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# Investigation into the Palatability of Lamb, Beef and Chicken Offal used in the Production of Pet Food

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## Abstract

This series of studies investigated the palatability of individual offals used in the production of pet food for cats from lamb, beef and chicken species.

Before initiating testing, a literature review was carried out to define palatability and identify possible drivers of palatability in both cats and dogs. Various palatability testing methods and the selection of suitable ingredients to analyse were also evaluated in the early stages of this study.

A standardised testing protocol was established and followed for palatability trials. These trials included the use of two-bowl acceptance tests to develop an overall ranking of offal within each species. Two-bowl preference tests between equivalent beef and lamb offals were also conducted to observe whether the panel showed preferences for one species over the other whilst also evaluating the meal size, frequency and rate of consumption. The final three-bowl preference tests between the top and bottom ranked beef, lamb and chicken offals were used to observe whether there were differences in the species of offal first approached, first consumed and first/most completed by the panel.

Acceptance testing revealed that within each species, liver was the most palatable offal presented, with kidney equivalent to it in the lamb acceptance testing. In all three sources of offal, liver possessed the highest amounts of protein compared to the other offals, which was identified in literature as a positive driver for palatability in cats due to their high requirements for protein. In addition, MDM was the least accepted offal, although heart was equivalent to it in the chicken acceptance testing. Furthermore, preferences for lamb over equivalent beef offals, with the exception of heart and liver, were also demonstrated.

The final three-bowl preference tests between the top and bottom ranked beef, lamb and chicken offals revealed that cats showed high palatability for liver with no preference for one species of liver over the other. However, of the bottom ranked MDM ingredients, chicken was consumed preferentially over beef and lamb MDM. Compositional data for the MDM showed that chicken had the highest protein content of the three MDM varieties.

As well as detecting difference in palatability between offals, this study suggested the amount of protein within individual offals may play a role in influencing offal acceptance and preference in cats.



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## Introduction

Pet food palatability in cats has been extensively studied as evidenced by literature in regard to complete diets. However, there is currently little research on the palatability of individual ingredients used in the production of pet food.

In the pet food industry, meat and meat organs make up the majority of ingredients in raw or canned diets and are necessary for cats who are obligate carnivores and have a high requirement for animal derived protein (Stasiak, 2002; Zaghini & Biagi, 2005). Species of meat and meat organs commonly used in pet food include beef, lamb, and chicken. Assumptions are commonly made by manufacturers and pet owners that various meat offals are more palatable than others, although no statistical evidence or known research has been conducted to evaluate palatability on an individual ingredient based level.

This study, therefore, aimed to identify whether palatability differences exist between the following offals: of lung, heart, kidney, tripe, MDM and liver in beef and lamb as well as heart, gizzard, MDM and liver in chicken, with the aim of identifying the likely drivers for palatability.

To do this, it was necessary to firstly develop a robust testing protocol by evaluating commonly used palatability testing methods and selecting those which would be most suited for this research. The methods that were adopted included two-bowl acceptance and two-bowl preference tests, as well as three-bowl preference tests. Furthermore, it was important to identify factors that are known to drive, as well as hinder, palatability from previous literature and where possible, relate these findings to the current research.

The purpose of this research was to firstly develop a ranking of offal acceptance within a single species. Following this, comparing equivalent offals across species was conducted to evaluate whether cats show preferences for one species over the other. Finally, macronutrient, fatty acid and amino acid analyses were carried out and used to determine if observed palatability differences can be attributed to the nutritional composition of the ingredients.

The structure of this thesis includes a literature review in chapter one, followed by the study's aims and hypotheses in chapter two. Development of the testing protocol and refinements to the cat palatability panel are presented in chapters three and four. Testing for the acceptance of offals within lamb, beef and chicken species are presented in chapters five, six and seven, respectively. Furthermore, the preference between equivalent offals from beef and lamb and the final three bowl preference test between the top and bottom ranked beef, lamb and chicken

offals are given in chapters eight and nine. Finally, a general discussion of the findings are presented in chapter ten.

The study will be the first of its kind to evaluate the palatability of individual meat offal ingredients used in commercial pet foods. The findings will likely be of great value to pet food manufacturers when formulating or looking to reformulate products to improve their overall palatability.

# 1. Literature Review

## 1.1 Introduction

Palatability is the number one determinant of the price of pet food in the market and can often determine the success or failure of a product. It is the initial hurdle for manufacturers to overcome and is often used throughout the product development process to identify which diets are and are not going to sell well. In general, the more palatable the diet, the more likely it can be sold at a higher price point.

This purpose of this review is to firstly define what palatability entails, determine the drivers of palatability for both cats and dogs, with greater emphasis on cats, and describe the various palatability testing methods that are most commonly used in the industry. This should enable suitable testing protocols to be selected for palatability trials in this study.

After considering all the fundamental information, the second half of this review will aim to identify the appropriate selection of ingredients, particularly those with by-product streams, to be considered for palatability testing. Assessing the nutritional components in each ingredient, such as the fatty acid composition and amino acid profile, will be used as a starting point for identifying possible nutrient drivers for palatability in cats.

## 1.2 Defining Palatability

Palatability has been interpreted in numerous ways in the literature. The National Research Council, as cited by Aldrich and Koppel (2015), describes palatability as the physical and chemical properties of the diet, which are linked with promoting or suppressing feeding behaviour during the pre-absorptive period. 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). It can be measured in regard to the attractiveness of the food and the amount of product consumed, in which a food that is readily accepted is indicative of a food that is palatable (Stasiak, 2002; Tobie, Péron, & Larose, 2015).

## 1.3 Key Drivers for Palatability

Palatability is believed to be a function of both sensory factors including aroma, taste, texture and consistency and metabolic inputs, which are controlled by experience, age and possible health and genetic factors (Bradshaw et al., 1996; Watson, 2011). In this section, a discussion of the main drivers for palatability in both cats and dogs will be discussed.

Cats are able to detect small differences in the composition of food they are offered (Bradshaw et al., 1996). The preference for food in kittens is often strongly influenced by the food

preferences exhibited in their mothers (Bradshaw, 2006). This includes the flavours kittens are exposed to during their mother's pregnancy and lactation via amniotic fluid and milk, as well as in the flavours that kittens themselves experience from four weeks to six months of age and may track along family lines (Aldrich & Koppel, 2015; Bradshaw, 2006; Watson, 2011; Zaghini & Biagi, 2005). Exposure to dietary flavours in early life can result in preference for that flavour, which is referred to as the primacy effect (Stasiak, 2002). In the future, when pet owners make a range of experiences available to their cats, the novelty effect can be displayed. This is defined as the persistent preference to eat a novel diet rather than a pet's accustomed diet (Stasiak, 2002). It was also found that when cats are presented with two foods that are both familiar and abundant, they tend to go for the less abundant of the two which was seen as a strategy to obtain a mixed diet (Bradshaw et al, 1996)

As with cats, dogs that eat the same diet for a long period can also display the novelty effect and show enhanced preference for other diets (Bradshaw, 2006). Odour preference in dogs was identified as the main driver for palatability in dogs, with dogs showing preference for food odour over no food odour. In a study carried out by Hall and others (2017), when presented with two diets, 89% of dogs did not need to taste each food before selecting the preferred diet but consumed more of the food they chose first. This test was repeated with the bowl positions being swapped to remove bias but yielded the same outcome. These results, therefore, indicated that product selection for dogs is likely based on odour as well as visual appearance of the food. Furthermore, odour is also believed to play a role in anosmic dogs as they showed reduced discrimination between different types of meat (Bradshaw, 2006).

Literature has revealed that exposure to flavours in early life and the significance of odour preference are key drivers of palatability in cats and dogs, respectively. It is important to consider these main palatability drivers during trials as this may help explain why an overall preference for one diet is displayed, or may help to justify why the less preferred diet to the majority of subjects is more preferred by some animals.

#### 1.4 Biological and Behavioural Influences on Palatability

As well as the identified key drivers for palatability previously discussed, many authors have analysed the biological adaptations and behavioural patterns as being contributors to food preferences in cats and dogs. The following will highlight the similarities and differences between the two species looking at how their biology and behaviour may drive food choice.

### 1.4.1 Hunting Strategies

Cats are commonly known as solitary hunters that will wait for their prey to show themselves before making their kill. Once caught, food is eaten quickly, as cats prefer freshly killed carcass as opposed to carrion (Becques et al., 2014). 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 will be consumed (Aldrich & Koppel, 2015). Cats are also classified as intermittent feeders, which means they consume small meals throughout the day (Becques et al., 2014; Watson, 2011; Zaghini, & Biagi, 2005).

Compared with cats, dogs originated as hunters that searched for prey in organised packs and are known to be able to consume large amounts of food in a short period of time (Aldrich & Koppel, 2015). Dogs often eat food in a gluttonous manner and do not spend much time chewing their food, as they are likely to regurgitate and re-consume it later on when away from other members of the pack (Aldrich & Koppel, 2015; Hall et al., 2017). It is believed that the competitive feeding display in dogs is a legacy of the wolf ancestors as well as a possible adaptation to scavenging during the early stages of domestication (Bradshaw, 2006).

### 1.4.2 Factors Influencing Food Choice

In both cats and dogs, the development of food preference may be dependent on innate, social and/or experiential factors (Stasiak, 2002).

Olfaction, a major sense in animals, plays a vital role in the sensory experience of eating and food choice (Hall et al., 2017). The olfactory senses of cats, although not as well developed as dogs, are active and used 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). Although important, it was revealed that flavour is in fact more dominant in influencing the food preference of cats as opposed to colour and ortho-nasal aroma (Pickering 2009).

However, taste is an important consideration in determining food preference, only when strictly combined with olfaction. The two work together to stimulate salivary, gastric and intestinal secretion, which allows the animals the opportunity to reject potentially toxic food (Zaghini, & Biagi, 2005). Cats and dogs also learn to avoid repeating disadvantageous feeding experiences associated with nutritionally incomplete or a potentially toxic prey/diet (Bradshaw, 2006). An example of this is given by Bradshaw et al. (1996), when lithium chloride was added to a single

meal, this resulted in cats refusing to eat that food for three days, with aversions to the same flavour of unadulterated food continuing for up to 40 days.

As well as flavour and taste, the size, shape and texture of food can also influence palatability in both cats and dogs (Zaghini, & Biagi, 2005). It was found that kibbles with sharp edges are unfavourable, particularly to cats as these can cause abrasions in the mouth and stomach (Zaghini, & Biagi, 2005). As well as this, stickiness and viscosity are important factors to consider in the production of wet foods (Watson, 2011). Pet food palatability can also be enhanced by coating the outside of kibble with fat as this was seen as having a positive impact on food texture rather than contributing to flavour (Zaghini and Biagi, 2005). Finally, the temperature at which food is served is also important for cats with rejection being observed if the temperature of the food is below 15°C or above 50°C (Zaghini, & Biagi, 2005). Cats will often refuse otherwise palatable foods if served chilled and tend to prefer food at blood, or if not, room temperature where possible (Bradshaw et al., 1996).

Unlike cats, dogs are often described as opportunistic eaters, as they will consume anything seemingly edible to them. As facultative carnivores, dogs have been known to eat animal faeces, insects, berries and grass as well as carrion and enjoy chewing on bones, hides, and other animal parts (Aldrich & Koppel, 2015). The ability for dogs 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 requirements (Bosch et al., 2015). The ability to do so allowed for the change from canivory to omnivory to take place during domestication. Dogs being able to consume foods of both animal and plant sources compared to cats selectivity shows great contrast between the two species of animals. The reason for such differences will be outline further in Section 1.5.

#### 1.4.3 Behavioural Response to Food

Behavioural response to various food through the development of taste reactivity patterns has also been evaluated in cats in sensory tests conducted by Van den Bos et al., (2000). It was found that certain physical responses in cats could indicate a liking or aversion to different foods. For example, licking and sniffing the feeding bowl, licking of the lips and grooming of their face indicated a liking towards the food. However, licking and sniffing of the food and licking their nose were associated with an aversion.

As well as these movements identified by Van den Bos et al. (2000), the time cats spent sniffing the food was also used to assess palatability (Tobie et al., 2015). In a separate study, two kibble diets were made available for 20 hours each day. These diets were classified as very palatable kibble (VPK) and less palatable kibble (LPK) (Becques et al., 2014). It was discovered that cats

spent more time sniffing the LPK on day one, showing hesitation to consume the less palatable diet. Furthermore, consumption of the VPK was higher than the LPK throughout the duration of the study, indicating preference for the VPK over the LPK.

When testing the preference of various diets in dogs, the time spent sniffing food was also used as an assessment of a food's palatability. In contrast to cats, who sniffed the less preferred food for longer, dogs sniffed the more preferred food for longer (Tobie et al., 2015). When dogs were given a choice of two foods, they showed exclusive or near exclusive preference for one over the other (Hall et al. 2017). Only when the bowl containing the more preferred food was empty did the subjects move to the next bowl.

In addition, when given *ad libitum* access to three diets of high fat, high protein and high carbohydrate over a ten day period, dogs initially demonstrated a "feast or famine" mentality, in which energy from fat was preferred over protein (Roberts et al., 2017). As day ten of the study approached, the dogs showed movement towards a more balanced energy contribution from both protein and fat, with carbohydrates showing a minimal contribution of energy to the diet.

