great selectivity towards food compared with dogs (Aldrich & Koppel, 2015). When presented with chemosensory stimuli as kittens at 9 to 10 weeks, and at 6 months of age, cats prefer familiar diets over unfamiliar ones (Hepper et al., 2012). It was found that cats will consume one food exclusively over another if they find the odour significantly more attractive. However, if the attractiveness of the foods presented cannot be distinguished based on smell, then cats will taste the foods and make their decision based on both senses (Hullár et al., 2001).

Although taste and smell are both important in food selection, taste is the more dominant sense in influencing the food preference of cats as opposed to colour and orthonasal olfaction (Aldrich & Koppel, 2015; Bradshaw et al., 1996; Pickering, 2009). As a result, more research has been published on the taste system than olfaction in cats (Bradshaw, 1991).

1.7.2 Taste Receptors

Most mammalian animals categorise taste into five main groups; sweet, bitter, sour, salty and umami/savoury (Yarmolinsky et al., 2009). Cats exhibit three groups of chemoresponsive tongue receptor units, all of which respond to different compounds. All three groups of units innervate fungiform papillae positioned in different but overlapping areas of the tongue (Boudreau, 1977). Group I units respond to acids in general (particularly citric and malic acid), as well as certain nitrogen compounds when consumed at a neutral pH and compounds with an imidazole ring (Boudreau, 1977; Boudreau & Alev, 1973). Group II units respond to amino acids, di- and triphosphate nucleosides, and some inorganic salts (Boudreau, 1977). Group III unit stimuli are less well defined but were maximally sensitive to nucleotides (Boudreau, 1977; Boudreau & Alev, 1973). In general, the sense of taste in cats is like that of other mammals, responding to salty, sour, and bitter stimuli as well as to amino acids and nucleotides, but showing no response to many sugars (Li et al., 2006).

In cats, the most abundant taste receptors are those which respond to amino acids and cats do show a preference for selected amino acids (Boudreau et al., 1985; Bradshaw et al., 1996; Watson, 2011 Zaghini, & Biagi, 2005; White & Boudreau, 1975). When 50 mM L-proline, L-lysine, and L-histidine were compared against 50 mM L-tryptophan, L-isoleucine with 50 mM saline as a control, an increase in spike output from geniculate ganglion chemoresponsive group II units was observed in response to L-proline, L-lysine, and L-histidine which compared to a decrease group II discharge towards L-tryptophan, L-isoleucine (White & Boudreau, 1975). Cats, therefore, appear to reject amino acids regarded as 'bitter' to human such as L-arginine, L-isoleucine, L-phenylalanine, L-tryptophan and prefer amino acids that are identified as 'sweet' including L-proline, L-cysteine, L-ornithine, L-lysine, L-histidine, and L-alanine (Beauchamp et al., 1977; Bradshaw et al., 1996; Zaghini, & Biagi, 2005). Although preferring 'sweet' amino acids to 'bitter' ones (where 'sweet' and 'bitter' is defined by humans), cats do not have any functional sweet taste receptors (Watson, 2011), but must have some means of differentiating between the two types of amino acid.

Generally, cats are drawn to foods with a strong umami/savoury flavour, which is often related to a high concentration of amino acids (Alegría-Morán et al., 2019; Salaun et al., 2016). The abundance of amino acid taste receptor units in cats is linked to meat-eating and are used to discriminate between meats of different quality (Bradshaw, 1991). Cats reject monophosphate nucleotides which are abundant in mammalian tissue after death, which may explain their preference for freshly killed prey and their dislike for carrion (Bradshaw et al., 1996; Zaghini, & Biagi, 2005).

Recent research also indicates that kokumi, described as the sensation of enhanced sweet, salty and umami tastes, is an important taste modality for carnivores that drives palatability of meat derived compounds such as amino acids and peptides (Laffitte et al., 2021; Ohsu et al., 2010; Rhyu et al., 2020). The Calcium Sensing Receptor (CaSR) has been designated as the putative kokumi taste receptor in humans and is also expressed in the circumvallate papillae of cats (Oshu et al., 2010; Maruyama et al., 2012). Various L-amino acids, L-amino acid derivatives, biogenic amines, glutathione (GSH) and its derivates, as well as β-aspartyl and γ-glutamyl peptides and certain aminoglycoside antibiotics studied by Laffitte et al. (2021) have been identified as agonists of the CaSR in cats. The study provides initial insight into certain components within a food that may be showing a direct link to palatability in cats. For example, γ-glutamyl peptides are of particular interest in cats as they are linked to GSH metabolism, and both are found in different meats. In addition, the biogenic amine spermidine is found in chicken liver, a food source that is palatable to cats and utilised in pet food formulations.

1.7.3 Structural Changes to Meat Due to Age of Animal at Slaughter

Regarding human consumption of meat, texture and meat tenderness play a vital role in consumer acceptance (Tornberg, 1996; Warner et al., 2021). Collagen is an abundant connective tissue and contributes greatly to the variation in meat texture and tenderness (Weston et al., 2002). Studies have shown that certain beef muscles decrease in tenderness as the animal gets older, particularly for muscles with high connective tissue strength, such as Bicep femoris, which trebled in toughness between young versus older beef, compared with the Psoas major which had no effect on meat tenderness with the animal’s age at slaughter (Shorthose and Harris, 1990). It is known that the strength and number of cross-links of intramuscular collagen in older animals increases and the collagen becomes less heat soluble with age, therefore resulting in greater perceived toughness (Hill, 1966; Warner et al., 2021; Weston et al., 2002).

In the pet food industry, humanisation, defined as the circumstance in which owners consider their pet, and their relationship with their pet, as if it was human in nature, along with the premiumisation of pet foods have remained two of the most dominant trends in the market (Forbes et al., 2018). Although not studied in the pet food industry to date, the age of animal by-products included in pet food may be a factor to consider in the production of premium pet foods, particularly as owners remain increasingly aware of what they feed their pets and look to seek the best for them.

1.7.4 Palatability of Meat and Meat By-Products

While animal-sourced proteins are essential for cats, little published information exists on the relative palatability of different types of meat and meat by-products and the factors that drive preference for one meat over the other. Bradshaw et al. (1996) acknowledges that limited research in this area is likely due to factors such as the freshness, nutritional status of the animal at slaughter, and subsequent processing affecting the meat.

Studies in dogs have shown a preference for different meats when fed raw, with beef the most preferred, followed by lamb, then chicken and horsemeat the least preferred (Lohse, 1974). Preference for canned or cooked over fresh meat was also observed, along with minced meat over chunks of meat, and canned meat over fresh meat (Lohse, 1974). In addition, free-ranging dogs seemingly following the rule of thumb that "if it smells like meat, eat it" as a means of maximising the utilisation of resources that contain any quantity of protein (Bhadra et al., 2016; Sakar et al., 2019). However, it has also been shown that this rule of thumb is not innate and needs to be learned by pups (Bhandra and Bhandra, 2014).

In cats, studies have revealed preferences for fish, specifically salmon, over commercial cat food (fish, liver, chicken, or beef flavoured) and rats (Adamec, 1976; Houpt & Smith, 1981). Additionally, the geniculate ganglion, a sensory structure of the facial nerve of the cats consisting of three distinct neural populations; 1) ear units, 2) regular discharge units and 3) tongue units, are all influenced by different types of stimulation (Boudreau et al., 1971). For meat products that had been diluted in distilled water, it was found that pork liver, pork kidney, tuna and chicken were the most effective food stimuli on the tongue units, with egg white and sucrose being the least effective (Boudreau et al., 1971).

As well as these classical studies, preliminary research investigating the palatability of commonly used beef and lamb offals when fed raw to cats has also been reported by Watson et al. (2020). Although the study was successful in determining the acceptance of offal, with liver being the most palatable and mechanically deboned meat (MDM) being the least palatable within beef and lamb, as well as a consistent preference for lamb over equivalent beef offal based on food intake and percentage consumption data, the underlying nutritional factors contributing to differences in palatability remains unclear.

The palatability of meat and meat by-products, both in their raw and processed forms, remains an area that is overlooked, particularly in feline nutrition studies. As the pet food industry continues to grow and utilise animal by-products in pet food formulations, further investigation is required to understand what drives preference for certain by-products over others. Such information could be used by manufacturers to optimise ingredient inclusion levels to deliver a product that is highly palatable and cost effective to manufacture.

1.7.5 Specific Nutrients

Nutrient components of diets including dry matter, crude protein, crude fibre, ether extract, nitrogen free extract, ash, calcium, phosphorus, total lipids and metabolisable energy have been evaluated to determine their influence on palatability in the cat (Alegría-Morán et al., 2019). Using principal component analysis and linear regression, the dietary fibre content along with the mineral components, calcium, phosphorus, and ash were identified as constituents which negatively affect food preference in cats. Diets that did not include appreciable levels of these components were therefore presumably more palatable than the diets with higher levels of them.

Other than this study, limited work has been done to identify nutrient drivers and inhibitors of palatability. For this reason, more research is required to bridge the gap between identifying complete foods that are more palatable and understanding the nutritional factors within them driving intake. Modern analytical techniques such as metabolomics, which is defined by Clish (2015) as the comprehensive analysis of metabolites in a biological specimen, may provide greater insight into the compounds responsible for characterising the nutritional and sensory properties within key ingredients in pet food (Muroya et al., 2020).

1.7.6 Physical Properties of Food

Dry and wet diets are the food formats most purchased by pet owners in New Zealand but differ significantly in their nutritional composition. Wet diets have a protein content which is closer to a cat's 'target intake', with more fat and minimal carbohydrates. Whereas dry foods often have less protein, similar fat levels to wet diets and carbohydrates can be as high as 40% (Watson, 2011). This may explain why wet food, which has a similar nutritional composition and water content as meat, may be more palatable than other semi-moist and dry foods (Zaghini and Biagi, 2005).

Processing:

The temperature and time for which pet foods are processed also has an influence on acceptance and palatability. Hagan-Plantinga et al (2017) studied the effect of retorting temperature on palatability using two-bowl testing. Three retort temperatures were used with different times to ensure equal lethality for the different temperatures. Retorting canned food at 113°C for 232 minutes resulted in a less viscous, less firm, and less adhesive product with greater particle size compared with two other canned foods processed at 120°C for 103 minutes and 127°C for 60 minutes. In addition, a greater preference ratio was shown for the 113°C diet, 0·38 compared to 0·31 for 120°C and 0.31 for 127°C. It was concluded by the authors that the higher temperatures disrupted the binding properties, negatively affecting texture and negatively affected palatability compared to the longer processing times that were necessary for the lower temperatures.

As previously mentioned, Maillard products in wet food have a positive influence on palatability in cats (Zaghini & Biagi, 2005; Tamanna & Mahmood, 2015). In contrast, lipid oxidation results in decreased palatability, as the off notes are easily detected by cats (Hagen-Plantinga et al., 2017; Zaghini & Biagi, 2005). To combat this problem, antioxidants are added to pet foods to prevent the oxidation of lipids, preserve nutrient quality, and maintain product freshness (Chanadang, Koppel & Aldrich, 2016; Gross et al., 1994, Hilton 1989).

Shape and texture:

Kibbles with sharp edges are known to be unfavourable to cats as these can cause abrasions in the mouth and stomach (Zaghini, & Biagi, 2005). Coating the outside of kibble with fat has a positive impact on food texture rather than contributing to flavour (Zaghini and Biagi, 2005). In terms of wet foods, the stickiness and viscosity are important factors to consider in the production of wet foods (Watson, 2011).

Serving temperature:

Rejection of food by cats is observed if the temperature of the food is below 15°C or above 50°C (Zaghini, & Biagi, 2005). Cats will often refuse palatable foods if served chilled and tend to prefer food at a temperature equivalent to the body temperature of live prey, or if not at least room temperature (Bradshaw et al., 1996). A study by Eyre and colleagues (2022) which examined specific serving temperatures of wet food to older (>7 years) domestic short-haired cats found that they preferred food served at 37°C (i.e. body temperature) compared to room temperature of 21°C, with food chilled to 6°C being the least preferred. Volatile compounds were also analysed in this study, with the hypothesis that warming food may help enhance the flavour profile for aging cats and help encourage those that have lost interest in eating to consume enough to maintain a healthy body weight, which could be examined in future studies and used to create more robust feeding guidelines for older and/or fussy cats (Eyre et al., 2021).

1.8 Conclusions

Pet food palatability, particularly for cats, continues to be of great importance to both manufacturers and owners. Currently, traditional palatability testing methods are used to assess the acceptance and preference of complete and balanced pet food, as well as treats. However, limited studies have used these traditional methods to assess the palatability of individual diet components, specifically meat and its by-products, which are important for the carnivorous cat. It is known that cats show differences in palatability for selected by-products, however, more work is required to determine the fundamental drivers responsible for these differences, which may be identified using modern techniques such as metabolomics. As a result, a collective approach using traditional palatability testing methods and modern analytical testing may help to not only determine the optimal inclusion level of ingredients to maximise palatability, but also the nutrients responsible for driving preference which, to date, has been understudied at the fundamental level.

1.9 Scientific Aims and Hypotheses

The overall aim of this thesis was to examine the palatability of selected beef and lamb by-products commonly used in the production of cat food in New Zealand and determine the compounds and/or nutrient drivers responsible for any palatability differences between ingredients. In detail, I aimed to address the following research questions:

1. *Whether or not palatability differences exist between beef and lamb by-products that have undergone heat treatment (specifically air-drying) when fed to cats?*
   Currently, palatability differences have been observed between beef and lamb by-products when fed raw to cats. However, majority of pet foods typically undergo heat treatment during manufacture. Therefore, the aim of this study was to evaluate whether cats showed varying levels of acceptance for different heat-

treated by-products from lamb and beef, as well as to examine their preference for the air-dried by-product between the meat-producing species to deliver a minimally processed, heat treated final product (Chapter 3).

As part of this study, both acceptance testing of individual lamb and beef by-products, as well as preference testing between equivalent by-products from lamb and beef was conducted. Additionally, the nutritional and physical properties of the samples were analysed and correlated to intake to determine possible drivers or inhibitors of palatability.

2. *Do cats show a preference for animal by-products of different ages? (Calves, young bulls and steers, lamb, and mutton comparison). If so, what nutrients and/or compounds within the ingredients are responsible for differences in palatability?*

   The age of the animal at slaughter defines meat tenderness for human consumption. To date, no studies have examined the effect of the age of an animal at slaughter on the palatability of the by-products in pet food. A difference in palatability for by-products from animals of different age would provide petfood manufactures with a mechanism to maximise palatability and further distinguish pet food products.

   This study was divided into two parts, firstly, preference testing was used to examine if cats show within-species differences in intake for sheep and beef by-products obtained from animals at two different ages. Secondly, the nutrient composition, as well as metabolomic and lipid oxidation (TBARS) analyses was used to identify specific nutrients and/or compounds which could be driving preference within each by-product (Chapter 4).

3. *How palatability influences the macronutrient self-selection by cats when diets formulated with the inclusion of highly and poorly palatable ingredients are used?*

   Using the results from the two previous research questions, the final aim of this project was to develop a series of poorly palatable and highly palatable diets from a limited set of ingredients that varied in protein, fat, and carbohydrate content. This study was conducted to examine the macronutrient selection by cats when given *ad libitum* access to three air-dried diets simultaneously (high protein, high fat, and high carbohydrate). Previous work in this area has not assessed a single (air-dried) diet format, nor examined palatability by using naturally poorly palatable or highly palatable ingredients as the base of the diet (Chapter 5).

Chapter 2 Air-Drying Method Development

2.1 Introduction

Thermal processing is one of the most utilised methods for preservation in the production of pet food (Ghani et al., 2001; Santana et al., 2013). In most commercial pet foods, heat treatment is used to kill bacteria and minimise microbial activity and growth to deliver safe final products, with the mechanism of preservation varying slightly between dry and wet pet foods. For example, in commercial canned pet food, ingredients are placed in a hermetically sealed container and thermally processed to deliver a commercially sterilised product, which upon opening and inappropriate storage conditions the product will undergo rapid microbial growth (Lambertini et al., 2016). In contrast, extruded pet foods undergo a cooking step with the aim to reduce possible pathogens and lower water activity (Lambertini et al., 2016). The low moisture content of extruded pet food is the main mechanism used to prevent growth during storage. However, unlike canned food, extruded pet food is not commercially sterile so may contain low levels of a range of microorganisms.

Pet food remains the largest area of spending under the pet care sector of the market (Euromonitor International, 2021). When examining cat food in particular, wet foods continue to dominate in terms of both volume and value compared to dry food. In 2020 alone, the volume of wet cat food sales in New Zealand reached 34,645.7 tonnes compared to 11,513.6 tonnes for dry, with this correlating to $252.3 million in sales of wet food compared to $115.7 million for dry food. (Euromonitor International, 2021). However, consumers do show a great appreciation for dry cat food which is likely driven by the convenience, longer shelf-life and cheaper cost associated with dry pet food compared to wet food which requires minimal, but some effort to prepare and is slightly higher in cost (New Zealand Companion Animal Council, 2016).

While traditional pet food formats (i.e., canned/wet or extruded/kibble) continue to dominate the market, there is growing interest in less processed pet food formats – including freeze-dried, air-dried, and frozen pet foods. Many pet owners are willing to spend more for these minimally processed foods compared to dry biscuits (Gyles, 2017). The focus of this thesis is to understand the palatability of air-dried animal by-products therefore, it was important to develop a standardised air-drying methodology before any palatability trials could take place.

Although pet food manufacturers have standardised processes in place to manufacture air-dried diets, these processes have been established for their specific formulations which consist of blends of multiple ingredients. The binding properties and palatability performance of a complete diet versus individually prepared by-products are likely to be different. Therefore, the main aim of this work was to start by replicating the temperature-time profiles used by industry in the manufacture of air-dried diets. However, this was then adjusted so that all by-products between species were processed as similarly as possible, as there are multiple factors that can influence palatability in cats, with cooking temperature and time being identified as key processing factors in pet food palatability.

Due to these two important criteria, this chapter provides a description of the method development work, as well as the final air-drying process that was used in the preparation of all air-dried by-products required for palatability trials in the following experimental chapters.

2.2 Raw Materials

Beef and lamb by-products (lung, heart, kidney, tripe, mechanically deboned meat (MDM), spleen, and liver) were commercially sourced through Ministry for Primary Industries (MPI) accredited meat processors in New Zealand. The by-products were delivered in approximately 15 to 25 kg frozen blocks.

2.3 Ingredient Preparation

The frozen by-products blocks were cut into smaller pieces using a band saw and packed into approximately 1.25 to 2 kg portions. All prepared bags of by-product were refrozen in a -30°C freezer.

2.4 Air-Drying Trials

A series of trials were used to determine the equipment and temperature-time cooking profiles needed to deliver an air-dried product consisting of water activity (α_w) of ≤ 0.60 and/or a moisture content of $\leq 10\%$, a final product thickness of 3mm, and a final product size of ~1.5 cm x 1.5 cm.

2.4.1 Trial One

Sub-samples of beef lung (1.95 kg) and beef MDM (1.5 kg) were used to compare the difference between preparing and air-drying beef organ meat which is generally collected as whole organs and provided frozen to pet food manufacturers, verses MDM, which is already in a frozen minced form as a final product.

Beef lung and MDM were separately minced through an 8 mm hole plate using a Dynasty Meat Grinder HL-GL12SS (Feng-Yuan, Taiwan) and then individually mixed in a Kenwood PM900 Professional Major benchtop mixer (Hampshire, England) for 10 minutes. Mincing was used as way to deliver consistency across all by-products and was seen as a more practical and safer way to deliver the desired thickness of 3 mm for the final air-dried product, as opposed to slicing or cubing the frozen by-product blocks. The mixed samples were then each placed into a handheld extruder which was used to produce strips approximately 10 cm wide and 3 mm thick. Sheets were then laid out onto a pre-weighed perforated deep oven tray and dried for one hour each at 60°C, 70°C, 80°C and then 90°C in an Inoxtrend Combi oven XBP-120E (Santa Lucia di Piave, Italy). Samples are shown in Figure 2.1 after drying.

Figure 2.1. Beef lung (left) and beef MDM (right) after drying at 60°C, 70°C, 80°C and 90°C for 1 hour at each temperature.

The samples were then cooled and placed in a 5°C chiller overnight. The following day, the beef lung strips and MDM strips were cut into 1.5 x 1.5 cm pieces using a knife and placed in deep oven trays lined with baking paper to undergo further drying at 70°C, as shown in Figure 2.2.

Figure 2.2. Beef lung (left) and beef MDM (right) following cutting prior to drying at 70°C

The beef lung and MDM samples were dried until they achieved a final water activity reading (α_w) of 0.60 or less. Subsamples of each product were removed from the oven after every hour of drying, cut into small pieces, and their water activity was determined using a benchtop AQUALAB 4TE water activity meter (Washington, USA).

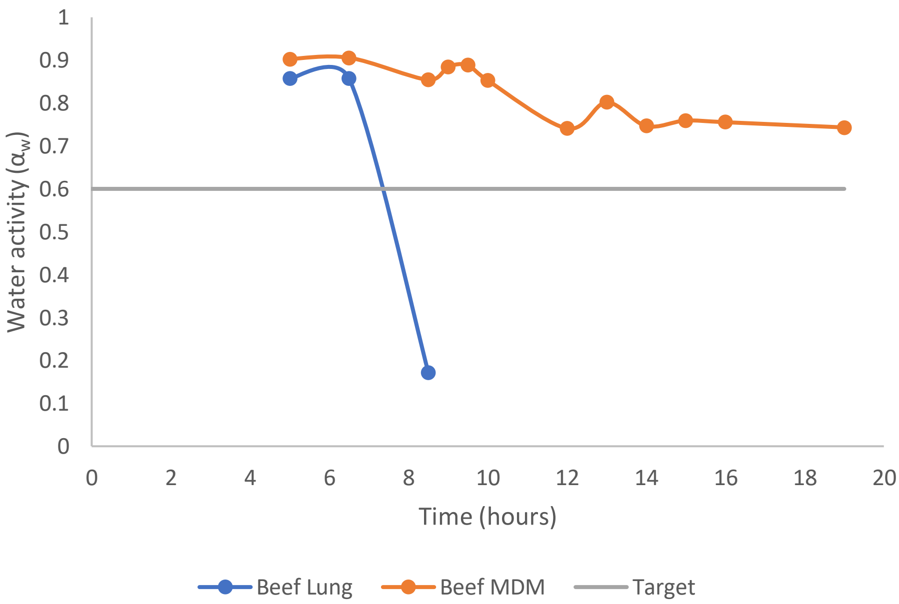


Figure 2.3. Drying curve for beef lung and beef MDM

The water activity reading of beef MDM fell to 0.74 after 12 hours of drying at 70°C and stabilised at this level during the next 6 hours, so drying was therefore discontinued. There are a few reasons that may explain why drying did not reduce the water activity further. Firstly, it is possible that MDM was displaying hygroscopic properties and gaining moisture before measurement of water activity (Srikiatden & Roberts, 2007). Secondly, the beef lung and MDM samples were, in hindsight, double (approx. 6 mm) the desired thickness of the final product causing case-hardening. This is where the increased thickness may have meant that the outer layers dried and hardened making it difficult for the moisture at the centre to diffuse to the surface in order to dry (Achanta et al., 1997; Gulati & Datta, 2015). Therefore, the decision was also made to address the issue of product thickness before developing complete batches needed for palatability trials.

In addition to the potential thickness issue, extruding the samples directly onto the stainless-steel tray resulted in an intermediate product after the first day of drying that had an undesirable case-hardened exterior and raw underside, rather than an intermediate product of similar consistency throughout the whole strip.

In conclusion, further refinements were required to not only address the product thickness issue, but also determine the most suitable surface needed to deliver even heat transfer to the upper and lower layer of the products during cooking to prevent case-hardening and allow the product to achieve a water activity below 0.60.

2.4.2 Trial Two

Sample portions of lamb kidney (1.9 kg) and lamb MDM (1.9 kg) were used to investigate improvements in preparation and air-drying, as these products have observable differences in their fluidity when minced. This also provided the opportunity to test the use of racks to hold the samples during drying rather than the use of trays. The use of racks was investigated in an attempt to increase the rates of heat and mass transfer both above and below the strips during cooking to reduce the moisture gradients between the surface and inside of the product and prevent the case-hardening that was observed in Trial One.

As in Trial One, lamb kidney and MDM were separately minced and then mixed for 10 minutes. By-products were extruded into strips and placed on two overlapping 41 cm x 25 cm cooling racks placed across a deep oven tray. However, mixing the lamb kidney resulted in a more liquid slurry which did not hold well on the cooling racks and began to sag through the cross hatching of the rack as seen in Figure 2.4. The decision was made to abandon the kidney and proceed with only the MDM using the tray drying.

Figure 2.4. Comparison of lamb MDM extruded strips (left) compared to lamb kidney (right).

During the first day of drying, lamb MDM was dried for four hours - one hour each at 60°C, 70°C, 80°C and 90°C. After drying, it was observed that the MDM strips adhered to the racks and needed to be scraped from them following cooking before being cut into small pieces and cooled overnight in preparation for drying the following day, as shown in Figure 2.5. This resulted in unnecessary product loss, which was minimised with minor improvements such as lining trays with baking paper in subsequent work.

Figure 2.5. Lamb MDM following day one drying (left) and residual product on tray following scraping (right).

The next day, lamb MDM was dried at 70°C, with water activity and moisture content determined during the cooking cycle, as shown in Table 2.1. This was done to determine whether drying to a consistent water activity of ≤0.60 (Labuza and Altunakar, 2020), or to a moisture content of ≤10% (Ministry for Primary Industries, 2018) using an Ohaus moisture analyser (New Jersey, USA), should be utilised as the standard drying parameter.

Table 2.1. Corresponding water activity and moisture content reading at the designated day two drying times a 70°C for trial two.

Day two drying time at 70°C (hours)	Water activity (α_w)	Moisture content (%)
2	0.84	7.55
4	0.51	5.81
5	0.27	3.69

The sample product was left to cool at ambient room temperature for 10 minutes, bagged and stored in ambient conditions overnight. On the following morning, the water activity level and moisture content were recorded after allowing the sample to reach equilibrium when stored in the bag overnight. The lamb MDM gave water activity readings of 0.70 and 0.79 and a moisture content of 5.38%. Due to the deviation from the final water activity reading of 0.2733 after five hours of drying at 70°C the previous day, these results confirm that the hygroscopic property of MDM was limiting its ability to achieve the target water activity of 0.60 or less.

Therefore, the decision was made to use a moisture content of ≤10% as the target drying parameter. This aligned with the standard used by the pet food industry in the manufacturing of dry food. Products reaching the target moisture content of ≤10%, were left to cool in ambient temperatures before being bagged to prevent condensation accumulating in the bag. The bags of product were then stored at -30°C until required for palatability studies and laboratory analyses.

2.4.3 Trial Three

The next stage of the work investigated the arrangements of racks and trays in the oven including (1) the position of the rack in the tray, (2) the position of the rack across the top of a tray and (3) the use of trays with no racks for sub-samples of beef heart (2.1 kg) and lamb kidney (1.25 kg).

Beef heart was prepared using the extrusion method previously described; however, lamb kidney was processed by rolling the product into a large sheet using a flat tray with a 7 mm lip on each long side and a rolling pin with a 4 mm grove to deliver a product with a 3 mm thickness, as shown in Figure 2.6.

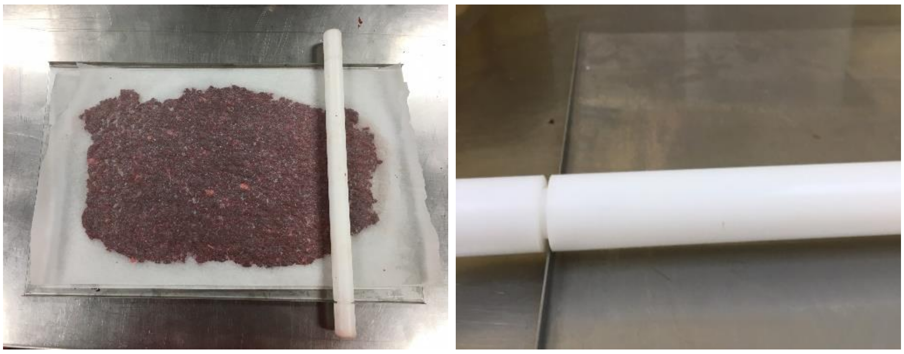


Figure 2.6. Left: Rolling pin set up with product. Right: Close up of the 4 mm groove in the rolling pin sitting in the 7 mm lip on the tray to deliver product with a 3 mm thickness.

All trays were lined with baking paper to minimise product loss. The beef heart and lamb kidney were then dried for three hours - one hour each at 60°C, 70°C and 80°C, with the products turned over after the 70°C stage of the process. Figure 2.7 shows the lamb kidney samples after they were turned over using three different drying methods.

Figure 2.7. Set ups from left to right (1) Rack in tray, (2) rack on tray, and (3) no rack set ups used for lamb kidney

The products were then cut into shape and cooled overnight in preparation for continued drying the next day. Drying times on day two and the corresponding moisture contents are provided in Table 2.2

Table 2.2. Moisture content readings of beef heart and lamb kidney after drying at 70°C for up to three hours in trial three.

Drying time at 70°C	Moisture content (%)	
(hours)	Beef heart	Lamb kidney
1	> 30.00	9.33
2	17.19	-
3	8.88	-

This trial demonstrated that positioning the rack across the top of a tray (2) allowed greater heat flow and mass transfer above and below the product during drying, as shown in Figure 2.7. This resulted in a more evenly dried product than observed in set ups (1) and (3), which continued to show rawness on the underside of the sheets. Therefore, the rack on tray set up was adopted for the manufacture of samples for palatability testing and laboratory analyses.

2.4.4 Trial Four

The final drying trial was used to examine whether a shorter mixing time would improve the handling of kidney during sample preparation, as 10 minutes of mixing in Trial Two may have been too long for the lamb kidney samples and resulted in a slurry that was too thin (Booren et al., 1981). For this trial, sample portions of lamb kidney were mixed for one minute which resulted in a more viscous paste than in trial two (Figure 2.8).

Figure 2.8. Lamb kidney after one minute of mixing

Lamb kidney samples were again prepared using the handheld extruder and were then dried for three hours - one hour each at 60°C, 70°C, 80°C with the products turned over after an hour at 80°C as the product was too wet to be turned over after the 70°C period of drying which was standard for the other by-products. The product was then dried at 80°C for an additional hour, resulting in a very flaky product with a moisture content of 12.81%. Images of the product before drying, after the standard drying process and after the additional hour of drying at 80°C are shown in Figure 2.9.

Figure 2.9. Lamb kidney from left to right (1) before drying, (2) after being turned over at the end of the standard drying process and (3) at the end of drying in trial 4

The kidney was then cut and dried at 70°C directly after for an additional 15 and 30 minutes, in which moisture readings of 10.31 and 8.21% were given, respectively.

This work showed that even with a shorter mixing time the kidney product was remained flaky rather than the desired jerky-like squares. Additionally, as only individual by-products were being processed at a time (e.g. no combining of different by-products to create a mixture), it was determined that there was no need to mix the by-products following mincing.

2.4.5 Extrusion Trial

An additional non-drying trial was carried out to examine the use of an extruder head attachment that was made to fit the sausage stuffer to streamline the production process, as seen in Figure 2.10.

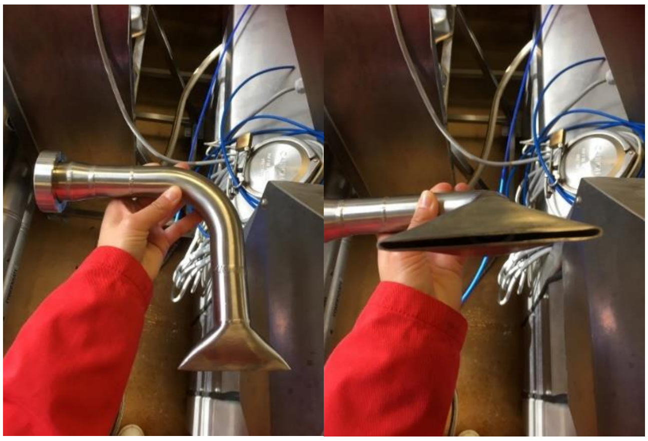


Figure 2.10. Extruder head attachment fabricated to fit the sausage stuffer

However, a few key issues were found with the use of this process and are detailed below:

- Increased product loss: A substantial amount of raw product was lost in the L bend of the extruder head. This resulted in greater product loss compared to the more labour-intensive process previously adopted. It was also quite difficult to clean and remove the trapped by-product.

- Flowability issues: MDM did not flow through the attachment well at all due to the low moisture content of the raw material and its fibrous nature, as seen in Figure 2.11. In contrast, kidney would flow straight through and not hold the desired strip shape.
- Two-person process: Two people are required for effective use of the sausage stuffer with the extruder head attachment. One person is needed to turn the handle on the sausage stuffer, and the other is needed to catch the product underneath using the tray.
- Insufficient use of tray space: The stand the sausage stuffer is on blocked the tray from maximising the number of strips that could be laid.

Figure 2.11. Left: Resulting MDM 'strips' following extrusion using sausage stuffer. Right: Kidney strips which flowed extremely quickly out of the extruder head.

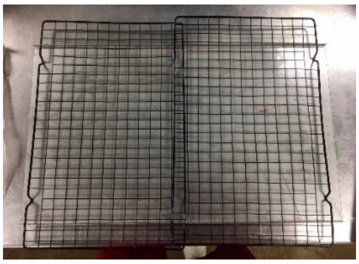
Rather than streamlining the preparation process, the use of the sausage stuffer with the extruder head attachment was more time-consuming and problematic than helpful, so was not pursued for use in the batch samples required for palatability testing.

2.5 Final Air-Drying Method

The series of drying trials resulted in the following process (outlined in Table 2.3) being developed as the final standardised air-drying method to cook the by-products.

Table 2.3. Finalised air-drying process

Inputs	Process Description	Outputs
Raw by-product	1. Each by-product was minced through an 8 mm hole plate. 	Minced by-product
Minced by-product	2. Approximately 350 g of minced by-product was placed on baking paper lined tray with 7 mm lip at each long edge. A second sheet of baking paper was placed on top of the by-product before it was rolled flat using a rolling pin with a 4 mm groove.  (Top layer of baking paper was peeled back after rolling)	Sheet of raw by-product with a thickness of 3 mm
Sheets of raw by-product	3. Sheet of by-products on the baking paper were placed onto two overlapping racks lining the tray. The top layer of baking paper was removed, and the trays	

	were placed in the oven in preparation for day one of drying.   The same by-product from different species were processed for the same length of time. Different by-products were processed for different lengths to time to achieve the same target moisture level (as described in Table 2.4).	
	4. Two hours through the first day of drying, all trays of product were turned over onto the rack and placed back in the over for further drying.  	
	5. Following day one of drying, the cooked by-product sheets were then cut into small ~1.5 cm x 1.5 cm squares and stored in a sealed plastic bag in the 5°C	Semi-cooked sheet of by-product into jerky squares

	chiller overnight in preparation for day two. 	
Semi-cooked jerky squares	6. The cut jerky squares were evenly divided into deep perforated oven trays and placed in the oven for day two drying at a set temperature of 70°C.  	
	7. Moisture readings: After every hour of drying, approximately 3 g of product was removed from the trays, cut into fine pieces, and used to check the moisture reading of the samples.	