#### 1.4.4 Taste Receptors

The taste receptor units in mammalian animals can be categorised into four main groups. These include, Type A units which respond to amino acids, as well as mono- and disaccharides in dogs, Type B which respond to acids, particularly carboxylic, phosphoric, other Brønsted acids (Bradshaw, 2006). Type C mainly respond to nucleotides, particularly those associated with the umami taste in humans and finally, Type D, which respond to fruity-sweet compounds (Bradshaw, 2006).

The units that are most abundantly exhibited in cats and dogs are those that respond to amino acids as they are able to detect food rich in protein (Bradshaw et al., 1996; Watson, 2011). Cats are known to reject amino acids that are regarded as 'bitter' 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 (Bradshaw et al., 1996; Zaghini, & Biagi, 2005). It was identified that the L forms of amino acids were more stimulatory than the D forms (Boudreau, 1985). The abundance of amino acid unit may be related to meat-eating in cats, allowing them to distinguish varying qualities of meats (Bradshaw et al., 1996).

Cats also possess taste receptor units that respond to acids, particularly carboxylic, phosphoric, other Brønsted acids. These units are also activated by sulphur-containing amino acids,



particularly L-cysteine and L-taurine, but are repressed by inosine monophosphate (Bradshaw et al., 1996)

Taste receptor units A, B and C are fairly similar between cats and dogs; however Type D units differ between the two species. Unlike dogs, cats do not have any functional sweet taste receptors and cannot detect foods based on their sugar or salt content (Watson, 2011). The author states that this inability to detect sweetness offers better appreciation of the essential amino acid balance within food by removing the masking effect from sugars. Cats are also unable to detect differences between water and a 1.0M sugar solution, therefore it should be noted that previous studies involving the use of sugar solutions resulted in severe gastro-intestinal disturbances in cats and resulted in the discontinuation of the study by Rofe, & Anderson (1970).

Rather than detecting sweetness, cats have a type of receptor that responds optimally to quinine, tannic acid and alkaloids, which is similar to the bitter taste humans' experience (Bradshaw, 2006; Rofe, & Anderson, 1970). Cats are highly sensitive to quinine in comparison to dogs, with cats being able to detect quinine present in food at 1.3mM compared to 10.3mM in dogs (Rofe, & Anderson, 1970)

#### 1.4.5 Similarities in Cats and Dogs

When cats and dogs are first exposed to new food, they tend to display neophilic behaviour as opposed to neophobia (Péron & Tobie, n.d.). Both cats and dogs prefer novelty to their accustomed diet as they can become bored to long term feeding of a single food over time (Watson, 2011). 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, n.d.).

Animals can also display a tendency to eat from the left or right bowl regardless of content. Such individuals are known as position eaters (Péron & Tobie, n.d.). Although this behaviour is more common in cats, dogs can also show side bias. It is therefore important to screen out position eaters before carrying out a palatability test and remove unwanted bias as soon as possible.

This information shows that cats and dogs exhibit different biological and behavioural adaptations which may further influence food preference. The omnivorous nature of dogs allows for greater acceptance of a wide variety of foods, compared to cats who are fussier eaters. They also have distinct feeding habits as well as strict nutritional requirements (Aldrich & Koppel, 2015; Bosch et al., 2015). For this reason, cats should therefore be considered as the species to use for palatability testing as they would provide clear results compared to dogs who are likely to show more subtle preference patterns and consume all foods presented.

## 1.5 Nutritional Needs for Cats

From a nutritional perspective, cats are known as prey-driven animals, formally termed obligate carnivores (Aldrich & Koppel, 2015). They have very high protein requirements and need food of animal origin in order to obtain some essential nutrients that can only be found in animal tissue (Stasiak, 2002; Zaghini, & Biagi, 2005). As well as the requirement for individual nutrients, research has found that cats are obligate carnivores in regards to their methods of ingesting, digesting and metabolising such nutrients (Bradshaw et al., 1996).

Without animal derived protein, severe nutrition deficiencies can occur in cats. At times cats can refuse to eat a diet and thus starve until they develop clinical consequences (Stasiak, 2002; Zaghini, & Biagi, 2005). Some key nutrients that cats require can only be sourced from animal tissue. These include preformed vitamin A, arachidonic acid, taurine, niacin, methionine, cysteine and arginine as well as EPA and DHA (Aldrich & Koppel, 2015; Watson, 2011; Zaghini, & Biagi, 2005).

Taurine is the only amino acid able to bind to bile acids in cats; they are unable to use glycine like other mammals (Zaghini, & Biagi, 2005). Taurine is required for cats to maintain retinal function and structure, and has roles in cardiac function, sight and reproduction. As cats, and particularly kittens, are unable to synthesise enough taurine to meet their needs, it has been identified as an essential amino acid (Knopf et al., 1978).

Arginine is another essential amino acid required for growth and the production of urea from ammonia (Morris & Rogers, 1978). It is of great importance to cats, as severe and near instant ammonia intoxication can result if they are fed diets lacking in arginine (Anderson et al., 1979).

As well as these key essential amino acids, cats also require a dietary source of pre-formed vitamin A due to the deletion of enzymes that starts the conversion of carotenoids to retinal, (Morris, 2001; Zaghini, & Biagi, 2005). Additionally, cats prefer diets with adequate methionine over methionine free diets and avoid those lacking in isoleucine (Watson, 2011).

Rather than choosing foods solely on sensory properties, it was identified that cats also seek nutritional adequacy to achieve a 'target intake' of protein, fat and carbohydrates (Watson, 2011). In a study evaluating the geometric analysis of macronutrient selection in cats, it was found that the optimal levels were 26g/day protein, 9g/day fat and 8g/day carbohydrate, which gave a macronutrient energy composition of 52% protein, 36% fat and 12% carbohydrate (Hewson-Hughes et al., 2011). 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).

In terms of the quality of food, 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)

The format of foods also has implications on palatability in cats. Wet foods are seen to have a similar protein content to their 'target intake', slightly more fat and minimal carbohydrates. Whereas dry foods often have less protein, similar fat and carbohydrates can be as high as 40% (Watson, 2011). It was also found that cats are less able to handle high levels of carbohydrate in their diet unlike dogs due to differing carbohydrate metabolism. Cats, when on high carbohydrate diets, appear to be in a constant state of gluconeogenesis (Legrand-Defretin, 1994). 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).

The AAFCO nutrient requirements (2017) provides information on the minimum, and where applicable the maximum requirements of specific nutrients for cat maintenance as well as during growth and lactation. On a dry matter basis, cat food diets must contain a minimum of 26% crude protein for maintenance and a minimum of 30% for growth and lactation.

The amino acids that make up the protein include arginine, histidine, isoleucine, leucine, lysine, methionine-cysteine, phenylalanine-tyrosine, phenylalanine, threonine and valine. These must be present in cat food at varying minimum levels. Methionine and tryptophan are amino acids that show both a minimum as well as maximum intake level. Additionally, taurine is an essential amino acid for cats and must be included in canned cat food at 0.2% compared to 0.1% in extruded cat food. Reasons for the difference in taurine levels will be discussed further under Section 1.6.

As well as protein, the AAFCO regulations outline a minimum crude fat requirement of 9.0% in cat food, with a minimum of 0.6% linoleic acid and 0.02% arachidonic acid required for both maintenance as well as during growth and lactation. Levels of alpha-linolenic as well as eicosapentaenoic and docosahexaenoic acid also need to be present at 0.02% and 0.012% respectively for growth and lactation (AAFCO, 2017).

Vitamin and mineral levels are also nutrients included in the AAFCO requirements. A summary of all nutrients and their levels of inclusion in cat food can be found in Appendix A.

There is also a strong correlation between pet food palatability and the amount of protein from animal origin used such as red meat, liver and blood, as well as fish (Zaghini, & Biagi, 2005). The use of such ingredients is therefore vital for improving the acceptance of food to both dogs and to a greater extent in cats.

Research by Houpt and Smith (1981) also evaluated different types of meat preparations for dogs and found that they showed greater preference for canned meat compared to fresh meat, minced meat compared to chunks of meat, and cooked meat compared to raw meat. Such studies have not been carried out to this extent in cats so may be investigated during this study, particularly to determine the most suitable format to present ingredients during palatability testing.

## 1.6 Food Processing Techniques

As well as the biological palatability drivers in cats and dogs, the way in which pet foods are processed also has a number of implications on the acceptance of foods. The production of Maillard products via a chemical reaction between amino acids and reducing sugars to produce different flavours and a brown colour during heating is associated with a positive influence on palatability in cats (Zaghini & Biagi, 2005; Tamanna & Mahmood, 2015). In contrast, lipid oxidation results in a decrease in palatability, as the off-notes are easily detected by cats.

When retorting canned foods, high temperature and pressure can result in desirable flavour compounds via Maillard reactions. However, increased retorting time may contribute negatively to palatability for cats, again due to the formation of lipid peroxides that give undesirable flavours (Hagen-Plantinga et al., 2017).

Hagen-Plantinga et al., 2017 studied the effects of different retort temperature and time regimes on palatability. The authors tested three different temperatures but used a similar level of lethality ( $F_0$  value = 30) by varying the retort time. It must be noted that this level of lethality is rather extreme, but the authors wanted to see the effect. The results showed that 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 food processed at 120°C for 103 minutes and 127°C for 60 minutes. In addition, the 113°C diet showed a greater preference ratio of 0.38 compared to 0.31 for 120°C and 127°C equally. It was concluded by the authors

that undesirable food textures may also result from excessive heating temperature and time, which may disrupt the binding properties and also negatively affect palatability.

Furthermore, the process of cooking can also result in reduced amounts of essential nutrients delivered in a food, such as the amount of taurine in animal ingredients (Zaghini and Biagi, 2005). The authors state that taurine supplementation generally needs to be higher in canned food than dry food, to account for these processing implications.

As previously mentioned, taurine is the only amino acid that cats can use to form bile salts. In canned cat food, the formation of Maillard product gives a food that is more palatable however, the protein becomes less digestible (Morris et al., 1994). It is believed that Maillard products may reduce protein digestibility and increase taurine exposure to bacteria in the caecum allowing them to get to the protein before taurine can be recycled back and conserved by the bile salts. In order to deliver sufficient amounts of taurine to cats, canned diets require as much as 2500mg taurine/kg dry matter compared to 1000mg taurine/kg diet in dry food (Morris et al., 1994). If not achieved, taurine deficiencies, which affect electrical impulse transmission across membranes can cause severe cardiac, retinal and reproductive issues in cats (Morris et al., 1994).

The formation of desirable and undesirable products during processing have been found to have implications on the palatability of pet food. As well as this, varying time and temperature profiles can deliver different end-products which may further effect palatability results. It is therefore important that all ingredients are prepared and processed in the same way in this study, as this will help eliminate factors that may drive possible bias.

## 1.7 Palatability Testing

The following section will focus on firstly describing the two classes of palatability testing that are available, as well as identifying the correct test type to use and provide information on the suitability of the panels and the number of subjects required to obtain reliable results.

In palatability testing, two classes of testing exist: non-consumption and consumption testing. Non-consumption testing consists of autonomic or conditioned response tests (Aldrich and Koppel, 2015). These can include the Pavlovian response of a dog to a meal, the Skinner box test where the animal learns to associate an action with a reward, as well as the cognitive palatability assessment protocol that relies on discrimination learning by animals for three foods at a time. Consumption testing is the most commonly used technique for palatability testing in the pet

food industry that measures food intake. It can include the use of a single-bowl (acceptance) and/or a two-bowl (preference) test (Aldrich & Koppel, 2015).

### 1.7.1 Test Methods

Depending on the aim of the research, different testing methods can be adopted. In this section, a discussion of the two most commonly used testing methods, the one- bowl and two-bowl tests, will be discussed, and an analysis of the advantages and disadvantages of each will be provided. Two additional, and different, approaches that have been used in recent studies will also be examined as potential tests to consider.

#### *One-Bowl Test*

The one-bowl test solely measures the daily intake of the test food when only one food product is presented to an animal. This method involves the use of multiple cats or dogs 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 generally aren't given a choice of what to eat. Any breed and size of animal can be used. Kennel or home 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 use of between eight to ten animal subjects 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 number of limitations also exist and have been identified by Aldrich and Koppel (2015). Firstly, it should be emphasised that this method of testing is only suitable for determining the acceptance of a food, however, no information on the preference or degree of liking can be obtained. In addition, this method does not take into account species-specific differences, nor does it provide sufficient justification for a company to use such information to develop marketing claims or product improvements. It is also important that the amount of food offered does not exceed the animal's daily calorie intake, as overeating can cause animals to become overweight. Finally, the results from home animals are likely to vary more than kennel animals due to their differences in prior feeding. To overcome these differences, it is recommended that home 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.

### *Two-Bowl Test*

The two-bowl test involves presenting two diets simultaneously to the subjects for a defined period of time (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 type of test used in expert panels for palatability assessment studies in both cats and dogs. It can also be used on in-home panels although the inability to control the testing environment can result in less precise findings. The differences between in-home and expert panels will be discussed further in Section 1.7.2.

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 time period (Tobie et al., 2015). The author also highlights that tests are normally run again and the two bowl positions are switched in order to remove the effect of side preference and evaluate the repeatability 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 have been frequently used for studies at Massey University (Tartellin, 1997). The two-hour testing period and subsequent 40 measurements over five days is seen as being able to deliver a sufficient number of observations as well as consistently reliable results.

In recent times, researchers have also moved to using 20 animals for two or four days of palatability testing. It has been indicated that conducting the same test for a greater number of days on a smaller number of subjects gave repeated observations per animal for the same measure (Aldrich and Koppel, 2015). In contrast, the use of a greater number of subjects over a lesser number of days provided more true observations of the animals and revealed more quickly whether the animals preferred one food over the other.

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

This method of testing is beneficial for evaluating new flavour systems and product enhancements. It is used for competitive analysis or new product development as identified by Aldrich and Koppel (2015). The main limitations of two bowl testing have also been identified by

Aldrich and Koppel (2015) and Tobie et al. (2015). These include only being able to rank between the two foods tested, so all paired comparisons should 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 liked in a particular food.

#### *Cognitive Palatability Assessment Protocol (CPAP)*

The Cognitive Palatability Assessment Protocol (CPAP) is a modern method of testing that has been used as a reliable measure of food preference, as well as the classical one and two bowl-test. This method is based on discrimination learning in which dogs are presented with three objects, one of which is associated with no reward and the other two are associated with a given food. However, the dogs are only able to respond to one (Araujo et al., 2004).

In a study carried out by the authors, CPAP was identified as a more reliable measure of food preference requiring less test subjects compared to the two-bowl test, which showed greater variability in data. Araujo and Milgram (2004) also conducted a separate study using CPAP to test the palatability of two diets. They were able to determine a clear link to dogs being able to associate a particular object with a preferred food. As a result, CPAP could also be used as another objective measure to test for palatability and thus establish food preferences in dogs.

Further advantages of CPAP are that it is able to control for factors that influence feeding such as satiety and the testing method can also be modified to determine the input of various factors such as age, hormonal state, and dietary experience on food preference in dogs (Araujo and Milgram 2004).

#### *Three-Bowl Test*

A three-bowl test was used to assess three diets processed at different temperature-time profile (Hagen-Plantinga et al., 2017). The test was completed over ten days using ten cats that were placed in individual cages for 16 hours, during which time the cats were provided with pre-weighed amounts (400g) of each diet.

The first choice for each cat was recorded, and a scoring system was adopted where +3 was given to the diet first consumed, -3 given to those not selected and a score of 0 if no clear preference was observed, all in the first two minutes after the cats were placed in the cage. Bowl placements were again switched each day throughout the ten day trial. At the completion of each test, the food was weighed and the intake ratio was calculated based on the following equation;  $(A/(A+B+C)) \times 100$  for diet A,  $(B/(A+B+C)) \times 100$  for diet B and  $(C/(A+B+C)) \times 100$  for diet C (expressed as a %) with A, B and C being the individual daily food consumption of each diet (Hagen-Plantinga et al., 2017).



This method shows similarities to both the one and two bowl testing procedures as well as CPAP without subjects needing to associate an object with a preferred food. However, few studies involving three-bowl testing have taken place, therefore little information regarding the advantages and disadvantages of this method can be found in the literature.

As shown, multiple palatability testing methods exist and any of which may be used depending on the aims of the study. Traditionally, the two-bowl method is most commonly used throughout the pet food industry to test for preference, however, use of more modern methods, particularly the three-bowl test, may prove to be of great value when looking to compare multiple ingredients against each other. Nonetheless, it is possible that more than one method may need to be adopted to ensure a comprehensive evaluation of ingredients has been carried out.

### 1.7.2 Naïve vs Expert Panels

As well as selecting the correct palatability test, it is also important that an appropriate panel be used in the study. Depending on the aims of the palatability testing, there is the ability to select either a naïve (in-home) panel or an expert panel. The advantages and disadvantages of both panels, as derived from Tobie et al. (2015) and Péron and Tobie, (n.d.) are provided in Table 1.1.

Table 1.1: Advantages and disadvantages of naïve and expert panels

Subject Type	Naïve (in-home)	Expert
Advantages	<ul style="list-style-type: none"> <li>+ Data is representative of the final market</li> <li>+ Obtain real-life feedback</li> <li>+ Evaluate the owners' reaction of a product and the perception of palatability to their pets</li> </ul>	<ul style="list-style-type: none"> <li>+ Perform palatability tests on a regular basis</li> <li>+ Can be specialised in one type of food (wet/dry)</li> <li>+ More reliable and accurate</li> </ul>
Disadvantages	<ul style="list-style-type: none"> <li>- Do not have any training</li> <li>- Lower testing frequency and testing conditions are less controlled</li> <li>- Feeding history can be vague and lack diversity</li> </ul>	<ul style="list-style-type: none"> <li>- Need intensive training to be exposed to a wide range of foods</li> <li>- Quality tests should be carried out to control for any side bias</li> </ul>
Subjects required	<p><i>~100 subjects required to ensure pet owners' perceptions have not biased objective measurements</i></p>	<p><i>Minimum of 30 subjects required to avoid bias and obtain accurate palatability measurements whilst ensuring statistical robustness</i></p>

From the information summarised above, measuring the acceptance of a food is seen as being well adapted to naïve panels, as they do not require extensive training and exposure to food to undergo this type of testing (Tobie et al., 2015). However, it is strongly recommended that the preference test be conducted using only an expert panel to control bias. The authors go on further to say that the preference of naïve panels may be more stable, but expert panels; due to the animals level of training, feeding history and testing environment, are better at discriminating small differences between foods.

### 1.7.3 Palatability Testing Sources of Variation

Palatability testing allows for rapid and relatively inexpensive use of animal models to evaluate pet foods in order to determine the products success or failure (Aldrich & Koppel, 2015). However, it should be noted that several factors need to be taken into account when carrying out preference tests.

Rofe and Anderson (1970) indicated that some of the key factors which include: individual variation, where some individuals prefer a diet that the majority reject; the influence of an animal's previous diet also has an impact on palatability, as cats and dogs tend to prefer new

food to one that is familiar to them. In addition, the presence of lateral bias can skew results. This is characterised when an animal prefers the left or right hand bowl regardless of what is presented in each bowl (Rofe, & Anderson, 1970). Other factors that have been identified by the author includes the level of hunger or satiety when the animal approaches a test.

To demonstrate the possible variation due to the difference in degree of hunger, Rofe and Anderson (1970) used two groups of dogs in a study. One group were given *ad lib* access to food and the other were fed once daily. It was found that hungry dogs, those fed once daily, were more selective with what they ate and avoided bitter tasting food compared to the less hungry *ad lib* dogs, which ate bitter tasting food. The results showed that when dogs are in energy balance or surplus that the metabolic properties of the ingested food are important for regulating intake, as shown by the dogs given *ad lib* access to food. However, when energy balance is lacking, as in the case of the dogs fed once daily, the sensory properties of food become a key factor in regulating intake (Rofe & Anderson, 1970). Consumption variability is another factor that also has implications in a one bowl test and includes; daily variation, for example dogs eat more food in the afternoon than in the morning and seasonal effects, where cats eat less during winter and dogs have a lower food intake ratio in summer (Tobie et al., 2015; Péron & Tobie, n.d.).

### 1.8 Other Important Palatability Considerations

While there are many frequently reoccurring drivers for palatability that have been identified throughout the literature for many years, a shift in recent times has been identified. Pet food manufacturers aim to provide a nutritionally complete and balanced diet, but are realising that it does not matter how well developed a diet is if a pet does not eat it (Becques et al., 2014), which indicates the importance of palatability over composition (Zaghini, & Biagi, 2005).

There is also increasing awareness of palatability no longer being solely focused on organoleptic properties, (i.e. the use of the sense organs, and nutritional aspects), but encompassing an emotional element of palatability performance. This is expressed through the three-way relationship of behavioural expression of pets, the interaction with their owners and the owners' perception of their pet's enjoyment as described by Tobie et al. (2015).

### 1.9 Ingredient Selection

In order to identify the main ingredients to test for palatability, the United Kingdom Pet Food Manufacturers' Association (2018) has provided a prioritised list of ingredients that are commonly found in pet food. Animal derivatives are used as good sources of highly digestible protein and have been positively associated with palatability as previously described by Zaghini

and Biagi (2005). Fish was identified as the second most commonly used ingredient in pet food and was seen mainly as a good source of high quality protein. Fish bones were also identified as a good source of calcium and phosphorous, and their flesh contributing vitamin A and D as well as omega 3 fatty acids.

As well as these two main ingredients dairy products and eggs, vegetables, cereals and cereal by-product, fats and oils from both plant and animal sources, vitamin and mineral supplementation, sodium chloride, various sugars and additives were also identified as other ingredients and components that may be included in pet food to deliver a complete and balanced diet.

The United Kingdom Pet Food Manufacturers' Association (2018) states that egg, meat and organs such as heart, kidney, liver and lung, fish and cereal glutes are all sources of dietary protein with high biological value as they show good digestibility and a high content of essential amino acids. Additionally, because of the carnivorous nature of cats and with both cats and dogs having an abundance of taste receptors that respond to amino acids, it is suitable to evaluate the palatability of individual high protein ingredients such as meat, meat organs and fish in this study.

Previous studies throughout the literature have been highly focused on assessing the palatability of complete diets, however, no published research was identified on individual ingredients. Identifying the ingredients that drive or hinder palatability as well as the nutritional components in each ingredients responsible for such effects would be seen as the first study of this kind and therefore be of great significance in the pet food industry.

In terms of identifying individual ingredients to test, Ziwi Ltd, a pet food manufacturer and supplier of raw materials for the project, provided a list of possible ingredients of interest. These included liver, lung, kidney, tripe, heart and mechanically deboned meat (MDM) from both beef and lamb, as well as chicken heart, gizzard, liver and MDM.

### 1.9.1 Meat Ingredients

In terms of meat ingredients, Marti et al., (2011), were able to identify the essential vitamins and nutrients that are most abundant in beef liver, heart, tripe and kidney. Liver was found to be high in vitamin A, iron, zinc, B vitamins, vitamins C and D as well as copper and fatty acids. Hearts have a high content of iron, are a good source of selenium, zinc, phosphorous, niacin, and riboflavin, and have a low sodium content. Tripe was seen as being abundant in protein and vitamin B12, and finally kidneys showed high amounts of protein and contain niacin and riboflavin (Marti et al., 2011).

In addition to these, Waltham (n.d) states that liver also contains taurine, vitamin K, choline and some vitamin E; heart also has taurine, vitamin B12 and choline, and tripe has vitamin B5. Kidneys also showed the presence of taurine as well as vitamins B7 and B12, with lung also having vitamin B12 as it is only found in animal products.

To support this information, a study carried out by Purchas and Wilkinson (2013) has identified the specific fatty acid profile and the vitamin and mineral content of 23 beef and 25 lamb cuts and offal items. Information on the offals of interest for this study were collated and are provided in Appendix B. The limitation of this study is that the specific amino acids present in each offal item were not evaluated, only the overall protein content was analysed. Information regarding the content of essential amino acids for humans in various beef and lamb offals including heart, liver, kidney and lung have been reported by Ockerman and Hansen (2000) and is given in Appendix C.

With meat proteins being an essential part of cat diets and also important in dogs, an assessment of the amino acid profiles of beef and lamb meats and offals may prove to be vital for this work, particularly when evaluating what drives the preference for certain ingredients over another.

## 2. Study Aims and Hypotheses

In the pet food industry, the development of products are often based on trial and error approaches, as little is known about the impact of individual ingredients on the overall palatability. The aim of this thesis is to therefore provide an in-depth evaluation of the palatability of offals within single species and between equivalent offals of different species using controlled testing methods. The results from these series of studies may then be used by manufacturers as a beneficial guide when looking to formulate new products or reformulate existing diets.

After evaluating the drivers of palatability, the methods available for palatability testing and understanding the purpose of this study, the following aims and hypotheses for this project have been determined.

Preliminary studies will look to determine the form ingredients should be delivered in for palatability testing. Cats are known to reject food whose temperature is below 15°C or above 50°C (Zaghini, & Biagi, 2005). Raw ingredients will be evaluated to limit variability in palatability due to the production of desirable compounds via Maillard reaction or the formation of lipid peroxides to give undesirable flavours (Hagen-Plantinga et al., 2017). To be consistent with Houpt and Smith (1981), minced vs cubed ingredients at room temperature will be compared against each other.

The first stage of palatability testing will involve comparing the intakes of selected offal from the same species. This will be carried out to determine whether certain offals are more palatable than others. It is expected that palatability differences will be observed between the various offals.

From here, an analysis of the amino acid and fatty acids profiles of chosen ingredients will be undertaken, and the results will be used to identify the nutrient components that either drive or hinder palatability. The fatty acid profiles of beef and lamb offals have been evaluated by Purchas and Wilkinson (2013), however their amino acid contents were not investigated in the study. With cats showing preference for 'sweet' amino acids and rejecting those considered 'bitter' (Bradshaw et al., 1996 and Zaghini and Biagi 2005), evaluating the amino acid composition of each ingredients may provide further evidence on why some offals may be more palatable than others and provide data that does not currently exist about ingredients in pet food.