	 	
	8. Once a moisture reading of 10% or less was observed, products were removed from the oven and left to cool before being bagged and frozen at -30°C until required for palatability testing and laboratory analyses. 	Finished product – air-dried by-product.

While the same by-products from beef and lamb were processed to similar day one cooking temperatures and times, there were significant differences in how long it took for each of the by-products to achieve the 10% or less moisture reading needed following the second day of drying at 70°C. The full time-temperature profiles of all the by-products are therefore described in Table 2.4.

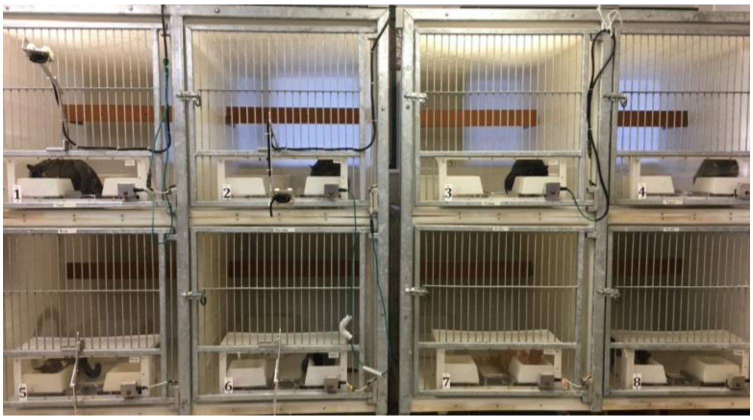
Table 2.4. Air-drying time-temperature profiles for the different by-product varieties.

By-products	Day one drying profile	Day two drying profile at 70°C	Total drying time
Lung			
Lamb	60°C - 1 hour, 70°C - 1 hour, 80°C - 30 minutes	5.5 hours	8 hours
Beef			8 hours
Heart			
Lamb	60°C - 1 hour, 70°C - 1 hour, 80°C - 1 hour	8.5 hours	11.5 hours
Beef		6 hours	9 hours
Kidney			
Lamb	60°C - 1 hour, 70°C - 1 hour, 80°C - 1 hour	6 hours	9 hours
Beef	60°C - 1 hour, 70°C - 1 hour, 80°C - 1.5 hours	6 hours	9.5 hours
Tripe			
Lamb	80°C - 2 hours, 90°C – 1 hour	3 - 4.5 hours	6 - 7.5 hours
Beef		2.5 - 3.5 hours	5.5 - 6.5 hours
MDM			
Lamb	60°C - 1 hour, 70°C - 1 hour, 80°C - 1 hour	6 hours	9 hours
Beef		7 hours	10 hours
Liver			
Lamb	60°C - 1 hour, 70°C - 1 hour, 80°C - 1 hour	9.5 hours	12.5 hours
Beef		8.5 hours	11.5 hours
Spleen			
Lamb	80°C - 2 hours, 90°C – 1 hour	3 - 4.75 hours	6 - 7.75 hours
Beef		2.75 - 3.75 hours	5.75 - 6.75 hours

2.5.1 Protein Conversion Factor

In addition to processing temperature, calculations for crude protein content throughout this thesis was calculated by multiplying nitrogen by a correction factor of 5.6 instead of the traditional 6.25 nitrogen-to-protein conversion factor. The 6.25 conversion factor assumes the nitrogen content of proteins to be 16% (Mariotti et al., 2008). However, the value 5.6 is given for food sources of meat, fish and eggs as they contain less non-alpha amino nitrogen and is therefore considered more accurate to use for the purpose of this thesis (Mariotti et al., 2008).

Chapter 3 Within- and Between-Species Comparison of Air-Dried Beef and Lamb By-Products



3.1 Introduction

In New Zealand, cats are identified as the most common household pet. Along with their popularity, cat food accounts for nearly half of pet food expenditure by pet owners (New Zealand Companion Animal Council Inc., 2016). Within the cat food market, wet foods (e.g. canned and pouch) makes up 44.8% of the value share of the main pet food categories, followed by dry food at 34.8%, and chilled at 11% (The New Zealand Pet Food Manufacturers Association Inc., 2021).

Feeding raw meat-based diets is becoming increasingly popular among pet owners, particularly as they are becoming more conscious of what they are feeding their pets (Schlesinger & Joffe, 2011). Accordingly, there has been a dramatic increase in sales of freeze-dried, air-dried, and frozen pet foods in recent years, with owners willing to spend more for minimally processed food compared to dry kibble (Schlesinger and Joffe, 2011).

While interest is growing for raw foods, particularly meat-based diets, there is still concern over the safety of these diets, primarily in relation to the associated microbiological hazards, particularly the improper handling of these pet foods by owners and the human health hazards of raw feeding (Davies et al., 2019; Knight & Leitsberger, 2016; Marks et al, 2011). However, in terms of palatability, previous work by Watson et al. (2020) evaluated the acceptance and preference of selected beef and lamb by-products (lung, heart, kidney, tripe, mechanically deboned meat (MDM), and liver) when fed raw to cats. From this, cats showed a clear ranking of acceptance for lamb and beef by-products, with liver being the most palatable by-product and MDM the least accepted within each meat species. Cats also showed a general preference for lamb compared to beef.

While the studies using raw, fresh product (Watson et al., 2020) identified differences in acceptance and preference, it did not examine the impact heat treatment would have on the ranking of by-product acceptance within beef and lamb nor the preference between species. The majority of pet food is manufactured using some form of heat treatment, such as baking, retorting, and drying. For example, drying beef and lamb by-product using a heating process has the advantage of killing bacteria, as well as removing water and reducing the water activity which helps to minimise microbial activity and growth, improving both the safety and shelf life of the product (Lambertini et al., 2016).

Therefore, the aim of this chapter was to evaluate whether cats showed varying levels of acceptance for different heat-treated by-products within lamb and beef, as well as to examine their preference for the air-dried by-product between the meat species as a way to deliver a minimally processed, heat treated final product.

As well as determining the level of acceptance and preference for air-dried beef and lamb by-products, this trial examined the physical and nutritional properties within the samples to begin to account for possible differences in palatability. Texture is known to be an important physical aspect in pet food acceptance for cats (Case et al., 2010). Texture research has been used to examine behavioural characteristics, such as grasping and biting of kibbles by pets, as well as determine the most suitable size and shape that would allow easier ingestion of kibble for cats and dogs (Koppel, 2014). Similarly, work by Alegría-Morán et al. (2019) revealed that specific nutrients, namely crude fibre, as well as calcium, phosphorus, ash and hydroxyproline have a negative influence of food preference in cats. Using the knowledge from literature, the secondary aims of this trial were to examine the relationship between by-product hardness (as a measurement of texture) and palatability, as well correlate nutrient contents to palatability to determine possible drivers or inhibitors of palatability.

3.2 Materials and Methods

All animal procedures described in this chapter were approved by the Massey University Animal Ethics Committee (Protocol MUAEC 18/16).

3.2.1 Test Animals

A trained panel of eight domestic short-haired cats were used to test the acceptance of seven air-dried by-products (lung, heart, kidney, tripe, MDM, liver and spleen) within lamb and beef, as well as the preference between the same air-dried by-products across lamb or beef. The cats in this trial were all healthy and consisted of four neutered males, one spayed female and three entire females aged from two to 11 years of age (average age of 5.30 ± 0.99 years), as shown in Table 3.1. Cats were trained and selected based on their ability to feed from small bowls without excessive mess and accept temporary isolation from their cage mates during testing time (Tarttelin, 1997).

Table 3.1. Information on the panel of cats used for the palatability trial.

Cat	Name	Gender	Neutered	Date of birth	Age* (years)
1	Craig	Male	Yes	12 January 2016	4.08
2	Orca	Male	Yes	10 March 2015	4.93
3	Zoro	Male	Yes	25 February 2014	5.96
4	Devon	Female	No	29 October 2008	11.29
4a	Mint	Female	No	1 February 2018	2.03
4b	Tiger	Female	No	29 September 2014	5.37
5	Pania	Female	No	28 February 2017	2.95
6	Sarah	Female	Yes	14 February 2015	4.99
7	Chewy	Male	Yes	9 February 2018	2.00

* Age of the cats at the start of testing (10th February 2020)

Due to persistent vomiting during lamb kidney acceptance testing, Devon (cat 4) was replaced with another female cat, Mint (Cat 4a), for the beef versus lamb kidney preference testing week and reinstated the following week. Vomiting was also observed from Devon during beef spleen acceptance testing. Therefore, the decision was made to replace her for the final two weeks of the trial (lamb spleen acceptance testing and beef versus lamb spleen preference testing) with a different female, Tiger (Cat 4b), as Mint was unavailable for use as the same replacement cat.

Acceptance and preference testing were carried out from Monday 10th February to Friday 20th March 2020. Testing was paused due to Covid-19 lockdown and recommenced on Monday 1st July and was completed on Friday 11th September 2020. All testing was carried out at the Centre for Feline Nutrition at Massey University, Palmerston North.

3.2.2 Testing Methods

During testing days, cats were fed a reduced amount (80%) of their standard canned diets in their communal pen. The standard canned diet was fed to the cats after testing from 11:00am and any refusals were removed at 8:00am the following morning. On non-testing days, the cats were given their full standard canned diet amount.

During testing, the eight cats were placed in individual testing booths for one hour a day for five days for each by-product. This was carried out to obtain 40 measurements for each test.

Acceptance testing

Two-bowl testing was used to determine the total food intake of each lamb and beef by-product to develop a ranking of by-product acceptance within each species. All cats were presented 20 g of the same by-product in each bowl, giving a total of 40 g offered at each sitting.

Preference testing

Two-bowl testing was used to determine the total food intake of the same by-product from lamb and beef to examine a species of by-product preference in cats. All cats were presented 20 g of lamb by-product in one bowl and 20 g of the same beef by-product in the second bowl. Bowl positions were alternated each day to remove any possibility of cats showing a positional bias and preference for one bowl over the other (Tarttelin, 1997; Péron and Tobie, 2018).

Load cells were also used to record the real-time intake by cats to observe the number of meals consumed, as well as the visits made to the respective bowls. A meal was defined as a bout of feeding lasting 80 seconds or longer (Thomas et al., 2018). Whereas a visit was classified as an occasion on which food was eaten during a period of feeding less than 80 seconds long, so it does not have such a rigorous definition as a meal (Thomas et al., 2018; Mugford and Thorne, 1980).

Meal and visit results were analysed for seven of the eight cats due to only one load cell recording intake data for Cat 8. Comparative intakes between beef and lamb by-products for this cat were, therefore, unable to take place.

Texture analysis

The maximum force required to pierce the by-products was measured using a Texture Analyser TA XT Plus (Stable Micro Systems, Godalming, Surrey, United Kingdom). Seven samples for each by product were used to obtain force measurements. The average value was then used for correlation analyses.

A compression test using a 2 mm diameter texture analyser probe with a rounded end attachment combined with a base containing a 4 mm hole plate was used to penetrate the samples at a test speed of 1.00 mm/sec using a 5 kg load cell, as pictured in Figure 3.1.

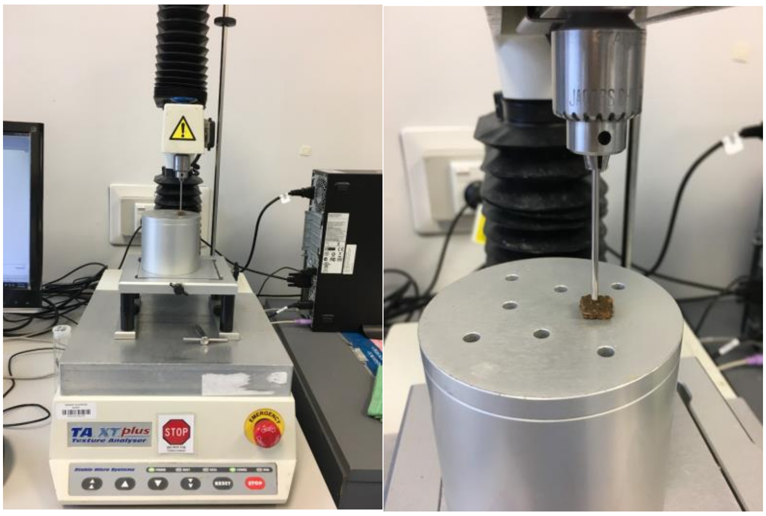


Figure 3.1. Set up of the texture analyser to measure the hardness of air-dried by-products.

Nutritional analysis

Standard Association of Official Analytical Chemist (AOAC) methods were used on all by-products in triplicate to examine the moisture, protein and fat content as well as the concentration of constituents that are likely to affect food preference in cats namely: ash, crude fibre, calcium, phosphorus and hydroxyproline (Alegría-Morán et al., 2019).

The methods used were moisture (method 950.46B), crude protein (Dumas method 968.06, N-P = 6.25), fat (Mojonnier, Acid, 954.02), ash (furnace 550°C, 920.153, 923.03), crude fibre (962.09/978.10 (modified)), calcium (968.08D preparation followed by colourimetric analysis), phosphorus (968.08D preparation, ISO6491.1998E Modified (In-house method) and hydroxyproline (HCl hydrolysis followed by RP HPLC separation using AccQ Tag derivatization, 994.12).

3.2.3 Data Collection and Statistical Analyses

Palatability Analysis

The weight of the bowl and food before and after feeding to the cats was recorded. Acceptance and preference were determined as food intake (g), using the following equations:

$$Food\ intake\ (g) = weight\ of\ bowl\ before\ (g) - \ weight\ of\ bowl\ after\ (g)$$

The average by-product intake of all eight cats on each day of testing was also calculated to evaluate the panels' overall acceptance of each by-product.

All statistical analyses were carried out using Minitab 19 (Minitab Inc., State College, PA, USA).

For acceptance testing, Tukey analysis was carried out using a statistical model that included the interaction between by-product intake and days of testing to determine differences between the food intake results of all the possible pairings of by-products. A significance level of $p < 0.05$ was used. Grouping from the Tukey analysis was also used to develop a final rank of by-product acceptance within each species.

The total intake of all lamb by-products and beef by-products during acceptance testing combined was analysed using paired t-tests using a significance level of $p < 0.05$ to determine whether the cats showed greater intake for one species over the other, regardless of by-product.

For preference testing, paired t-tests were used to determine whether there were differences between the overall intake of equivalent beef and lamb by-products for each test using a significance level of $p < 0.05$.

Interaction between by-product and species were also analysed within meal and visit intake results using a general linear model and a significance level of $p < 0.05$.

Correlation Analysis Within Species

Pearson correlations of the within species by-product intake versus texture and within species by-product intake versus nutritional content were also conducted using a significance level of $p < 0.05$. Definitions for the strength of the Pearson correlations at given values are provided in Table 3.2.

Table 3.2. Definition of the Person correlation values (Ratner, 2009)

Correlation value	Definition
0	No linear relationship
1	A perfect positive linear relationship
-1	A perfect negative linear relationship
Between 0 and 0.3 (0 and −0.3)	A weak positive (negative) linear relationship
Between 0.3 and 0.7 (-0.3 and −0.7)	A moderate positive (negative) linear relationship
Between 0.7 and 1.0 (−0.7 and −1.0)	a strong positive (negative) linear relationship

Hardness Correlation Between Species

A paired t-test was used to determine if the average hardness (as a predictor of texture) of the same beef and lamb by-products was different using a significance level of $p < 0.05$.

3.3 Results

3.3.1 Lamb Acceptance Testing

All cats were offered 200 g of air-dried lamb by-product each week (40 g per day). The mean total intake over the testing period for each by-product (± SEM) was 71.6 ± 1.2 g for lung, 76.1 ± 0.6 g for heart, 95.7 ± 0.6 g for kidney, 85.5 ± 0.8 g for tripe, 34.5 ± 0.5 g for MDM, 129.0 ± 1.4 g for liver and 101.8 ± 1.9 g for spleen (Figure 3.2).

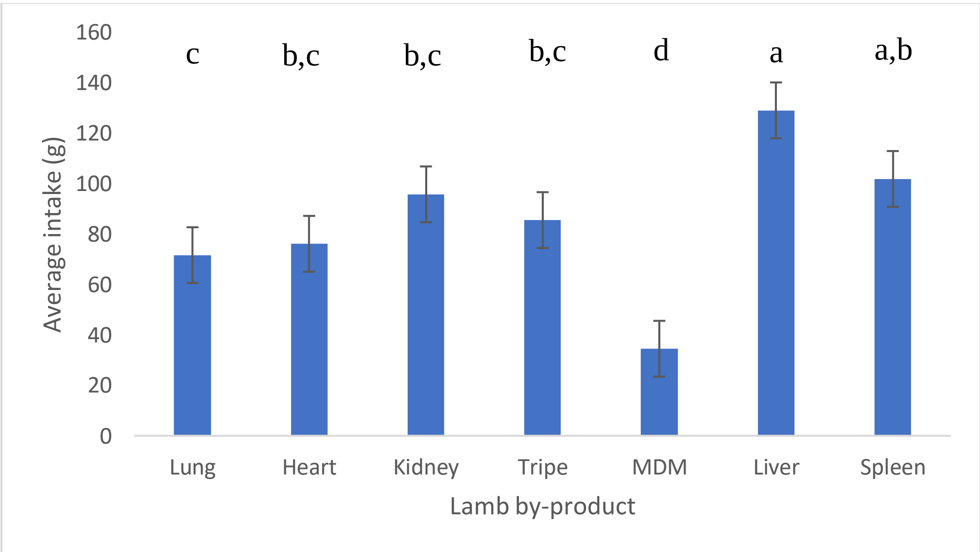


Figure 3.2. Average intake of the seven air-dried lamb by-products out of the possible 200 g served throughout the week.

Liver showed the highest intake along with spleen ($p > 0.05$; Figure 3.2). Spleen was also equally palatable to kidney, tripe, and heart ($p > 0.05$); however, kidney, tripe and heart were less palatable than liver ($p < 0.05$; Figure 3.2). Additionally, lung was also equally palatable to kidney, tripe, and heart ($p > 0.05$) but less palatable than spleen ($p < 0.05$; Figure 3.2). Finally, MDM showed the lowest intake of all by-products ($p < 0.05$). Within each by-product, the day of testing had no influence on the intake results ($p > 0.05$).

3.3.2 Beef Acceptance Testing

All cats were offered 200 g of air-dried beef by-product each week (40 g per day). The mean total intake over the testing period for each by-product (± SEM) was 66.7 ± 0.4 g for lung, 82.9 ± 1.5 g for heart, 86.6 ± 1.3 g for kidney, 38.3 ± 1.1 g for tripe, 38.5 ± 0.5 g for MDM, 108.0 ± 2.2 g for liver and 71.3 ± 1.4 g for spleen (Figure 3.3Figure 3.3).

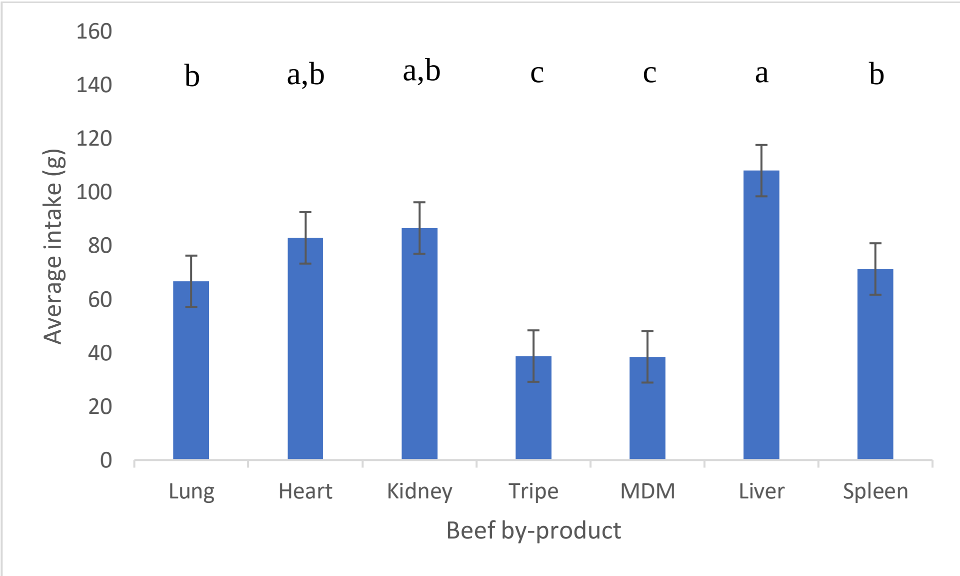


Figure 3.3. Average intake of the seven air-dried beef by-products out of the possible 200 g served throughout the week.

Liver had the highest intake, with kidney and heart also equivalent to it ($p > 0.05$). Kidney and heart were also equally palatable to spleen and lung ($p > 0.05$); however, spleen and lung were less palatable than liver ($p < 0.05$). Tripe and MDM had the lowest intake of all the air-dried beef by-products ($p < 0.05$). Day 5 tripe intake was less than day 4 ($p < 0.05$), but overall, the day of testing had no influence on the intake results ($p > 0.05$).

3.3.3 Correlating Acceptance Intake Results to Texture and Nutrients

In addition to assessing the acceptance of lamb and beef by-products, the weekly food intake results were correlated to texture (Table 3.3) and selected nutrients (Table 3.4) to determine their influence on palatability (Table 3.5).

Table 3.3. Average hardness of air-dried by-products

By-Product	Average Hardness ± SEM[1] (N)	
	Lamb	Beef
Lung	26.9 ± 2.4	35.1 ± 5.0
Heart	20.5 ± 2.5	48.9 ± 2.2
Kidney	15.0 ± 3.0	11.5 ± 1.1
Tripe	22.8 ± 3.9	37.2 ± 6.9
MDM[2]	11.4 ± 1.8	11.2 ± 2.0
Liver	25.2 ± 4.5	31.4 ± 7.3
Spleen	26.5 ± 1.4	15.9 ± 2.2

[1]SEM defined as standard error of mean, [2] MDM defined as mechanically deboned meat

Table 3.4. Nutritional analysis of air-dried lamb and beef by-products (as fed basis)

By-product	Moisture (%)	Ash (%)	Crude Protein (%)	Fat (%)	Crude Fibre (%)	Calcium (mg/g)	Phosphorus (mg/g)	Vitamin A (IU/kg)	Hydroxy-proline (mg/100g)
Lamb									
Heart	8.3	2.9	39.8	45.4	1.2	ND	4.6	-	0.35
MDM[2]	5.6	12.0	31.2	48.2	1.2	36.6	19.7	-	1.43
Liver	9.3	6.6	56.7	13.9	0.2	0.4	10.0	690,278	0.56
Lung	4.8	5.4	67.1	10.2	1.5	0.2	9.4	-	1.50
Kidney	6.3	5.5	56.0	25.7	0.3	0.2	8.8	-	0.77
Tripe	9.8	4.2	49.6	32.5	1.0	2.9	6.3	-	1.40
Spleen	10.5	5.5	64.3	12.6	1.9	0.3	11.3	164.5	0.73
Beef									
Heart	8.7	4.9	60.8	18.7	0.7	1.2	7.9	-	0.69
MDM[2]	7.6	18.6	23.9	44.4	2.0	60.1	30.3	-	1.68
Liver	10.1	6.6	58.7	5.6	0.4	0.1	9.8	791,613	0.41
Lung	6.8	6.1	68.0	7.1	3.6	0.4	7.5	-	2.49
Kidney	8.9	3.7	43.5	36.2	0.4	0.2	6.4	-	0.88
Tripe	10.3	7.6	67.6	8.6	1.6	8.4	9.0	-	2.50
Spleen	8.7	7.7	66.6	8.6	3.9	2.8	9.6	123.1	0.76

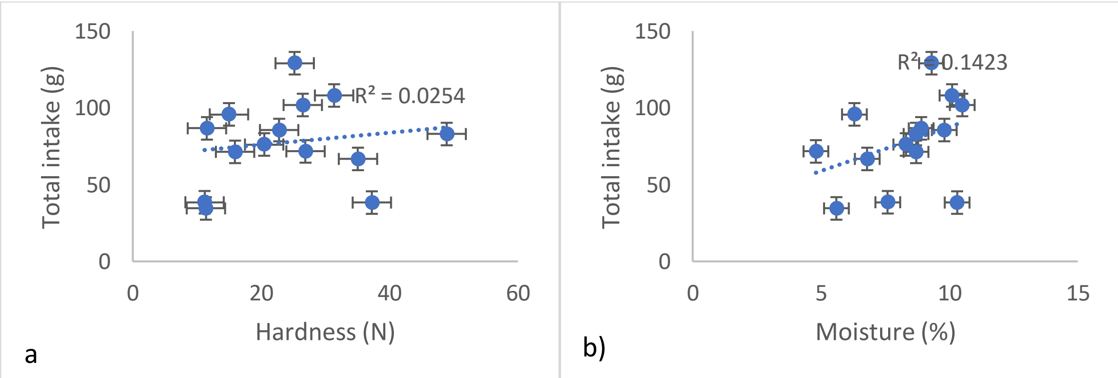
[2] MDM defined as mechanically deboned meat *Crude Protein calculated using a

Table 3.5. Correlation table of combined by-product intake versus texture and nutrient variables.

Variable	Pearson correlation coefficient	P value
Hardness	0.159	0.586
Moisture	0.377	0.185
Ash	-0.582	0.029
Protein	0.356	0.211
Fat	-0.371	0.192
Fibre	-0.436	0.119
Calcium	-0.665	0.010
Phosphorus	-0.509	0.063
Hydroxyproline	-0.683	0.007

There was no correlation between intake and by-product hardness ($r = 0.159$; $p > 0.05$, Figure 3.4a). Similarly, in terms of the nutrients, there was no correlation between intake and moisture, protein, fat, fibre and phosphorus ($r = 0.377$, 0.356, -0.371, -0.436 and -0.509, respectively; $p > 0.05$; Figure 3.4b, 4d to 4f and 4h).

In contrast there was a moderate negative correlation between intake and ash, calcium and hydroxyproline were statistically significant ($r = -0.582$, -0.665 and -0.683, respectively; $p < 0.05$, Figure 3.4c, 4g and 4i).



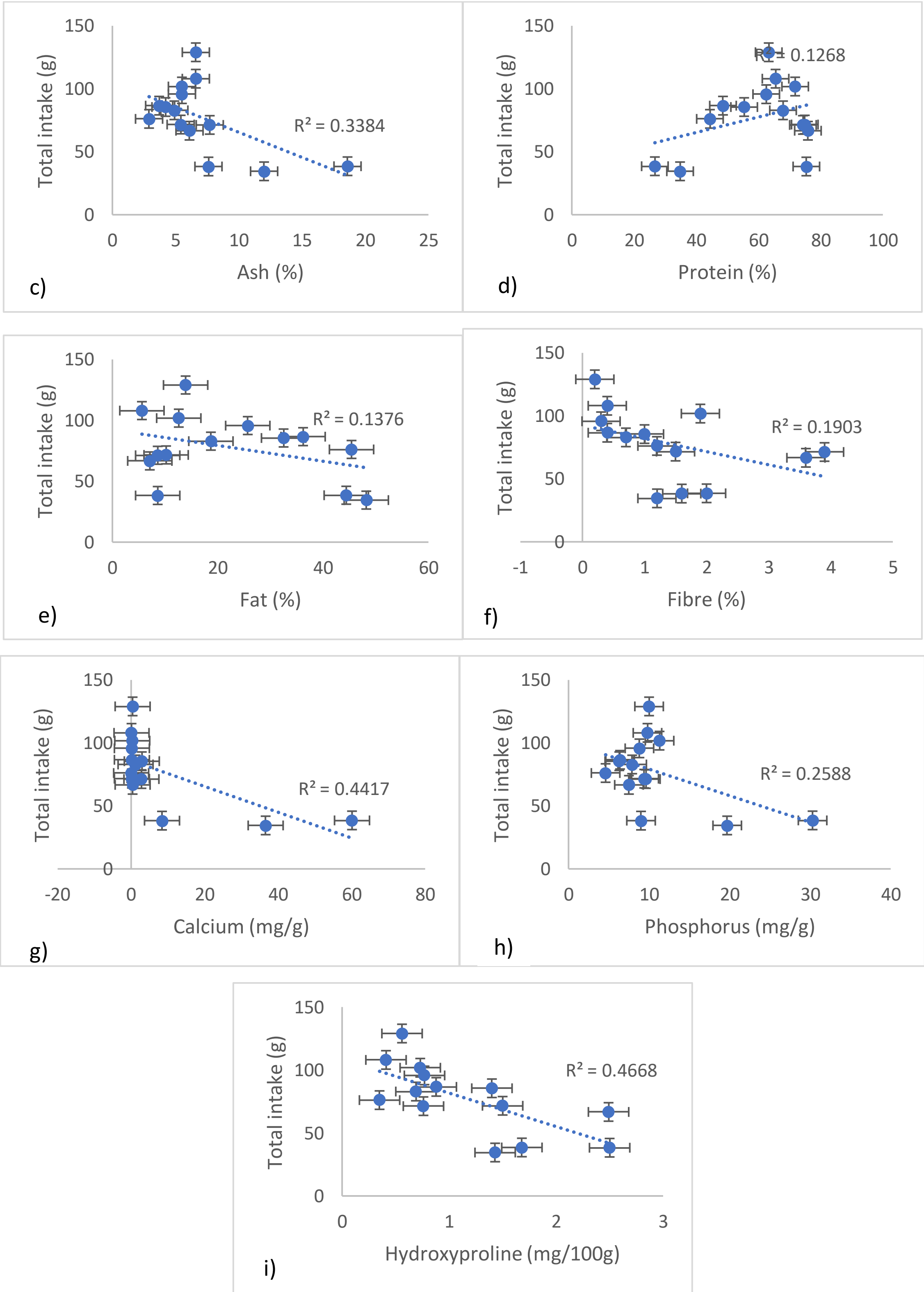


Figure 3.4. Correlation plots of lamb and beef acceptance intake versus hardness (a) and the selected nutrients (b-i)

3.3.4 Total Acceptance Testing Intake Results

The total intake results from all lamb by-products and all beef by-products during acceptance testing combined were analysed for each cat to examine whether the cats preferred one species over the other, as shown in Table 3.6.

Table 3.6. Total intake of all lamb by-product and beef by-products combined

Cat	Combined intake (g)	
	Lamb	Beef
1	781.9	557.2
2	923.8	879.2
3	665.7	557.2
4	660.6	566.0
5	521.0	404.7
6	286.1	211.8
7	283.8	263.7
8	629.4	504.9
Average ± SEM[1]	594.0 ± 79.2[a]	493.1 ± 73.5[b]

[1]superscripts that are different indicate significant differences ($p<0.05$)

Overall, the cats showed a higher average total intake of lamb compared to beef ($p < 0.05$), indicating a likely species effect or a possible age effect on palatability to be examined in greater detail using preference testing.

3.3.5 Lamb versus Beef Preference Testing

Food intake

All cats were offered 100 g of each equivalent beef and lamb by-product each week of preference testing (20 g per day), shown in Figure 3.5.

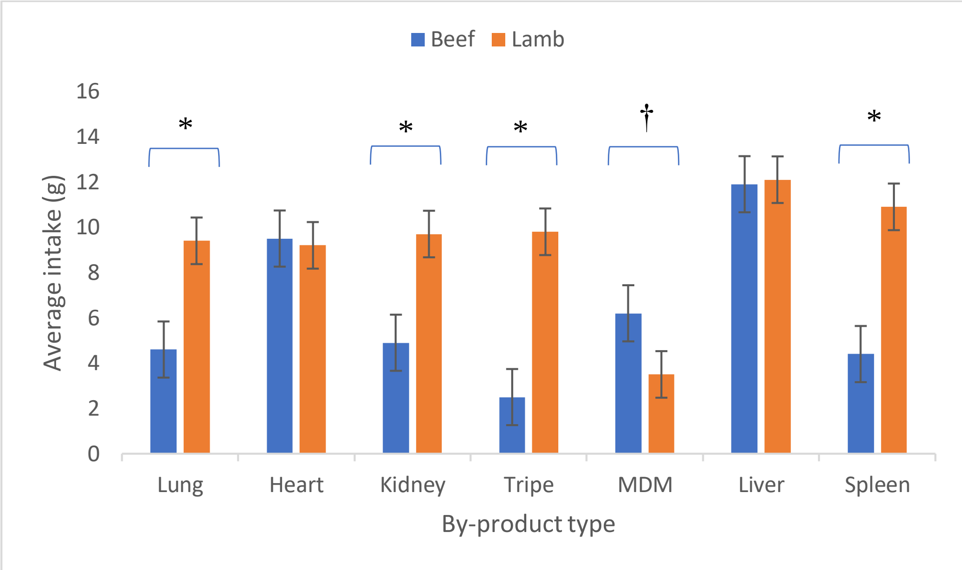


Figure 3.5. Average intake of equivalent beef and lamb by-products offered in the two-bowl preference test (indicates a preference for lamb over the same beef by-product, † indicates a preference for beef over the same lamb by-product). Significance level of p < 0.05.*

The cats had a higher intake of lamb lung, kidney, tripe, and spleen compared to the beef by-products ($p < 0.05$). However, similar intakes of heart and liver were observed between lamb and beef ($p > 0.05$). Beef MDM intake was greater than lamb MDM ($p < 0.05$).

Meal and Visit Frequency and Sizes

Table 3.7. Average number of visits and meals per cat per day for each lamb and beef by-product during each testing week

By-Product	Visits (per cat per day)		P value	Meals (per cat per day)		P value
	Lamb	Beef		Lamb	Beef	
Lung	0.5 ± 0.1	0.8 ± 0.1	0.242	1.7 ± 0.1	1.0 ± 0.1	<0.001
Heart	1.1 ± 0.2	0.4 ± 0.1	<0.001	1.3 ± 0.2	1.4 ± 0.1	0.851
Kidney	0.6 ± 0.1	0.9 ± 0.2	0.174	1.8 ± 0.2	1.2 ± 0.2	0.026
Tripe	0.6 ± 0.1	0.5 ± 0.1	0.556	1.6 ± 0.1	0.7 ± 0.1	<0.001
MDM	1.1 ± 0.2	0.9 ± 0.2	0.346	0.5 ± 0.1	0.9 ± 0.2	0.075
Liver	0.3 ± 0.1	0.3 ± 0.1	1.000	1.5 ± 0.1	1.7 ± 0.2	0.524
Spleen	0.2 ± 0.1	0.4 ± 0.1	0.205	1.4 ± 0.2	0.8 ± 0.1	0.001

In terms of visits, cats frequented lung, kidney, tripe, MDM, liver and spleen bowls a similar number of times between lamb and beef ($p > 0.05$; Table 3.7). However, cats visited bowls containing lamb heart more than those with beef heart ($p < 0.05$; Table 3.7). Within lung, kidney, tripe and spleen preference testing, cats consumed a greater number of meals of lamb compared to beef ($p < 0.05$; Table 3.7). However, within heart, liver and MDM preference testing, cats showed similar meal numbers for lamb and beef ($p > 0.05$; Table 3.7).