Finally, once identifying the offals that are most palatable within a species has been completed, comparing the same offal type from different species can be carried out. This will be used to determine whether the ingredient species has an influence on palatability or whether palatability is dependent solely on the offal type.

### 3. Developing the Palatability Testing Protocol

Before undergoing palatability testing, it is important to establish a robust testing protocol. In order for this to take place, identifying the factors that have a positive influence on palatability and putting measures in place to maximise these effects need to be carried out.

Along with developing a standardised testing protocol, this trial aims to identify whether or not the format in which ingredients are presented to cats has an impact on palatability. A study by Houpt and Smith (1981) revealed preference for canned meat versus fresh meat, minced meat versus chunks of meat, and cooked meat versus raw meat were observed in dogs. Such studies of this kind have not been carried out in cats and may be of great importance, particularly as the size, shape, texture and serving temperature of food can play a vital role in the palatability of foods to cats.

In this study, the preference of cats for minced lamb kidney versus cubed lamb kidney was evaluated. The result was used to determine the format in which ingredients were presented in further palatability testing.

In addition to selecting the best meat preparation, identifying other ingredient preparation and testing factors that may impact palatability will also be evaluated and controlled where possible in order to develop a standardised testing protocol.

#### 3.1 Materials and Methods

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

##### 3.1.1 Test Animals

A designated panel of eight domestic short hair cats were used for the study to test the preference for lamb kidney presented in two different forms, minced and cubed. The cats used in this trial were healthy and consisted of four entire females and four castrated male cats aged 18 months to 13 years. Information on the gender and age of each individual cat can be found in Appendix D.

Testing was carried out from Monday 23<sup>rd</sup> April until Friday 27<sup>th</sup> April 2018 at the Feline Nutrition Unit at Massey University, Palmerston North.

##### 3.1.2 Ingredient Used

Lamb kidney was selected as the ingredient to use for determining the most suitable preparation method to use in future palatability testing. Lamb kidney is a common ingredient in pet food



manufactured in New Zealand and was readily available for this trial. A 15kg frozen block of lamb kidney used in the trial was provided by Wilbur Ellis, based in Longburn, New Zealand.

### 3.1.3 Ingredient Preparation

Half of the frozen block of lamb kidney was cut into 2 x 2 x 2cm cubes using a band saw and these were vacuum packed into 1kg portions. The remaining half was minced through an 8mm hole plate and vacuum packed into 1kg portions.

All bags of product were refrozen in a -27°C freezer. On each day prior to testing, one bag of minced kidney and one bag of cubed kidney was placed in a 7°C fridge to thaw overnight.

On the day of testing, the thawed minced and cubed kidney were placed on a 1.00mm steel sieve to separate the solid matter and the excess purge. Once separated, the solids were evenly distributed into eight bowls containing cubed kidney and an additional eight bowls containing the minced preparation. An example of the meat preparations presented to three cats are shown in Figure 3.1.

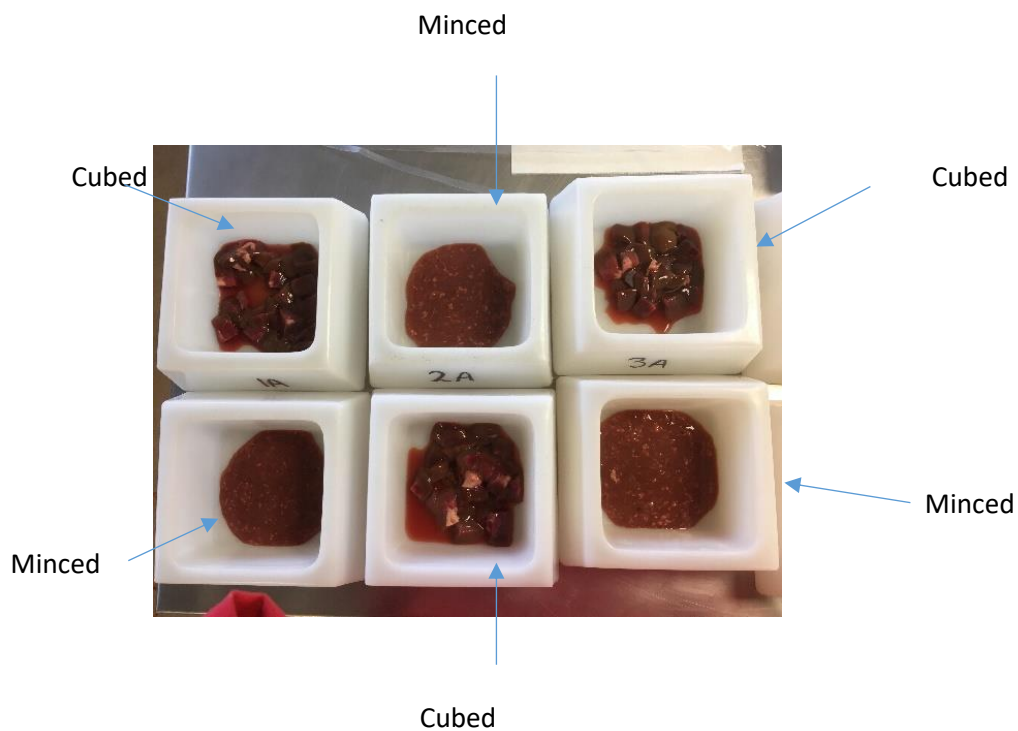


Figure 3.1: Presentation of the bowls showing the minced and cubed lamb kidney for three cats

On the first day of testing, temperature probes were used to test the meat at 15-minute intervals until a temperature of 18°C was obtained, the temperature at which the palatability testing room was set to for this trial. This process was carried out to ensure the cats would not reject

the kidney due to the ingredient being too cold, as cats are known to be sensitive to the temperature at which food is served (Zaghini & Biagi, 2005).

### 3.1.4 Purge Loss

The first parameter measured in the ingredient preparation stage was the amount of purge present in each 1kg bag of the thawed minced and cubed kidney preparations. This was carried out to determine how much solid kidney could be used for testing and identify the amount of product lost as purge. Table 3.1 shows the amount of purge found in each sample of kidney expressed in both grams and as a percentage of the original product weight. Complete data showing the solid and liquid amounts and percentages are provided in Appendix D.

*Table 3.1: Amount of kidney lost as purge expressed in both grams and as a percentage*

		Day 1	Day 2	Day 3	Day 4	Average
Minced	Product weight (g)	1072.9	956.7	1039.8	957.6	1006.8
	Liquid and losses (g)	772.7	699.8	832.2	725.6	757.6
	Percentage purge (%)	72.0	73.1	80.0	75.8	75.2
Cubed	Product weight (g)	1046.8	968.9	1014.9	1004.1	1008.7
	Liquid and losses (g)	312.2	217.9	254.2	283.2	266.9
	Percentage purge (%)	29.8	22.5	25.0	28.2	26.5

The results show that for an approximate 1kg bag of minced kidney, between 72.0% to 80.0% of the kidney, with an average ( $\pm$  SEM) of  $75.2 \pm 1.8\%$ , was lost as purge. This allowed for 25g to 62g of minced product to be distributed over eight bowls. In contrast, the cubed kidney showed a lower range of purge loss (22% to 30%), with an average ( $\pm$  SEM) of  $26.5 \pm 1.8\%$ . This allowed for a greater amount of cubed kidney to be distributed across the eight bowls (ranging from 89g to 106g), compared to the minced kidney. From the purge alone, use of the cubed kidney would be seen as advantageous as less product is lost in the preparation stage.

In future trials, it is also important that equivalent amounts of raw material be presented in both bowls. It should be noted that prior to testing, 100g portions were selected to be the desired serving size in each bowl. If the minced product was tested in future studies, the batch size for a single week-long test would be 35kg compared to 12kg for the cubed, therefore it is evident that the cubed product is more efficient going forward. However, due to the high amount of product lost as purge and this only being determined during the ingredient preparation stage, adjustments needed to be made to ensure each cat was presented with a minimum of 25g minced and 89g of cubed kidney.

### 3.1.5 Thaw Time

The time required for the bowls of kidney to reach an ambient temperature of 18°C was the next factor to determine as part of the testing methodology. Recording the temperature of the kidney every 15 minutes until three consecutive readings of 18°C were displayed was used to find the necessary standing period before presenting the bowls to the cats.

Figure 3.2 shows the time taken for 18°C to be achieved for the minced and cubed kidney. It was found that the minced option was at the desired temperature 70 minutes after being removed from the fridge. The cubed kidney took two hours (120 minutes) to reach 18°C after being removed from the fridge.

The longer thawing time for the cubed is expected as temperature readings were taken at the centre of the cubes. In comparison, the thin layer of minced kidney was enough to cover the surface of the bowl allowing the desired temperature to be achieved faster.

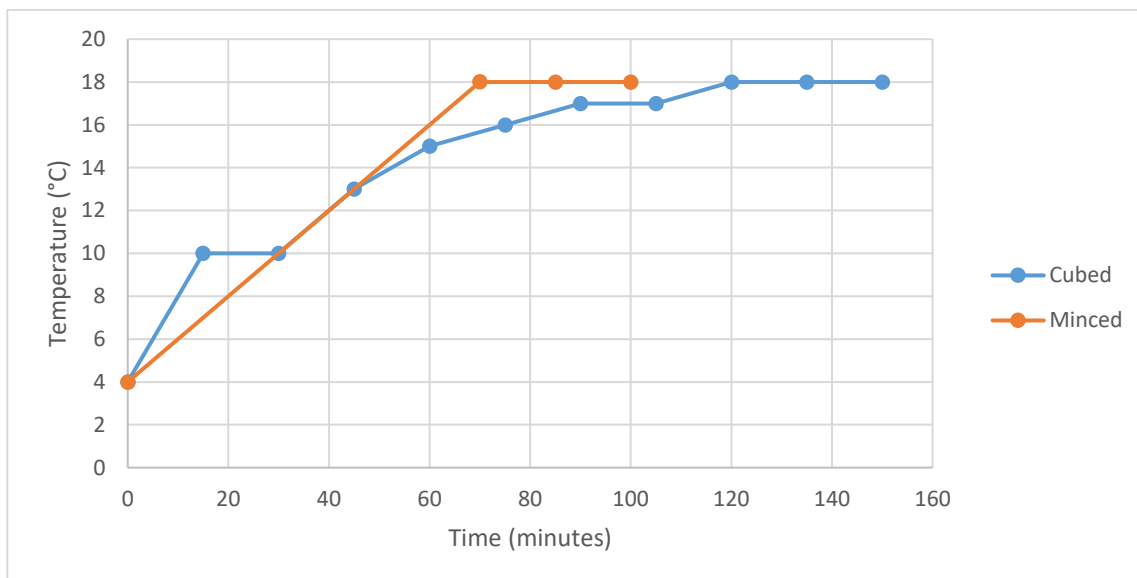


Figure 3.2: Time taken for the minced and cubed kidney to achieve an ambient temperature of 18°C

Two hours was therefore used as the standing period throughout the remainder of the week during preparation to ensure the all samples were at room temperature before palatability testing. This time period was adopted for all future palatability testing.

### 3.1.6 Testing Methods

A two-bowl test was used to determine the preference for cubed or minced kidney. Each cat was placed in an individual testing booth for one hour and was presented with two bowls, one containing cubed kidney and the other containing minced kidney.

The amount of minced kidney presented each day ranged from approximately 25g to 62g, with cubed ranging from 89 to 106g. Variation was observed due to the amount of purge present in each bag of raw material (see Section 3.1.4)

The position of the minced and cubed kidney bowls was switched each day of testing throughout the four day period to remove side bias.

The cats were given the offal for testing at the same time each day, 10:00 am. Following testing, the cats were given their usual canned diets that were available until the following morning when they were removed at 8:00am. This provided a two hour fast prior to feeding the offals for testing.

### 3.1.7 Data Collection and Statistical Analyses

Palatability was determined as both the food intake (g) and was converted to a percentage consumption (%) using the following equations:

$$\text{Food intake (g)} = \text{weight of bowl before consumption (g)} - \text{weight of bowl after consumption (g)}$$

$$\text{Percentage consumption (\%)} = \frac{\text{food intake (g)}}{\text{initial weight of food (g)}} \times 100$$

This was carried out as the amounts of minced and cubed kidney presented to the animals each day were not equivalent. As a result, the amount of food consumed relative to the amount initially presented needed to be evaluated.

## 3.2 Results and Discussion

### 3.2.1 Food Intake

Thirty two measurements were collected over the four day period to reveal whether or not the cats showed preference for one meat preparation over the other, no preference or whether they disliked both options.

The weight of each bowl before and after testing each day was recorded and converted to a percentage consumption as shown in Table 3.2. As eight cats were each presented with two bowls, the bowls were coded using the number corresponding to the test subject, followed by C for cubed and M for minced.

Table 3.2: Percentage consumption (%) of lamb kidney throughout the four day testing period

Bowl	Percentage consumption (%)				
	Day 1	Day 2	Day 3	Day 4	Average $\pm$ SEM
1C	50	96	99	98	85.8 $\pm$ 11.9
1M	63	47	59	56	56.3 $\pm$ 3.4
2C	1	100	99	100	75.0 $\pm$ 24.7
2M	2	98	100	99	74.8 $\pm$ 24.3
3C	1	100	99	100	75.0 $\pm$ 24.7
3M	4	98	97	99	74.5 $\pm$ 23.5
4C	1	7	33	99	35.0 $\pm$ 22.4
4M	2	6	98	87	48.3 $\pm$ 25.7
5C	99	99	98	99	98.8 $\pm$ 0.3
5M	94	97	96	98	96.3 $\pm$ 0.9
6C	1	99	99	100	74.8 $\pm$ 24.6
6M	14	97	94	97	75.5 $\pm$ 20.5
7C	57	23	5	8	23.3 $\pm$ 11.9
7M	1	78	2	2	20.8 $\pm$ 19.1
8C	96	98	99	100	98.3 $\pm$ 0.9
8M	79	98	97	97	92.8 $\pm$ 4.6

All information on the weight of the bowls before and after testing as well as the initial weight of food can be found in Appendix D.

When analysing results, the difference between the minced and cubed percentage consumption for each cat was used to identify whether or not a preference was observed. If a difference of greater than or equal to 5% was shown, this indicated a difference in preference between the two preparations. If the difference in percentage consumption was less than 5%, this indicated no preference between the minced or cubed kidney. Finally, any cat that consumed 5% or less of food from both bowls in a single day were deemed to dislike both options provided.

Individual results from all cats on each day of testing can be found in Appendix D, however a summary of the overall preference from the 32 measurements are shown in Table 3.3.

Table 3.3: Overall preference for each meat preparation option

	Total
Minced	4
Cubed	9
No preference	15
Dislike both	4

Almost half of the measurements recorded showed that the cats, when given the option between minced and cubed product, do not show a distinct preference for one over the other. On comparing the preference for minced to cubed, cubed was preferred on nine occasions compared with four occasions for the minced. On four occasions, some cats ate less than 5% of the food from both bowls which showed that they did not like either option. Three of these occasions occurred on the first day of testing, indicating that some of the cats experienced neophobia when presented with a diet they are not familiar with (Péron & Tobie, n.d.).

When considering the overall performance of each cat, six showed no preference for minced or cubed kidney and two preferred the cubed. This results indicate that the cats showed no distinct preference for one meat preparation over the other. In most cases, the cats consumed both the minced and cubed varieties as shown in Figure 3.3.

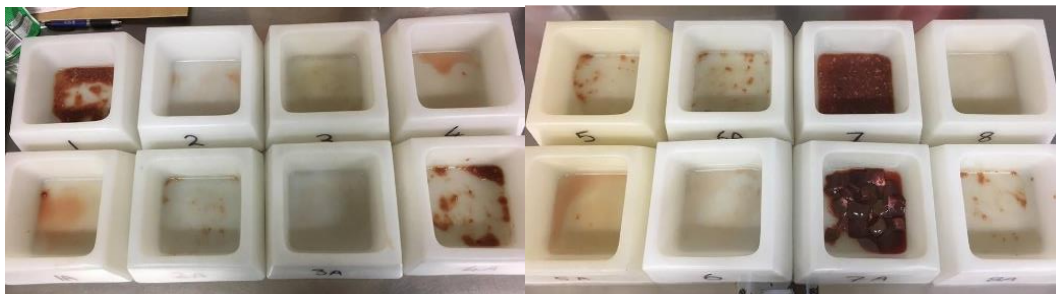


Figure 3.3: Images showing the bowls after the final day of palatability testing

From this data, when deciding on the best way to present the ingredients for palatability testing either format could be used. However, on comparing the minced and cubed measurements, cubed was the most preferred, so may be considered the better option to use in further trials.

In terms of preparation, cutting the meat into cubes was slightly easier and less time consuming compared to mincing. This was mainly due to fact that cutting cubes required only the use of the band saw, whereas the minced kidney required the frozen block to be cut into pieces first with the band saw to ensure they were small enough to fit in the mincer, which was an additional

step. In addition, less product was lost as purge when using cubed kidney compared to minced (see 3.1.4), providing further justification for using cubed over minced offal for future palatability testing.

Overall, the main findings from the trial was that cats showed no preference between the minced or cubed preparations. In most cases the cats consumed all of the kidney from both bowls so either meat preparation could be used for palatability testing, however there is a greater preference for the cubed preparation compared to the minced.

By considering all the findings from this trial as a whole, it was possible to identify parameters that need to be controlled before carrying out palatability testing. The results showed that no distinct preference was observed between minced and cubed, so future palatability testing will take place using 2 x 2 x 2cm cubes of product as it is the easiest to prepare and less product is lost as purge. In addition, plated samples containing 100g of product in each bowl will be left for two hours to reach a temperature of 18°C before being presented to the test cats for palatability assessment. As well as the results obtained, a detailed palatability testing protocol was able to be developed and will act as the foundation for future trials in this study.

## 4. Refinements to Cat Palatability Panel

Before undergoing full palatability trials, an alteration was made to the original panel of eight cats. Throughout the development of the testing protocol trial, it was observed that Cat 7 (Leo) showed a considerable decrease in intake of both the minced and cubed kidney on each day of testing compared to the other cats.

The old age of the cat, at 13 years, was identified as a potential contributing factor to his declining intake of both meat preparations so a decision was made to substitute Leo with another cat.

In order to find an appropriate replacement, a single two-bowl test was used to examine the preference for minced and cubed lamb kidney using four male cats aged between two and six years. The aim of this trial was to identify cats that showed preference for the cubed meat preparation or no preference between the two, as it was previously determined that the cubed format would be used in future palatability testing.

*Table 4.1: Information on the four possible male cats to replace Cat 7*

Cat	Name	Neutered	Date of Birth	Age* (years)
1	Ninja	Yes	14 December 2015	2.36
2	Orca	Yes	10 March 2015	3.12
3	Muse	Yes	4 January 2012	6.30
4	Paddy	Yes	17 March 2015	3.10

\* The age of the cats as at 23<sup>rd</sup> April 2018

The methods and materials outlined in Chapter 3 were followed in this trial using 400g portions of minced and cubed kidney. Data collection and statistical analyses also followed the same procedures previously mentioned.

### 4.1 Results and Discussion

A single two-bowl preference test was used to examine the preference for minced and cubed kidney. The results from the trial are given in Table 4.2, showing both the food intake and percentage consumption of each diet. The initial amount of food presented, as well as the weight of the bowls before and after testing are given in Appendix E.



Table 4.2: Results showing the food intake (g) and percentage consumption (%) of minced and cubed kidney for the four possible cats that may be used to replace Cat 7

Bowl	Food intake (g)	Percentage consumption (%)
1C	10.6	14
1M	18.0	95
2C	74.2	99
2M	18.3	98
3C	73.5	99
3M	18.0	97
4C	10.5	15
4M	0.4	2

It can be seen in Figure 4.1 that Cat 1 (Ninja) showed a clear preference for the minced kidney consuming 95% of the option provided compared to 14% of the cubed. Cats 2 and 3 (Orca and Muse) showed no preference between the two options, with 98 and 97% of the minced being consumed respectively, and both cats consuming 99% of the cubed variety. Finally, Cat 4 (Paddy) showed preference for the cubed option, although the percentage consumption of both minced and cubed was still relatively low in comparison to the other cats at 2% for the minced and 15% for the cubed.



Figure 4.1: Images showing the intake of minced and cubed kidney in the single palatability trial to replace Cat 7

Taking the results into account, Paddy was seen as an inappropriate replacement for Leo due to his overall low intake of both meat preparations. Ninja was also excluded as a possible replacement due to its preference for the minced over the cubed kidney preparation.

Orca or Muse were seen as the best possible replacements for Leo as both showed no preference between the two meat preparations, so Orca was chosen as the replacement for Leo from the original panel.

*Table 4.3: Information on the amended panel of eight cats used for palatability trials in this study*

Cat	Name	Gender	Neutered	Date of Birth	Age* (years)
1	Jetty	Female	No	1 December 2013	4.39
2	Kaia	Female	No	17 October 2016	1.52
3	Nyssa	Female	No	2 February 2016	1.22
4	Heka	Male	Yes	25 November 2013	4.41
5	Token	Female	No	1 December 2013	4.39
6	Fox	Male	Yes	28 December 2011	6.32
7	Orca	Male	Yes	10 March 2015	3.12
8	Gerrit	Male	Yes	25 November 2013	4.41

\* The age of the cats as at 23<sup>rd</sup> April 2018

It should be noted that in future trials, Orca will now be identified as Cat 7 for the remainder of the study. The information on the finalised panel of eight cats for the study are given in Table 4.3.

## 5. Acceptance of Lamb Offal

### 5.1 Materials and Methods

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

#### 5.1.1 Test Animals

A designated panel of eight domestic short hair cats was used to test the acceptance of six lamb offals. The cats used in this trial were healthy, and consisted of four entire females and four castrated male cats aged from 18 months to six years of age (average age of  $3.7 \pm 0.6$ ).

Information on the gender and age of each individual cat used in the first two weeks of testing is given in Section 4.3. After the first two weeks of testing, a final change to the composition of the test panel was made (see Table 5.1). Heka (Cat 4) was replaced with Pango due to persistent vomiting following consumption of the food.

*Table 5.1: Information on the panel of cats used for the remaining four weeks of lamb offal acceptance testing.*

Cat	Name	Gender	Neutered	Date of Birth	Age* (years)
1	Jetty	Female	No	1 December 2013	4.39
2	Kaia	Female	No	17 October 2016	1.52
3	Nyssa	Female	No	2 February 2016	1.22
4	Pango	Male	Yes	26 February 2014	4.16
5	Token	Female	No	1 December 2013	4.39
6	Fox	Male	Yes	28 December 2011	6.32
7	Orca	Male	Yes	10 March 2015	3.12
8	Gerrit	Male	Yes	25 November 2013	4.41

\* The age of the cats at the start of testing (23<sup>rd</sup> April 2018).

Acceptance testing was carried out from Monday 28<sup>th</sup> May until Friday 13<sup>th</sup> July 2018, with a final preference test between the top and bottom ranked lamb offal taking place from Monday 29<sup>th</sup> October until Friday 2<sup>nd</sup> November. All testing was carried out at the Feline Nutrition Unit at Massey University, Palmerston North.

#### 5.1.2 Ingredient Used

Six lamb offal varieties were evaluated in this block of acceptance tests. These consisted of: lung, heart, kidney, tripe, mechanically deboned meat (MDM) and liver, and they were tested in this

order. All ingredients were provided by MPI-accredited meat processors through Zivi Ltd (Mount Maunganui, New Zealand), and were delivered in approximately 20kg frozen blocks.

### 5.1.3 Ingredient Preparation

All the frozen blocks of lamb offal were cut into 2 x 2 x 2cm ( $\approx$ 5g) cubes using a band saw and were all separately vacuum packed into 2kg portions, except for the MDM. The MDM showed signs of crumbling once cut, so it was cut into larger blocks of roughly 250g before vacuum packing into 2kg portions.

Additional 250g samples of each offal were also collected and vacuum packed separately in preparation for future nutritional analyses.

All prepared bags of by-product were refrozen in a  $-27^{\circ}\text{C}$  freezer. On each day prior to testing, one bag of offal was placed in a  $7^{\circ}\text{C}$  refrigerator to thaw overnight.

On the day of testing, the offal was placed in a 1.00mm aperture steel sieve to separate the solid matter from the excess purge. Once separated, all testing bowls were filled with 100g of offal.

An example of the lamb lung preparations for four cats is shown in Figure 5.1.



*Figure 5.1: Presentation of the bowls showing 100g portions of lamb lung for four cats*

Following allocation, all bowls were left to stand for two hours to ensure that all samples were at room temperature before palatability testing commenced.

Due to the heterogeneity of the lung samples, pieces of hard bronchioles, as shown in Figure 5.2, were removed to ensure that the lung offal was consistent and textural differences did not influence palatability.



*Figure 5.2: Examples of the hard bronchioles that were removed prior to testing*

#### 5.1.4 Testing Methods

A two-bowl test was used to determine the total food intake and percentage consumption of each lamb offal (Tarttelin, 1997; Tobie et al., 2015). The eight cats were placed in individual testing booths for one hour a day for five days for each offal. This was carried out to obtain 40 measurements for each offal variety. It should be noted that cats were removed before one hour had elapsed if they had consumed all offal from both bowls.



*Figure 5.3: Set up of the cages for the two-bowl acceptance test*

All cats were presented with two bowls, each containing 100g of the offal being tested with an exception for lamb liver. Liver is known to have a high vitamin A content (Purchas and Wilkinson, 2013) and a safe maximum daily intake of vitamin A for cats is 333,300 IU/kg of diet (equivalent to 99.99 $\mu$ g/g as retinol) (AAFCO, 2017).

Therefore the amount of liver presented to the cats was adjusted as follows: Given the recommended maximum vitamin A content of a diet for cats is given by AAFCO (2017) as 99.99  $\mu$ g/g as retinol, this value was divided by the level of vitamin A level found in raw lamb liver from Purchas and Wilkinson (2013) of 154.34  $\mu$ g/g and multiplied by one hundred which gave a maximum intake of 65g/day. So, 65g of liver was presented to each cat.

The cats were given the offal for testing at the same time each day, 10:00 am. Following testing, the cats were given their usual canned diets that were available until the following morning when they were removed at 8:00am. This provided a two hour fast prior to feeding the offals for testing.