Table 3.8. Average visit intake amounts per cat per day for each lamb and beef by-product during each testing week, with fixed effects and interaction results ($p < 0.05$)

By-Product	Intake per visit (g)		P value	Pr > F		
	Lamb	Beef		By-Product	Species	By-Product * Species
Lung	1.2 ± 0.2	0.8 ± 0.1	0.130	0.123	0.718	0.400
Heart	1.3 ± 0.2	1.4 ± 0.3	0.200			
Kidney	1.2 ± 0.2	1.0 ± 0.1	0.258			
Tripe	1.0 ± 0.1	1.4 ± 0.6	0.494			
MDM	1.2 ± 0.3	1.1 ± 0.1	0.755			
Liver	2.2 ± 0.3	1.5 ± 0.3	0.089			
Spleen	1.3 ± 0.3	1.5 ± 0.2	0.649			

For visit intake amounts, there were no differences observed between lamb and beef intakes for any of the by-product varieties ($p > 0.05$; Table 3.8). Additionally, the fixed effects of by-product and species, and the interaction between by-product and species had no significant impact on visit intake amounts ($p > 0.05$).

Table 3.9. Average meal intake amounts per cat per day for each lamb and beef by-product during each testing week, with fixed effects and interaction results (p < 0.05)

By-Product	Intake per meal (g)		P value	Pr > F		
	Lamb	Beef		By-Product	Species	By-Product * Species
Lung	5.1 ± 0.4	3.4 ± 0.4	0.007	<0.001	0.001	0.003
Heart	4.8 ± 0.5	5.7 ± 0.5	0.214			
Kidney	5.1 ± 0.4	3.4 ± 0.3	0.004			
Tripe	5.6 ± 0.5	2.9 ± 0.3	0.001			
MDM	3.4 ± 0.5	4.3 ± 0.4	0.176			
Liver	7.2 ± 0.6	6.7 ± 0.5	0.556			
Spleen	7.6 ± 0.7	5.1 ± 0.7	0.022			

Meal intakes of lung, kidney, tripe, and spleen were greater for lamb compared to beef ($p < 0.05$). However, no difference in the meal intakes were observed for heart, MDM and liver between the two species ($p > 0.05$; Table 3.9). Overall, the fixed effect of by-product and species, and the interaction between by-product and species had a significant impact on meal intake amounts ($p < 0.05$).

Texture Analysis

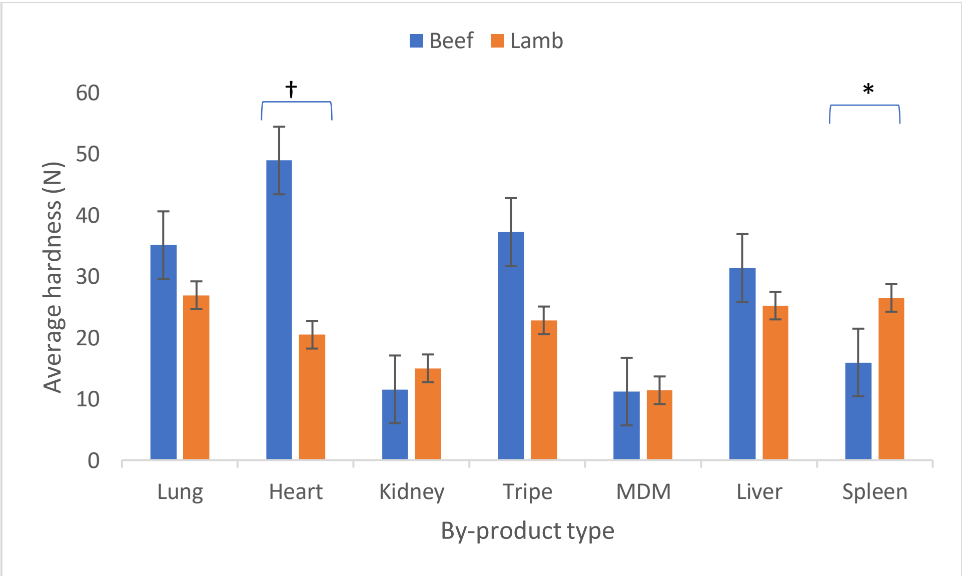


Figure 3.6. Average hardness of equivalent beef and lamb by-products offered in the two-bowl preference test (indicates a harder lamb product over the same beef by-product, † indicates a harder beef over the same lamb by-product).*

There was no difference in hardness between the air-dried lung, kidney, tripe, MDM and liver when comparing beef and lamb ($p > 0.05$). However, beef heart was harder than lamb heart ($p < 0.05$) and lamb spleen was harder than beef spleen ($p < 0.05$), shown in Figure 3.6.

3.4 Discussion

This trial investigated the palatability of commonly utilised beef and lamb by-products in pet food. The by-products were thermally processed using air-drying to deliver a heat-treated final product to cats. The objective of the work was to develop a ranking of by-product acceptance within lamb and beef, as well as evaluate whether cats show a preference for the same by-product of one species over the other using a similar approach to previous work (Watson et al. 2020). However, unlike the previous study by Watson et al. (2020), this work looked at examining the acceptance and preference of by-products which had undergone thermal processing, with additional analyses used to correlate palatability results to both textural and nutritional properties of the by-products.

In the lamb by-product acceptance testing, liver had the greatest acceptance, along with spleen. Spleen was also equally palatable to kidney, tripe, and heart, followed by lung. In contrast to the lamb organ meats, MDM had the lowest intake and was therefore the least accepted by-product for cats.

Like the lamb acceptance testing, beef liver also showed the greatest acceptance, with kidney and heart having equivalent intake levels. Kidney and heart also showed similar acceptance to that of spleen and lung. Finally, tripe and MDM were the beef by-products which were least accepted by cats.

The acceptance tests highlights that, MDM, an ingredient which is readily available and commonly utilised in large quantities in commercial pet food formulations, is not appealing to cats in terms of palatability from beef and lamb sources. Instead, the cats in this trial showed significantly greater acceptance for organ meats, particularly liver and spleen from lamb and liver, kidney, and heart from beef. This pattern of consuming organ meats preferentially over MDM was also previously observed in raw ingredient testing (Watson et al., 2020). Consumption of these by-products maybe reflective of the feeding behaviour of wild cats, that have been observed to preferentially consume organ meats over muscle tissue of their prey (Thompson, 2008, Laflamme et al., 2014).

When expressed as percentage consumption, results of air-dried by-products were like that obtained in the same by-products in raw form (Watson et al., 2020), air-dried by-products showed significantly lower intakes compared to the raw by-products when adjusted to the same dry matter. These results are expected as foods with a higher moisture content are known to be positively associated to palatability in cats (Pekel et al., 2020). Additionally, these findings show that in contrast to dogs who prefer heat processed meat by-products over raw (Houpt and Smith, 1981), cats prefer raw over heat-processed, specifically air-dried in this case.

In addition to assessing the overall palatability of each by-product within each species, intake was correlated to both texture and selected nutrients to determine their influence on palatability. There was no evidence to suggest any correlation between intake and hardness of the by-products in this study. However, findings by Kozuchowicz (2018) for extruded kibbles has revealed that biting rate was positively associated with hardness. This suggests that harder kibbles require more biting to break down the product into smaller sizes that are easy to digest. However, in terms of physical properties of the kibbles tested in their study, palatability was mainly driven by the shape of the kibble over the hardness. The disc shape with mid-range hardness was most palatable, followed by the cross shape and triangle with hole shape with the lowest hardness score, and the cylinder shape being the least palatable with the highest hardness score (Kozuchowicz, 2018). Although having an influence on palatability, this study was not concerned with selecting the most palatable shape for by-product consumption. However, the shape of all air-dried by-products in this trial were consistent with one another.

Additionally, while all by-products were dried to a moisture content of 10% or less, slight differences were still observed in moisture readings between samples. However, there was no evidence to suggest correlation between intake and moisture within the air-dried samples. Greater differences would likely be observed between different food formats, with cats generally preferring wet food over dry foods due to moisture levels being like that of meat (Zaghini and Biagi, 2005). This work, however, was focused on air-dried by-products and not the comparing of different food types.

Regarding macronutrients, there was no evidence to suggest a correlation between intake and protein content, nor between intake and fat content. For protein, these results contradict those reported in the literature by Zaghini and Biagi (2005), who found that food palatability was positively correlated to the amount of protein, particularly if ingredients of animal origin were used. The same trend has also been reported for the fat contents in pet food, with reports that palatability of food increases proportionally as the fat content increases and that animal fats are one of the flavours that are highly palatable to cats (Pekel et al., 2020; Thombre, 2004). However, the Pearson correlation value obtained indicated the opposite of what is reported in the literature. As the air-dried samples were 100% meat by-products with no addition of antioxidants or other preservatives, it is possible that lipid oxidation had taken place resulting in the off-notes that are easily detected by cats, and the subsequent weak negative correlation between palatability and fat content, although the p value was too large to warrant an association between the two variables (Hagen-Plantinga et al., 2017; Zaghini & Biagi, 2005).

Ash, crude fibre, calcium, and phosphorus are nutrients reported by Alegría-Morán et al. (2019) to be negatively associated with palatability of complete and balanced diets. In the current work, statistically significant moderate negative correlations were reported between the intake and ash content, and also intake and calcium content. This correlation is likely due to MDM which is known to have a greater bone content compared to the other by-products, which is measured by estimating the calcium content (Field et al., 1977). Furthermore, the size of bone particles and the total bone content within MDM may contribute to poor textural properties, particularly as it was the least accepted by-product from both lamb and beef. A possible explanation for the differences between calcium contents of the selected by-products may also be due to processing differences between the organ meats and MDM. For example, Ang and Hamm (1982) found that mechanically deboning of broiler parts showed a 350% increase in calcium content compared to hand boning, the likely source of by-product collection for the organ meats in this study. By-product collection, however, was not examined in detail throughout this work but may be explored in future.

In addition to ash and calcium, a statistically significant moderate negative correlation between intake and hydroxyproline was observed. Across all by-products, beef showed a slightly higher hydroxyproline values than the same lamb by-product. Hydroxyproline is a major component of the fibrous connective tissue protein known as collagen (Engelking, 2015). As animals age, the number and strength of the cross linkages of intramuscular collagen increases (Hill, 1966). Although the ages of these animals these by-products were sourced from was not recorded, lamb is typically processed at 4 to 9 months of age for meat and its by-products, however, cull cows (the likely source of beef components) are typically five to eight years of age at slaughter. Differences in age at the time of processing could therefore be investigated in further detail in future to determine the influence of animal age on palatability.

Overall, when examining the total combined intake of all lamb by-products versus all beef by-products from acceptance testing, a significant difference in intake was observed. The cats showed a greater general preference for lamb over beef. This revealed a species effect on palatability that was again examined in more detail using preference testing for comparable by-products between lamb and beef.

In terms of preference testing, the ranking of offal palatability was similar to previously determined for raw by-products (Watson et al., 2020). For instance, lamb lung, kidney, tripe, and spleen were more preferred than the beef equivalents, lamb and beef heart and liver showed no significant difference in intake. However, unlike the raw preference testing, beef MDM was more palatable than the lamb MDM when fed air-dried. It is known in the industry that the mechanical separation of meat can result in significant variation in finished product quality (Miller, 2018). For example, differing processing parameter used during collection, as well as the variability of ingoing material from different portions of a carcasses can result in significant differences particularly regarding the fat content as well as the connective tissue and collagen content of the MDM (Miller, 2018). This may explain why differing outcomes were observed between species in the raw work compared to the air-dried testing. Overall, the cats showed a greater preference for lamb over the same beef by-products, indicating a species preference.

In addition to the overall intake, the visit and meal intake patterns of the cats were also analysed in this study. In terms of visit results, the cats made a similar number of visits to lamb and beef within lung, kidney, tripe, MDM, liver, and spleen, which were also similar in size. Heart was the only by-product where cats displayed more visits to lamb than beef, however, no difference was seen in the amount consumed between the two species during the visits ($p > 0.05$). In contrast, the meal results showed a greater number of lamb meals than beef meals within lung, kidney, tripe, and spleen preference testing, which were also greater in terms of grams consumed. For heart and kidney, that cats consumed a similar number of lamb and beef meals which were similar in size. For MDM, the number of individual lamb and beef meals consumed were similar to each other and were also similar in size.

It is known throughout the literature that cats use both smell and taste in the detection and selection of foods and will generally consume one food exclusively over another if they find the odour significantly more attractive (Pickering, 2009; Alegría-Morán et al., 2019; Hullár et al., 2001). During visits, it is likely that smell was used as the main determinant in assessing the attractiveness of the beef and lamb by-products. However, the attractiveness of the foods was indistinguishable during visit time and therefore visits had little relation to overall palatability. In contrast, meals would have encompassed the use of both smell and predominantly taste, which is the most dominant scent used in influencing the food preference of cats (Aldrich & Koppel, 2015; Bradshaw et al., 1996; Pickering, 2009).

As well as assessing total food intake, the hardness of each beef and lamb by-product was compared to determine whether texture was influencing the preference for lamb over beef. Lung, kidney, tripe, MDM, and liver showed no difference in hardness between beef and lamb samples. However, lamb lung, which was more palatable than beef lung, was softer. Conversely, lamb spleen, which was again more palatable than beef, was harder than beef spleen. In summary, although texture is one of the factors that play a role in food preferences by cats as described by Hullár et al. (2001), the effect of hardness between the same beef and lamb by-product when fed alongside each other does not appear to be influencing the preference for lamb over beef. Instead, preference for lamb over beef by-products is likely to be driven by chemical and nutritional properties of the food, particularly in terms of odour and taste, rather than the physical properties of the by-products (National Research Council, 2006; Aldrich and Koppel, 2015).

3.5 Conclusion

The results in this chapter demonstrate that after heat treatment (i.e. air-drying) of by-products, cats were able to show behaviours indicating differences in palatability between by-products from lamb and from beef as well as between the same by-products from the two species. In acceptance testing, the cats ranked all organ meats preferentially over MDM, with liver being the top ranked by-product and MDM the least palatable within both species. Additionally, correlation analyses between all lamb and beef by-product intakes and nutrient contents revealed a negative relationship between intake and ash, calcium, and hydroxyproline content. The final assessment of acceptance testing results showed that, in terms of the combined intake of all lamb by-products and all beef by-products, a higher average intake of lamb was observed. This indicated a possible species effect on palatability that was explored further using preference testing. In preference testing, cats showed a general preference for lamb over the same beef by-products except for beef MDM which was more palatable than lamb MDM, and no difference being observed between beef and lamb heart and kidney intakes. In addition, individual meal intakes generally reflected the total intake results. However, the number of visits to the bowls was not predictive of overall palatability. Finally, intake versus hardness of the same by-product across species were compared and revealed that texture does not appear to be affecting preference for lamb over beef by-products. It is more likely that the chemical and nutritional properties of the food have a greater influence on palatability; however, more analytical testing will need to be adopted in future to confirm this assumption.

Chapter 4 Age of Animal By-Product Influence on Palatability in Cats

4.2 Introduction

Domestic cats are obligate carnivores and have a higher daily protein requirement compared to dogs (26% versus 18% on a dry matter basis respectively) (AAFCO, 2020; Pekel et al., 2020; Salaun et al., 2017). Due to specialised nutritional and physiological features, cats must consume animal sources to obtain various essential nutrients that cannot otherwise be obtained in their diet (Pekel et al., 2020). Nutritionally, taurine is an essential amino acid for cats, and can only be obtained by cats through the consumption of animal sources such as heart (Knopf et al., 1978). Physiologically, due to the loss of functionality of various enzymes, cats cannot convert enough beta-carotene to vitamin A (Aldrich & Koppel, 2015; Stasiak, 2002; Zaghini & Biagi, 2005; Corbin, 1992). As a result, cats need to obtain vitamin A from direct consumption of animal sources such as liver (Schweigert et al. 2002).

Along with their biological need for meat, cats are known to show a strong positive correlation between protein content and palatability, particularly when animal sources of protein are used (Zaghini and Biagi, 2005). Previous research investigating palatability of animal protein sources within formulated diets indicated that cats prefer fish, specifically salmon, over commercial cat food (fish, liver, chicken, or beef flavoured) and rats (Adamec, 1976; Houpt & Smith, 1981). However, this research did not look to compare the palatability of specific meat by-products included in formulations, nor the reasons for the observed differences in palatability.

With this being considered and following on from Chapter 3 and previous work by Watson et al. (2020) using red meat by-products, it is apparent that cats show a greater preference for lamb by-products over the same beef by-products both in its raw form and when heat treated using air-drying. These results suggest a possible species and/or age effect on palatability which will be studied further in this chapter, with a focus on potential within-species age of animal differences in palatability.

To my knowledge, no previous studies have examined the influence of the age of animal by-product on palatability in terms of pet food research. With pet owners becoming increasingly conscious of what they feed their pets, fundamental analysis of common meat by-product and selection of the most palatable ingredients used in pet food formulations may further substantiate the trend in the industry and ensure only the best is provided to their pets.

Using a series of palatability studies and analytical testing, the aims of this study have been divided into two parts. Firstly, palatability testing will be used to examine if cats show within-species differences in intake between ovine and bovine by-products of two different ages. Secondly, the nutrient composition, as well as metabolomic (with a focus on hydrophilic interaction liquid chromatography - HILIC compounds) and lipid oxidation (Thiobarbituric acid reactive substances – TBARS) analyses will be used to investigate each of the by-products and begin to determine factors which are likely driving preference for certain by-products over others.

I hypothesised that young animal by-products are likely to be more palatable than the older animal by-products. This is largely based off similar findings in the literature in humans where the flavour of young animals is more desirable than older ones, specifically with the acceptance of lamb being greater than mutton (Sink & Caporaso, 1977).

4.3 Materials and Methods

All animal procedures described in this chapter were approved by the Massey University Animal Ethics Committee (Protocol MUAEC 18/16).

4.2.1 Test Animals

The same trained panel of eight domestic short-haired cats that was used in Chapter 3 tested the within-species preference of seven air-dried by-products (lung, heart, kidney, tripe, mechanically deboned meat (MDM), liver, and spleen) from ovine and bovine sources of two different ages. Specifically, sheep less than one year of age (lamb) versus sheep older than two years of age (mutton) of over two years in age, and calves of four days old versus young bulls and steers of two and a half to three years of age (beef).

One cat from the panel was replaced part-way through this trial compared to Chapter 3:

- Ewok (cat 8), was put down due to health reasons unrelated to this study and was replaced by Tiger in week 6 of testing - lamb versus mutton lung testing.

Testing was carried out from Monday 8th March 2021 to Friday 21st May 2021 for the lamb versus mutton preference testing and Monday 4th October 2021 to Friday 19th November 2021 for the calf versus beef testing at the Centre for Feline Nutrition at Massey University, Palmerston North.

4.2.2 Palatability Testing

The same general procedure and methods for preference testing outlined in Chapter 3, Section 3.2.2 were used in the age of animal trial to examine food intake, number of meals and visits made, the amount consumed during these periods and the correlation between food intake and nutrients.

Data Collection and Statistical Analyses

The weight of the bowl and food before and after feeding to the cats was recorded. Acceptance and preference were determined as food intake (g), using the following equations:

$$Food\ intake\ (g) = weight\ of\ bowl\ before\ (g) - \ weight\ of\ bowl\ after\ (g)$$

All statistical analyses were carried out using Minitab 19 (Minitab Inc., State College, PA, USA).

For preference testing, paired t-tests were used to determine whether there were differences between the overall intake of lamb versus mutton, and calf versus beef by-products for each test using a significance level of $p < 0.05$.

Interaction between by-product and species were also analysed within meal and visit intake results using a general linear model and a significance level of $p < 0.05$ and up to up $p < 0.10$.

Pearson correlations of by-product intake versus nutritional content were also conducted using a significance level of $p < 0.05$.

A principal component analysis (PCA), with the removal of MDM data due to extremely high calcium and phosphorus values, was performed in R Studio (R version 4.2.1, 2022) to evaluate how nutritional components of the by-products (moisture, ash, crude protein, fat, crude fibre, calcium, phosphorus, hydroxyproline, TBARS) may explain food preference and if their components were grouped, using R packages ‘ggplot2’ and ‘ggpubr’. Nutritional components were expressed in percentages. Following PCA, a multiple linear regression was performed between the most important variables grouped in the PCA.

4.2.3 Analytical Testing

Lipid Oxidation (TBARS) Analysis

Freeze-dried samples of the by-products were analysed according to the methods by Buege and Aust (1978) with modifications.

Briefly, approximately one gram of ground sample was homogenised with 15 mL of MilliQ water using an ultra-turrax (IKA Labortechnik, Germany) at 14,000 rpm for 30 seconds over ice slurry. Homogenates were then centrifuged at 5,000 x *g* for five minutes at 4°C. 1 mL of supernatant was removed and mixed with 50 µL of butylated hydroxytoluene (BHT) and 2 mL of thiobarbituric acid – trichloroacetic acid (TBA-TCA) reagent. The mixture was incubated in a water bath at 90°C for 15 minutes to develop colour, followed by cooling on ice for 10 minutes. Following centrifugation at 10,000 x *g* at 4°C for 5 minutes, supernatant was measured at 531 nm against a blank prepared with 1 mL MilliQ water and 2mL TBA-TCA reagent.

An 8-point standard curve with malondialdehyde (MDA) concentrations from 0 M to 20 $\times 10^{-6}$ M to was prepared using 1,1,3,3-tetra-ethoxypropane (TEP, Sigma, T9889, MW 220.31, 97% purity, d=0.919 g/mL). The amount of TBARS was calculated based on the standard curve and expressed as mg of MDA/kg freeze-dried meat. All the measurements were performed in duplicate.

Pearson correlations were performed between intake and TBARS, as well as between fat content and TBARS using a significance level of $p < 0.05$.

Metabolomic Analysis

Metabolomics analysis included a comprehensive coverage of polar and semi-polar compounds using liquid chromatography-mass spectrometry (LC-MS) systems set up for data independent acquisition (DIA), in both positive and negative ionisation modes.

<u>Sample preparation</u>

Briefly, 50 ± 5 mg of freeze-dried and ground by-product samples (n=4) were weighed in a 2 mL microcentrifuge tube. A ceramic bead and extraction solvent made up of chloroform and methanol (1:1, v/v) was added, followed by water. After disrupting the sample in a bead mill, samples were centrifuged, and the upper/aqueous phase was used for analysis of polar and semi-polar compounds. Supernatants were dried down in a vacuum concentrator (Vacuumbrand, Wertheim, Germany), and reconstituted in appropriate solvents with corresponding internal standards to monitor sample degradation.

Pooled quality control (QC) samples for each stream, HILIC positive and HILIC negative, were made by adding remaining supernatants of all samples, and sub-aliquoting from the pool for analysis. A dilution series of QC samples were established and analysed prior to running samples, to determine ideal concentrations for analysis without saturating the detector (Bilbao et al., 2018).

Chromatography

Samples were analysed using a Nexera X2 ultra high-performance liquid chromatography (UHPLC) system (Shimadzu, Japan) consisting of a SIL-30AC autosampler coupled to a LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer (Shimadzu, Japan) equipped with an electrospray ionization source.

A sample (2 µL) was injected into a normal phase Ascentis® Express HILIC UHPLC column (2.1 x 100 mm, 2 µm particle size; Sigma, USA) and eluted at 30°C over a 20 min gradient with a flow rate of 400 µL/min. The mobile phase solvent A was 10 mM ammonium formate in water and solvent B consisted of acetonitrile with 0.1% formic acid. The solvent gradient program started at 97% solvent B from 0 to 0.5 min, decreased to 70% within 11.5 min and further to 10% from 11.5 to 13.5 min, held at 10% for 1.5 min, increased to 97% B within 1 min and held at that concentration until the end of the elution run.

Mass spectrometry

Full scan (m/z 55-1100 for HILIC), and MS/MS scans for windows spanning m/z 20, were setup for analyses in positive and negative ionisation modes (Appendix 1). A total of 42 events were setup with a loop time of 0.85 secs. Spray voltage was 4.0 and -3.0 kV for positive and negative ionisation modes, respectively, and collision energy (CE) was set at 23 ± 15 V (Appendix 1). The ion source was operated under an optimal condition: nebulizing gas flow, 3.0 L/min; heating gas flow; 10.0 L/min; interface temperature, 300°C; drying gas flow, 10.0 L/min; desolvation line temperature, 250°C and heat block temperature, 400°C.

Batch sequence

All samples were run in one batch starting with the positive ionisation mode. Five blanks were run first, followed by an external standard to verify system performance (Amino acid standard, A9906, Sigma, USA for HILIC). This sequence was followed by a couple of QC pooled samples, and then samples in a randomised order with QC samples interspersed once for every 8 samples. The same order was followed for negative ionisation mode.

Data Analysis

Pre-processing

Raw data files (.lcd) were converted to a common file format mzML using LabSolutions software (Ver 5.99 SP2, Shimadzu, Japan). MS-Dial (Tsugawa et al., 2015) was used for peak detection, MS2 deconvolution, alignment of samples and compound identification. Appropriate adducts were selected for positive and negative ionisation modes.

4.2.4 Statistical Analysis

Statistical analysis was completed using MetaboAnalysts v5.0 – one factor statistical analysis. In the data filter settings, data was filtered if the relative standard deviations (RSD) were greater than 30% in the QC samples and analysed on an interquartile range (IQR). QC values were then removed from the sample set in the data editor setting and data was log transformed and auto scaled in the normalisation setting.

Focus was placed on examining compounds named in the literature which have been shown to have an influence on palatability in cats. For the HILIC negative compounds these were L-glutamic acid, γ-glutamylleucine (γ-glu-leu) and γ-glutamylmethionine (γ-glu-met), as well as carnosine. For the HILIC positive compound these were alanine, choline [M]+, glutamic acid, L-phenylalanine, and tryptophan, as well as anserine.

A general linear model was run in Minitab 19 to examine the species effect on compounds known to have an influence on palatability, using a significance level of $p < 0.05$.

From MetaboAnalyst, boxplots of the normalised concentration for the above compounds were obtained within each species from the one-way ANOVA analysis in the program. The PCA of HILIC positive and HILIC negative ionisation mode data matrix, showing differences between by-products from ovine and bovine sources were also carried out.

From here, a “proc glimmix” model was run on the log transformed data in SAS 9.4 to examine the fixed effect of by-product and age, as well as the interaction between by-product and age on the compounds of interest within each species. The standard error and p values of the least squares means (LSM) for the interaction between age and by-product were reported, along with the grouping of relative amounts of the compounds within each by-product.

4.4 Results

4.3.1 Lamb versus Mutton Testing

Food Intake

All cats were offered 100 g of each equivalent lamb and mutton by-product for each week of preference testing (20 g per day), the daily intake is shown in Figure 4.1.

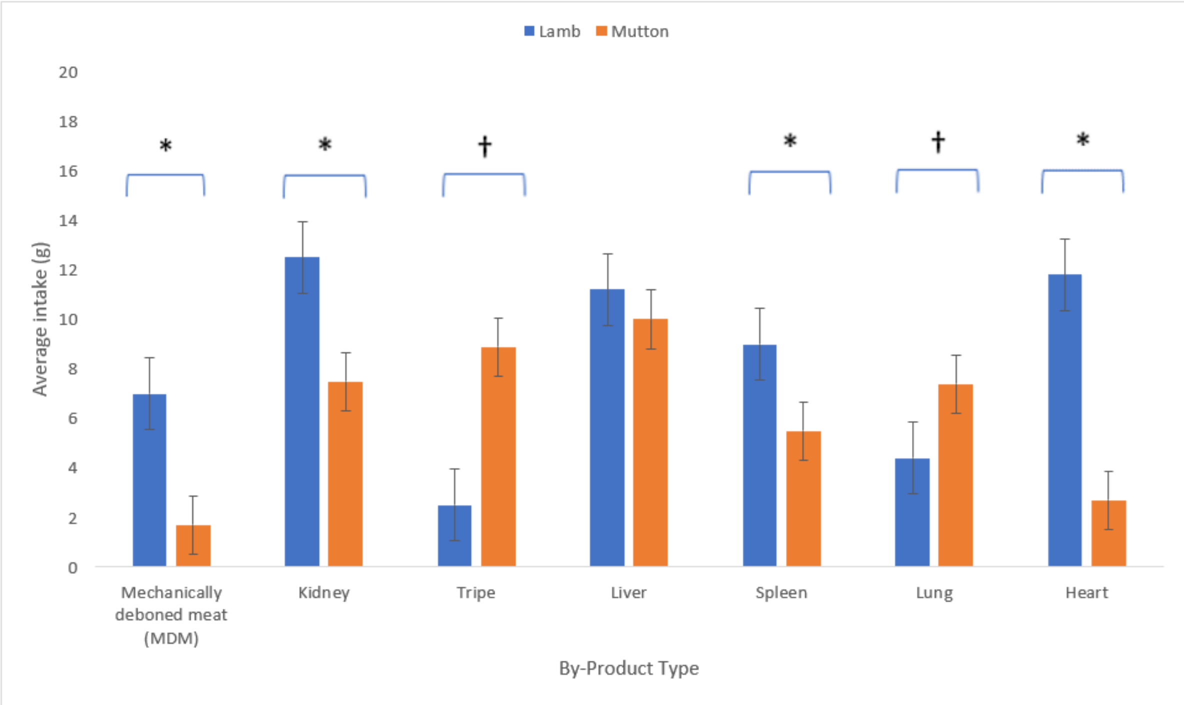


Figure 4.1. Average daily intake of equivalent lamb and mutton by-products offered over the two-bowl preference test (indicates a preference for lamb over the same mutton by-product, † indicates a preference for mutton over the same lamb by-product)*

The cats had a higher intake of lamb MDM, kidney, spleen, and heart compared to the mutton by-products ($p < 0.05$). In contrast, the cats showed higher intake of mutton tripe and lung compared to the lamb by-products ($p < 0.05$). Similar intakes of liver were observed between lamb and mutton ($p > 0.05$)

Meal and Visit Frequency and Meal Size

Table 4.1. Average number of visits and meals per cat per day for each lamb and mutton by-product during each testing week

By-Product	Visits (per cat per day)		P value	Meals (per cat per day)		P value
	Lamb	Mutton		Lamb	Mutton	
Lung	0.4 ± 0.1	0.3 ± 0.1	0.686	1.2 ± 0.2	1.3 ± 0.1	0.882
Heart	0.1 ± 0.0	0.5 ± 0.1	0.001	1.5 ± 0.1	0.5 ± 0.1	<0.001
Kidney	0.6 ± 0.1	0.6 ± 0.2	0.888	1.7 ± 0.1	1.4 ± 0.2	0.274
Tripe	0.6 ± 0.1	0.5 ± 0.1	0.680	0.5 ± 0.1	1.5 ± 0.2	<0.001
Mechanically deboned meat (MDM)	0.6 ± 0.1	0.7 ± 0.1	0.459	1.6 ± 0.2	0.3 ± 0.1	<0.001
Liver	0.2 ± 0.1	0.3 ± 0.1	0.626	1.4 ± 0.1	1.3 ± 0.1	0.601
Spleen	0.2 ± 0.2	0.3 ± 0.1	0.610	1.5 ± 0.1	1.1 ± 0.2	0.069

In terms of visits, cats frequented the bowls containing the lamb and mutton lung, kidney, tripe, MDM, liver, and spleen a similar number of times ($p > 0.05$; Table 4.1). However, cats visited bowls containing mutton heart more than those containing lamb heart ($p < 0.05$; Table 4.1). Within heart and MDM preference testing, cats consumed a greater number of meals of lamb compared to mutton. However, within tripe preference testing, cats consumed a greater number of meals of mutton compared to lamb ($p < 0.05$; Table 4.1). A similar number of meals within lung, kidney, liver, and spleen were observed between lamb and mutton ($p > 0.05$; Table 4.1).

Table 4.2. Average intake amounts each visit per cat per day for each lamb and mutton by-product during each testing week, with fixed effects and interaction results (p < 0.05)

By-Product	Intake per visit (g)		P value	Pr > F		
	Lamb	Mutton		By-Product	Age	By-Product * Age
Lung	0.8 ± 0.1	1.0 ± 0.2	0.215	0.295	0.289	0.509
Heart	1.3 ± 0.5	1.3 ± 0.2	0.953			
Kidney	1.4 ± 0.2	1.1 ± 0.1	0.325			
Tripe	0.9 ± 0.1	1.0 ± 0.2	0.807			
MDM	1.0 ± 0.2	1.1 ± 0.1	0.956			
Liver	1.4 ± 0.4	0.9 ± 0.2	0.293			
Spleen	1.8 ± 0.9	1.0 ± 0.1	0.307			

For the intakes per visit, there were no differences observed between lamb and mutton for any of the by-product varieties ($p > 0.05$; Table 4.2). Additionally, the fixed effects of by-product and age, and the interaction between by-product and age had no significant impact on intake amounts per visit ($p > 0.05$).

Table 4.3. Average meal intake amounts per cat per day for each lamb and mutton by-product during each testing week, with fixed effects and interaction results (p < 0.05)

By-Product	Intake per meal (g)		P value	Pr > F		
	Lamb	Mutton		By-Product	Age	By-Product * Age
Lung	3.4 ± 0.4	5.8 ± 0.7	0.003	<0.001	0.047	<0.001
Heart	8.7 ± 1.1	3.0 ± 0.6	0.003			
Kidney	6.8 ± 0.6	4.3 ± 0.3	0.001			
Tripe	2.6 ± 0.5	5.6 ± 0.6	0.003			
MDM	3.6 ± 0.4	2.3 ± 0.4	0.177			
Liver	7.6 ± 0.7	7.5 ± 0.8	0.971			
Spleen	6.7 ± 0.7	4.9 ± 0.7	0.074			

Meal intakes of heart and kidney were greater for lamb compared to mutton ($p < 0.05$). In contrast, meal intakes for lung and tripe were greater for mutton compared to lamb ($p < 0.05$). However, no difference in meal intake was observed between MDM, liver, and spleen ($p > 0.05$). Overall, the fixed effect of by-product and age, and the interaction between by-product and age had a significant impact on meal intake amounts ($p < 0.05$; Table 4.3). It should be highlighted that mutton lung and tripe showed higher intakes than lamb. However, lamb heart and kidney showing higher intake than mutton ($p < 0.05$), resulting in opposing drivers of intake. Although the age effect was found to be significant, it only falls just below the 0.05 significance level at 0.047.

4.3.2 Calf versus Beef Testing

Food Intake

All cats were offered 100 g of each equivalent calf and beef by-product for each week of preference testing (20 g per day), average intakes are shown in Figure 4.2.