#### 5.1.5 Data Collection and Statistical Analyses

On an individual cat basis, acceptance was determined initially as the food intake (g) and this figure was converted to a percentage consumption (%) using the following equations:

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

$$\text{Percentage consumption (\%)} = \frac{\text{food intake (g)}}{\text{initial weight of food (g)}} \times 100$$

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

The average amount of offal consumed each day was then divided by the total amount of offal consumed over the week to calculate the percentage daily intake of offal during the week.

The weekly percentage consumption of each offal by each cat was also calculated. This was used to determine the average percentage consumption of each offal by the panel of cats.

To account for the difference in the amount of liver presented to the cats, only the average percentage consumption of the other offals over the week were compared with the liver intake values.

Tukey analysis was carried out in Minitab 18 (Minitab Inc., State College, Pennsylvania, USA) and used to determine statistical differences between the percentage consumption of all the possible pairings of offal using a significance level of  $P < 0.05$ . Grouping from the Tukey analysis was also used to develop a final rank of offal acceptance.

Interactions between offal intake and days of testing, as well as cats and offal intake were also analysed using a PROC mixed model in SAS 9.4 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA).

## 5.2 Results and Discussion

Forty measurements for each of the six lamb offals were obtained over each five-day testing period using the eight cat panel to evaluate the acceptance of lamb lung, heart, kidney, tripe, MDM and liver.

### 5.2.1 Food Intake

All cats were offered 1kg of lamb offal each week (200g a day), except in the case of liver where 325g (65g a day) was presented. The total intake over the testing period for each offal ( $\pm$  SEM) was 727.1  $\pm$  69.9g for lung, 704.4  $\pm$  73.1g for heart, 912.5  $\pm$  53.8g for kidney, 696.0  $\pm$  65.6g for tripe, 342.8  $\pm$  53.1g for MDM and 307.8  $\pm$  9.7g for liver.

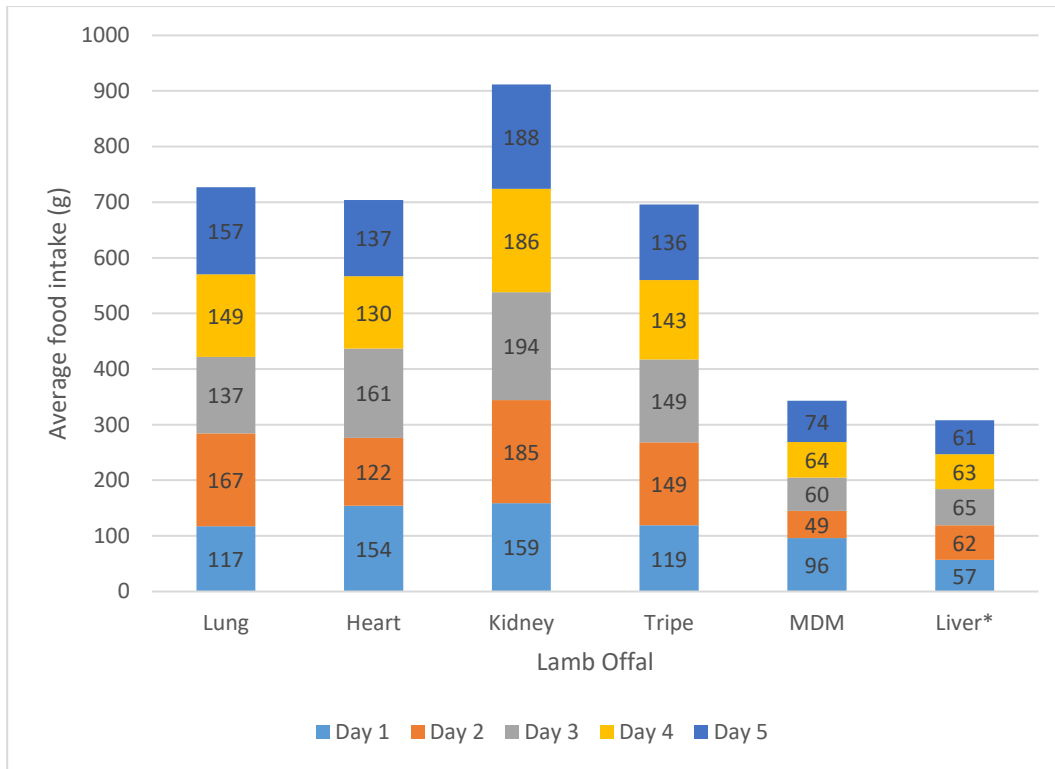


Figure 5.4: Average food intake of the six lamb offal out of the possible 1000g served throughout the week (\*maximum possible intake of liver was 325g compared to 1000g for the other offal varieties)

Figure 5.4 shows the average food intake on each day of acceptance testing. The consumption appears lower on Day 1 for lung, kidney, tripe and liver, with lung showing lower intakes on Day 1 compared to Day 2 ( $P < 0.05$ ). The cats displayed neophobic effects, defined by Bourgeois et al., (2006) as the avoidance of a new food, when fed lung as it was the first offal presented in the study.

In contrast, lamb heart intake was high on the first day of testing (154g) then fluctuated during the rest of the week, although no statistical significance between daily intakes were observed ( $P > 0.05$ ). For liver, no difference between daily intakes were observed ( $P > 0.05$ ) as the panel members consistently consumed 100% of the liver presented throughout the week, as shown in Figure 5.5c and d.

Intake of MDM was lower than the other offals ( $P < 0.05$ ). Although the greatest amount of MDM was consumed on Day 1 (96g), the intake on Day 2 (49g) was significantly lower ( $P < 0.05$ ). When considering the intake of MDM in isolation, the Day 1 intake is consistent with findings by Péron & Tobie (n.d.). They found that cats (and dogs) tend to display neophilic behaviour, which is defined by Bourgeois et al., (2006) as the preference for a food that has never been encountered before. However, when compared with lamb lung, heart and kidney intakes on Day 1 of testing, the amount of MDM consumed was significantly lower ( $P < 0.05$ ).

The results from acceptance testing, therefore, highlights the findings from Bradshaw et al (1996) that cats can detect small differences in the composition of food they are offered. As a result, the macronutrient composition is shown in Table 5.2, and the amino acid and fatty acid profiles of each offal presented in this study can be found in Appendix F.

*Table 5.2: As fed moisture, crude fat, crude protein and ash content in the six lamb offal varieties*

Lamb Offal	Moisture (%)	Crude Fat (%)	Crude Protein (%)	Ash (%)
Heart	73.7	15.3	10.8	0.6
Kidney	73.5	4.3	20.2	1.6
Liver	63.4	5.6	25.4	1.7
Lung	80.4	2.8	15.2	1.0
MDM	64.2	20.8	11.1	3.5
Tripe	76.2	6.4	16.0	1.0

MDM protein levels were the second lowest (11.1% as fed) and it had the highest amount of fat (20.8% as fed) relative to the other offals presented. With the exception of heart, which had the lowest amount of protein (10.8% as fed) and the second highest fat content (15.3% as fed), all remaining offal showed favourable high protein contents (15.2% to 25.4% as fed) and considerably low fat contents (2.8% to 6.4% as fed). Throughout the week of MDM testing, the intake remained well below 100g while the intake of lung, heart, kidney and tripe were well into the 120 to 180g region. Additionally, all lamb liver presented was consumed on each day of testing.

Previous research by Stasiak (2002) and Zaghini & Biagi (2005) indicated that cats have very high protein requirements and are also able detect off-notes due to lipid oxidation, which is known to decrease palatability. As an ingredient with a high fat and relatively low protein content, MDM was identified as having a relatively unfavourable macronutrient composition compared to the



other offals for cats. This was supported by the intake patterns observed with the cats simply consuming some MDM on Day 1 as it was still novel to them before displaying near complete refusal to eat any MDM at all (as shown in Figure 5.5a and b) on the remaining days.

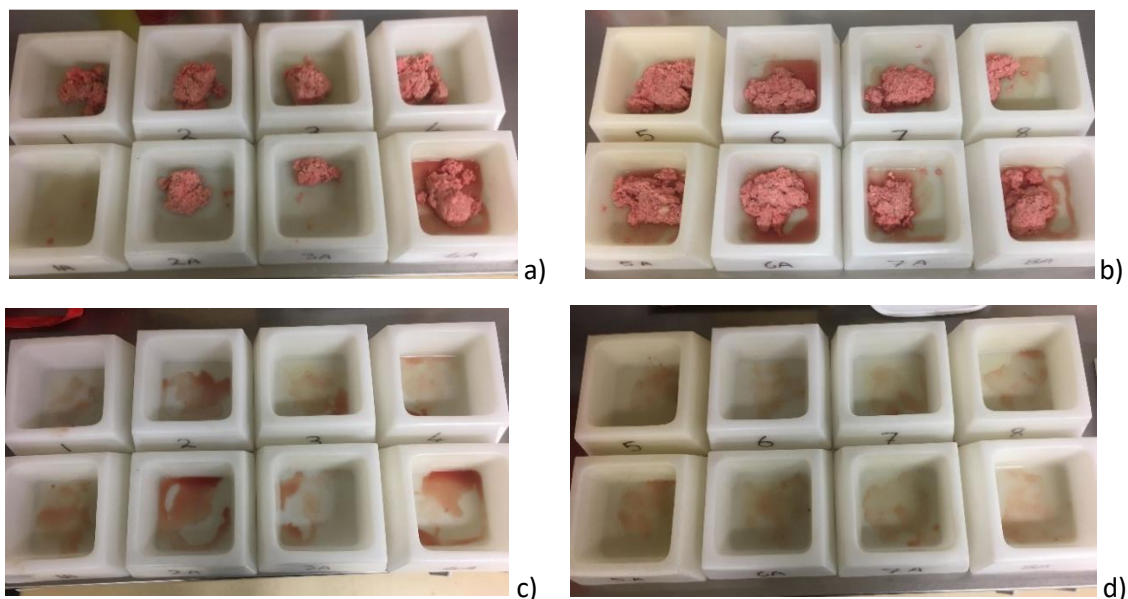


Figure 5.5a-d: Top: Remaining MDM after Day 3 of lamb acceptance testing.

Bottom: Remaining liver after Day 3 of lamb acceptance testing

Overall, with the exception of lung on Day 1 which showed a lower intake than that on Day 2 ( $P < 0.05$ ) and particularly MDM which showed a high intake on Day 1 compared to Day 2 ( $P < 0.05$ ), the fixed effect of day and the interaction between the day and offal had no significance on the intake results ( $P > 0.05$ ). However, the fixed effect of offal had significance on the intake results ( $P < 0.05$ ). This therefore indicated that cats demonstrated clear preferences for different lamb offals.

### 5.2.2 Distribution of Food Intake

Intake patterns were investigated further by determining the proportion of food eaten each day as a percentage of the total intake over the week (see Figure 5.6). This corrected for the lower amounts of liver offered compared to the other offals.

It was clearly shown that lung, kidney, tripe and liver had a slightly lower percentage consumption on the first day at 16.1, 17.4, 17.1 and 18.5%, respectively, than the expected 20% if intake was consistent over the entire week.

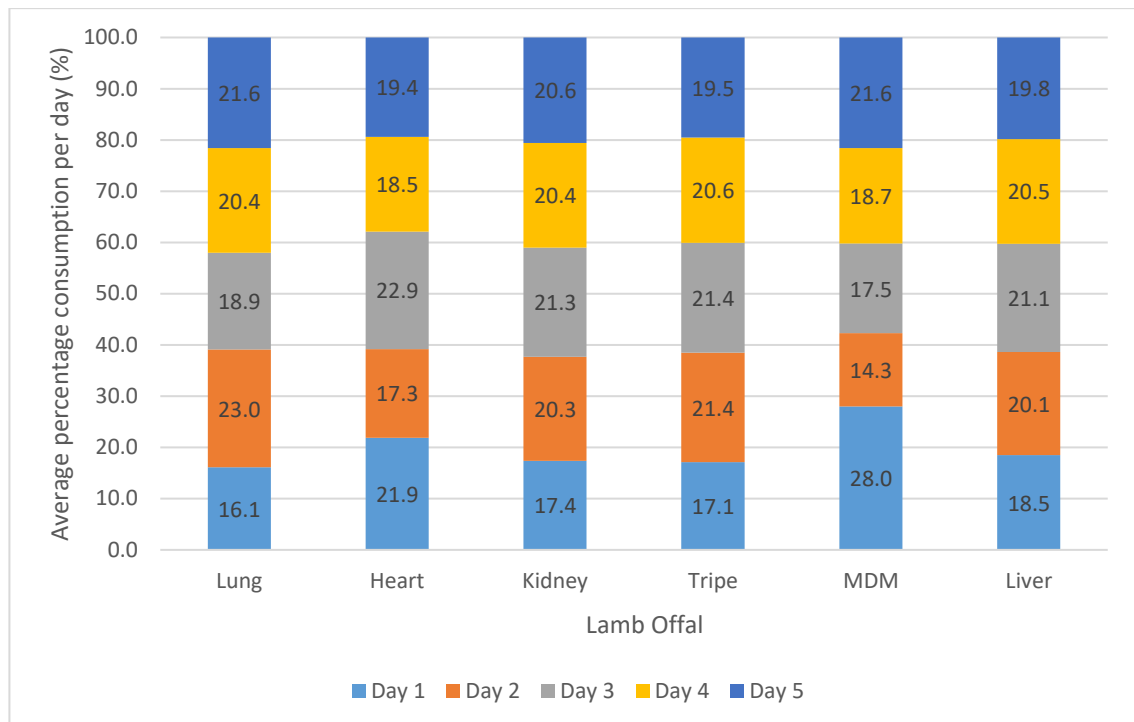


Figure 5.6: Average percentage consumption distribution of food consumed each day relative to the amount of food eaten throughout the testing week

MDM was the only ingredient that showed a significant ( $P < 0.05$ ) percentage intake decline between Day 1 and 2 (28% versus 14.3% of the weekly consumption). The Day 1 percentage consumption of MDM at 28% of the weekly intake (96g of 343g) was much higher than that of the remaining offals (which ranged from 16.1% to 21.9%) before falling for the remainder of the week. The difference between the overall intake of MDM versus the other lamb offal highlights that the panel consumed some MDM on Day 1 as it was a novel food to them, however, as the week of testing went on, the intakes of MDM declined and it was a less palatable ingredient than the other offals.