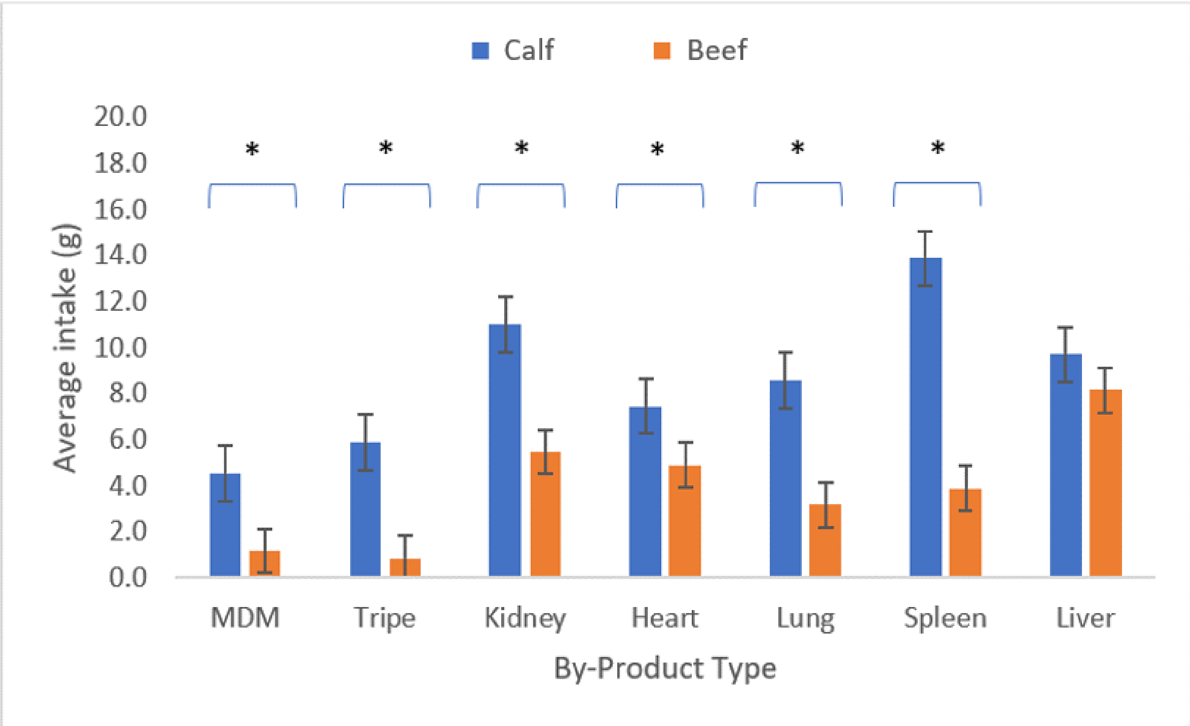


Figure 4.2. Average intake of equivalent calf and beef by-products offered over the two-bowl preference test (indicates a preference for calf over the same beef by-product)*

The cats had a higher intake of calf MDM, tripe, kidney, heart, lung, and spleen compared to the beef by-products ($p < 0.05$). Similar intakes of liver were observed between calf and beef ($p > 0.05$).

Meal and Visit Frequency and Meal Size

Table 4.4. Average number of visits and meals per cat per day for each calf and beef by-product during each testing week

By-Product	Visits (per cat per day)		P value	Meals (per cat per day)		P value
	Calf	Beef		Calf	Beef	
MDM	0.4 ± 0.1	0.6 ± 0.1	0.300	1.4 ± 0.1	0.2 ± 0.1	<0.001
Tripe	0.8 ± 0.2	0.7 ± 0.1	0.821	1.4 ± 0.1	0.2 ± 0.1	<0.001
Kidney	0.3 ± 0.1	0.6 ± 0.1	0.134	1.5 ± 0.2	1.1 ± 0.1	0.062
Heart	0.3 ± 0.1	0.5 ± 0.1	0.228	1.3 ± 0.1	0.8 ± 0.1	0.009
Lung	0.1 ± 0.1	0.2 ± 0.1	0.753	1.5 ± 0.1	0.6 ± 0.1	<0.001
Spleen	0.2 ± 0.1	0.4 ± 0.1	0.240	1.6 ± 0.1	0.9 ± 0.1	<0.001
Liver	0.3 ± 0.1	0.1 ± 0.0	0.050	1.2 ± 0.1	1.4 ± 0.1	0.333

In terms of visits, cats frequented the bowls containing the calf and beef MDM, tripe, kidney, heart, lung, and spleen a similar number of times ($p > 0.05$; Table 4.4). However, cats visited bowls containing calf liver more than those containing beef liver ($p = 0.05$; Table 4.4). Within MDM, tripe, heart, lung and spleen preference testing, cats consumed a greater number of meals of calf compared to beef. A similar number of meals within kidney and liver were observed between calf and beef ($p > 0.05$; Table 4.4).

Table 4.5. Average intake amounts each visit per cat per day for each calf and beef by-product during each testing week, with fixed effects and interaction results (p < 0.05)

By-Product	Intake per visit (g)		P value	Pr > F		
	Calf	Beef		By-Product	Age	By-Product * Age
MDM	0.7 ± 0.1	0.7 ± 0.1	0.961	0.017	0.403	0.554
Tripe	0.9 ± 0.1	0.7 ± 0.1	0.316			
Kidney	1.2 ± 0.2	1.2 ± 0.2	0.807			
Heart	1.0 ± 0.1	0.9 ± 0.1	0.519			
Lung	0.6 ± 0.2	0.6 ± 0.1	0.983			
Spleen	0.8 ± 0.1	1.0 ± 0.2	0.608			
Liver	0.7 ± 0.2	1.3 ± 0.3	0.071			

For the intake amounts per visit, there were no differences observed between calf and beef intakes for any of the by-product varieties ($p > 0.05$; Table 4.5). The fixed effects of by-product had a significant impact on intake amounts per visit ($p < 0.05$). However, the age, and the interaction between by-product and age had no significant impact on visit intake amounts per visit ($p > 0.05$; Table 4.5).

Table 4.6. Average meal intake amounts per cat per day for each calf and beef by-product during each testing week, with fixed effects and interaction results ($p < 0.05$)

By-Product	Intake per meal (g)		P value	Pr > F		
	Calf	Beef		By-Product	Age	By-Product * Age
MDM	2.3 ± 0.5	2.9 ± 0.3	0.406	<0.001	<0.001	0.003
Tripe	3.5 ± 0.3	1.4 ± 0.3	0.030			
Kidney	7.0 ± 0.6	4.1 ± 0.6	0.001			
Heart	5.1 ± 0.6	5.1 ± 0.8	0.971			
Lung	5.3 ± 0.5	5.0 ± 0.8	0.729			
Spleen	8.3 ± 0.7	3.4 ± 0.5	<0.001			
Liver	7.4 ± 0.7	5.6 ± 0.6	0.077			

Meal intakes of tripe, kidney and spleen were greater for calf compared to mutton ($p < 0.05$; Table 4.6). No difference in meal intake was observed between MDM, heart, lung, and liver ($p > 0.05$). Overall, the fixed effect of by-product and age, and the interaction between by-product and age had a significant impact on meal intake amounts ($p < 0.05$; Table 4.6).

4.3.3 Correlating Intake Results to Nutrients

In addition to assessing the preference for lamb versus mutton and calf versus beef by-products, selected nutrients shown in Table 4.7 for ovine and Table 4.8 for bovine and the difference nutrients within the species was examined between young and old.

Table 4.7. Nutritional analysis of lamb and mutton by-products (as fed basis), and the difference between the two by subtracting lamb from the mutton nutrient percentages

By-product	Moisture (%)	Ash (%)	Crude Protein (%)	Fat (%)	Crude Fibre (%)	Calcium (mg/g)	Phosphorus (mg/g)	Hydroxyproline (mg/100g)	TBARS (MDA/kg freeze-dried meat)
Lamb									
Heart	5.5	3.1	47.0	34	0.9	ND	5.1	0.37	1.67
MDM	9.6	12.1	36.9	38.4	0.7	38.6	20.1	1.75	4.77
Liver	10.5	4.3	55.6	14.4	0.1	0.1	10.3	0.18	17.43
Lung	13	4.2	57.1	18.1	2	0.2	7.2	1.2	13.08
Kidney	11	5.4	61.6	10.5	0.2	0.3	10.5	0.4	5.81
Tripe	10.9	2.4	52.2	29	1.4	0.5	3.2	1.61	0.42
Spleen	3.9	6.2	69.7	9.8	2.2	0.2	10.4	0.65	9.15
Mutton									
Heart	7.9	2.5	41.5	43	0.7	0.1	4.4	0.27	1.54
MDM	10.4	6.2	34.8	45.9	0.8	15.1	9.4	0.97	1.95
Liver	11.5	4.1	53.7	14.7	0.1	0.1	9.7	0.23	17.13
Lung	6.9	4.8	70.5	5.6	1.1	0.3	8.9	0.99	15.98
Kidney	7.3	4.6	60.9	18.3	0.3	0.3	9.3	0.66	2.93
Tripe	14.2	2.3	54.6	23.8	1.6	0.2	3.1	1.92	0.4
Spleen	7.1	5.1	63.1	14	1.4	0.8	9.2	0.73	12.91
Difference									
Heart	2.4	-0.6	-5.5	9	-0.2	ND	-0.7	-0.1	-0.13

MDM	0.8	-5.9	-2.2	7.5	0.1	-23.5	-10.7	-0.78	-2.82
Liver	1	-0.2	-2.0	0.3	0	0	-0.6	0.05	-0.3
Lung	-6.1	0.6	13.4	-12.5	-0.9	0.1	1.7	-0.21	2.9
Kidney	-3.7	-0.8	-0.7	7.8	0.1	0	-1.2	0.26	-2.88
Tripe	3.3	-0.1	2.3	-5.2	0.2	-0.3	-0.1	0.31	-0.02
Spleen	3.2	-1.1	-6.6	4.2	-0.8	0.6	-1.2	0.08	3.76

1 TBARS defined as Thiobarbituric acid reactive substances
2 MDA defined as malondialdehyde

The target moisture level for the air-dried by-products throughout this work was 10%. Moisture levels of the ovine samples were variable, ranging from 3.9% in lamb spleen to 14.2% in mutton heart. For ash, most mutton by-products had lower percentages compared to lamb. For macronutrients, protein was generally higher in the lamb by-products than mutton. In contrast, mutton showed higher values for fat than the lamb by-products. Crude fibre levels were similar between the lamb and mutton samples as were calcium, phosphorus, hydroxyproline and TBARS, except for the calcium and phosphorus levels in lamb MDM which were 23.5% and 10.7% higher than in mutton, respectively.

Moisture levels of the bovine samples were variable, ranging from 5.6% in calf MDM to 15.3% in beef kidney. For ash, most beef by-products had lower percentages compared to calf. For macronutrients, protein was generally higher in the calf by-products than beef, except for beef lung and beef tripe which were higher in protein than beef. For fat, beef by-products showed higher levels compared to calf, except for calf lung and calf tripe which showed higher fat percentages than beef. Crude fibre levels were similar between the calf and beef samples as were calcium, phosphorus, hydroxyproline and TBARS, except for calf MDM having 58% higher calcium and 30.3% higher phosphorus than beef MDM, and beef lung and spleen having 11.97% and 14.61% higher TBARS percentages than calf, respectively.

Table 4.8. Nutritional analysis of calf and beef by-products (as fed basis), and the difference between the two by subtracting calf from the beef nutrient percentages

By-product	Moisture (%)	Ash (%)	Crude Protein (%)	Fat (%)	Crude Fibre (%)	Calcium (mg/g)	Phosphorus (mg/g)	Hydroxyproline (mg/100g)	TBARS (MDA/kg freeze-dried meat)
Calf									
Heart	5.8	7.4	61.3	21.7	1.9	0.3	6.9	0.49	1.03
MDM	5.6	20.7	46.5	18.3	0.4	68.1	38	1.77	3.47
Liver	10.8	7.0	56.4	12.6	0.3	0.1	10.2	0.28	38.86
Lung	7.0	7.1	63.0	16.9	6.1	0.3	9.6	0.26	5.08
Kidney	14.3	5.4	52.5	20.9	1.1	0.2	8.5	0.49	2.79
Tripe	9.5	2.8	55.9	24.1	1.2	0.4	5.1	2.24	0.43
Spleen	11.6	6.2	68.8	7.1	3.2	0.2	11.7	1.02	26.62
Beef									
Heart	8.7	3.7	49.1	30.1	0.4	0.1	6.0	0.37	0.99
MDM	9.8	4.4	31.3	51.1	0.7	10.1	7.7	0.30	1.73
Liver	12.1	9.2	55.5	13.9	1.3	0.0	8.0	0.18	40.17
Lung	6.8	4.3	70.9	9.5	3.4	0.3	8.0	2.00	17.05
Kidney	15.3	3.9	51.0	23.2	0.8	0.2	8.0	0.57	2.03
Tripe	5.9	2.2	74.0	13.2	1.7	0.3	3.5	3.74	0.80
Spleen	13.6	4.7	57.4	17.3	2.8	0.7	9.1	0.70	41.23

Difference									
Heart	2.9	-3.7	-12.2	8.4	-1.5	-0.2	-0.9	-0.12	-0.04
MDM	4.2	-16.3	-15.2	32.8	0.3	-58	-30.3	-1.47	-1.74
Liver	1.3	2.2	-0.9	1.3	1	-0.1	-2.2	-0.1	1.31
Lung	-0.2	-2.8	7.9	-7.4	-2.7	0	-1.6	1.74	11.97
Kidney	1	-1.5	-1.5	2.3	-0.3	0	-0.5	0.08	-0.76
Tripe	-3.6	-0.6	18.1	-10.9	0.5	-0.1	-1.6	1.5	0.37
Spleen	2	-1.5	-11.4	10.2	-0.4	0.5	-2.6	-0.32	14.61

The weekly food intake results were then correlated to selected nutrients to obtain Pearson correlation coefficients and determine their influence on palatability.

Table 4.9. Pearson correlation coefficients for intake versus nutrients using a significance level of p < 0.05

Variable	Pearson correlation coefficient	P value
Moisture	0.134	0.496
Ash	0.060	0.761
Protein	0.277	0.153
Fat	-0.463	0.013
Fibre	-0.013	0.948
Calcium	-0.192	0.337
Phosphorus	0.067	0.736
Hydroxyproline	-0.426	0.024
TBARS	0.251	0.198
Fat vs TBARS	***-0.494***	***0.008***

There was a moderate negative correlation between intake and fat, as well as intake and hydroxyproline ($r = -0.463$ and -0.426, respectively; $p < 0.05$; Table 4.9). Additionally, a moderate negative correlation between fat and TBARS was observed within the samples tested.

4.3.4 Effect of Nutrient Composition on Cats' Food Preference

Variances (i.e. eigenvalues) for the first three principal components (PC1-PC3) were greater than one (>1) and explained 74.0% of the variability found in the original variable (i.e., nutrients). The value from these components (expressed as percentages) were 36.2%, 25.3% and 12.5%, respectively. Eigenvectors from these three components confirm that some nutrients are correlated. This included ash, fat, P in PC1, protein and hydroxyproline in PC2 and moisture in PC3, as shown in Table 4.10 and Figure 4.3.

Table 4.10. Summary of PCA analysis, indicating the importance of each nutritional component of by-products by the test cats, standard deviation (SD) and the percentage explanation of variation linked to each principal component

Nutritional Components[1]	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Principal Component Eigenvectors								
M	0.036	0.305	0.753	-0.056	0.263	-0.437	0.173	-0.032	-0.210
ASH	-0.438	0.230	-0.226	-0.177	0.148	0.109	0.765	0.228	0.052
CP	-0.370	-0.458	-0.071	0.251	0.024	-0.097	0.061	-0.129	-0.745
F	0.484	0.167	-0.143	-0.367	0.007	0.183	-0.033	0.481	-0.562
CF	-0.219	-0.298	-0.18	-0.685	0.505	-0.176	-0.284	-0.072	0.100
Ca	-0.086	-0.351	0.381	-0.472	-0.677	0.107	0.174	-0.007	0.043
P	-0.469	0.212	-0.049	0.060	-0.286	-0.374	-0.402	0.587	0.033
HYD	0.132	-0.558	0.271	0.270	0.296	0.118	0.120	0.591	0.244
TBARS	-0.377	0.219	0.355	0.008	0.160	0.747	-0.306	-0.003	-0.095
	Principal Component Eigenvalues								
SD	3.2612	2.275	1.1291	0.8828	0.7053	0.4409	0.2492	0.0407	0.0154
% of Variance	36.2	25.3	12.5	9.8	7.8	4.9	2.8	0.5	0.2
Cumulative %	36.2	61.5	74.0	83.8	91.6	96.5	99.3	99.8	100.0

[1]Principal component (PC), moisture (M), crude protein (CP), fat (F), crude fibre (CF), calcium (Ca), phosphorus (P), hydroxyproline (HYD), and standard deviation (SD).

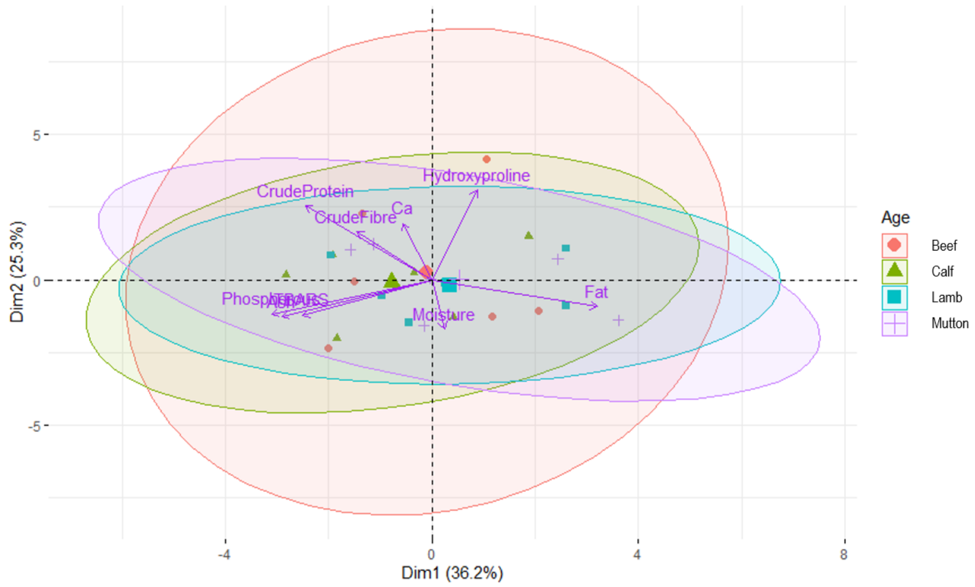


Figure 4.3. A principal component analysis (PCA) plot showing the distribution of the nutritional components of the by-products on the first two principal components differentiated by age.

The linear regression analysis between principal components and intake by cats revealed significance for the effects of PC1 (ash, fat, P) and PC2 (protein and hydroxyproline) and no significance for PC3 (moisture) ($p = 0.027$, 0.013 and 0.543, respectively).

In terms of the different ages of bovine and ovine by-products used as ingredients in this study, there was no evidence suggesting an age of animal driver on nutrient composition, as indicated in Figure 4.3, with all ages being superimposed on top of each other. However, when looking at the effect of by-products on nutrients initially examined, as displayed in Figure 4.4, there are distinct separations between by-products, particularly with tripe being very different to spleen and liver. This was further investigated using metabolomic analysis.

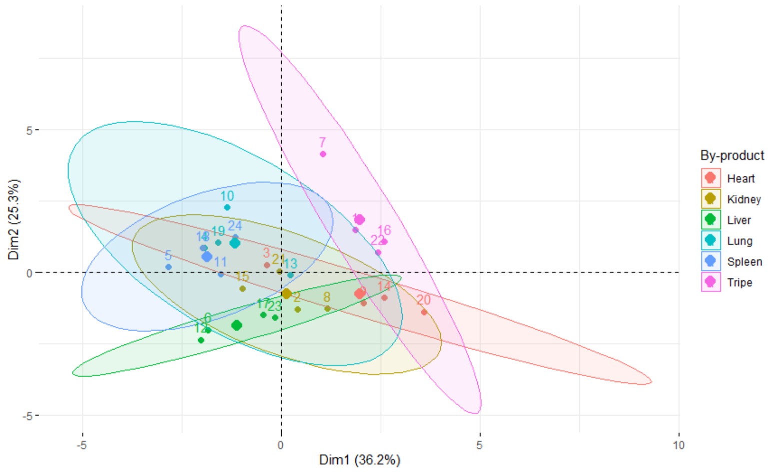


Figure 4.4. PCA plot showing the distribution of the nutritional components of the by-products on the first two principal components differentiated by by-product type.

4.3.5 Metabolomic analysis of by-products

Species effect on compounds associated to palatability in cats

The effect of species, with a focus on components known to influence palatability in cats, was analysed using a GLM with a significance level of 0.05, as shown in Table 4.11.

Table 4.11. The between species effect and associated p values of components known to influence palatability in cats

Compounds	Species effect (p value)
HILIC negative*	
L-Glutamic acid	0.430
γ-glu-leu	0.571
γ-glu-met	0.638
HILIC positive	
Alanine	0.368
Choline [M]+	0.109
Glutamic acid	0.595
L-phenylalanine	0.278
Tryptophan	0.545

* HILIC defined as hydrophilic interaction liquid chromatography

Within the HILIC negative components, L-Glutamic acid, γ-glu-leu and γ-glu-met are known to be positively associated with palatability in cats, however, no difference was observed between the relative amounts of these compounds between species ($p > 0.05$).

Similarly, within the HILIC positive compounds, alanine, choline [M]+ and glutamic acid have positive associations to palatability in cats, and L-phenylalanine and tryptophan have negative associations with palatability in cats. However, no difference was observed between the relative amounts of these components between bovine and ovine ($p > 0.05$)

Within lamb and mutton effect on compounds associated to palatability in cats

The fixed effects of by-product, age, and the interaction between age and by-products was analysed to determine the potential nutritional drivers of palatability within sheep, as shown in Table 4.12.

Within the HILIC negative compounds, the fixed effects of age and by-product, and the interaction between age and by-product also had a significant impact on the relative amounts of the compounds known to have an influence on palatability in cats ($p < 0.05$), except for the fixed effect of age on the relative amounts of γ-glu-leu ($p = 0.483$).

In terms of HILIC positive compounds, the fixed effects of age and by-product, and the interaction between age and by-product had a significant impact on the relative amounts of the compounds known to have an influence on palatability in cats ($p < 0.05$), except for the effect of age on the relative amounts of alanine and glutamic acid ($p = 0.506$ and 0.064, respectively).

Table 4.12. The within ovine fixed effects of age and by-product and the interaction of age and by-product p value results ($p < 0.05$)

Compounds	Age	By-Product	Age * By-Product
HILIC negative			
L-Glutamic acid	<0.001	<0.001	<0.001
γ-glu-leu	0.483	<0.001	0.001
γ-glu-met	0.007	<0.001	<0.001
HILIC positive			
Alanine	0.506	<0.001	0.020
Choline [M]+	<0.001	<0.001	<0.001
Glutamic acid	0.064	<0.001	<0.001
L-phenylalanine	<0.001	<0.001	<0.001
Tryptophan	<0.001	<0.001	<0.001

The interaction between age and by-product within ovine samples was further analysed to examine the grouping of by-products, as detailed in Table 4.13, in terms of the relative amounts of each compound to determine its potential effect on palatability.

Table 4.13. The relative amounts of HILIC positive and HILIC negative ionisation compounds known to have an influence on palatability in cats in lamb and mutton by-products. Log transformed mean values denoted with different letters differ significantly at p < 0.05.

Age	**By-Product**	**HILIC negative**			**HILIC positive**				
		L-Glutamic acid	γ-glu-leu	γ-glu-met	Alanine	Choline [M]+	Glutamic acid	L-phenylalanine	Tryptophan
Lamb	Liver	11.20^{e}	8.04^{c}	4.80^{c}	7.95^{g}	$11.62^{d,e}$	$10.74^{b,c,d}$	10.56^{d}	9.48^{c}
	Lung	11.41^{d}	6.46^{e}	3.24^{d}	8.07^{f}	11.74^{a}	$10.40^{d,e}$	8.91^{h}	$7.73^{e,f}$
	Kidney	12.14^{a}	10.50^{a}	8.62^{a}	8.46^{d}	11.74^{a}	11.64^{a}	10.65^{c}	10.65^{a}
	Heart	10.22^{f}	2.58^{g}	2.62^{d}	8.99^{a}	11.47^{g}	9.01^{f}	8.87^{h}	$7.36^{f,g}$
	Tripe	$11.30^{d,e}$	$6.83^{d,e}$	6.03^{b}	8.53^{d}	11.67^{b}	10.27^{e}	9.37^{g}	$7.50^{e,f,g}$
	Spleen	$12.06^{a,b}$	9.42^{b}	8.93^{a}	$8.55^{c,d}$	11.68^{b}	11.69^{a}	10.43^{e}	9.45^{c}
	MDM	8.25^{i}	3.81^{f}	2.62^{d}	8.78^{b}	$11.64^{c,d}$	6.73^{h}	8.66^{i}	6.41^{h}
Mutton	Liver	11.19^{e}	8.28^{c}	$5.57^{b,c}$	7.86^{g}	11.60^{e}	$10.81^{b,c}$	10.76^{b}	10.03^{b}
	Lung	11.70^{c}	7.18^{d}	6.00^{b}	8.17^{f}	11.74^{a}	10.88^{b}	9.66^{f}	8.72^{d}
	Kidney	12.06^{a}	11.24^{a}	8.61^{a}	8.34^{e}	11.72^{a}	11.42^{a}	$10.69^{b,c}$	10.52^{a}
	Heart	9.89^{g}	2.58^{g}	2.62^{d}	8.94^{a}	11.37^{h}	8.68^{f}	8.87^{h}	7.31^{g}
	Tripe	11.43^{d}	7.08^{d}	$5.47^{b,c}$	8.65^{c}	$11.66^{b,c}$	$10.48^{c,d,e}$	9.61^{f}	7.85^{e}
	Spleen	11.95^{b}	9.17^{b}	8.98^{a}	8.48^{d}	$11.67^{b,c}$	11.34^{a}	11.01^{a}	9.99^{b}
	MDM	9.43^{h}	2.58^{g}	2.62^{d}	8.79^{b}	11.57^{f}	7.76^{g}	8.54^{j}	6.16^{h}
SEM (LSM)		0.063	0.202	0.283	0.041	0.011	0.123	0.030	0.132
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Regarding HILIC negative compounds, L-glutamic acid, γ-glu-leu and γ-glu-met showed varying levels within different by-products, as shown in the boxplots in Figure 4.5 and confirmed by the groupings obtained in Table 4.13. Generally, the lamb and mutton organ meats contained higher ($p < 0.05$) amounts of these compounds which are associated with a positive influence on palatability in cats and lower amounts ($p < 0.05$) in the MDMs (and heart for γ-glu-leu, and lamb heart and lamb lung in γ-glu-met) reflecting their lower palatability in the rankings.

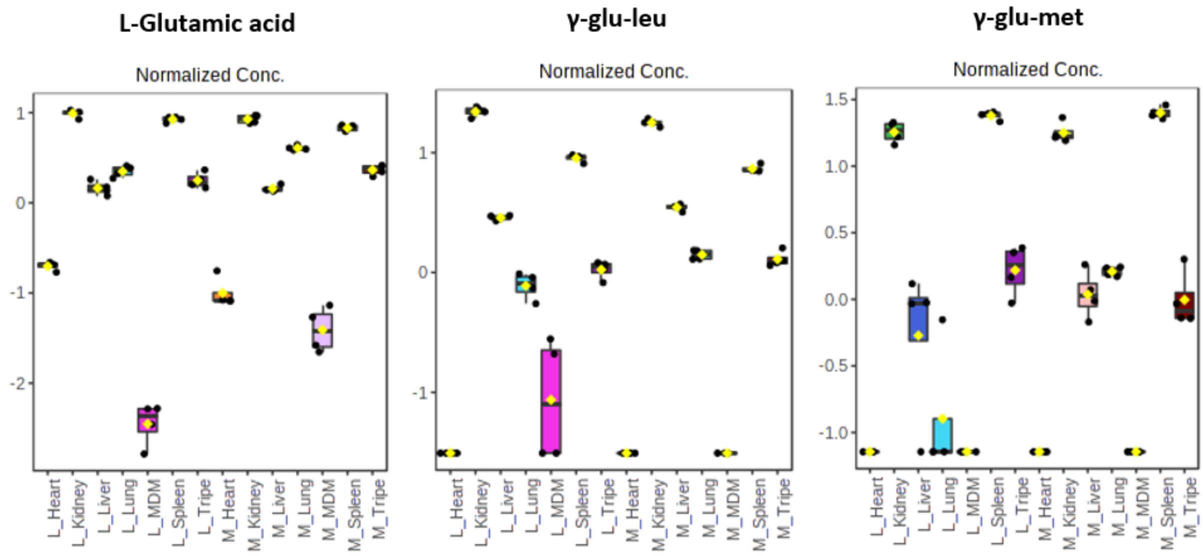


Figure 4.5. Interquartile range boxplot showing the relative amounts of HILIC negative compounds between the by-products (n=4) within each age group of lamb (L) and mutton (M)

In terms of the HILIC positive compounds, as shown in Figure 4.6, alanine content was highly variable among all by-products ($p < 0.05$) with lamb heart showing the highest level compared to mutton liver with the lowest level. Choline content was also variable between by-products, with both lamb heart and mutton heart showing the lowest level ($p < 0.05$; as confirmed by the superscripts in Table 4.13) of all the by-products tested. Glutamic acid in its positive ionisation state showed similar outcomes to L-glutamic acid in the HILIC negative results with the organ meats showing higher levels than the MDMs ($p < 0.05$). Finally, for L-phenylalanine and tryptophan, the organ meats with high palatability in previous testing, including liver, kidney and spleen showed the highest levels of these amino acids although differences were still observed between these organ meats ($p < 0.05$). In contrast, the less palatable MDMs contained significantly lower levels ($p < 0.05$) of these amino acids known to be negatively associated to palatability in cats, suggesting a less extensive effect of these amino acids when presented in a complex food matrix than when diluted in water.

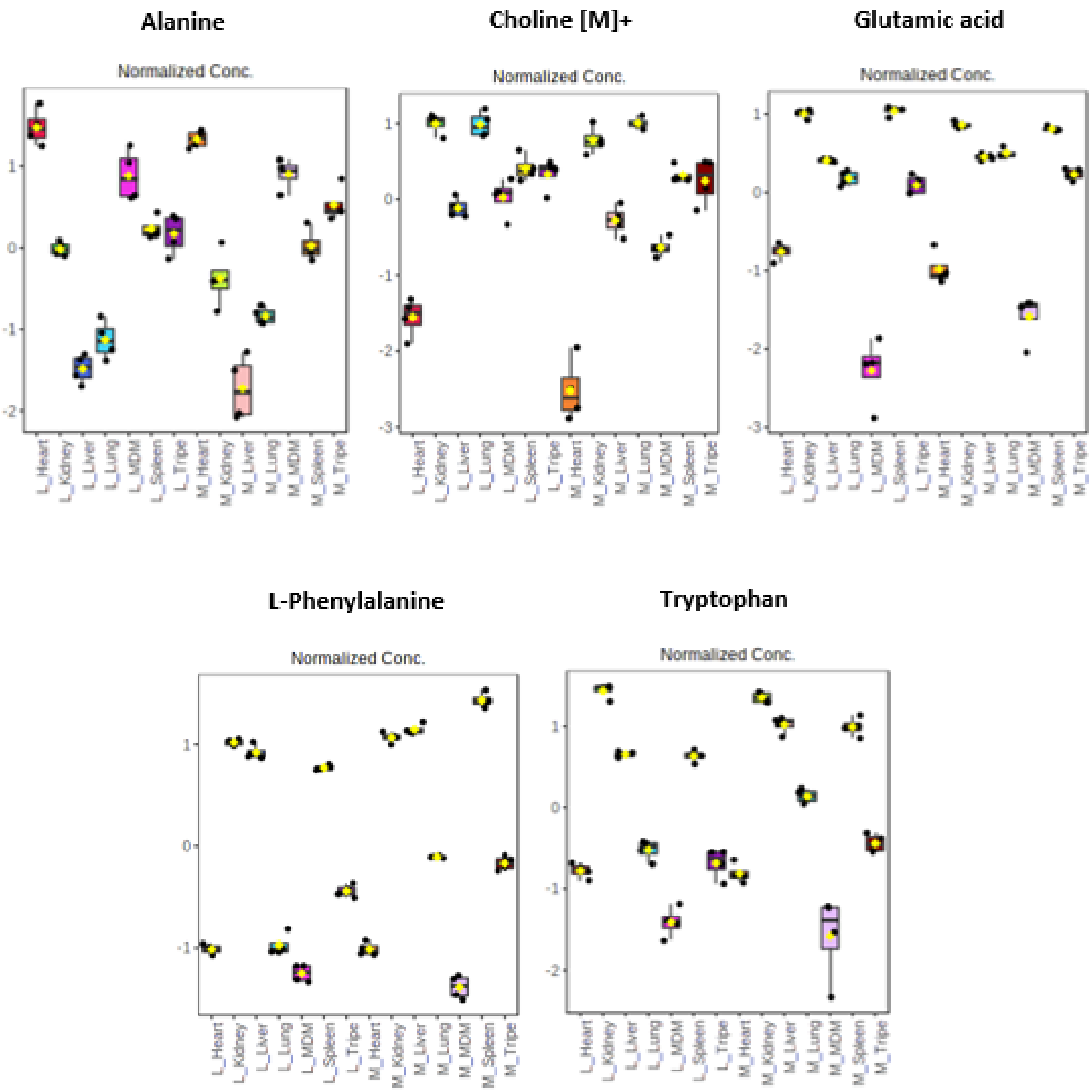


Figure 4.6. Interquartile range boxplot showing the relative amounts of HILIC positive compounds between the by-products (n=4) within each age group of lamb (L) and mutton (M)

Principal component analyses were also conducted to show the separation of by-product samples in both the HILIC negative and positive ionisation modes. PCA of the negative ionisation mode data alone, as depicted in Figure 4.7 showed that PC1 accounted for 33.6% (maximum) variation in the dataset and that by-products from lamb and mutton clustered according to by-product, not age of animal. This highlights the differences between by-products but no significant age effect, as previously suggested in Figure 4.3 and Figure 4.4. Similar results were also observed in the positive ionisation mode in

Figure 4.8 showing that PC1 accounted for 35.9% (maximum) variation in the dataset and that by-products also clustered according to their like by-product.

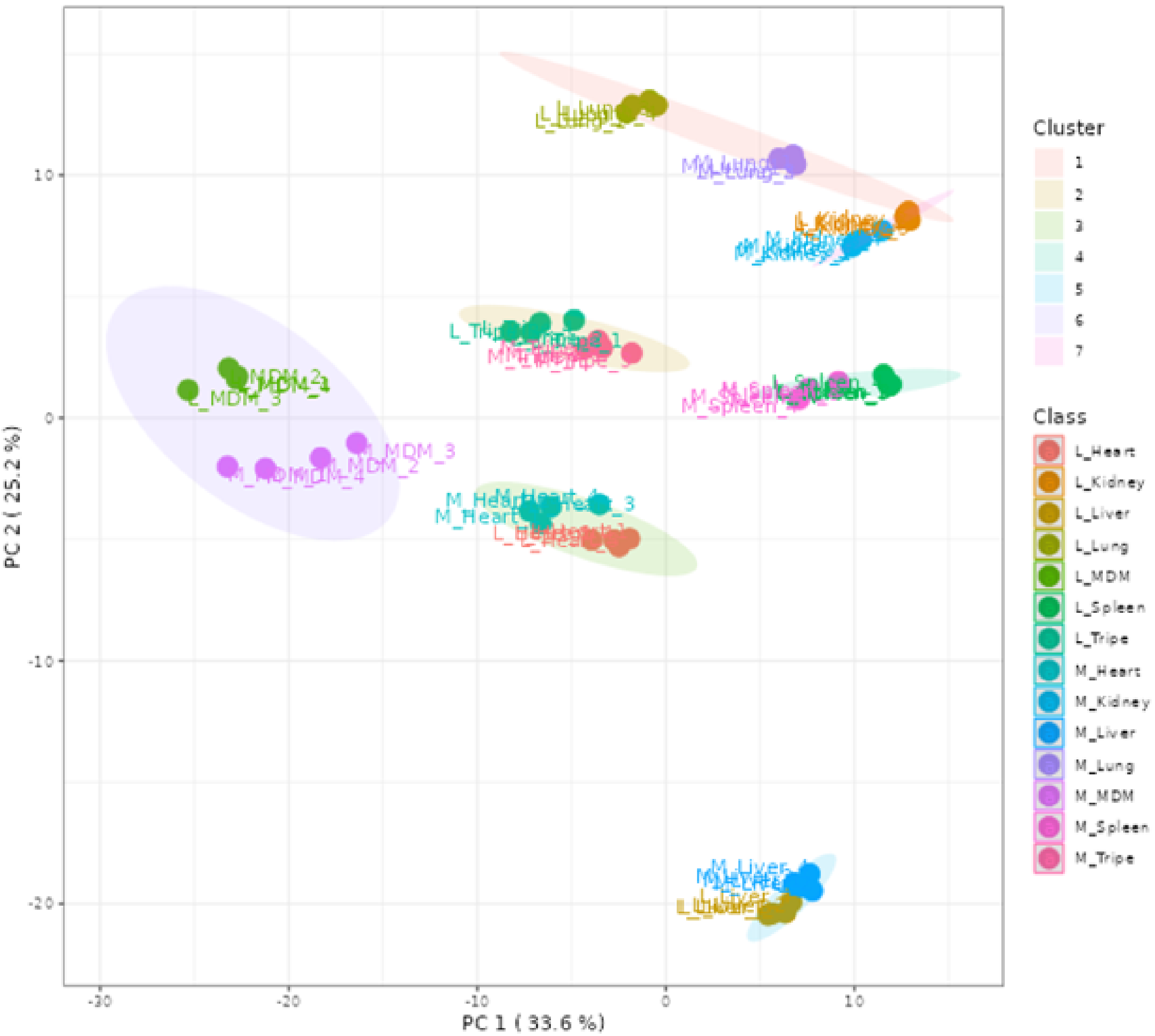


Figure 4.7. 2D-PCA plots of HILIC negative ionisation mode data matrix, showing differences between by-products within ovine

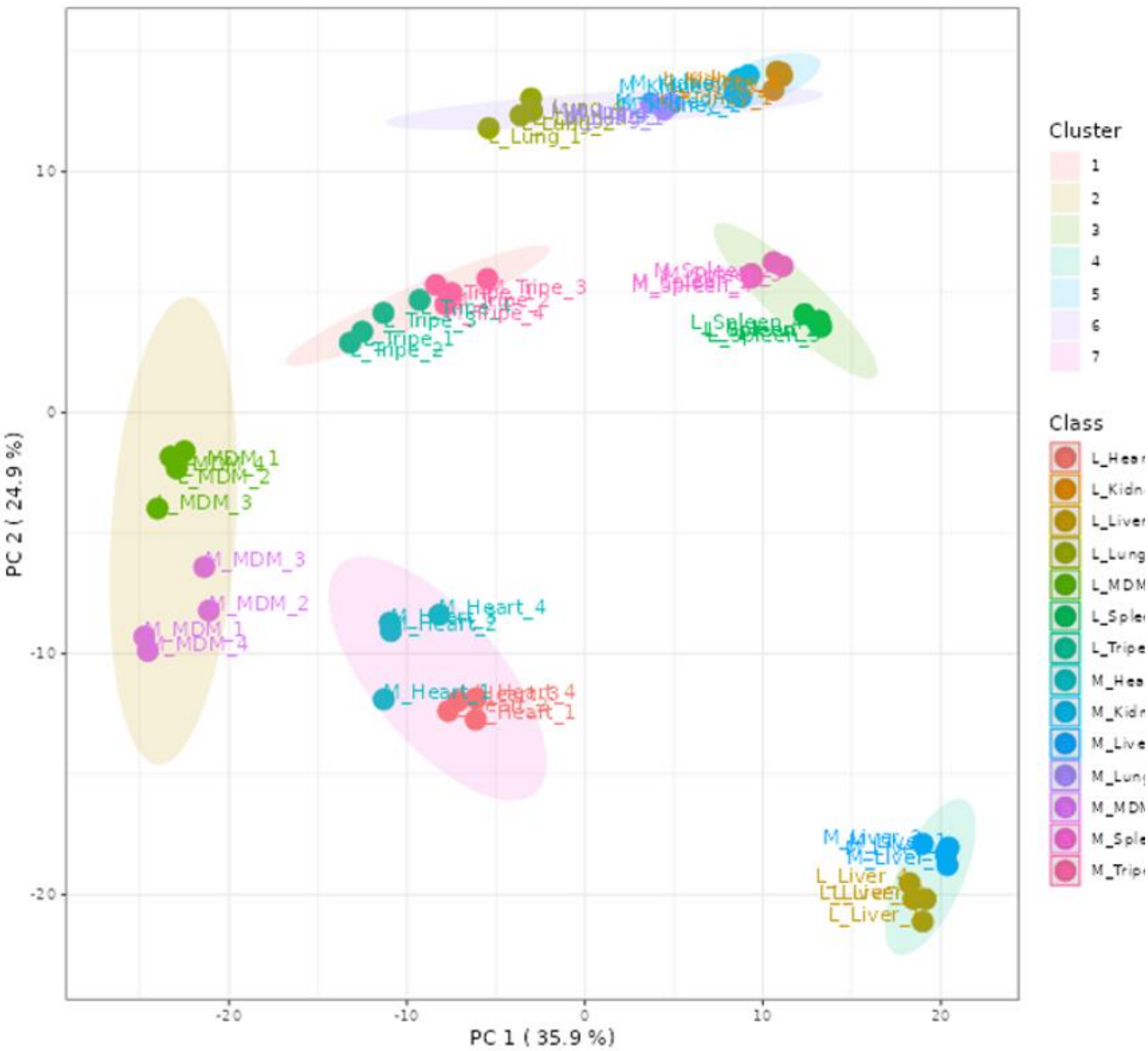


Figure 4.8. 2D-PCA plots of HILIC positive ionisation mode data matrix, showing differences between by-products within ovine

Within beef and calf effect on compounds associated to palatability in cats

The fixed effects of by-product, age, and the interaction between age and by-products was analysed to determine the potential nutritional drivers within beef, as shown in Table 4.14.

Within the HILIC negative compounds, the fixed effects of age and by-product, and the interaction between age and by-product had a significant impact on the relative amounts of the compounds known to have an influence on palatability in cats ($p < 0.05$), except for the fixed effect of age on the relative amounts of L-glutamic acid ($p = 0.825$).

In terms of HILIC positive compounds, the fixed effects of age and by-product, and the interaction between age and by-product also had a significant impact on the relative amounts of the compounds known to have an influence on palatability in cats ($p < 0.05$), except for the effect of age on the relative amounts of alanine and glutamic acid ($p = 0.229$ and 0.855, respectively).

Table 4.14. The within beef fixed effects of age and by-product and the interaction of age and by-product p value results (p < 0.05)

Compounds	Age	Offal	Age * offal
HILIC negative			
L-Glutamic acid	0.890	<0.001	<0.001
γ-glu-leu	<0.001	<0.001	<0.001
γ-glu-met	<0.001	<0.001	<0.001
HILIC positive			
Alanine	0.229	<0.001	<0.001
Choline [M]+	<0.001	<0.001	<0.001
Glutamic acid	0.855	<0.001	<0.001
L-phenylalanine	<0.001	<0.001	<0.001
Tryptophan	<0.001	<0.001	0.001

Regarding HILIC negative compounds, L-glutamic acid, γ-glu-leu and γ-glu-met showed varying levels within different by-products, as shown in the boxplots in Figure 4.9 and confirmed by the groupings obtained in Table 4.15.

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Table 4.15. The relative amounts of HILIC positive and HILIC negative ionisation compounds known to have an influence on palatability in cats in calf and beef by-products. Log transformed mean values denoted with different letters differ significantly at p < 0.05.

Age	**By-Product**	**HILIC negative**			**HILIC positive**				
		L-Glutamic acid	γ-glu-leu	γ-glu-met	Alanine	Choline [M]+	Glutamic acid	L-phenylalanine	Tryptophan
Calf	Liver	11.45[d]	8.33[b]	7.04[c]	8.21[g]	11.66[d,e,f]	10.89[b]	10.39[c]	9.71[a,b,c]
	Lung	11.64[c,b]	7.67[b]	7.00[c]	8.34[e,f]	11.70[b,c]	10.81[b,c]	9.83[e]	9.17[c,d]
	Kidney	12.07[a]	9.31[a]	8.20b	8.45[d,e]	11.7[b,c]	11.51[a]	10.44[c]	10.17[a,b]
	Heart	11.12[e]	5.14[c]	4.79[d]	8.96[a]	11.47[g]	10.30[e]	9.21[g]	7.94[e,f]
	Tripe	10.78[f]	5.59[c]	4.97[d]	8.29[f,g]	11.63[f]	9.40[f]	9.03[h]	7.35[f]
	Spleen	12.05[a]	9.46[a]	8.85[a,b]	8.53[c,d]	11.65[e,f]	11.60[a]	11.11[a]	10.37[a]
	MDM	9.04[i]	3.19[d]	2.62[e]	8.79[b]	11.68[c,d,e]	7.42[h]	8.74[i]	7.04[f]
Beef	Liver	11.20[e]	8.27[b]	5.46[d]	8.28[f,g]	11.72[b]	10.57[c,d]	10.28[d]	9.43[a,b,c,d]
	Lung	11.51[c,d]	8.07[b]	6.94[c]	8.21[g]	11.75[a]	10.54[d,e]	9.57[f]	8.58[d,e]
	Kidney	11.81[b]	8.09[b]	6.77[c]	8.28[f,g]	11.76[a]	10.92[b]	9.83[e]	9.33[b,c,d]
	Heart	10.55[g]	2.58[d]	2.62[e]	9.06[a]	11.43[h]	9.51[f]	9.08[h]	7.71[e,f]
	Tripe	11.56[c,d]	2.58[d]	2.62[e]	8.74[b]	10.94[j]	10.91[b]	7.27[k]	4.95[g]
	Spleen	12.00[a]	9.74[a]	9.34[a]	8.56[c]	11.69[b,c,d]	11.45[a]	10.97[b]	10.24[a,b]
	MDM	9.57[h]	2.58[d]	2.62[e]	8.63[c]	11.37[i]	7.96[g]	8.05[j]	4.42[g]
SEM (LSM)		0.065	0.318	0.299	0.038	0.011	0.087	0.030	0.344
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Generally, the calf and beef organ meats contained higher ($p < 0.05$) amounts of these compounds associated with a positive influence on palatability in cats and lower ($p < 0.05$) amounts in the MDMs (and beef heart and beef tripe for γ-glu-leu and γ-glu-met) reflecting their lower palatability in the rankings.

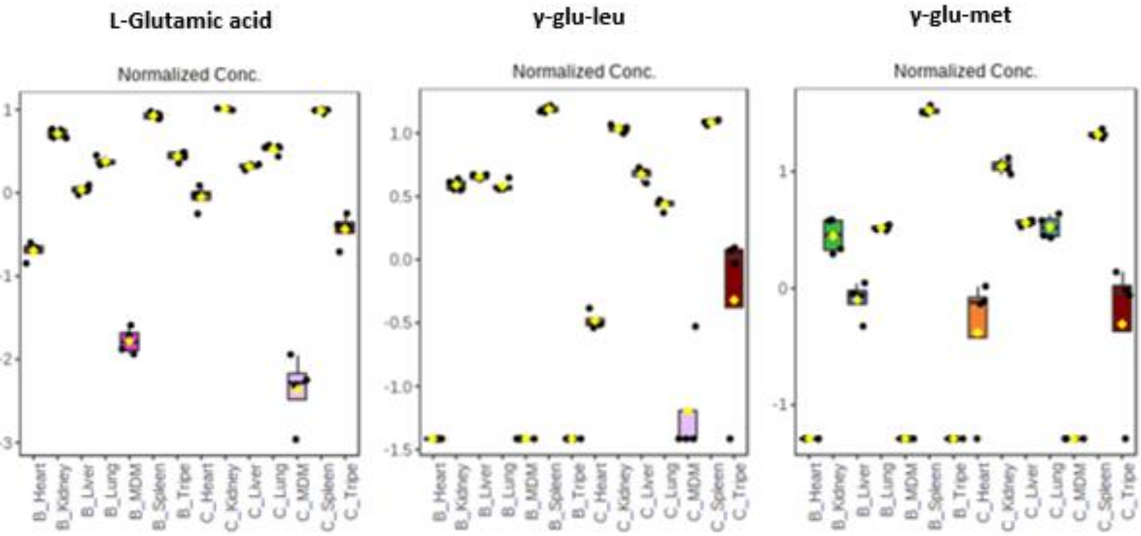

Figure 4.9. Interquartile range boxplot showing the relative amounts of HILIC positive compounds between the by-products (n=4) within each age group of beef (B) and calf (C)

In terms of the HILIC positive compounds, as shown in Figure 4.10, alanine content was considerably variable among all by-products ($p < 0.05$) with beef heart showing the highest level and calf liver with the lowest level. Choline content was also variable between by-products, with beef kidney and beef lung showing the highest level ($p < 0.05$) and beef tripe showing the lowest level ($p < 0.05$; as confirmed by the superscripts in). Glutamic acid in its positive ionisation state showed similar outcomes to L-glutamic acid in the HILIC negative results with the organ meats showing higher levels than the MDMs ($p < 0.05$). Finally, in terms of L-phenylalanine and tryptophan, the organ meats with high palatability in previous testing, including liver, kidney and spleen showed the highest levels of these amino acids although differences were still observed between these organ meats ($p < 0.05$). In contrast, beef tripe followed by beef MDM showed significantly lower levels ($p < 0.05$) of L-phenylalanine and tryptophan of the amino acids known to be negatively associated to palatability in cats, suggesting a less extensive effect of these amino acids when presented in a complex food matrix than when diluted in water.

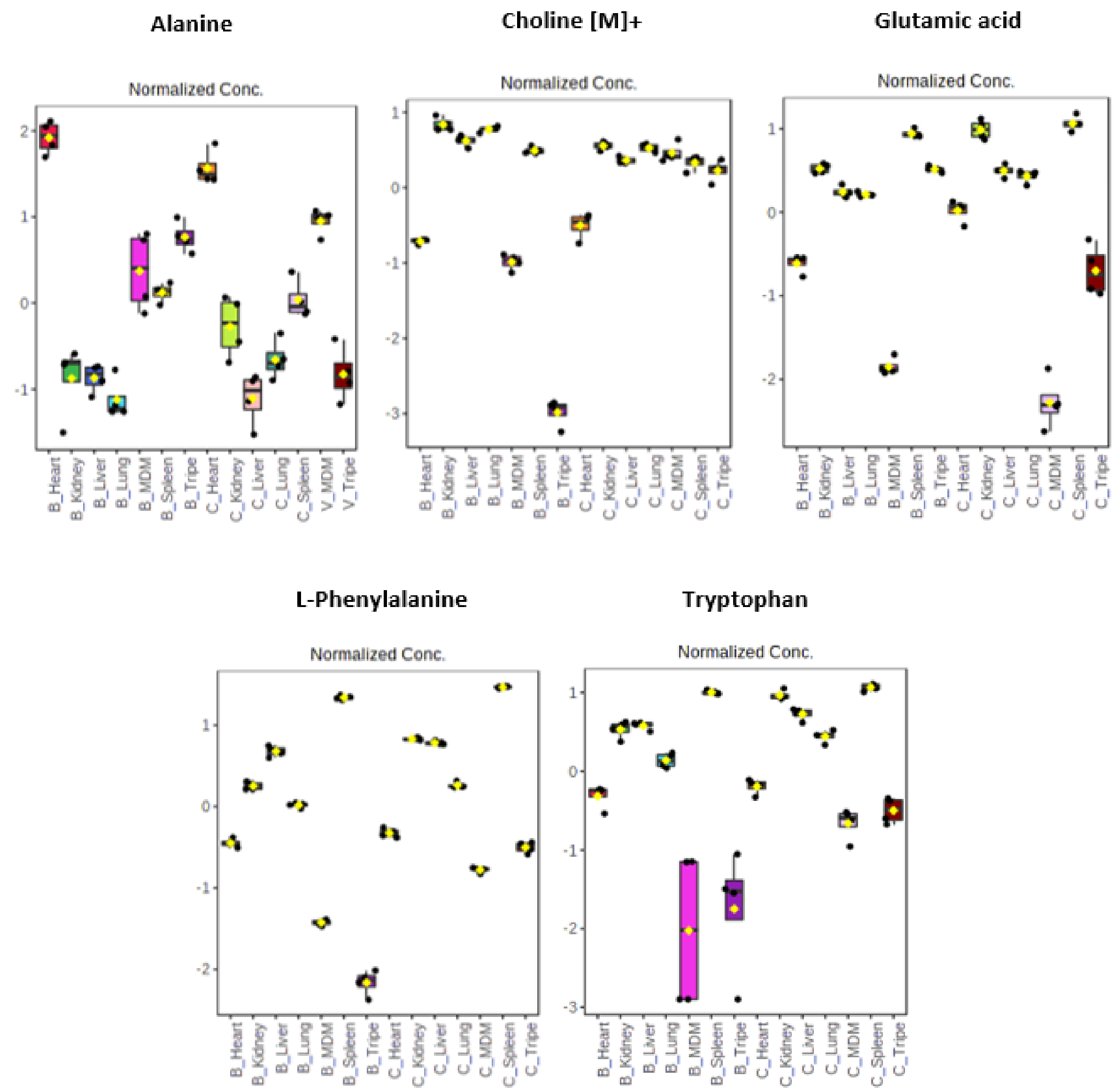

Figure 4.10. Interquartile range boxplot showing the relative amounts of HILIC positive compounds between the by-products (n=4) within each age group of beef (B) and calf/veal (C/V)

Principal component analyses were also conducted to show the separation of by-product samples in both the HILIC negative and positive ionisation modes. PCA of the negative ionisation mode data alone, as depicted in Figure 4.11 showed that PC1 accounted for 41% (maximum) variation in the dataset and that liver, spleen and heart from calf and beef clustered according to by-product, not age of animal. However, separation was observed between beef MDM and beef tripe clustering as individual by-products, with calf MDM and calf tripe clustering together and finally, the kidney and lung of both calf and beef clustering together. Similar results were also observed in the positive ionisation mode in Figure 4.12 showing that PC1 accounted for 36.8% (maximum) with by-products following the same clustering as observed in the negative ionisation mode. This pattern of a slight overlapping of by-products in the bovine results was not observed in the previous ovine results which showed clear distinctions between by-products with no difference due to age.

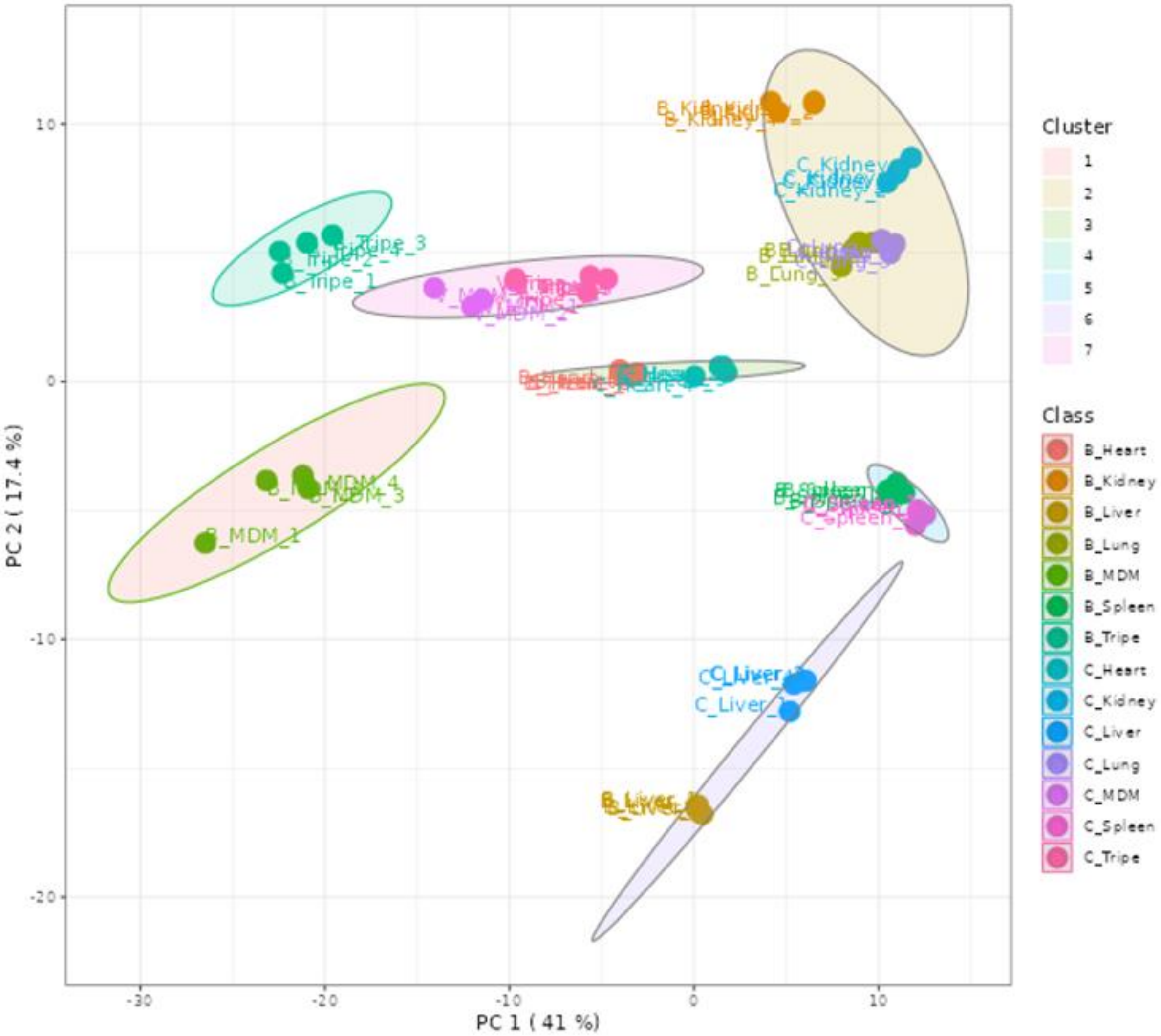

Figure 4.11. 2D-PCA plots of HILIC negative ionisation mode data matrix, showing differences between by-products within bovine

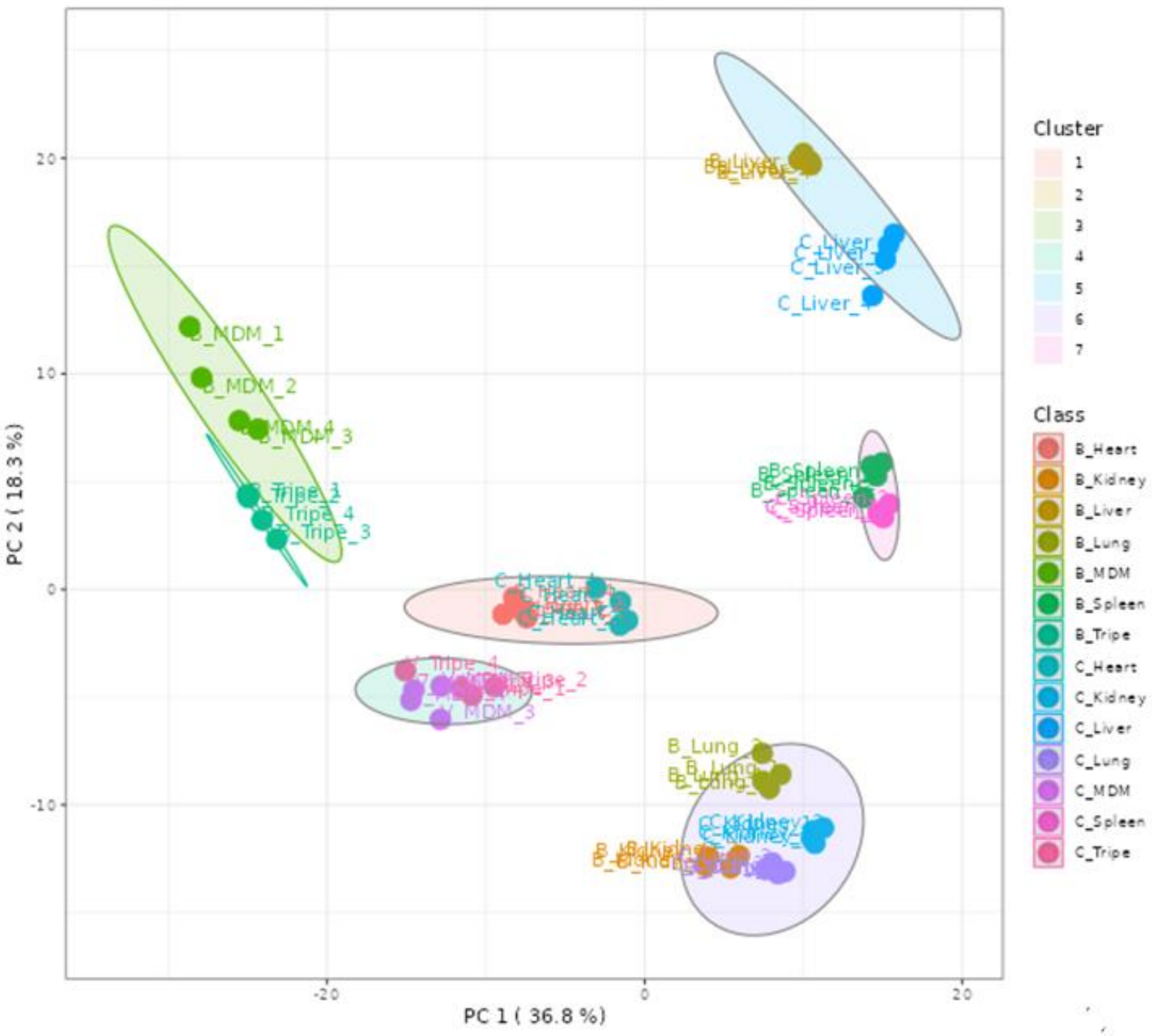

Figure 4.12. 2D-PCA plots of HILIC positive ionisation mode data matrix, showing differences between by-products within bovine

Effect of meat dipeptides carnosine and anserine on ovine and bovine by-product palatability

The levels of carnosine (HILIC negative) and anserine (HILIC positive) were also examined as they are key dipeptides known to be present in meat.

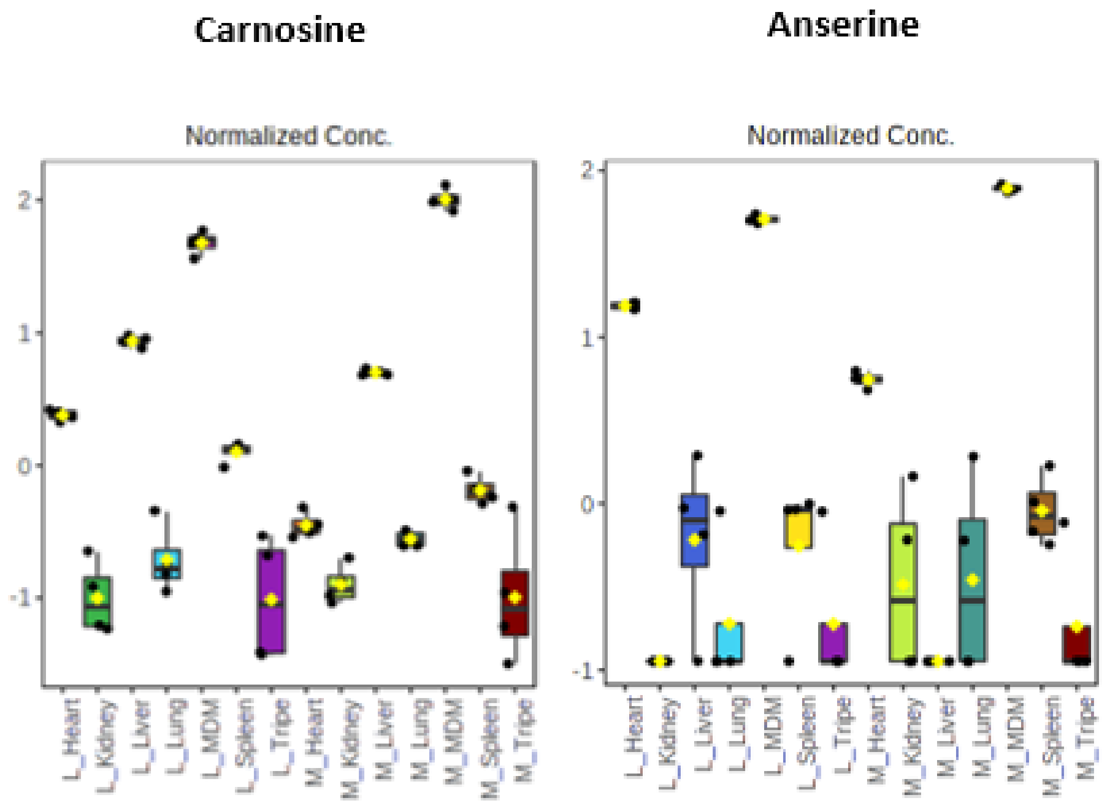

Figure 4.13. Interquartile range boxplot showing the relative amounts of carnosine and anserine between the lamb (L) and mutton (M) by-products (n=4)

Within the ovine results, carnosine levels were highest in the lower palatability by-products of mutton MDM ($p < 0.05$) and lamb MDM ($p < 0.05$), as pictured in Figure 4.13 and confirmed by the groupings provided in Table 4.16. However, lamb and mutton liver were the by-products containing the next highest levels of carnosine ($p <0.05$), and these by-products consistently had the highest palatability. Carnosine levels were lower and more variable in the remaining organ meats. In comparison, anserine levels were again highest in the MDM samples ($p < 0.05$), followed by heart ($p < 0.05$), with variable levels in the remaining organ meats ($p < 0.05$), as given in Table 4.16

Table 4.16. Groupings of the log transformed data of the interaction between age and by-product on the relative amounts of carnosine and anserine. Superscripts are to be compared within each column for all lamb and mutton results, using a significance level of 0.05

Age	**By-Product**	**HILIC negative** Carnosine	**HILIC Positive** Anserine
Lamb	Liver	9.97[c]	4.90[c,d]
	Lung	7.76[g,h]	3.25[d,e,f]
	Kidney	7.37[h]	2.51[f]
	Heart	9.22[d]	9.52[b]
	Tripe	7.67[h]	3.24[d,e,f]
	Spleen	8.85[d,e]	4.78[c,d,e]
	MDM	10.96[b]	11.23[a]
Mutton	Liver	9.66[c]	2.51[f]
	Lung	7.97[g]	4.11[c,d,e,f]
	Kidney	7.51[h]	4.02[c,d,e,f]
	Heart	8.11[f,g]	8.06[b]
	Tripe	7.38[h]	3.19[e,f]
	Spleen	8.46[e,f]	5.48[c]
	MDM	11.41[a]	11.84[a]
SEM (LSM)		0.1494	0.586
P value		<0.001	<0.001

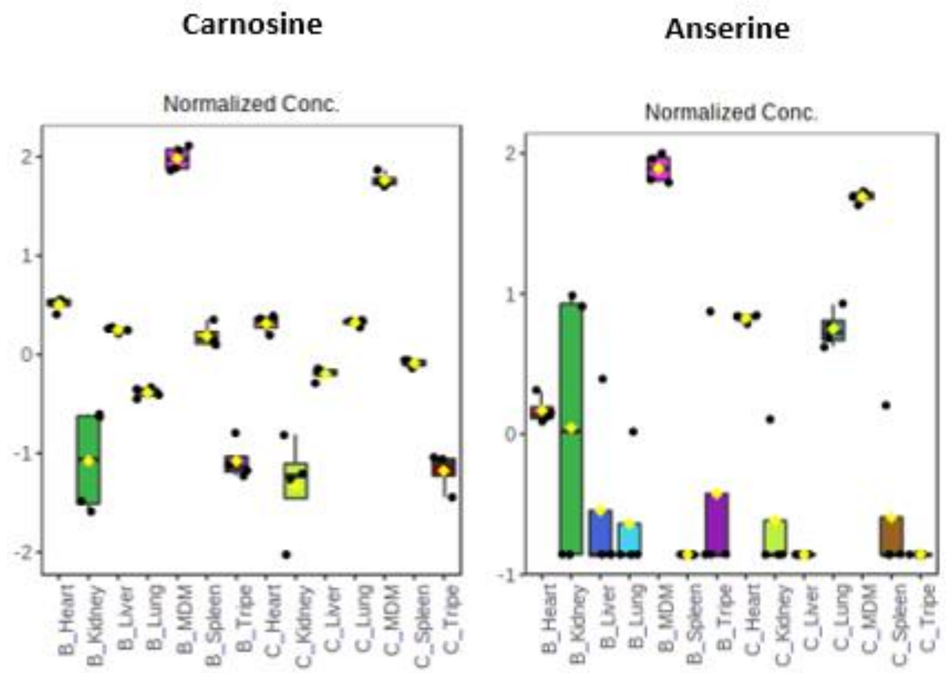

Figure 4.14. Interquartile range boxplot showing the relative amounts of carnosine and anserine between the beef (B) and calf (C) by-products (n=4)

Within the bovine results, carnosine levels were again highest in the lower palatability by-products of beef and calf MDM ($p < 0.05$), as shown in Figure 4.14 and confirmed by the groupings provided in Table 4.17. Carnosine levels were again variable in the remaining organ meats ($p < 0.05$). Similar findings were also found regarding anserine, with the highest levels in the MDM samples ($p < 0.05$), and more variable levels in the remaining organ meats ($p < 0.05$), as given in Table 4.17.

Table 4.17. Groupings of the log transformed data of the interaction between age and by-product on the relative amounts of carnosine and anserine. Superscripts are to be compared within each column for all calf and beef results, using a significance level of 0.05

Age	**By-Product**	**HILIC negative** Carnosine	**HILIC Positive** Anserine
Calf	Liver	8.46^{e}	2.51^{e}
	Lung	$9.21^{b,c}$	6.85^{b}
	Kidney	6.83^{f}	3.16^{e}
	Heart	$9.19^{b,c}$	7.06^{b}
	Tripe	7.05^{f}	2.51^{e}
	Spleen	$8.62^{d,e}$	$3.22^{d,e}$
	MDM	11.28^{a}	9.39^{a}
Beef	Liver	$9.10^{b,c}$	$3.35^{d,e}$
	Lung	8.19^{e}	3.10^{e}
	Kidney	7.19^{f}	$4.95^{c,d}$
	Heart	9.48^{b}	$5.28^{b,c}$
	Tripe	7.19^{f}	2.51^{e}
	Spleen	$9.01^{c,d}$	$3.68^{c,d,e}$
	MDM	11.60^{a}	9.94^{a}
SEM (LSM)		0.158	0.623
P value		<0.001	<0.001

4.5 Discussion

This trial examined the within species age of animal palatability differences of air-dried ovine and bovine by-products. This work aimed to further investigate the findings from the raw work by Watson et al. (2020) and the results from Chapter 3 for air-dried lamb and beef by-products which suggested that there may be an age or species effect on palatability. Specifically, the objective of this work was to determine whether cats showed a preference for ovine or bovine by-products from young or older animals. Palatability was assessed in this trial using a series of preference testing between the same by-products from lamb and mutton, as well as calf and young bulls and steers, referred to as beef, in this work.