In contrast to the MDM results, lung showed the opposite pattern of intake. Day 1 showed the lowest relative intake at 16.1% (117g) with an increase to 23.0% (167g) on Day 2 ( $P < 0.05$ ). The percentage consumptions of liver, kidney and tripe were also lower on Day 1, however, the relative consumption was more consistent throughout the week with fluctuations of only 2.6, 3.9 and 4.3% being observed ( $P > 0.05$ ).

Finally, the percentage consumption of heart varied from day to day by up to 5.6%, with intake lowest on Day 2 (17.3%; 122g) and highest on Day 3 (22.9%; 161g), although not statistically significant ( $P > 0.05$ ).

### 5.2.3 Average Percentage Consumption of Offal

In order to observe the difference between the percentage consumption of individual cats in the panel, the weekly average intakes of each offal are shown in Table 5.3. For percentage consumption results on each day of testing, please refer to Appendix F.

*Table 5.3: Weekly percentage consumption results of the six lamb offal varieties for each cat in the panel during acceptance testing (Note: superscripts are to be compared within a column)*

Cat	Lamb Offal					
	Lung	Heart	Kidney	Tripe	MDM	Liver
1. Jetty	63.4 <sup>a,b,c</sup>	64.8 <sup>a,b</sup>	95.2 <sup>a</sup>	72.6 <sup>a,b</sup>	38.4	99.1 <sup>a</sup>
2. Kaia	94.0 <sup>a</sup>	69.8 <sup>a,b</sup>	99.0 <sup>a</sup>	53.2 <sup>a,b</sup>	35.8	85.8 <sup>a,b</sup>
3. Nyssa	63.0 <sup>b,c</sup>	82.1 <sup>a</sup>	85.9 <sup>a</sup>	60.6 <sup>a,b</sup>	38.4	98.5 <sup>a</sup>
4. Heka/Pango	36.3 <sup>c</sup>	25.4 <sup>b</sup>	55.3 <sup>b</sup>	36.9 <sup>b</sup>	3.3	77.2 <sup>b</sup>
5. Token	88.2 <sup>a,b</sup>	80.3 <sup>a</sup>	99.1 <sup>a</sup>	91.5 <sup>a</sup>	21.2	99.4 <sup>a</sup>
6. Fox	92.1 <sup>a,b</sup>	80.2 <sup>a</sup>	96.9 <sup>a</sup>	72.2 <sup>a,b</sup>	40.9	99.7 <sup>a</sup>
7. Orca	81.2 <sup>a,b</sup>	89.9 <sup>a</sup>	99.5 <sup>a</sup>	81.3 <sup>a</sup>	46.5	98.8 <sup>a</sup>
8. Gerrit	63.5 <sup>a,b,c</sup>	85.5 <sup>a</sup>	99.1 <sup>a</sup>	88.5 <sup>a</sup>	39.3	99.1 <sup>a</sup>
Average ± SEM	72.7±3.7	71.0±4.2	91.3±2.9	69.6±4.1	34.3±4.1	94.7±1.9

Cat 4 (Heka in weeks one and two when the lung and heart acceptance tests were carried out, and Pango for the remaining four weeks) consistently showed the lowest percentage consumption of all offals, with intakes ranging from 3.3% for MDM to 77.2% for liver. Intakes of lung, heart and kidney by these cats were lower than all other cats ( $P < 0.05$ ). For tripe and liver, intake from cat 4 was similar to cat 2 (Kaia) ( $P > 0.05$ ), and for MDM intake was similar to cat 5 (Token) ( $P > 0.05$ ). The remaining seven cats showed more consistent patterns of consumption of each offal.

For lung, cats 1 (Jetty), 2 (Kaia), 5 (Token), 6 (Fox), 7 (Orca) and 8 (Gerrit) showed similar intakes (63.4% to 94.0%;  $P > 0.05$ ) for the week. Cats 1, 3 (Nyssa), 5, 6, 7 and 8 also showed similar intakes (63.0% to 92.1%;  $P > 0.05$ ), as did cats 1, 3, 4 (Pango) and 8 (36.3% to 63.4%;  $P > 0.05$ ). However differences were observed between cat 4 versus cats 2, 5, 6 and 7 ( $P < 0.05$ ).

For heart, cats 1, 2, 3, 5, 6, 7 and 8 showed similar intakes (59.8% to 89.9%;  $P > 0.05$ ) for the week, as did cats 1, 2 and 4 (25.4% to 64.8%;  $P > 0.05$ ). However, differences were observed between cats 3, 5, 6, 7 and 8 versus cat 4 ( $P < 0.05$ ).

For kidney, the remaining seven cats showed similar intakes (85.9% to 99.5%;  $P>0.05$ ) for the week, with only cat 4 showing a lower intake of 55.3% to the rest of the panel ( $P<0.05$ ).

For tripe, cats 1, 2, 3, 5, 6, 7 and 8 showed similar intakes (53.2% to 91.5%;  $P>0.05$ ) for the week, as did cats 1, 2, 3, 4 and 6 (36.9% to 72.6%;  $P>0.05$ ). However, differences were observed between cats 5, 7 and 8 versus cat 4 ( $P<0.05$ ).

For MDM, lower intakes were observed for all cats compared to the other offals. All eight cats showed similar intakes (3.3% to 48.8%;  $P>0.05$ ) for the week.

For liver, cats 1, 2, 3, 5, 6, 7 and 8 showed similar intakes (85.8% to 99.7%;  $P>0.05$ ) for the week, as did cats 2 and 4 (77.2% and 85.8%, respectively;  $P>0.05$ ). However, differences were observed between cats 1, 3, 5, 6, 7 and 8 versus cat 4 ( $P<0.05$ ).

After removing the results for cat 4, the variability in percentage consumption, defined as the difference between the highest and lowest percentage consumption value of each offal was greatest for tripe, lung, MDM and heart with values of 38%, 31%, 27.6% and 25% observed respectively. In addition to the highest consumption levels, variability was lowest in kidney at 13%, followed by liver at 14%.

Overall, the fixed effects of cat and offal and the interaction between cat and offal had significance on the intake results ( $P<0.05$ ). This therefore indicates that individual cats showed differences between one another and also showed preferences for different offals.

To account for the lower amount of liver fed to cats (due to concerns regarding the high vitamin A content), the percentage consumption was calculated to determine an overall ranking of lamb offal (as displayed in the bottom row of Table 5.3). Liver was the offal most accepted by cats with 94.7% consumption followed closely by kidney at 91.3%. The third, fourth and fifth ranked offal were lung, heart and tripe at 72.7%, 71.0% and 69.6%, respectively. Although not an offal variety, MDM was least accepted by cats with a percentage consumption of 34.2%.

When comparing the mean consumption of all possible lamb offal pairings, all differences in means except for heart and lung, tripe and lung, tripe and heart and liver and kidney (highlighted in red) were significant ( $P<0.05$ ), as shown in Figure 5.7.

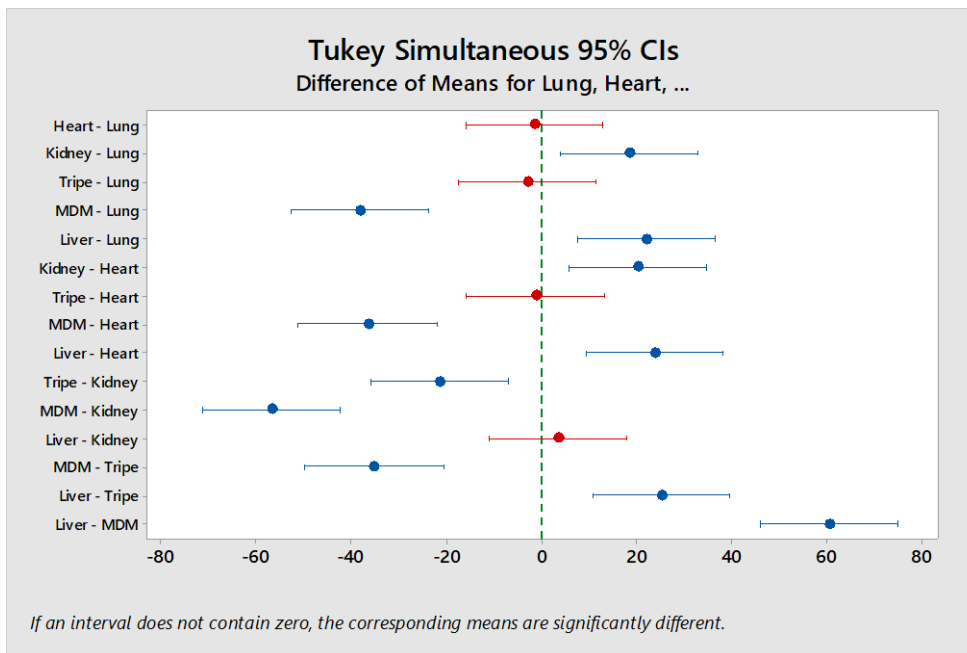


Figure 5.7: Tukey Simultaneous 95% Confidence Interval plot for all lamb offal pairings

The grouping information from the Tukey analysis was used to develop a final rank of offal acceptance and is shown in Table 5.4. In summary, kidney and liver were identified by the panel of cats as being equally highly palatable ( $P > 0.05$ ). Lung, heart and tripe were also identified as equally palatable ( $P > 0.05$ ) but less palatable ( $P < 0.05$ ) than kidney and liver. Finally, MDM was ranked the least palatable of all the ingredients with intakes lower ( $P < 0.05$ ) than all of the other offals.

Table 5.4: Tukey Analysis and final ranking of lamb offal

Offal	Mean Percentage Consumption (%)	Final Ranking
Liver	94.7 <sup>a</sup>	1
Kidney	91.3 <sup>a</sup>	
Lung	72.7 <sup>b</sup>	2
Heart	71.0 <sup>b</sup>	
Tripe	69.6 <sup>b</sup>	
MDM	34.3 <sup>c</sup>	3

#### 5.2.4 Preference Test between Top and Bottom Ranked Lamb Offal

A final two-bowl preference test was conducted to evaluate the intake patterns of the top and bottom ranked lamb offal following acceptance testing. With liver and kidney both being top

ranked, the decision was made to compare kidney to MDM to allow equivalent 100g portions to be evaluated side by side. Testing was carried out using load cells which recorded the real time feeding pattern of each cat.



Figure 5.8: Presentation of the bowls for the preference test (Note: this was the set up for Days 1, 3 and 5 of testing. For Days 2 and 4, offals were placed in the alternate bowls)

Forty measurements for lamb kidney and MDM were obtained over a five-day testing period using the same panel of cats. Each cat was presented with 100g of kidney and MDM offal each day (500g of each offal over the week). Throughout the five-day testing period, kidney was almost totally consumed on each day of testing for all cats, with the intake of MDM being significantly lower ( $P < 0.05$ ). The total intakes for kidney and MDM over the week were  $495.0 \pm 1.6$ g and  $148.5 \pm 37.1$ , respectively.