It was hypothesised that young animal by-products would be more palatable than older animal, and this was observed throughout the study in most by-products tested. Furthermore, metabolomic analysis identified a number of novel (in terms of what is known for the cat) compounds that were associated with high/low palatable by-products.

For lamb versus mutton preference testing, lamb MDM, kidney, spleen, and heart were more palatable than the mutton equivalents. However, mutton lung and tripe were more palatable than lamb, and no difference was observed between the intake of lamb and mutton liver with high intakes of both. In comparison to the lamb and mutton preference testing, the beef versus calf testing showed that calf MDM, tripe, kidney, heart, lung, and spleen were more palatable than beef. However, no difference was observed between the intake of beef and calf liver with again high intakes of both. When examining the series of preference tests, ten of the fourteen individual preference tests showed that cats preferred the younger over the older animal by-products, two tests showed greater preference for mutton over lamb, and within both species, liver, a highly palatable but limiting ingredient in pet food formulations, showed similar intakes between young and old animals. The reason for the limited inclusion in pet foods stems from significant levels of vitamin A being stored in the liver of animals (Scotter et al., 1992). As a fat-soluble vitamin, the consumption of excess vitamin A can be extremely toxic and result in a myriad of issues in cats including muscle soreness, tenderness of joints and hyperesthesia, particularly along the neck and forelimbs of cats due to the development of bony exostoses (Green and Fascetti, 2016; Hayes, 1982; Kantorosinski and Morrison, 1987).

In addition to comparative intakes, the number of visits (where cats spent less than 80 seconds at the bowl) and meals (when they spent longer) were also examined. The cats visited bowls containing lung, kidney, tripe, MDM, liver, and spleen a similar number of times whether it came from lamb or mutton, however bowls containing mutton heart were visited more than those containing lamb heart. For visits to bowls containing bovine offal, cats frequented bowls containing lung, heart, kidney, tripe, MDM, and spleen a similar number of times whether it came from calf or beef. However, bowls containing calf liver were visited more than those containing beef. In terms of meals within the ovine testing, a greater number of lamb heart and MDM meals were observed compared to the mutton counterpart, and a greater number of mutton tripe meals were consumed compared to lamb tripe. For the remaining by-products similar number of meals were observed between lamb and mutton. For meals within the bovine testing, a greater number of calf

MDM, heart, lung, tripe, and spleen meals were observed compared to their beef counterparts, with no difference being observed between calf and beef liver and kidney.

The amount (in grams) consumed during these visits and meals were also examined. Within both ovine and bovine testing, no significant difference was observed between the amount consumed during the visits between young and old by-products within each species. In terms of the fixed effects by-product, age and the interaction between by-product and age, only the by-product effect in the beef testing showed a significant impact on visit intake amounts. The intake amounts within meals for ovine testing showed that cats consumed more mutton lung and tripe than lamb, and more lamb heart and kidney compared to mutton and overall, the fixed effects by-product, age and the interaction between by-product and age had a significant effect on meal intake amounts. Similarly, the intakes during the bovine testing showed that calf tripe, kidney and spleen was consumed in greater amounts than the beef counterparts and overall, the fixed effects by-product, age and the interaction between by-product and age had a significant effect on meal intake amounts.

The within species bowl visits and by-product intake results show similar findings as seen previously between lamb and beef in Chapter 3. Specifically, bowl visits appear to be a poor predictor of preference for certain by-products over others. Whereas, meals, defined as a sustained bout of feeding lasting 80 seconds or longer (Thomas et al., 2018), and the differing amounts consumed between certain lamb and mutton, as well as beef and calf by-products more clearly demonstrate relative palatability. This likely occurs as a result of the consumption of meals involving the use of both smell and predominantly taste, which is the most dominant sense used in influencing the food preference of cats. This contrasts with the primary use of the olfactory senses as the main determinant in assessing the attractiveness of the by-products in the shorter visit (Aldrich & Koppel, 2015; Alegría-Morán et al., 2019; Bradshaw et al., 1996; Hullár et al., 2001; Pickering, 2009).

As there is little to no literature available on the acceptance of meat of different ages in cat studies, literature from human studies have been examined, with a focus on lean meat cuts. The nutritional composition of Spring and Autumn lambs, representing two age groups (4 to 4 and a half months and 8 to 9 months of age) regarding raw and cooked lean meat cuts has been investigated (Ono et al., 1984). Except for moisture, total lipid, riboflavin, niacin, zinc and iron levels, no practical differences in nutrients were observed between cuts or age group. In contrast, a study by Schönfeldt et al, (2010) examined the nutritional composition of three different age groups of carcasses and cuts of South African beef. The different ages included an age group: those with no permanent incisors or less than 2 years, those with 2 permanent incisors or greater than 2 years, and a final age group with 8 teeth or those greater than 4 years. In terms of physical composition, meat and bone were found to increase with increasing age. Compositional differences were also found between the different age classes, specifically palmitic acid, lysine, and iron were higher and linoleic acid lower in older compared to younger animal. Significant differences were found between the different cuts in terms of fat content (subcutaneous and proximate), meat, moisture, various fatty acids (palmitic, stearic, and oleic acids) and calcium. Finally, different cuts within a carcass were discriminated by varying hydroxyproline and glycine levels (Schönfeldt et al., 2010). In brief, several years difference in beef age contributed to greater nutritional differences between samples compared to the four-month age difference in the earlier lamb study of Ono et al (1984). This provides justification as to why the age extremes within ovine and bovine sources were examined in this work.

The intake results obtained in the first part of the chapter were also correlated to selected nutrients present in the offal and revealed a negative correlation between intake and fat, intake and hydroxyproline content and a negative correlation between TBARS and fat. Whilst the inclusion of fat is known to improve the texture of cat foods, cats are known to be sensitive to lipid oxidation as they can easily detect off-notes in foods, thus resulting in decreased palatability (Hagen-Plantinga et al., 2017; Zaghini & Biagi, 2005). With no inclusion of antioxidants in the air-dried meat samples tested, it is possible that the negative correlation may be driven by the lipid oxidation effect outstripping the positive influence of fat on the texture of the samples. When looking at the correlation of fat to TBARS (measure of lipid oxidation), a negative correlation was observed, which was consist with results examining the relationship of crude fat content to lipid peroxidation of beef during storage (Sasaki et al., 2001).

Furthermore, hydroxyproline content is an index of the total collagen content of the sample (Bergman & Loxley, 1963; Boccard et al., 1979; Hill, 1966; Schönfeldt et al., 2010). Collagen is known to be abundant in connective tissue and contributes greatly to the variation in meat texture and tenderness (Weston et al., 2002). The strength and number of cross-links of intramuscular collagen increases in older animals and the collagen becomes less heat soluble, resulting in greater perceived toughness in meat, as determined from human studies (Hill, 1966; Warner et al., 2021; Weston et al., 2002). The results in this chapter suggest that increased amounts of collagen in pet food ingredients may adversely affect preference and palatability potentially through the change in texture seen in human studies.

A PCA was also carried out looking at these nutrients and found that the variables in PC1 (ash, fat, and phosphorus) and PC2 (protein and hydroxyproline), explained 36.2% and 25.3% of the variability in the data set and that the components within each of the principal components were correlated ($p < 0.05$). Linear regression analyses between PCs and intake by cats revealed significance for the effects of PC1 (ash, fat and phosphorus; $p = 0.027$) and PC2 (protein and hydroxyproline; $p = 0.013$). Furthermore, there was no evidence suggesting an age of animal driver on nutrient composition. However, distinctions were seen between different by-products in the PCA plots. With these results being ambiguous, minimal research into by-product palatability in cats and no research into preference for meat ingredients, analytical testing was used to develop hypotheses as to what components could be driving differences.

With a focus on compounds known in the literature to have an influence on palatability in cats, metabolomic analysis was conducted to measure the relative amounts of HILIC negative compounds, specifically L-glutamic acid, γ-glu-leu and γ-glu-met, as well as carnosine. As well as alanine, choline [M]+, glutamic acid, L-phenylalanine, and tryptophan, as well as anserine for the HILIC positive compounds within all by-product samples.

No differences were observed between the relative amounts of any of the compounds known to have an influence on palatability in cats between species. However, when analysing the relative amounts of compounds within ovine and bovine sources, the by-product, age and interaction between age and by-product had a significant impact on the relative amount of the selected HILIC positive and negative compounds. These results suggest that differences in relative amounts of some compounds are similar to the nutrient content of by-products findings, where large differences were generally exhibited between different offal (Edney, 1982; Murray et al., 1997; Shariff & Mona, 2013). This was confirmed in the ovine PCA, in both the negative and positive ionisation states, where each of the seven by-products were clustered separately in their respective pairs of the young and old by-product. Similarities were also seen in the PCAs of beef by-products with liver, spleen, and heart clustering separately in their calf and beef by-product pairs. However, beef MDM and beef tripe clustered separately as individual by-products, and calf MDM and calf tripe clustered together and finally, kidney and lung of both calf and beef clustered together. This slight overlapping of bovine by-products was different to the distinct by-product groupings a seen in the ovine results.

In terms of the ovine analysis of specific compounds, the HILIC negative compounds of L-glutamic acid, γ-glu-leu and γ-glu-met showed differences in the relative amounts present between by-products. Specifically, the organ meats were higher in L-glutamic acid than the MDM, organ meats, except for lamb and mutton hearts and MDMs, were also high in γ-glu-leu, and γ-glu-met was again highest in organ meats, except for lamb and mutton heart and MDM and lamb lung. Similar results were also seen in the beef analysis of HILIC negative compounds where the organ meats were higher in L-glutamic acid than the MDM, organ meats, except for beef heart and tripe and the MDMs, were also high in γ-glu-leu, and γ-glu-met was again highest in organ meats except for beef heart and tripe and the MDMs. Glutamic acid in its positive ionisation state again reflected similar results to that obtained for the HILIC negative L-glutamic acid measurements of being higher in the organ meats than in MDM.

Organ meats, particularly liver, kidney and spleen have shown high palatability throughout this project and in this analysis, show the greatest level of glutamic acid and γ-glutamyl peptides. Glutamic acid is a ubiquitous amino acid present in most foods in either the free form or bound to peptides and proteins (Garattini, 2000). Whilst somewhat insoluble in water, the salts of glutamic acid are generally soluble and have a very distinct taste, known as umami which was discovered and identified by Kikunae Ikeda in 1908, used to describe the unique taste of kelp and meat (Kurihara, 2009; Yamaguchi & Ninomiya, 1998). Like humans, cats are known to be drawn to foods with a strong umami/savoury flavour, which is often related to a high concentration of amino acids (Alegría-Morán et al., 2019; Salaun et al., 2016).

In addition to umami, kokumi taste perception, described as the sensation of enhanced sweet, salty and umami tastes, is an important taste modality for carnivores that drives palatability of meat derived compounds such as amino acids and peptides (Laffitte et al., 2021; Ohsu et al., 2010; Rhyu et al., 2020). Studies have found that various L-amino acids and γ-glutamyl peptides (as well as other compounds) are agonists of the Calcium Sensing Receptor (CaSR) in cats (Laffitte et al., 2021). The current work provides initial insight into components within a food that may be showing a direct link to palatability in cats. For example, γ-glutamyl peptides are of particular interest in cats as they are linked to glutathione metabolism, and both are found in varying levels in different by-products.

For HILIC positive compounds, choline, a nutrient known to have a positive influence on the palatability and overall consumption on dry cat food when included at 0.3% by weight choline chloride (Lin et al., 1997), was highest in the more palatable organ meats and lowest in lamb and mutton heart and beef tripe. Alanine, an amino acid known to be accepted by cats when diluted in water, was considerably variable with lamb heart showing the highest level and the more palatable livers showing the lowest amount in the ovine analysis, and beef heart showing the highest level and calf liver showing the lowest amount in the beef analysis. Furthermore, phenylalanine and tryptophan, amino acids known to be rejected by cats when diluted in water were considerably higher in the more palatable kidney, liver, and spleen organ meats and lowest in MDM within the two species (Beauchamp et al., 1977; Bradshaw et al., 1996; Zaghini, & Biagi, 2005). These results suggest a less extreme effect of these “accepted” and “rejected” amino acids when presented in a complex food matrix than when diluted in water.

It is reported that animal proteins, emulsified meats, and animal fats are identified as flavours that are highly palatable to cats (Thombre, 2004). Studies have investigated carnosine and anserine, two key imidazole dipeptides that are particularly abundant in skeletal and cardiac muscle, that were present in the by-product samples (Gil-Agustí et al., 2008). The levels of carnosine and anserine within ovine and bovine by-products was highest in MDM, the ingredient known to be of low palatability throughout this project. This suggests that it is important to consider the type of meat included in cat food formulations as skeletal muscle, as indicated by the higher carnosine and anserine levels in MDM, is least preferred compared to most of the organ meats tested. The literature has reported the importance of umami and its relation to glutamic acid and more recently analysed kokumi taste perceptions in cats. Links to these moieties appear to be reflected at a more fundamental level in this study. In particular, the preference for organ meats over MDM may be related to the level of glutamic acid and γ-glutamyl dipeptides in the by-product samples. However, future work will need to examine the concentrations that positively trigger the geniculate ganglion chemoresponsive group II units, as previously done for the "sweet" and "bitter" amino acids in a more controlled experimental study (White & Boudreau, 1975).

4.6 Conclusion

This study showed that cats can detect differences in palatability of by-products from ovine and bovine sources at different ages, with a general preference for younger over older by-products observed. However, within both species, no difference was observed between the intake of young or old liver. Regarding the correlation of palatability to selected nutrients, intake was negatively affected by fat and hydroxyproline content. Along with these findings, initial PCA results of nutrients showed there was no evidence suggesting an age of animal driver on nutrient composition. However, distinctions were seen between different by-products. These finding were also apparent when analysing compounds of interest from metabolomic analyses which showed no species effect on the relative amounts of compounds, however, the effect of age, by-product and the interaction of age and by-product were significant when examining the within ovine and bovine results.

For the compounds of interest, glutamic acid and the two γ-glutamyl dipeptides showed higher levels in organ meats, which were of higher palatability, compared to MDMs, which were of lower palatability throughout this project. This suggests an important relationship between umami and kokumi compounds and the palatability of meat ingredients in cat food, which have been identified as important taste moieties in the literature but now may be seen as important at the fundamental ingredient level. In contrast, amino acids previously associated to being liked and disliked by cats were present in varying levels in the by-products, suggesting a less extensive effect of these when presented in a complex food matrix than offered singly diluted in water. Furthermore, levels of carnosine and anserine, dipeptides found abundantly in skeletal and cardiac meat, were high in MDMs and lower in organ meats. Although cats have a biological need for meat proteins, the type of meat selected has a great influence on palatability, with preferential consumption of organ meats over muscle meat.

Future work in a more controlled experimental setting could be used to examine varying concentrations of glutamic acid and the two γ-glutamyl dipeptides diluted in water. This would be useful for determining the optimal inclusion levels of these compounds that may have a positive influence on palatability in cats. Compounds could then be included as ingredients in palatability enhancers and/or digests or in pet food formulations to improve the palatability of diets once further research has been conducted.

Chapter 5 Macronutrient Selection of Cats When Given Ad Libitum Access to Air-Dried Diets Varying in Macronutrient Composition (Geometric Nutrition and Palatability Study)

5.1 Introduction

Interest in understanding macronutrient selection and determining the potential nutritional drivers within the specialised dietary system of obligate carnivores is an area that is often overlooked in pet food research and development. Palatability studies, often used in the latter stages of pet food research and development, have largely focussed on identifying what diets are preferred by cats. However, little emphasis has been placed on determining what is driving differences in palatability.

There are a few key studies that have examined palatability beyond assessing intake, by using a geometric framework, for studying the macronutrient selection by cats when given wet and dry diets varying in protein, fat, and carbohydrate.

Firstly, an extensive study using wet and dry foods found that regarding macronutrient selection, cats selected a dietary macronutrient target based on the metabolisable energy (ME) compositions of 52% protein, 36% fat and 12% carbohydrate (Hewson-Hughes et al., 2011). Another study using wet diets found that when fat was stabilised at 36% of total ME, cats were able to regulate their macronutrient intake to obtain 53% of ME from protein and 11% from carbohydrate (Salaun et al., 2016). Both studies show remarkably similar levels of protein-fat-carbohydrate intake which resembles the levels in prey consumed by free-roaming cats (52:46:2%) when reviewed in the literature (Plantinga et al., 2011). It could be hypothesised that the ME selection from protein between domestic and free-roaming cats were aligned, but, the fat and carbohydrate selection is not. The differences in the contribution from carbohydrates is likely to be driven by the considerable differences in nitrogen-free extract (NFE) content of their prey (0% and 12.9% on a DM basis in rats and invertebrates respectively (Plantinga et al., 2011). Additionally, the study by Hewson-Hughes et al. (2011) also revealed that domestic cats displayed a ceiling for carbohydrate intake of approximately 75 kcal/day (~20 g/day or 30% of ME requirements) as given by Farrow et al., (2013).

In terms of assessing palatability in these geometric framework studies, the study by Salaun et al. (2016) found that the addition of a palatability enhancer to the diets resulted in increased food intake but no change to protein or carbohydrate intake patterns. Further work by Hewson-Hughes et al. (2016) using steam sterilised and homogenised wet food also showed that when diets were masked with fish (positive), rabbit (neutral) and orange (negative) flavours, cats were initially able to distinguish between the flavours. However, in the longer term they selected similar protein and fat intake regardless of flavour combination, as reflected by consistent intake ratios and amounts consumed. This suggests that macronutrient balancing is key driver for longer term food selection and intake in the domestic cat.

Whilst successful in examining the target macronutrients by cats, the studies to date have only been carried out using traditional commercial dry (extruded or kibbled) diets which often have high carbohydrate inclusion levels or wet (retorted or canned) diets or a combination of these two. With the range of pet food products continuing to expand, it would be beneficial to look at the macronutrient selection by cats on more emerging "natural" diet formats which are developed to reflect the raw meat materials and have minimal processing. Such formats include; air-dried and freeze-dried. This could provide insight as to whether macronutrient selection is comparable between the studies already carried out, or whether intake selection using these newer formats more closely reflects that of free-roaming cats. Another limitation of the studies to date is that when assessing palatability, the diets consisted of flavours and palatants that were added to the diets rather than the diets themselves being formulated with ingredients of high and low palatability.

Chapter 3 showed differences in intake between air-dried beef and lamb by-products. In Chapter 4 the within species age of animal difference affected palatability of by-products. Therefore, it is of interest to understand whether macronutrient selection differs between high and low palatability diets formulated with highly palatable and less palatable meat by-products. Therefore, the aims of this study were to; 1) determine macronutrient selection in the cat fed a 'natural' diet and 2) examine the effect of high and low palatability diets on macronutrient selection. It is hypothesised that cats will consume more of the high palatability diet compared to the low, but that macronutrient selection will be similar across the two treatments with cats selecting a macronutrient energy composition close to 50% ME from protein, which has consistently been reported.

Cats will be provided with carbohydrate targets seen in both the free-roaming cat study by Plantinga et al. (2011) with considerably lower carbohydrate levels as reflected in their prey at 2% ME, as well as a higher level of carbohydrate as seen in the study by Hewson-Hughes et al. (2011) of 12% ME and traditionally included in commercially available pet food of up to 40% ME to give cats a wider macronutrient selection range than the studies in the literature. The air-dried diet format should allow me to reduce the carbohydrate content of the high protein and high fat diets so minimal amounts are included, unlike in traditional extruded kibble where carbohydrates can make up to 40% of the total metabolisable energy content. This will allow greater accuracy in determining whether the previously defined macronutrient intake targets for cats would remain if the carbohydrate content in dry foods was reduced.

5.2 Materials and Methods

Ethics approval was obtained from the Massey University Animal Ethics Committee (Protocol AEC 21/76) to carry out the study.

5.2.1 Test Animals

Eight domestic short-haired cats, consisting of four neutered males, three entire females and one spayed female, were used throughout this study. All cats were deemed healthy in terms of weight (3884.9 ± 338.2.3 g) and with body condition scores of 5.3 ± 0.4 prior to commencing the study. The mean age of the cats used in the study was 5.8 years ± 0.8. Cats were housed in two sets (Panel A and B) of four cats in their group pen during the pre-, post-, and between-data collection periods of the study. During the data collection periods (two experimental phases of 14 days), the cats were housed in individual cages as pictured in Figure 5.1. Water was provided *ad libitum* to all cats during the study.

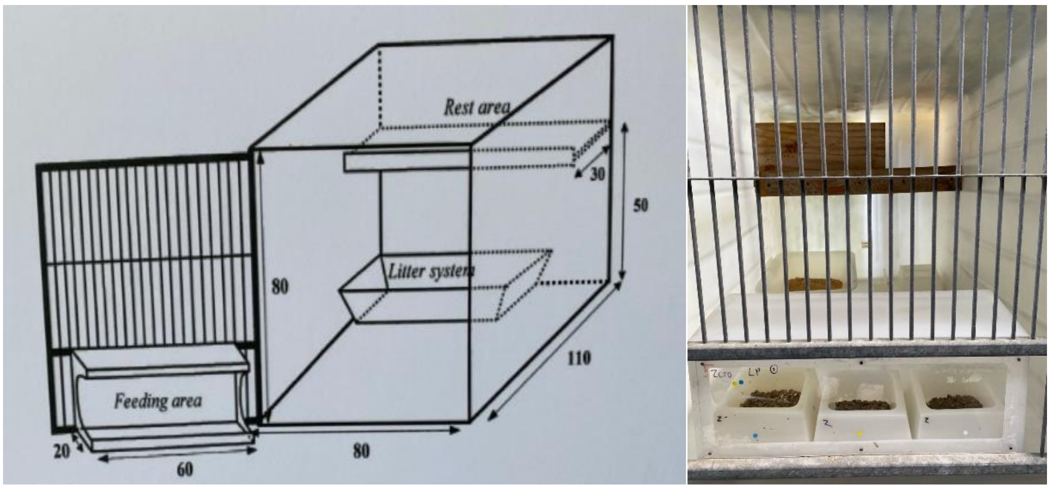

Figure 5.1. Left: A schematic diagram of the full dimensions (cm) of a single cage used in the geometric nutrition study (Hendriks et al., 1999) Right: complete set up of a single cage for the data collection period

5.2.2 Test Diets

The full diet formulation is provided in Table 5.1.Six air-dried diets in total - a high protein (HP), high fat (HF), and high carbohydrate (HC) of low and high palatability - were formulated to meet AAFCO Cat Food Nutrient Profiles for adult maintenance

(AAFCO, 2020), as outlined in Table 5.2. All diets within the low palatability and high palatability diets consisted of the same ingredients included at different levels. Namely lamb skirt, a leaner cut of meat that was readily sourced by the sponsoring company as a source of protein, lamb MDM, although lowest in palatability, it was used as a source of fat to the diets, maize for carbohydrate, vitamin and mineral premixes, and lecithin. In the low palatability diets, sheep heart was used as the ingredient of low palatability and lamb kidney replacing sheep heart in the high palatability diets with all other ingredients remaining the same.

Table 5.1. Percentage contribution (wet weight) of each ingredient to the six formulations of the test diets fed to the cats (n=8) at 600% maintenance energy requirements for 14 days.

	High Palatability			Low Palatability		
Ingredient (%)	Protein diet	Fat Diet	Carbohydrate diet	Protein diet	Fat Diet	Carbohydrate diet
Lamb MDM	15.0	67.5	12.3	1.6	34.8	1.6
Lamb skirt	14.0	1.7	16.2	28.4	1.7	26.4
Lamb kidney[1]	66.0	25.8	41.5	-	-	-
Sheep heart[2]	-	-	-	65.0	58.5	43.0
Maize	1.0	1.0	26.0	1.0	1.0	25.0
Premix	2.2	2.2	2.2	2.2	2.2	2.2
Lecithin	1.8	1.8	1.8	1.8	1.8	1.8

[1]Indicates the use of lamb kidney as the ingredient of high palatability in the series of diets
[2]Indicates the use of sheep heart as the ingredient of low palatability in the series of diets

Table 5.2. Formulated (predicted) target macronutrient composition of the high and low palatability diets

Nutrient % (DM basis)	High Palatability HP	High Palatability HF	High Palatability HC	Low Palatability HP	Low Palatability HF	Low Palatability HC
Protein	55.9	36.3	32.3	52.4	38.9	30.9
Fat	26.7	50.2	15.3	36.7	50.9	20.5
Ash (estimated)	3.5	3.5	8.9	3.1	4.3	9.0
Carbohydrate	12.0*	8.8*	42.0	6.9*	4.1*	37.1
Crude fibre (estimated)	0.9	1.2	1.5	0.9	1.8	2.5
ME (kcal/kg) †	4646	5846	3901	5195	5832	4123
Predicted ME% (P:F:C)	42:49:9	22:73:5	29:33:38	35:60:5	23:74:2	26:42:31

Note DM; dry matter, ME: Metabolisable energy. *Carbohydrate calculated by difference. †Calculated from modified Atwater factors (National Research Council, 2006), HP; high protein, HF; high fat and HC; high carbohydrate, (P:F:C); protein:fat:carbohydrate ratio

As part of typical husbandry procedures for the Massey University Feline Nutrition Unit the cats were maintained on a commercial wet diet, but just prior before the data collection period (day -5), cats adapted to the new diets over a 5-day period in their group pen. This involved presenting increasing amounts of an equal mixture HP, HF and HC diets to the cats. The cats in panel A were adjusted to the high palatability series of diets, and the cats in panel B adjusted to the low palatability series for phase 1 of the study. The process of dietary adaptation consisted of offering 20% air-dried and 80% wet food on day -5 and gradually increasing the proportion of air-dried diet until 100% air-dried and no wet food was presented on day -1. At this point, the cats were deemed to have fully transitioned onto the diets (day 0). At the end of experimental phase 1 of the study the cats were transitioned back onto the same commercial wet diet which was fed for two weeks until the panels were crossed over and adapted to the opposite mixtures of HP, HF and HC diets in preparation for experimental phase 2 of the study.

Nutritional analysis of diets

Diets were analysed for moisture, nitrogen, fat, ash, crude fibre, calcium, phosphorus and hydroxyproline in triplicate using the Association of Official Analytical Chemist (AOAC) methods described; moisture (method 950.46B), nitrogen (Dumas method 968.06), fat (Mojonnier, Acid, 954.02), ash (furnace 550°C, 920.153, 923.03), crude fibre (962.09/978.10 (modified)), calcium (968.08D preparation followed by colourimetric analysis), phosphorus (968.08D preparation, ISO6491.1998E Modified (In-house method) and hydroxyproline (HCl hydrolysis followed by RP HPLC separation using AccQ Tag derivatization, 994.12).

Crude protein content was calculated by multiplying nitrogen by a correction factor of 5.6 instead of the traditional 6.25 nitrogen-to-protein conversion factor. The 6.25 conversion factor assumes the nitrogen content of proteins to be 16% (Mariotti et al., 2008). However, the value 5.6 is given for food sources of meat, fish and eggs as they contain less non-alpha amino nitrogen and is therefore considered more accurate to use for the purpose of this work as the diets had a high meat content (Mariotti et al., 2008).

5.2.3 Experimental Protocol

The cats were weighed at the start (day 1), middle (day 7), and end (day 14) of each phase of the study.

To assess the self-selected macronutrient intake, three bowls each containing 200% (600% in total) of the daily energy requirements of the HP, HF, and HC diets were simultaneously presented to each cat for two 14-day data collection periods, as shown in Figure 5.2. The positions of each bowl were interchanged each day of feeding to prevent positional bias. Diets were offered to each cat from 10am until 8am the following morning, after which, a fresh ration of each diet was presented for the next day of testing.

Figure 5.2. Presentation of the low palatability diets (left) and high palatability diets (right). The high protein diet, high fat diet and high carbohydrate diet are shown from left to right within each picture.

5.2.4 Calculations

Intake of each diet (in grams) was calculated by subtracting the total weight of each diet provided to each cat from that remaining after each daily feeding period. Percentage consumption of each diet was calculated by dividing the intake of each diet by the total intake and multiplying it by 100.

The metabolisable energy (ME) provided by each macronutrient in each diet was determined by multiplying the total protein, fat, and carbohydrate content (expressed as a percentage) by the respective modified Atwater factors; protein and carbohydrate 3.5 kcal/g, fat 8.5 kcal/g and multiplying these values by 10 to get kcal/kg. The summation of the values from protein, fat and carbohydrate provided the total metabolisable energy content of the diet (National Research Council, 2006).

The total metabolisable energy (ME) consumed by each cat was determined as follows:

$$\text{Total ME (kcal/kg) per day} = \left(\frac{g\ of\ HP\ diet}{1000} \times ME\ HP\right) + \left(\frac{g\ of\ HF\ diet}{1000} \times ME\ HF\right) + \left(\frac{g\ of\ HC\ diet}{1000} \times ME\ HC\right)$$

Additionally, the macronutrient metabolisable energy ratios consumed by each cat was determined as the overall percentage energy contribution that each macronutrient made to each diet as follows for each macronutrient:

Protein:

$$\text{Protein ME (kcal/kg) per day} = \left(\frac{g\ of\ HP\ diet}{1000} \times Protein\ ME\ from\ HP\right) + \left(\frac{g\ of\ HF\ diet}{1000} \times Protein\ ME\ from\ HF\right) + \left(\frac{g\ of\ HC\ diet}{1000} \times Protein\ ME\ from\ HC\right)$$

$$\text{ME from Protein (\%)} = \left(\frac{\text{Protein ME}}{Total\ ME}\right) \times 100$$

Fat:

$$\text{Fat ME (kcal/kg) per day} = \left(\frac{g\ of\ HP\ diet}{1000} \times Fat\ ME\ from\ HP\right) + \left(\frac{g\ of\ HF\ diet}{1000} \times Fat\ ME\ from\ HF\right) + \left(\frac{g\ of\ HC\ diet}{1000} \times Fat\ ME\ from\ HC\right)$$

$$\text{ME from fat (\%)} = \left(\frac{\text{Fat ME}}{Total\ ME}\right) \times 100$$

Carbohydrate:

$$\text{Carbohydrate ME (kcal/kg) per day} = \left(\frac{g\ of\ HP\ diet}{1000} \times Carbohydrate\ ME\ from\ HP\right) + \left(\frac{g\ of\ HF\ diet}{1000} \times Carbohydrate\ ME\ from\ HF\right) + \left(\frac{g\ of\ HC\ diet}{1000} \times Carbohydrate\ ME\ from\ HC\right)$$

$$\text{ME from Carbohydrate (\%)} = \left(\frac{\text{Carbohydrate ME}}{Total\ ME}\right) \times 100$$

5.2.5 Statistical Analysis

The level of significance was set at $p < 0.05$ for all analyses in this chapter.

Effect of phase, day, and palatability on intake

A general linear model (GLM) was used to determine the effects of phase of the trial, day within phase, and palatability level on the total intake, as well as the intake of the HP, HF, and HC diets throughout this study using Minitab 19, as shown in Table 5.3.

Table 5.3. The p values obtained from the GLM examining the effects of phase, day, and palatability on the intake of the protein, fat, carbohydrate, and overall diets in study.

Variables	P values (α = 0.05)			
	HP	HF	HC	Total
Phase (block)	0.264	0.084	0.491	0.167
Day	0.361	0.759	<0.001	<0.001
Palatability (treatment)	0.827	0.585	0.001	0.002

No differences were observed between the intakes between the two phases (i.e., there was no block effect) of the study ($p > 0.05$). Therefore, the data from the two phases were combined and results in this chapter were examined in terms of high and low palatability treatments.

Palatability effect on intake and diet preference

Paired t-tests were used to determine whether intake differences were observed between the total intake, as well as the intake of HP, HF, and HC diets between cats on the high and low palatability diet series.

Sigmaplot 14 was used to generate ternary plots of diet intake, on a percentage basis, within the high palatability and low palatability treatments to examine selection and preference between the HP, HF and HC diets.

Effect of day of testing on intake

A general linear model using cat as a random variable, day as fixed variables and weight as a covariate was run to determine their effect on HP, HF, HC, and total intake using Minitab 19.

Energy Intake

Quadratic regression, as used by Roberts et al. (2018) for the macronutrient intake of dogs in a similar geometric framework study involving limited ingredient diets, was carried out in SigmaPlot 14 and used to predict energy intake by cats in the high and low palatability treatments.

Macronutrient metabolisable energy selection

The macronutrient energy selection by the cats, as determined from the calculations in section 5.2.5 were presented in ternary plots in SigmaPlot 14 for the high palatability and low palatability treatments on a DM basis, relative to the ME contribution provided by the HP, HF and HC diets within the low and high palatability treatments.

5.3 Results

5.3.1 Body weight and Body Condition Score (BCS)

The body weight of the cats increased from 3957.0 ± 312.4 g on day 1 to 4465.5 ± 321.0 g on day 14 ($p < 0.001$) within the high palatability treatment, an average weight gain of 508.5 g. Body condition score also increased from 5.9 ± 0.3 to 6.8 ± 0.4 ($p < 0.001$).

For the low palatability treatment, body weight increased from 3932.8 ± 335.5 g on day 1 to 4454.3 ± 345.9 g on day 14 ($p < 0.001$), an average weight gain of 521.5 g. Body condition score also increased from 5.9 ± 0.3 to 7.0 ± 0.3 ($p < 0.001$).

Table 5.4. Weight and body condition score (BCS) ± SEM of the cats at the start, middle and end of the study (presented on a palatability basis)

		Weight (g)				Body condition score (BCS)			
Palatability (Pal)		Day 1	Day 7	Day 14	p value	Day 1	Day 7	Day 14	p value
High	Mean	3957.0[a]	4298.9[b]	4465.5[c]	<0.001	5.9[a]	6.5[b]	6.8[b]	<0.001
	SEM	312.4	319.8	321.0		0.3	0.4	0.4	
Low	Mean	3932.8[a]	4278.4[b]	4454.3[c]	<0.001	5.9[a]	6.6[b]	7.0[b]	<0.001
	SEM	335.5	343.4	345.9		0.3	0.3	0.3	

	p value	0.956	0.965	0.981	1.0	0.763	0.563

*Superscripts used to compare within treatment to examine the day effects in weight and BCS, and between

Body weight and body condition score showed no difference between the two treatments on days 1, 7 and 14 ($p > 0.05$).

5.3.2 Predicted versus actual diet composition

Macronutrient compositions were analysed in triplicate across the entire study, and the average is presented in Table 5.5, with a comparison to the predicted values generated by a diet formulator (Table 5.2). The diets varied considerably from what was predicted given the ingredient inclusion levels. Furthermore, both the high and low palatability HC diets did not meet the minimum protein requirement of 26% DM (AAFCO, 2022).

Table 5.5. Measured (actual) macronutrient composition of the high and low palatability diets

Nutrient %	High Palatability			Low Palatability		
(DM basis)	HP	HF	HC	HP	HF	HC
Protein	34.8	31.2	24.7	28.1	27.9	21.9
Fat	41.9	47.8	37.3	44.9	46.2	32.9
Ash	6.7	8.6	6.3	5.6	5.9	4.2
Carbohydrate*	15.8	11.8	30.5	20.2	18.9	39.5
Crude fibre	0.9	0.6	1.2	1.2	1.2	1.5
ME (kcal/kg) †	5333	5571	5100	5508	5561	4950
Measured ME% (P:F:C)	23:67:10	20:73:7	17:62:21	18:69:13	18:71:12	16:57:28

Note DM; dry matter, ME: Metabolisable energy. *Carbohydrate calculated by difference. †Calculated from modified Atwater factors (National Research Council, 2006), HP; high protein, HF; high fat and HC; high carbohydrate, (P:F:C); protein:fat:carbohydrate ratio

5.3.3 Predicted versus actual ME composition

With a focus on ME in this Chapter, comparisons of the predicted versus the measured protein, fat, and carbohydrate ME percentages, are presented in Table 5.6. The differences from the predicted macronutrient contents are also summarised in Table 5.6.

Table 5.6. Predicted versus measured protein, fat and carbohydrate ME percentages on a DM basis

Pal	Diet	Predicted ME content (%)			Measured ME content (%)			Difference		
		Protein	Fat	CHO	Protein	Fat	CHO	Protein	Fat	CHO
High	HP	42	49	9	23	67	10	-19	18	1
	HF	22	73	5	20	73	7	-2	0	2
	HC	29	33	38	17	62	21	-12	29	-17
Low	HP	35	60	5	18	69	13	-17	9	8
	HF	23	74	2	18	71	12	-5	-3	10
	HC	26	42	31	16	57	28	-10	15	-3

Within the high and low palatability treatments, the protein ME content was consistently less than the predicted protein content. The formulations predicted a greater range of protein ME contents from 22% to 42%, however, the measured protein ME content range was less extensive, ranging from 16% to 23%. Within the high palatability diets, the HP diet did show the highest ME percentage of protein (23%) compared to the HF and HC diet (20% and 17%, respectively). However, in the low palatability diets, the HP and HF diets showed similar protein ME contents (18%) compared to 16% in the HC. As observed in Table 5.6, the protein ME contents in the high and low palatability HP diets were underestimated by 19% and 17% respectively, which restricted the maximum levels of protein that could be selected by the cats.

The fat ME content was generally higher than the predicted fat contents across most of the diets. The exceptions were the high palatability and low palatability HF diets, which showed equal values to the predicted fat ME for the high palatbility HF diet and slightly lower levels (-3%) for the low palatability HF diet. Rather than offering fat levels that ranged from 33% to 74% ME, as estimated in the predicted fat content, the measured fat ME content was relatively consistent and high among all diets, and only ranged from 57% to 73%. Within the high palatability diets, the HF diet did show the highest percentage ME of fat (73%) compared to 67% in the HP and 69% in the HC diet. In the low palatability diets, the HF diet also contained the highest percentage ME of fat 71%, compared to 69% and 57% in the HP and HC diets, respectively.

Finally, the measured carbohydrate ME contents of all diets were generally higher than the predicted values, apart from the high palatability HC diet which showed a lower measured ME value (21%) than was predicted (38%). The predicted dietary range of carbohydrate ME content was 2% to 38%, however an actual range of 7% to 28% was achieved. Within the high and low palatability HC diets, the carbohydrate ME contents were 21% in the high palatability diet and 28% in the low palatability carbohydrate diet which were both higher than the HP and HF diets within each series.

5.3.4 Palatability effect on diet intake

Paired t-tests, presented in Table 5.7, were conducted to determine whether differences in intake between the high palatability and low palatability diets were observed.

Table 5.7. Mean intake and p value of the HP, HF, HC, and overall diets of high and low palatability

	Diet intake (g)			
	HP	HF	HC	Total
High Palatability	51.75	10.54	30.69	92.97
Low Palatability	51.21	11.36	21.98	84.54
p value	0.813	0.473	0.001	0.005

The cats showed no difference between the intake of the HP and HF diets between the high and low palatability treatments ($p = 0.813$ and $p = 0.473$, respectively). However, the cats did show higher intake of the high palatability HC diet compared to the low palatability HC diet ($p = 0.001$), this also resulted in a higher total intake of high palatability diets over low ($p = 0.005$).

Although higher intakes were observed for the high palatability HC diet over the low palatability HC diet, when comparing the macronutrients from Table 5.6, the high palatability HC contained 17% ME from protein, 62% ME from fat and 21% ME from carbohydrate compared to the low palatability HC which contained 16% ME from protein, 57% ME from fat and 28% ME from carbohydrate. It is possible that the higher ME level of protein and fat, and the lower carbohydrate content in the high palatability HC may be what is driving greater intake (macronutrient driver), as opposed to the cats preferring the inclusion of kidney in the high palatability HC diet over sheep heart in the low palatability diet (ingredient as a palatability driver).

5.3.5 Selection by cats

5.3.5.1. High Palatability

a) Diet Selection

When diet intake was converted to a percentage intake basis for the high palatability series of diets, as shown in Figure 5.3, the cats selected 58% of their intake from the HP diet, followed by 31% from the HC diet and 11% from the HF diet ($p < 0.05$) for the high palatability series, indicating a difference in palatability between diets within the high palatability treatment.

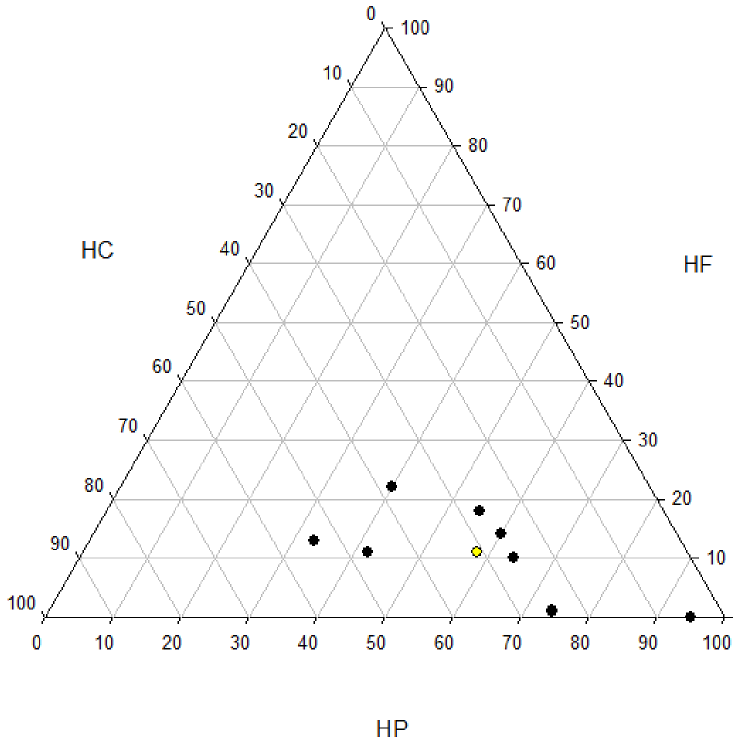

Figure 5.3. Individual diet intake selection by cats for the high palatability series on a percentage basis (black dots). The average selection by the cats denoted by the yellow circle

b) Effect of variables on intake

Cat weight and day did not have a significant impact on the intake within the HP and HF diets ($p > 0.05$). Cat weight also did not have a significant impact on the intake within the HC diet or total intake ($p > 0.05$). However, the day of testing did have an impact on the intake of the HC diet ($p < 0.001$) and total intake ($p < 0.001$) (see Table 5.8).

Table 5.8. Effects of day (fixed), cat (random) and weight as the covariate on diet and total intake in the high palatability treatment

Variable	P values (α = 0.05)			
	HP	HF	HC	Total
Weight	0.115	0.110	0.174	0.093
Day	0.071	0.689	<0.001	<0.001

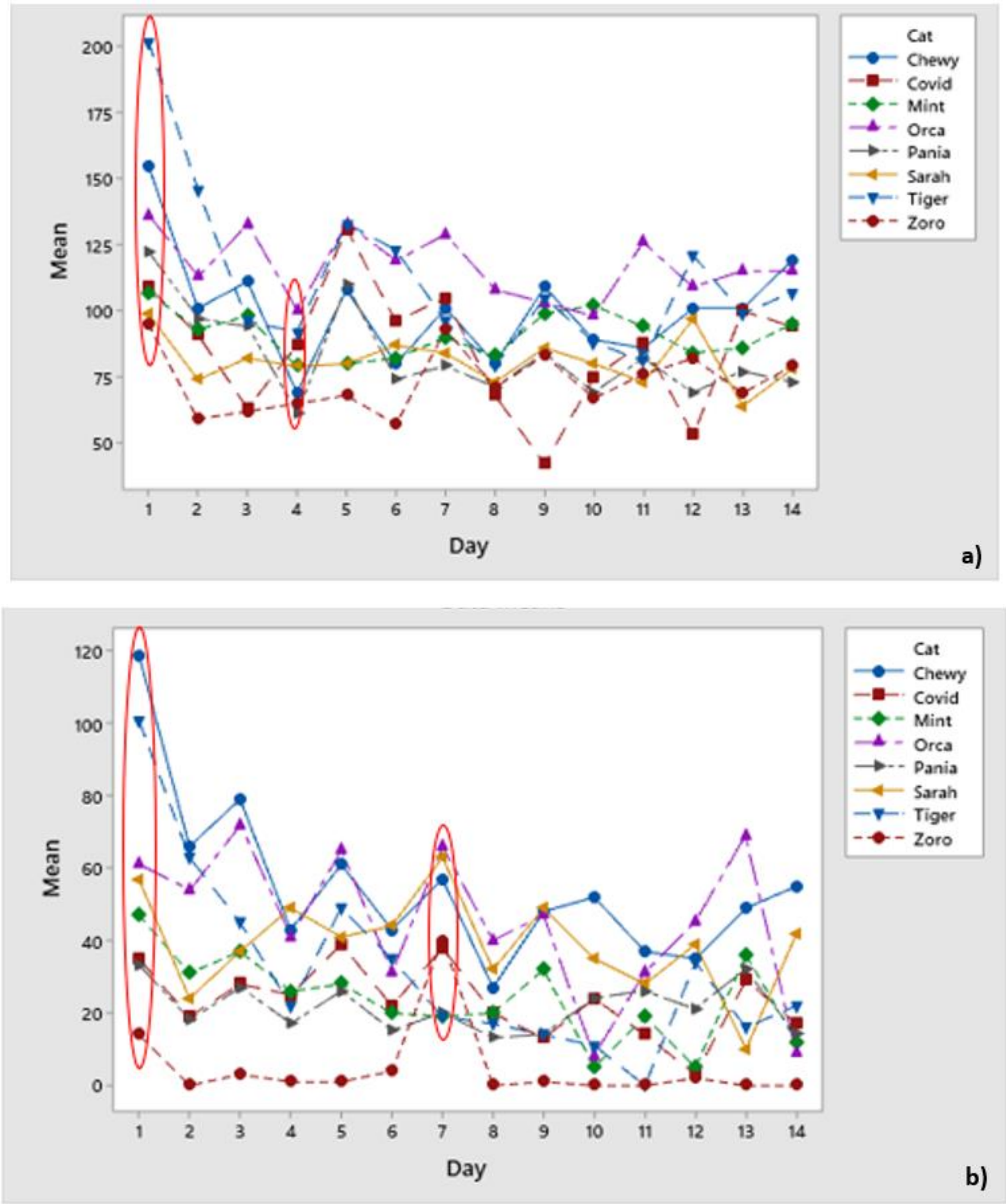

Figure 5.4. a) Total daily intakes of all diets by each cat in the high palatability series b) Intakes of the high palatability HC diet by each cat. Red rings indicate the data that needs to be removed for no day effect to be observed

The day effect on the HC and total intake, appeared to be driven by results from day 1 for both HC and total intake (p values of 0.005 and 0.002 in the model, respectively), as well as day 4 for the total intake ($p = 0.003$), and day 7 for the HC ($p = 0.014$) as circled in Figure 5.4. Removal of the data from these specific days removes the day and weight effect (Table 5.9).

Table 5.9. p values following removal of day 1 and 7 for high palatability HC diet and day 1 and 4 for total intake

Variable	P values ($\alpha = 0.05$)	
	HC	Total
Weight	0.070	0.053
Day	0.143	0.072

c) Energy Intake

The cats energy intake of the high palatability diet was reduced ($p < 0.001$) from 272% of maintenance requirements on day 1 to 201% on day 14 according to the quadratic formula ME% = 255.1 (± 19.3 SEM) – 16.6 (± 5.9 SEM) x day + 0.9 (± 0.4 SEM) x day^2, $R^2 = 0.479$, as pictured in Figure 5.5. The lowest energy intake was observed on day 8 (168%) which was still well above normal maintenance levels, with an average 197% energy consumption of 197% of maintenance for high palatability diet.

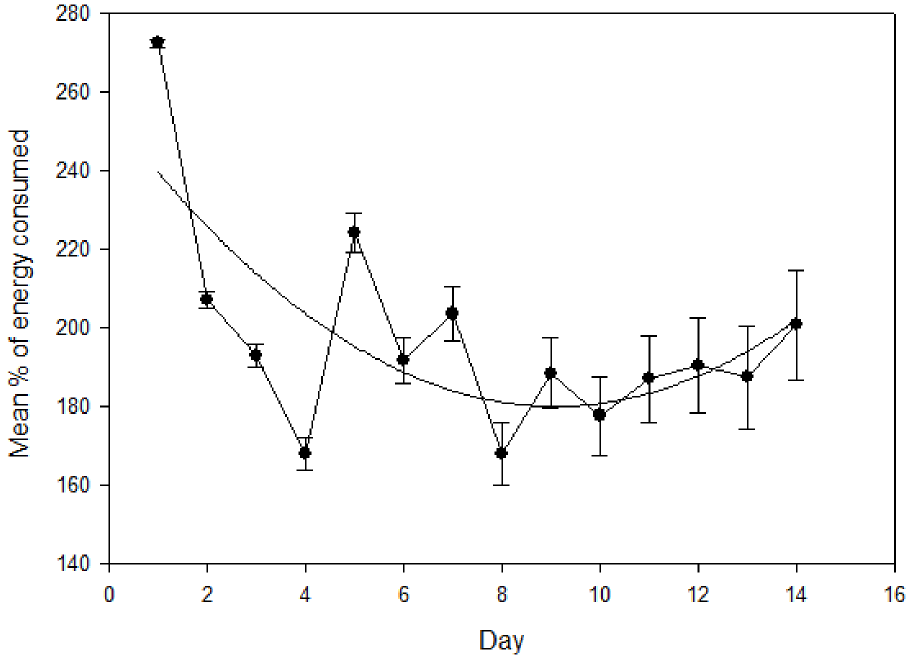

Figure 5.5. The percentage of energy consumed by the cats for the high palatability treatment (n=8) over the 14-days of testing when presented diets with macronutrient energy profiles protein:fat:carbohydrate (PFC) of (23%:67%:10% ME) for HP, (20%:73%:7% ME) for HF and (17%:62%:21% ME) for HC each offered at 200% maintenance energy requirements (600% maintenance energy in total).

d) Macronutrient Selection on a Metabolisable Energy Basis

For the high palatability treatment, the cats consumed on average 491 kcal/day, this encompassed 101 kcal/day of protein (21%), 324 kcal/day of fat (66%) and 66 kcal/day of carbohydrate (13%) on a DM basis, as indicated by the green point in Figure 5.6.

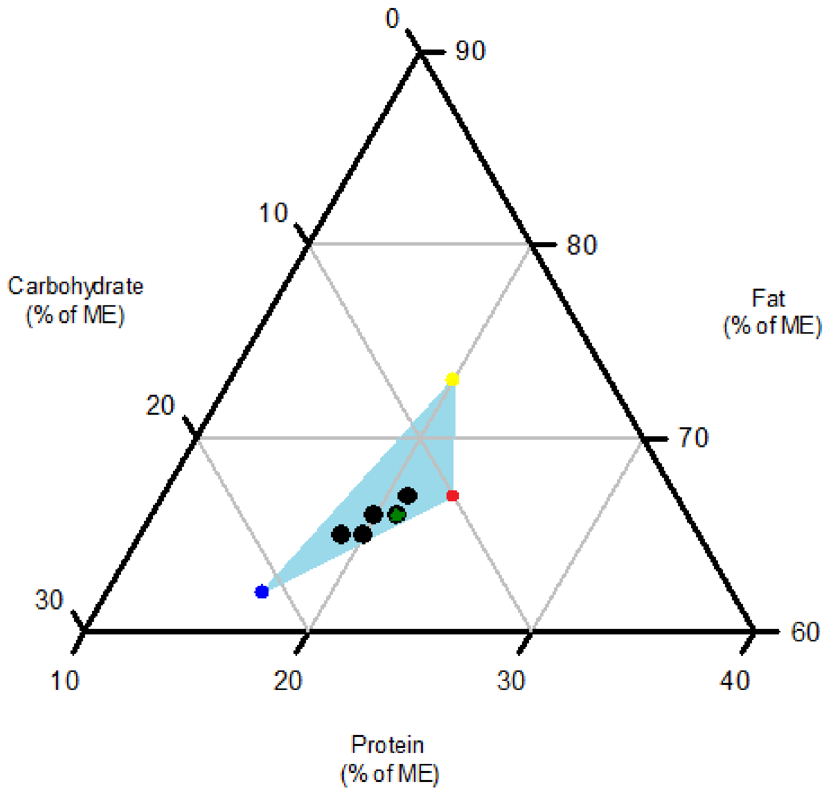

Figure 5.6. Macronutrient metabolisable energy selection by the cats from the overall macronutrient composition of protein, fat, and carbohydrate eaten from each high palatability diet. Yellow dot represents the HF diet (PFC 20%:73%:7% ME), red dot represents the HP diet (PFC 23%:67%:10% ME) and blue dot represents the HC diet (PFC 17%:62%:21% ME). Black dots show individual macronutrient selection on an ME basis. Green star represents the average macronutrient selection by the cats.

When comparing the macronutrient metabolisable energy composition, the 66% ME of fat selected by the cats was greater than the 21% ME selected from protein and 13% ME selected from carbohydrate ($p < 0.001$).

5.3.5.2. Low Palatability

<u>a) Diet Selection</u>

When diet intake was converted to a percentage intake basis for the low palatability series of diets, the cats selected 62% of their intake from the HP diet, followed by 25% from the HC diet and 13% from the HF diet (Figure 5.7; $p < 0.05$).

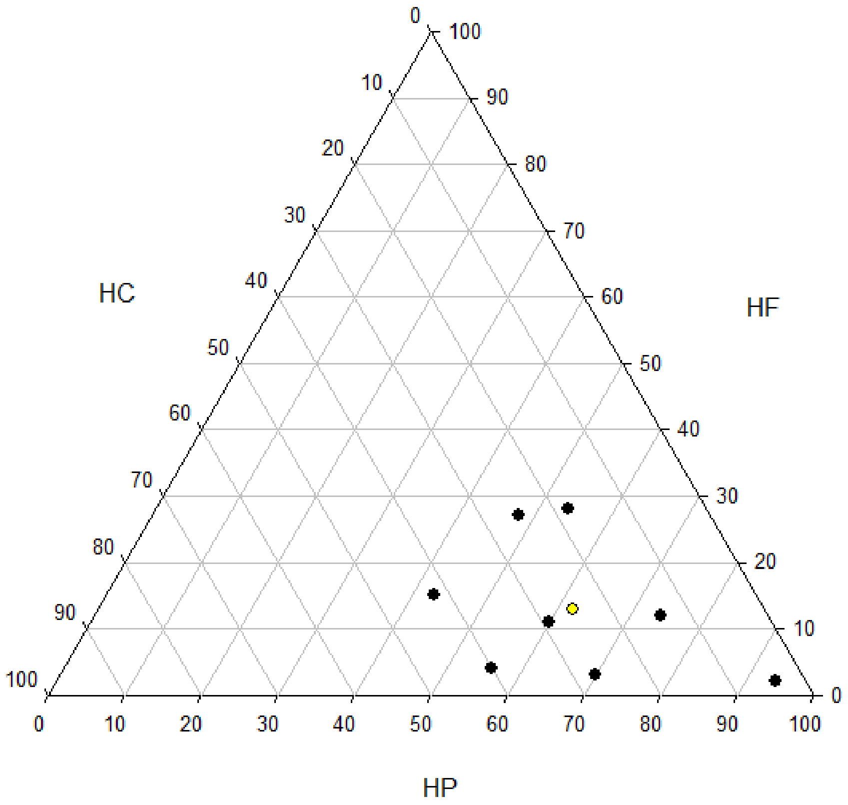

Figure 5.7. Individual diet intake selection by cats for the low palatability series on a percentage basis (black dots). The average selection by the cats denoted by the yellow circle

<u>b) Effect of variables on intake</u>

Cat weight and day did not affect the intake with the HP and HF diets ($p > 0.05$). Weight also did not affect the total intake ($p > 0.05$). However, weight did affect the intake of the HC diet ($p < 0.05$), and the day of testing affected HC and total diet intake ($p < 0.05$) (see Table 5.10).

Table 5.10. p values obtained from the GLM examining the effects of day (fixed), cat (random) and weight as the covariate on diet and total intake in the low palatability treatment

Variable	P values (α = 0.05)			
	HP	HF	HC	Total
Weight	0.121	0.407	0.003	0.824
Day	0.067	0.078	<0.001	<0.001

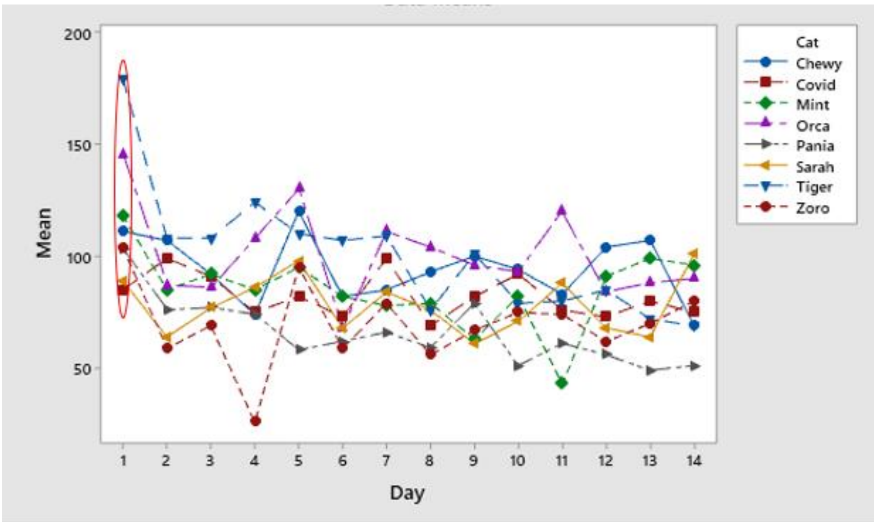

Figure 5.8. Total daily intakes of all diets in the low palatability series by each cat. Red ring indicates the data that needs to be removed for no day effect to be observed

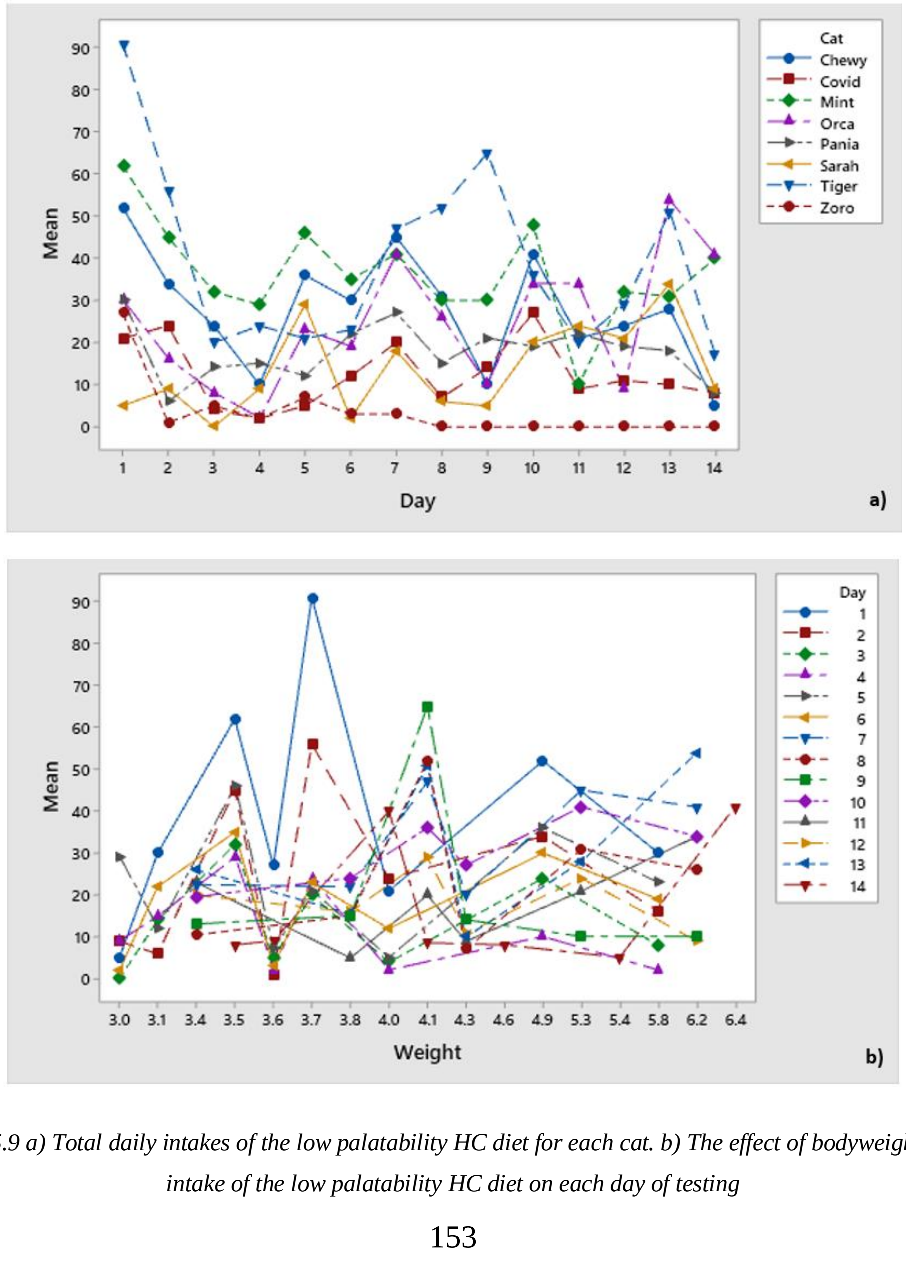

Figure 5.9 a) Total daily intakes of the low palatability HC diet for each cat. b) The effect of bodyweight on the intake of the low palatability HC diet on each day of testing

The total intake of the low palatability diets, appear to be driven by results from day 1 ($p = 0.005$), as circled in Figure 5.8. Removal of the day 1 data removes the day effect (Table 5.11).

The effect of experimental day on the intake of the low palatability HC diet was not dependent on day 1 results. Days 2, 5, 8, 9, 11 and 12 showed significantly different intakes compared to all other days ($p < 0.05$).

Table 5.11. p values following removal of day 1 results from low palatability HC and total intake

Variables	P values (α = 0.05)	
	HC	Total
Weight	0.001	0.759
Day	0.001	0.190

c) Energy Intake

In terms of the low palatability results, the cats energy intake was lower ($p < 0.001$) on day 14 at 170% of maintenance requirements compared today 1 at 245% of maintenance requirements (ME% = 229.5 (± 15.4 SEM) – 12.6 (± 4.7 SEM) x day + 0.6 (± 0.3 SEM) x day^2, R^2 =0.5709; Figure 5.10). The lowest energy intake was observed on day 8 (160%) which was still well above normal maintenance levels, with an average energy consumption of 178% of maintenance consumed across the treatment days for the low palatability diet.

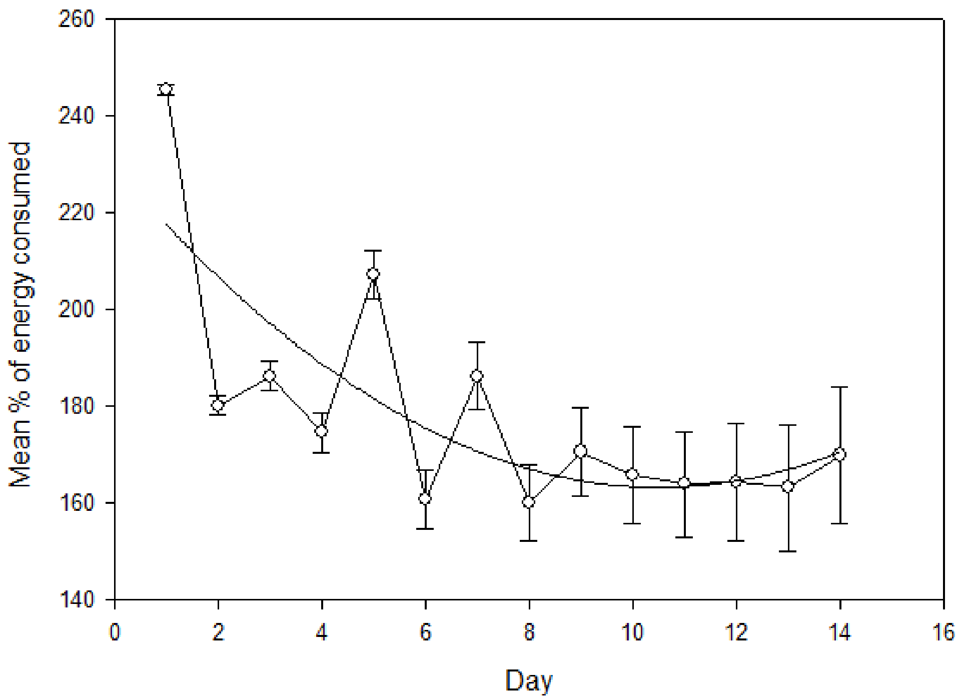

Figure 5.10. The percentage of energy consumed by the cats for the low palatability treatment (n=8) over the 14-days of testing when presented diets with macronutrient energy profiles protein:fat:carbohydrate (PFC) of (18%:69%:13% ME) for HP, (18%:71%:12% ME) for HF and (16%:57%:28% ME) for HC each offered at 200% maintenance energy requirements (600% maintenance energy in total).

d) Macronutrient Selection on a Metabolisable Energy Basis

In the low palatability treatment, the cats consumed on average 454 kcal/day which consisted of 78 kcal/day of protein (17%), 302 kcal/day of fat (67%) and 74 kcal/day of carbohydrate (16%), as indicated by the green point in Figure 5.11. When comparing the macronutrient metabolisable energy composition, the 67% ME of fat selected by the cats was greater than the 17% ME selected from protein and 16% ME selected from carbohydrate ($p < 0.001$).

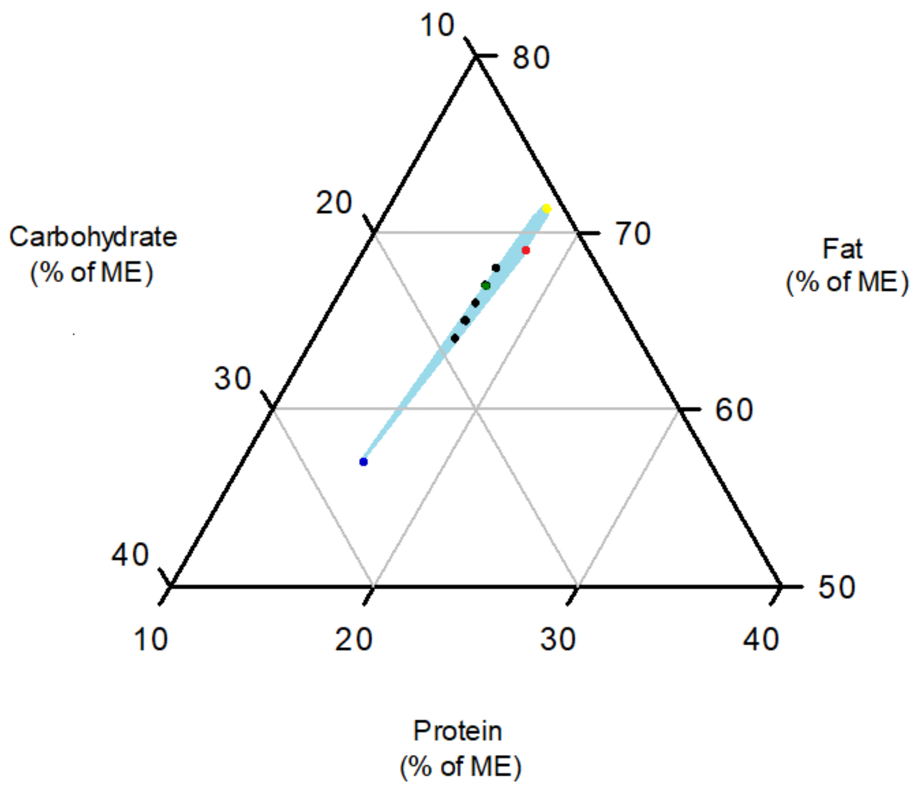

Figure 5.11. Macronutrient metabolisable energy selection by the cats from the overall macronutrient composition of protein, fat, and carbohydrate eaten from each low palatability diet. Yellow dot represents the HF diet (PFC 18%:71%:12% ME), red dot represents the HP diet (PFC 18%:69%:13% ME) and blue dot represents the HC diet (PFC 16%:57%:28% ME). Black dots show individual macronutrient selection on an ME basis. Green star represents the average macronutrient selection by the cats.

5.3.5.3. Comparison of High and Low Palatability Trials

a) Diet Selection

The percentage intake of protein and fat did not differ between high and low palatability diets (58% and 62%, p = 0.127 for protein and 11% and 13%, p = 0.259 for fat). However, the cats did show greater intake of the high palatability carbohydrate over the low palatability carbohydrate diet (31% and 25%, p = 0.010).

b) Effect of variables on intake

When the results from the high and low palatability treatments groups combined, the cat weight did not have a significant impact on the intake of each diet or the total intake (p > 0.05). The day of the experiment did not affect intake of the HP and HF diets (p > 0.05). However, the day effect particularly the higher intake on the first day of the two phases did have a significant impact on HC intake and total intake throughout the entire study (p < 0.05) (see Table 5.12).

Table 5.12. p values obtained from the GLM model examining the effects of day (fixed), cat (random) and weight as the covariate on diet and total intake in the collective results from the high and low palatability treatments

Variable	P values (α = 0.05)			
	Protein	Fat	Carbohydrate	Total
Weight	0.121	0.084	0.421	0.086
Day	0.114	0.238	<0.001	<0.001

The day effect on total intake, appear to be driven by results from day 1 (p value of < 0.001; Figure 5.12). Removal day 1 data removes the day effect on total intake.

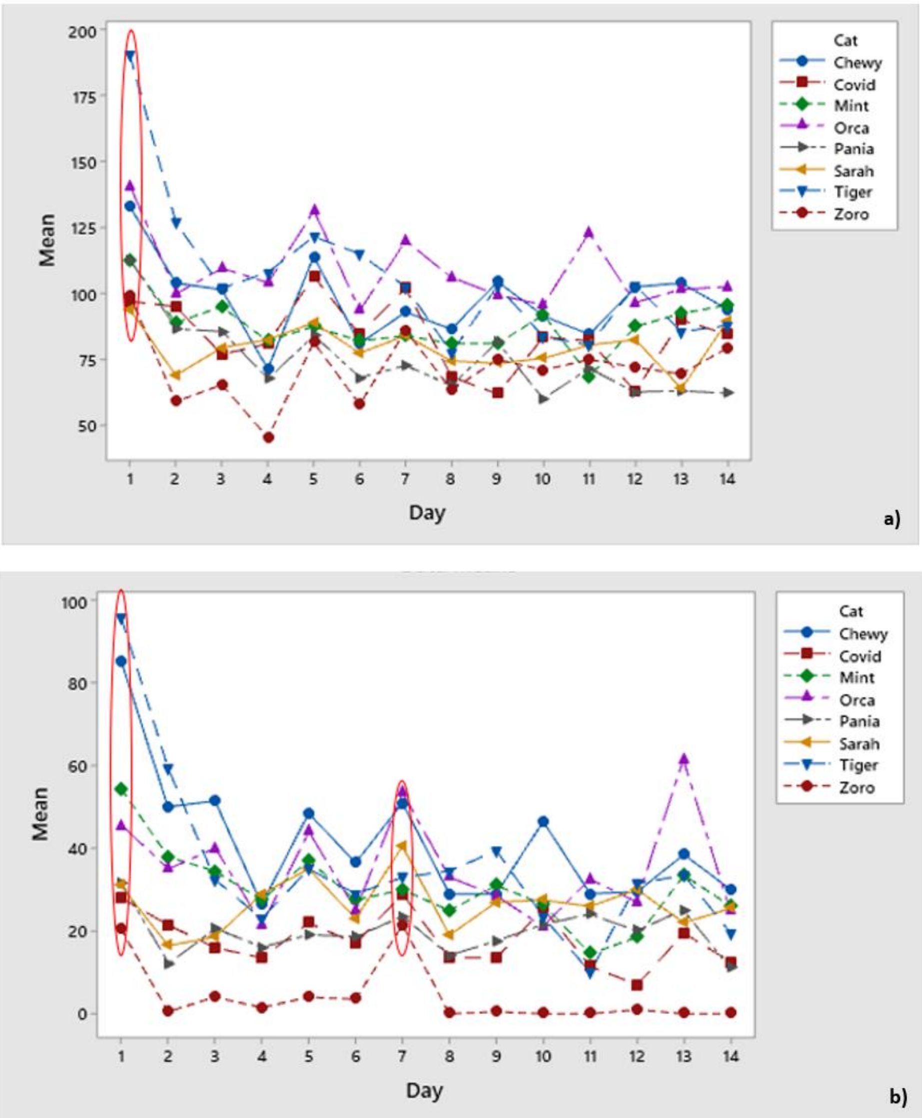

Figure 5.12. Top: Total daily intakes of all diets for each cat throughout the study. Bottom: Total daily intakes of the HC diets for each cat throughout the study. Red rings indicate the data that needs to be removed for no day effect to be observed.

The day effect on intake of HC remained when removing day 1 and 7 data (p = 0.173; Table 5.13) suggesting more than just a novelty effect. In addition to day 1 and 7, days 4, 5, 6 and 8 had a different intake of the HC diet, indicating variable intakes throughout the entire study.

Table 5.13. p values following removal of day 1 and day 7 results from the collective HC and removal of day 1 total intake collective results from the high and low palatability treatments

Variables	P values (α = 0.05)	
	HC	Total
Weight	0.669	0.061
Day	0.173	0.001

c) Energy Intake

For both the high and low palatability diets, cats consumed more than their 100% daily ME requirement, as shown in Figure 5.13. Specifically, the cats consumed an average 197% energy throughout the high palatability treatment which was significantly higher ($p < 0.001$) than the energy they consumed in the low palatability treatment (167%).

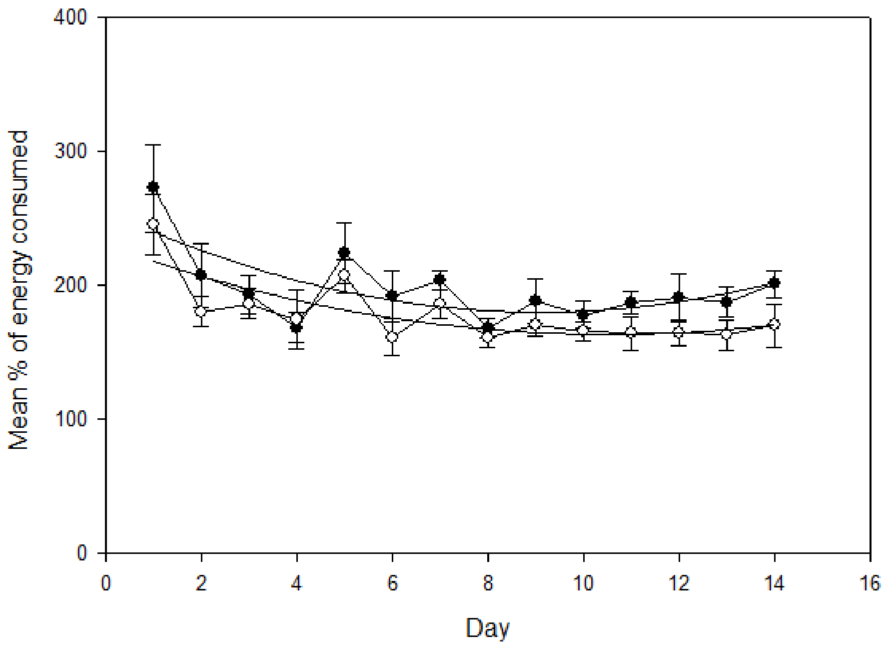

Figure 5.13 Comparison of the percentage energy consumed by the cats (n=8) for the high palatability treatment (black dots) and low palatability treatment (white dots) for over the 14-days of testing when presented with diets at 600% maintenance energy requirements

d) Macronutrient Selection on a Metabolisable Energy Basis

The cats consumed more protein in the high palatability diets than the low palatability diets ($p < 0.001$). Fat intakes were similar ($p = 0.058$) between the two sets of diets. Carbohydrate intake of the low palatability diet was higher than that of the high palatability diet ($p < 0.001$) (see Table 5.14).

Table 5.14. The metabolisable energy contribution from protein, fat, and carbohydrates between the high and low palatability diet treatments and the associated p values

	Metabolisable energy contribution (ME%)		
	Protein	Fat	Carbohydrate
High Palatability	21	66	13
Low Palatability	17	67	16
P value	<0.001	0.058	<0.001

5.4 Discussion

This study aimed to examine the effect of palatability on the macronutrient selection of cats when presented with diets varying in protein, fat, and carbohydrate content. A series of high and low palatable diets in this study were formulated from a limited set of ingredients. The major difference between the two sets of diets was that lamb kidney was used as a major ingredient of high palatability in the high palatability diets and sheep heart as the ingredient of low palatability in the low palatability diets, as determined from the results obtained in Chapter 3. It was hypothesised that the cats would consume more of the high palatability diet compared to the low, which was observed in this study. It was also hypothesised that macronutrient selection will be similar across the two treatments with cats selecting a macronutrient energy composition close to the reported 50% ME from protein, however, this was not the case and differences in macronutrient selection between the two treatments was observed.

Lamb was used as the protein source over beef in this experiment as previous work conducted by Watson et al. (2020) and in Chapter 3 found that cats showed greater general intake of lamb over beef by-products, with differences in intakes between by-products also apparent. With the cats being solely fed the air-dried diets during the two 14-day periods of the study, it was important to ensure diet consumption was sufficient to meet maintenance energy requirements (MER). In addition, formulating the diets using offal from sheep only, allowed me to create diets than were similar nutritionally and differed only in the degree of palatability (based on the previous work in Chapter 3), with no confounding factors due the species used.

Large discrepancies were observed between the predicted ME range of fat (33% to 73%) compared to what was measured (57% to 73%), resulting in higher levels in the HP and HC diets. Consequently, when compared to previous geometric nutrition studies where cats selected >50% ME from protein, ≥36% ME from fat and between 2 to 12% ME from carbohydrate (Hewson-Hughes et al., 2011; Plantinga et al., 2011; Salaun et al., 2016), in the high palatability treatment, this study found that cats selected a ME composition comprised of 21% ME from protein, 66% ME from fat and 13% ME from carbohydrate. In the low palatability treatment, cats selected a ME composition comprised of 17% ME from protein, 67% ME from fat and 16% ME from carbohydrate. When comparing between the two treatments, cats consumed more ME from protein in the high palatability than the low palatability treatment ($p < 0.001$), similar fat ME contributions were observed between the two treatments ($p = 0.058$) and cats consumed more carbohydrate in the low palatability than the high palatability treatment ($p < 0.001$), indicating differences in macronutrient selection when given access to diets varying in palatability.

Unfortunately, the actual macronutrient profiles of the diets differed to what was formulated based predicted protein, fat, and carbohydrate composition. This resulted in a major problem in this experiment as all diets contained high fat levels which was presumably a consequence of one or multiple ingredients having a unexpectedly high fat content, which made it difficult to obtain a high protein diet, which has been seen in the literature as being a main driver of macronutrient selection in cats (Hewson-Hughes et al., 2011; Plantinga et al., 2011; Salaun et al., 2016). This outcome is likely to be driven by the variable protein and fat levels of the lamb by-products used in the diets. It is known that MDM is an extremely variable product itself. For example, the boning method used, hand or mechanically deboning, will result in significantly different calcium contents being present in the final product (Ang and Hamm, 1982). Additionally, it is possible that the formulator data used to generate the diets contains values from online databases and literature values for certain by-products rather than laboratory measured results. Even if full nutritional specifications were analysed for each by-product, there is likely to be variation from batch to batch and between by-products collected during different seasons. Furthermore, the data may not be reflective of current practices where animals are finished on higher energy forages such as chicory resulting in decreased abundance of some myofibrillar proteins in lamb loins compared to those finished to traditional perennial ryegrass (Ye et al., 2022).

At the time of diet manufacture, New Zealand was amid a COVID outbreak, therefore my opportunity to manufacture the diets was severely constrained. This in conjunction with extended laboratory analysis time (for determining macronutrient composition) meant that I needed to start the study before I had my final nutritional analysis data. In an ideal scenario, macronutrient composition of the contrasting diets would be confirmed prior to starting the study, and re-formulation/manufacture undertaken if necessary.

Although the actual macronutrient contents of the diets were not as predicted, palatability was still able to be assessed in this study. Between the high and low palatability treatment, the cats consumed more of the high palatability treatment diets (92.97 g) compared to the low palatability diets (84.54 g; $p = 0.005$). There were no differences in intake of the high and low palatable HP and HF diets ($p = 0.813$ and 0.473, respectively) and overall intake differences were driven by greater consumption of the high palatability HC diet compared to the low palatability HC diet ($p < 0.001$). This was likely due to the diet having a higher ME content of protein and fat, and lower carbohydrate content compared to the low palatability HC diet. This result suggests a macronutrient driver effect, as opposed to the preference for the more palatable by-product (lamb kidney), being included in the high palatability diets over the less palatable by-product (sheep heart) in the low palatability diets. The HP and HC diets within both high and low palatability treatments exceeded the minimum 20 g threshold needed to define the diets as being accepted by the cats (Tarttelin, 1997). However, the HF diets fell below this threshold with average intakes of 10.54 g for the high palatability HF diet and 11.36 g for the low palatability HF diet.

Within the high palatability treatment, cats selected the majority (58%) of their total intake from the HP diet, followed by the HC diet (31%) and HF diet (11%), indicating a strong preference for this diet within the high palatability series. Almost identical results were obtained within the low palatability treatment, with cats again selecting majority of their intake from the HP diet (62%), followed by the HC diet (25%) and the HF (13%), indicating the same preference for diets within the low palatability series. When comparing between treatments, difference was observed ($p < 0.001$) between the HC diet with greater intake of the high palatability HC over the low palatability HC. This was likely due to the HC diet having a higher protein and fat content and lower carbohydrate diet than the low palatability HC diet, indicating a macronutrient effect as the driver for greater intake between these diets.

Within the high palatability treatment, the day effect on HC and total intake appeared to be driven by results from day 1, which indicated that cats displayed the novelty effect, the preference for a new food, in this case a series of air-dried foods (Bourgeois et al., 2006; Péron & Tobie, 2018). Removal of day 1 data from both the HC and total intake, as well as day 4 results from the total intake, due to a significant drop in intake from two cats, and day 7 results in the HC data adjusted the p values to result in no significant day effect. There was also an observed day effect on total intake within the low palatability treatment, with cats showing a novelty effect on day 1. Removal of day 1 total intake data adjusted the p values to result in no significant day effect. There was also an observed day and weight (covariate) effect on HC intake within the low palatability treatment. However, the original model indicated variable intakes of the HC diet throughout the low palatability treatment on multiple days and not solely driven by a novelty effect on day 1.

Within the high palatability treatment diets, cats consumed on average almost twice (197%) their MER. Similar findings were also observed within the low palatability treatment, with cats consuming slightly lower amounts on average (178% of MER). When comparing between the high and low palatability treatments, it is evident to see why substantial weight gains were observed as both sets of diets were highly palatable.

The data from this study supported the carbohydrate ceiling effect observed in previous feline studies. In terms of the high palatability treatment, cats consumed 66 kcal/day of carbohydrate which falls below the 75 kcal/day, or approximately 30% of maintenance energy requirements defined as the carbohydrate ceiling for cats (Farrow et al., 2013; Hewson Hughes et al., 2011; Hewson Hughes et al., 2013a; Verbrugghe & Hesta, 2017). For the low palatability treatment, cats consumed 74 kcal/day of carbohydrate, falling just below the ceiling value. These results suggest that the shortfall in protein, particularly in the low palatability treatments due to high fat across all diets, prompted the cats to seek nutrients elsewhere (Hewson Hughes et al., 2011). In this case, the cats shifted towards a higher intake of carbohydrate in the low palatability treatment.

The bodyweights of the cats increased significantly ($p < 0.001$) on both sets of diets during the feeding periods. In terms of BCS, cats were assessed at the start of the feeding periods and had an average BCS of 5.9 ± 0.3 in both the high and low palatability treatments on day 1. Based on the scoring system provided by the World Small Animal Veterinary Association (2013), a score of 5.9 indicated that cats were viewed as "over-ideal" prior to the feeding studies, with a score of 5 on the nine-point scale accepted as the "ideal". Body condition score then increased to 6.8 ± 0.4 within the high palatability treatment and 7.0 ± 0.3 within the low palatability treatment by day 14. Both of which were significantly different to their average starting BCS ($p < 0.001$). These results indicated that both sets of diets were very palatable and substantial weight gain was observed when cats were given *ad libitum* (600% MER) access to diets varying in protein, fat, and carbohydrate content and this also resulted in the subsequent increase in BCS.

While this experiment consisted of two short-term studies, it is important to note that being male, neutering in either sex, being middle age (over two years) and certain dietary factors, such as eating premium or therapeutic diets, are common risk factors of relevance to colony cats of being overweight, along with apartment dwelling, inactivity mixing of breeds, living in a house with one or two cats in common household cats (Colliard et al., 2009; Lund et al., 2005; Robertson, 1999; Scarlett et al., 1994). If a long-term follow up study were to take place, care must be taken to monitor weight gain and changes in BCS at regular intervals as this short-term study resulted in an average 500 g increase in weight within each treatment after two-weeks. However, it would be interesting to observe whether intakes declined with increasing exposure to the diets or if a less drastic change in BCS would have occurred had there been opportunity for the cats to select a high protein diet with lower fat content. There is evidence suggesting that high-protein diets may be able to promote increased lean tissue mass in cats, specifically adult neutered cats, that may be helpful in preventing or treating obesity (Nguyen et al., 2004).

This significant increase in body weight is likely the result of the finalised diets being much higher in fat and lower in protein, both on a DM and ME basis than initially predicted. In addition, the separation technique used to prepare MDM can result in variable connective tissue and collagen content of the MDM which has an impact on final product quality (Miller, 2008). Lamb skirt (for all diets), lamb kidney in the high palatability and sheep heart for the low palatability diets, were also used as dietary protein sources. Data from Chapter 4 indicated that lamb kidney contributed a theoretical percentage of 77.3% protein and 11.8% fat on a DM basis to the high palatability diets, whereas mutton heart contributed 49.9% protein and 46.7% fat on a DM basis to the low palatability diets. Lamb skirt was not a by-product examined in previous chapters, so I relied on the recorded product specification data provided by the company to generate the diet formulation. It is possible that the inclusion of lamb skirt contributed to the considerably high fat content across all diets. As such, analysis of the ingredient itself and comparison to the values included in the diet formulator model may be required to ensure closer estimates of predicted versus measured nutritional components, if looking to further work using limited high meat ingredient diets for geometric studies. However, meat by-products are known to show variation among different batches, which could explain why macronutrient selection of limited high meat content pet foods have not been examined in cats since this work.

5.5 Conclusion

This study clearly showed an effect of palatability on the intake and macronutrient selection by cats. Whilst the predicted formulations were considerably different to the measured diets in terms of DM content and ME content, differences in palatability were still observed between treatments with cats consuming a greater total intake of the high palatability diets compared to the low palatability diets, which was driven by greater intake of the high palatability HC diet. Along with this, a clear novelty effect was also displayed on day 1 of testing within each treatment group even after a two-week wash out period between treatments. In terms of energy consumed, cats exceeded their 100% MER within both treatments by a large margin, which was reflected in the significant weight gain and increase in BCS during each feeding phase. In terms of macronutrient selection, cats selected most of their ME from fat, followed by protein and then carbohydrate, a result which was not consistent with the literature due to the considerably higher fat contents in the diets than was predicted during formulation. This was clearly due to the high variability in macronutrient content of the by-product ingredients. Subtle differences in macronutrient selection on an ME basis were observed between the high and low palatability treatment diets. Specifically, more protein being consumed within the high palatability treatment, similar fat consumption between the two treatments, and greater carbohydrate consumption within the low palatability treatment. When related back to the literature, cats appeared to maximise their carbohydrate intake in the low palatability treatment compared to the high palatability treatment. This indicated that when cats were provided a diet formulated with high palatability ingredients, they will more actively seek a greater amount of protein. However, when diets were formulated with low palatability ingredients, the cats shifted towards a higher intake of carbohydrate in the low palatability treatment due to the shortfall in protein.

Whilst providing insight into the palatability of high meat based air-dried diets, the findings of this study were limited by having relatively high fat contents across all diets. Future work should involve careful selection of highly and poorly palatable meat ingredients and inclusion levels that would allow greater macronutrient targets to be achieved, whilst still meeting the defined protein and fat minimums required in commercial cat food.

General Discussion

Palatability remains an important criterion in the evaluation of pet food. It often determines the success or failure of a product in the market and is one of the top determinants used by owners when selecting food for their pets, particularly cats (Dodd et al., 2019; Knight and Satchell, 2021; Schleicher et al., 2019; Zaghini & Biagi, 2005). In New Zealand, cats are the most popular companion animal, with 41% of households home to at least one cat (Companion Animals New Zealand, 2020). Whilst popular as pets, domestic cats have retained much of their ancestral traits particularly in terms of their nutritional requirements and are known as obligate carnivores due to their methods of ingesting, digesting, and metabolising nutrients (Bradshaw et al., 1996). As such, cats have a requirement for animal-sourced ingredients in their diets as without animal derived protein, severe nutritional deficiencies can occur (Case et al., 2010).

Along with their biological requirement for animal-derived protein, it has been reported that food palatability in cats was positively correlated to the amount of protein, particularly if ingredients of animal origin were used (Zaghini and Biagi, 2005). As well as the inclusion of meat and its by-products in diets, animal protein hydrolysates have been used to create palatability enhancers via a Maillard reaction scheme that results in the production of different flavours and a brown colour (Nagodawithana et al., 2008; Tamanna & Mahmood, 2015; Zaghini & Biagi, 2005). Furthermore, animal proteins, emulsified meats, amino acids, animal fats, and acids are identified as food components that are highly palatable to cats (Pekel et al., 2020; Thombre, 2004).

Palatability studies to date have largely focused on assessing complete, manufactured diets, rather than individual ingredients. However, fundamental analysis of dietary ingredients, in this instance meat and its by-products, is important for the appropriate formulation of diets in the context of the specialised dietary system of obligate carnivores. However, little work has looked at analysing the palatability of by-products as ingredients or examined the nutritional drivers responsible for differences in intake of different by-products. With this being considered, the overall aim of this thesis was divided into two parts. Firstly, the primary aim was to examine the within and between species palatability of commonly utilised ovine and bovine by-products after undergoing heat-treatment by the process of air-drying. This was assessed through use of palatability studies involving cats, as well as examining the potential nutritional drivers of palatability at a fundamental ingredient level using basic nutritional analysis and more comprehensive metabolomic analyses – a method which has not been examined in detail in pet food research. Only a few key studies have examined palatability using a geometric framework and going beyond assessing palatability via intake (Hewson-Hughes et al., 2011; Plantinga et al., 2011; Salaun et al., 2016). However, palatability of these diets has been altered through the inclusion of palatability enhancers as opposed to being formulated from ingredients of varying palatability. Therefore, the secondary aim of this work was to determine whether macronutrient selection differed between high and low palatability diets formulated using the highly palatable or less palatable meat by-products identified from the initial phase of the work.

The acceptance and preference of selected raw beef and lamb by-products (lung, heart, kidney, tripe, mechanically deboned meat (MDM), and liver) when fed to cats has been assessed (Watson et al., 2020). Cats showed a clear ranking of acceptance for both the lamb and beef by-products, with liver being the most palatable by-product and MDM the least accepted within each meat species and showed a general preference for lamb compared to beef. Whilst this work did show differences in by-product palatability in raw form, most pet food is manufactured using some form of heat treatment, such as baking, retorting, and drying which is necessary to kill bacteria, minimise microbial activity and growth to deliver safe final products (Lambertini et al., 2016). In particular, air-drying has become popular as it reflects pet owner purchasing trends for a minimally processed product which retains the majority of the nutritive qualities of the raw product. With this being considered, Chapter 2 of the thesis details the methodology required to develop an air-drying process which was adopted to produce samples for Chapters 3 and 4.

Chapter 3 investigated the palatability of commonly utilised bovine and ovine by-products in pet food that were thermally processed using air-drying to deliver a heat-treated final product. In acceptance testing within both species, organ meats were preferentially consumed over MDM. This confirmed the pattern previously observed in raw ingredient testing (Watson et al., 2020). The pattern of consumption of these ingredients also reflects the feeding behaviour of larger wild cats who, when hunting prey, will often preferentially consume organ tissues (Thompson, 2008, Laflamme et al., 2014). When expressed as percentage consumption, results of air-dried by-products were like that obtained in the same by-products in raw form (Watson et al., 2020), however air-dried by-products showed significantly lower intakes compared to the raw by-products when adjusted to the same dry matter. These results are supported by previous research which showed foods with a higher moisture content are positively associated to palatability in cats (Pekel et al., 2020). For preference testing, lamb lung, kidney, tripe, and spleen were more preferred than the beef equivalents, lamb and beef heart and liver showed no significant difference in intake which agrees with those previously determined for raw by-products (Watson et al., 2020). However, unlike the raw preference testing, beef MDM was more palatable than the lamb MDM when fed air-dried. A potential reason for this difference is the process of mechanical separation of meat which results in significant variation in finished product quality between batches (Miller, 2018).

With a general preference being observed for lamb over beef, further work was done in Chapter 4 to examine the within-species age of animal (i.e., mutton vs lamb and calf vs beef) effects of palatability in cats, an analysis which has not been published in pet food research. In terms of palatability, within the ovine by-product comparison lamb MDM, kidney, spleen, and heart were preferentially consumed over the mutton variants, and mutton tripe and lung were preferentially consumed over the lamb variants. No difference was observed in liver intake between mutton and lamb. Within the bovine comparison, calf by-products were preferential consumed over beef, except for liver, which showed no difference between young and old. This again highlights that liver is a highly palatable ingredient regardless of its species of origin or age of animal at slaughter. These high levels of palatability for liver are supported by previous research which showed that when diluted in distilled water, pork liver was the most effective food stimuli (Boudreau et al., 1971). The current work also showed a slaughter age effect of by-product source on palatability in cats, which has yet to be reported in the literature in terms of pet food research. With premiumisation continuing to be a strong trend in the pet food industry, selection of the most palatable ingredients including the selection of by-product using a slaughter-age specification may become a step in the ingredient selection process within the industry.

Along with the assessment of palatability, Chapter 4 provided an array of by-products that were divergent in their palatability which allowed for an investigation of the nutrient composition of the by-products using metabolomic analysis, to understand the metabolite composition of individual ingredients used in pet food. This work focused on HILIC positive and negative ionisation mode data was used to identify compounds of interest that have previously been shown to have an influence on palatability in cats (Laffitte et al., 2021; Pekel et al., 2020). For the HILIC negative compounds these were L-glutamic acid, γ-glutamylleucine (γ-glu-leu) and γ-glutaymlmethionine (γ-glu-met), as well as carnosine. For the HILIC positive compound these were alanine, choline [M]+, glutamic acid, L-phenylalanine, and tryptophan, as well as anserine.

Previous studies have found that various L-amino acids, including glutamic acid, as well as γ-glutamyl peptides (along with other compounds) are agonists of the Calcium Sensing Receptor (CaSR) in cats (Laffitte et al., 2021). The current work in cats also provides initial insight into compounds within a food that may be showing a direct link to palatability at a fundamental ingredients level, with organ meats showing comparatively greater relative abundance of these three compounds than MDM. MDM is an ingredient used extensively in industry but showed consistently lower intakes throughout this research. Decreasing the amount of MDM in formulations for a greater inclusion level of organ meat may help increase the acceptance of diets.

It has been reported that cats prefer, and have a biological requirement for, animal derived protein (Zaghini and Biagi, 2005). However, key compounds associated with meat that are present in high concentrations in vertebrate tissue, carnosine and its methylated analog anserine (Uenoyama et al., 2019), were also examined out of interest. Levels of carnosine and anserine, dipeptides found abundantly in skeletal and cardiac meat, were high in the MDMs and lower in organ meats. This highlights that although cats have a biological need for meat proteins, the type of meat selected has a great influence on palatability, with a consistent and preferential consumption of organ meats over muscle meat.

After examining the palatability of individual by-products, a series of high and low palatability diets varying in protein, fat and carbohydrate levels were formulated using by-products of high and poorer palatability to assess the macronutrient selection by cats using a geometric framework in Chapter 5. With diets being manufactured from a limited set of meat ingredients, the major difference between the two sets of diets was that lamb kidney was used as the ingredient of high palatability and sheep heart was used as the ingredient of low palatability. At the completion of the study, it was evident that the predicted formulations were considerably different to the measured diets in terms of dry matter content and the metabolisable energy content. However, differences in palatability were still observed between treatments with cats consuming a greater total intake of the high palatability diets compared to the low palatability diets. Whilst defined as low and high palatability diets, intake results suggest both series of diets were highly palatable, which could be seen by the cats consuming over their 100% MER within both treatments by a large margin, which was reflected in the significant weight gain and increase in BCS during each feeding block. Cats also showed a clear novelty effect, defined as the preference for a new food, in this case both series of air-dried foods on day one of each treatment even after a two-week wash out period between treatments (Bourgeois et al., 2006; Péron & Tobie, 2018).

Finally, in terms of macronutrient selection, cats selected most of their ME from fat, followed by protein and then carbohydrate, a result which was not consistent with the literature due to the considerably higher fat contents in the diets than was predicted during formulation. This resulted in a major problem in this experiment as all diets contained high fat levels, which made it difficult for the cats to obtain a high protein diet, which has been seen in the literature as being a main driver of macronutrient selection in cats (Hewson-Hughes et al., 2011; Plantinga et al., 2011; Salaun et al., 2016). Between the high and low palatability treatments, subtle differences in macronutrient selection on an ME basis were observed between the high and low palatability treatment diets. Specifically, more protein was consumed within the high palatability treatment, similar fat consumption between the two treatments, and greater carbohydrate consumption within the low palatability treatment. When related back to the literature, cats appeared to maximise their carbohydrate intake in the low palatability treatment compared to the high palatability treatment. These results suggest that the shortfall in protein, particularly in the low palatability treatments due to high fat across all diets, prompted the cats to seek nutrients elsewhere (Hewson Hughes et al., 2011). In this case, the cats shifted towards a higher intake of carbohydrate in the low palatability treatment.

Summary and Main Conclusions

To summarise the results of each experimental trial, Chapter 3 examined the effect of heat treatment on the palatability of beef and lamb by-products and found that air-drying has a negative influence on by-product palatability, particularly when compared to the results obtained from previous raw work.

Chapter 4 assessed the effect of by-products sourced from ovine and bovine of two different ages and found that a general preference was observed for young over old within species. Additionally, organ meats were consumed preferentially over MDM and had higher relative concentrations of desirable umami and kokumi compounds. These compounds are sought after by cats, and may act as drivers for organ meats over non-organ MDM.

Finally, the palatability and geometric nutrition study in Chapter 5, although flawed in the macronutrient composition it provided, revealed that cats did show greater consumption of the high palatability diets over the low. It should be highlighted that both series of diets were deemed highly palatable with intakes exceeding 100% maintenance energy requirements and was reflected in the weight and body condition scores of the test cats. Furthermore, differences in macronutrient selection were observed between high and low studies, with the cats actively seeking a greater amount of protein in the high palatability study. Whilst cats shifted to a higher carbohydrate intake, which reflected their carbohydrate ceiling, due to the shortfall in protein within the low palatability diets.

Limitations of the Study and Future Directions

While this study provides an initial examination of by-product palatability and potential nutrient drivers, further work could be done in future to substantiate the findings.

For example, the individual by-product analysis has identified potential compounds of interest relating to higher (glutamic acid, as well as γ-glu-leu and γ-glu-met kokumi peptides), and lower (carnosine and anserine) palatability in cats. It is possible for a more controlled experimental protocols, such as those traditionally used by Boudreau et al. (1971) and White & Boudreau (1975) to examine the response of these compounds on the taste receptors of cats by varying the concentration of these compounds when diluted in water.

Additionally, focus was placed on compounds known to have an influence on palatability in the literature, however, hundreds were identified in the HILIC positive and negative analysis as untargeted metabolomic analysis was carried out. As such, there is the potential to examine other compounds identified that may not have a directly link to palatability in cats in the literature to date.

The geometric nutrition study in Chapter 5 was unsuccessful in providing diets of significantly varying macronutrient levels for cats to make their selection. All diets were extremely high in fat, which is likely related to the ingredients used being extremely variable between batches and I was unable to obtain a high protein diets within each treatment. It is possible that variation in the raw meat by-products themselves is reason for why work using high meat content diets for geometric framework studies has not been done in the literature to date. This may be due to formulator data used to generate the diets not reflecting current practices where animals are finished on higher energy forages that allow for greater intake such as chicory resulting in increased fat content in the carcass and non-carcass tissues compared to those finished to traditional perennial ryegrass (Ye et al., 2022). In future, reformulating the diets with varying levels of the ingredients used in this study or complete reformulation using highly and poorly palatable meat ingredients and inclusion levels that could allow greater macronutrient targets to be achieved. This would need to be done to ensure all diets meet the defined protein and fat minimums required in commercial cat food which would be confirmed by carrying out macronutrient analysis prior to feeding studies commencing, an error made in this study due to lab closures and delays from COVID

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Appendices

Appendix 1. MS parameter settings for HILIC runs.

Event	Pos / Neg	Type	Start (min)	End (min)	Precursor Ion m/z	TOF Start m/z	TOF End m/z	CE	CE Spread	Event Time (s)	Pulser Inj. Times
1	+	MS	1	15		55	1100			0.03	54
2	+	MS/MS	1	15	100	60	800	23	15	0.02	34
3	+	MS/MS	1	15	120	60	800	23	15	0.02	34
4	+	MS/MS	1	15	140	60	800	23	15	0.02	34
5	+	MS/MS	1	15	160	60	800	23	15	0.02	34
6	+	MS/MS	1	15	180	60	800	23	15	0.02	34
7	+	MS/MS	1	15	200	60	800	23	15	0.02	34
8	+	MS/MS	1	15	220	60	800	23	15	0.02	34
9	+	MS/MS	1	15	240	60	800	23	15	0.02	34
10	+	MS/MS	1	15	260	60	800	23	15	0.02	34
11	+	MS/MS	1	15	280	60	800	23	15	0.02	34
12	+	MS/MS	1	15	300	60	800	23	15	0.02	34
13	+	MS/MS	1	15	320	60	800	23	15	0.02	34
14	+	MS/MS	1	15	340	60	800	23	15	0.02	34
15	+	MS/MS	1	15	360	60	800	23	15	0.02	34
16	+	MS/MS	1	15	380	60	800	23	15	0.02	34
17	+	MS/MS	1	15	400	60	800	23	15	0.02	34
18	+	MS/MS	1	15	420	60	800	23	15	0.02	34
19	+	MS/MS	1	15	440	60	800	23	15	0.02	34
20	+	MS/MS	1	15	460	60	800	23	15	0.02	34
21	+	MS/MS	1	15	480	60	800	23	15	0.02	34
22	+	MS/MS	1	15	500	60	800	23	15	0.02	34
23	+	MS/MS	1	15	520	60	800	23	15	0.02	34
24	+	MS/MS	1	15	540	60	800	23	15	0.02	34
25	+	MS/MS	1	15	560	60	800	23	15	0.02	34
26	+	MS/MS	1	15	580	60	800	23	15	0.02	34
27	+	MS/MS	1	15	600	60	800	23	15	0.02	34
28	+	MS/MS	1	15	620	60	800	23	15	0.02	34
29	+	MS/MS	1	15	640	60	800	23	15	0.02	34
30	+	MS/MS	1	15	660	60	800	23	15	0.02	34
31	+	MS/MS	1	15	680	60	800	23	15	0.02	34
32	+	MS/MS	1	15	700	60	800	23	15	0.02	34
33	+	MS/MS	1	15	720	60	800	23	15	0.02	34
34	+	MS/MS	1	15	740	60	800	23	15	0.02	34
35	+	MS/MS	1	15	760	60	800	23	15	0.02	34
36	+	MS/MS	1	15	780	60	800	23	15	0.02	34
37	+	MS/MS	1	15	800	60	800	23	15	0.02	34
38	+	MS/MS	1	15	820	60	800	23	15	0.02	34
39	+	MS/MS	1	15	840	60	800	23	15	0.02	34

40	+	MS/MS	1	15	860	60	800	23	15	0.02	34
41	+	MS/MS	1	15	880	60	800	23	15	0.02	34
42	+	MS/MS	1	15	900	60	800	23	15	0.02	34