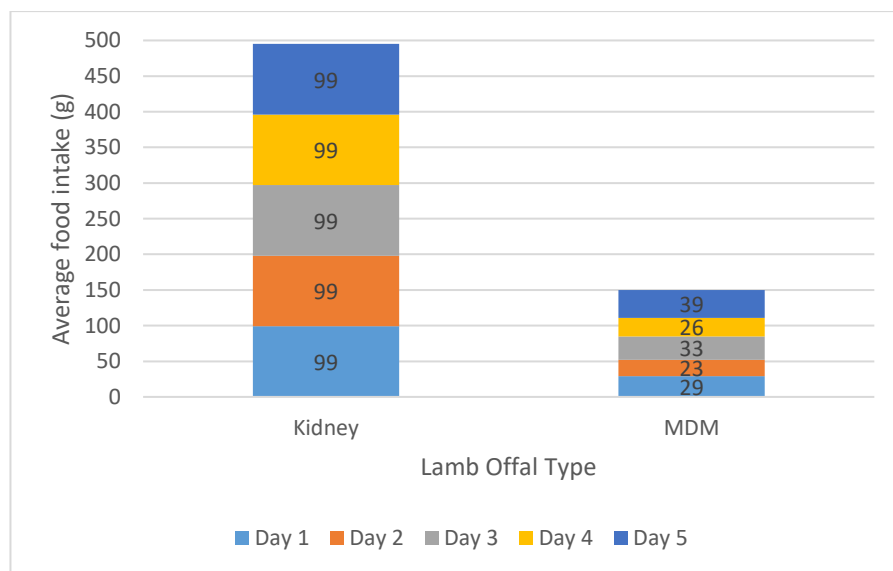


Figure 5.9: Average food intake of the top and bottom ranked lamb offal, kidney and MDM, on each day of preference testing

Figure 5.9 shows the intake of kidney and MDM on each day of testing. The cats showed clear preference for kidney on all days compared to MDM ( $P < 0.05$ ). Overall, the panel's average intake

of kidney and MDM showed statistical significance ( $P < 0.05$ ) indicating that kidney, at an average intake of 98.9%, was preferred over MDM with an intake of 29.7%.

When evaluating the percentage distribution each day (see Figure 5.10), no difference was observed between the distribution within kidney as near 100% consumption was observed on each day of preference testing and therefore resulting in equal 20% distributions being observed. Similarly, no significant difference were observed within MDM daily intakes ( $P > 0.05$ ).

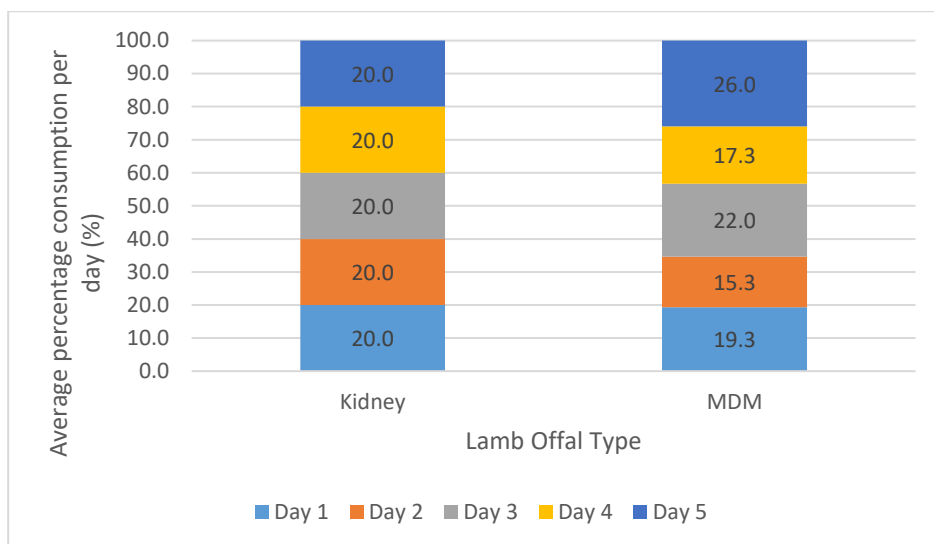


Figure 5.10: Average percentage consumption distribution of lamb kidney and MDM consumed each day over the preference testing week

The weekly average intake of both offals were also analysed to observe the difference in performance of individual cats in the panel. All cats in the panel had the same intake of kidney ( $P > 0.05$ ), one of the top ranked offals from acceptance testing. However, the intake of MDM varied amongst the cats in the panel. Cats 1, 2, 5, 7 and 8 showed similar intakes (34.7% to 57.8%;  $P > 0.05$ ) for the week, as did cats 1, 2, 5, 6 and 7 (17.88% to 45.7%;  $P > 0.05$ ) and cats 2, 3, 4, 6 and 7 (0.1% to 35.7%;  $P > 0.05$ ). However differences were observed between cats 1, 5 and 8 versus cats 3 and 4 ( $P < 0.05$ ), as well as between cat 8 versus cats 3, 4 and 6 ( $P < 0.05$ ). For percentage consumption results on each day of testing, please refer to Appendix F.

Further analyses were carried out by evaluating the intake pattern of lamb kidney and MDM as determined from the load cell results. All graphical outputs for each day of testing and for each cat can be found in Appendix F.

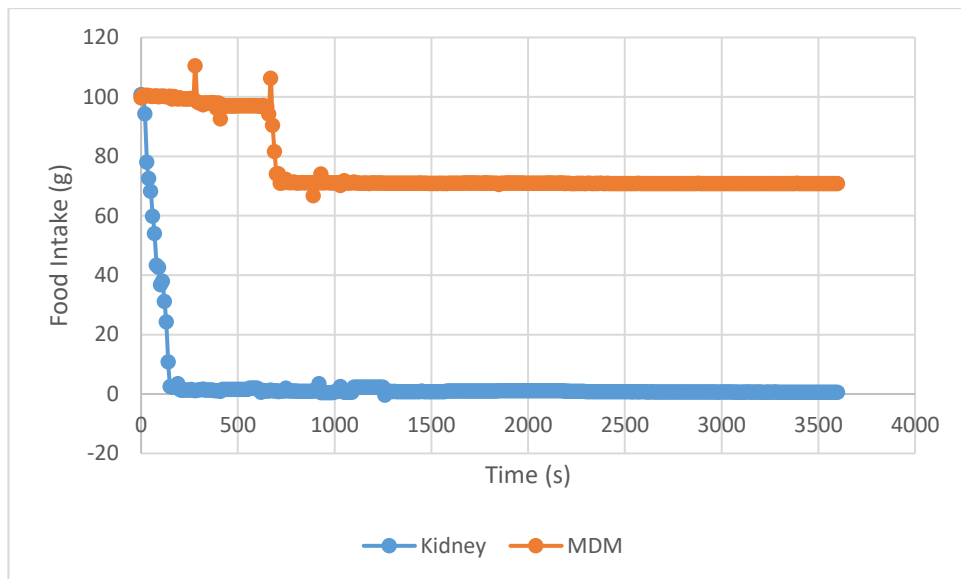


Figure 5.11: Example intake pattern of lamb kidney vs MDM (shown is the intake pattern of cat 7 intake on day 4 of testing)

A similar intake pattern was observed for all cats throughout the test. The cats consumed all of the kidney rapidly, before switching and subsequently consuming the MDM, as shown in Figure 5.11. Some cats, however, did not consume any MDM which indicated a strong preference for lamb kidney as an ingredient over MDM.

Pet food palatability and the amount of protein from animal origin has been found by Zaghini, & Biagi (2005) to be strongly correlated in cats (and dogs). The findings from this series of acceptance and final preference tests further indicate that the choice of offal within a single species also plays a role improving palatability.

When evaluating macronutrient composition of the top and bottom ranked offals (see Table 5.2), MDM contained the highest fat content (20.8%) and had the second lowest protein content (11.1%) of all lamb offals presented, making it unfavourable for cats due to their high needs for protein, as well as their sensitivity to lipid oxidation, which is known to decrease palatability (Stasiak, 2002 and Zaghini & Biagi, 2005). In contrast, kidney had the second lowest fat content of 4.3% and the second highest protein content of 20.2%, a more favourable macronutrient composition consisting of high protein and low fat.

It was clearly shown that MDM, an ingredient that is used extensively and in large volumes within pet food formulations, was not a highly palatable ingredient compared to the other offals. With such a clear drop in percentage consumption compared to the other ingredients, incorporating more offal into pet food may be a better option in future high value pet foods which will result in increased diet palatability.



## 6. Acceptance of Beef Offal

### 6.1 Materials and Methods

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

#### 6.1.1 Test Animals

The same cat panel as described in Section 5.1.1 was used for beef offal acceptance testing, except during week three of testing. During the final two days in week two of testing (beef heart analysis), there was a dramatic decrease in food intake throughout the colony. Kaia (cat 2) was severely affected and replaced with Muse for week three of testing (beef kidney analysis) before re-entering the panel the following week. Information on Muse's gender and age can be found in Section 4 in Table 4.1.

Acceptance testing was carried out from Monday 23<sup>rd</sup> July until Friday 7<sup>th</sup> September 2018, with a final preference test between the top and bottom ranked beef offal taking place from Monday 22<sup>nd</sup> October until Friday 26<sup>th</sup> November. All testing was carried out at the Feline Nutrition Unit at Massey University, Palmerston North.

#### 6.1.2 Ingredient Used

Six beef offal varieties were evaluated in this block of acceptance tests. These consisted of: lung, heart, kidney, tripe, mechanically deboned meat (MDM) and liver, and they were tested in this order. All ingredients were provided by MPI-accredited meat processors through Ziwi Ltd (Mount Maunganui, New Zealand), and were delivered in approximately 20kg frozen blocks.

#### 6.1.3 Ingredient Preparation

All the frozen blocks of beef offal were prepared in the same manner as the lamb offal (see Section 5.1.3).

#### 6.1.4 Testing Methods

The testing methods outlined in Section 5.1.4 were followed for the acceptance of beef offal, with two amendments.

- 1) The amount of beef liver that was presented

The recommended maximum vitamin A content of a diet for cats is given by AAFCO (2017) as 333,300 IU/kg (equivalent to 99.99µg/g as retinol), so the retinol value was divided by the level of vitamin A found in raw beef liver from Purchas and Wilkinson (2013) of 283.19 µg/g and

multiplied by one hundred which gave a maximum intake of 35g/day. So, 35g of liver was presented to each cat.

## 2) Number of days used for analysing beef heart acceptance

As food intake decreased severely in the last two days of testing, an additional three days of acceptance testing was conducted at the end of the beef offal testing to replace those two days. Rather than obtaining 40 measurements for one test, the beef heart analysis was evaluated based on 48 measurements, the first three days of the original heart testing and the three days of repeated testing.

### 6.1.5 Data Collection and Statistical Analyses

Please refer to Section 5.1.5 for the data collection and statistical analyses.

## 6.2 Results and Discussion

Forty measurements for beef lung, kidney, tripe, MDM and liver were obtained over five day testing periods using the eight cat panel (forty eight measurements over six days for beef heart) to reveal the acceptance of each of the six beef offals.

### 6.2.1 Food Intake

All cats were offered 1kg of beef offal each week (200g a day), except in the case of heart and liver. The maximum amount of heart was 1.2kg (200g over six days) and for liver it was 175g (35g a day). The total intake over the testing period for each offal ( $\pm$  SEM) was 716.0  $\pm$  67.6g for lung, 697.5  $\pm$  110.7g for heart, 750.6  $\pm$  106.2g for kidney, 622.1  $\pm$  100.4g for tripe, 239.4  $\pm$  40.0g for MDM and 172.9  $\pm$  0.4g for liver.

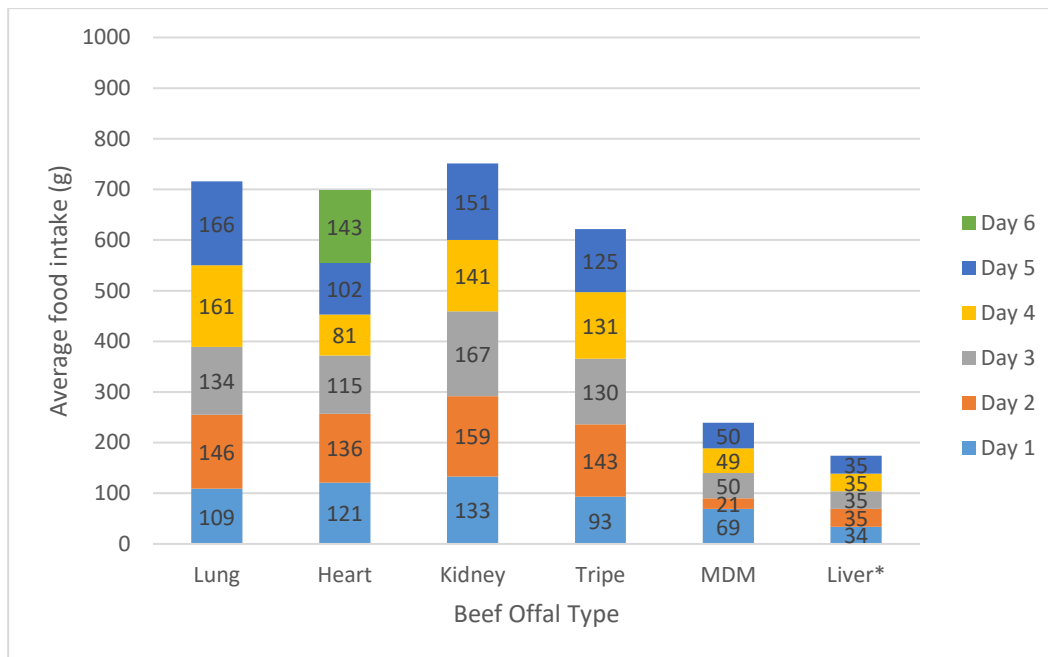


Figure 6.1: Average food intake of the six beef offal out of the possible 1000g served throughout the week (\*maximum possible intake of liver was 175g compared to 1000g for the other offal varieties and heart had a maximum intake of 1200g over six days)

Figure 6.1 shows the average food intake on each day of acceptance testing. The cats displayed clear neophobic behaviour when fed tripe on Day 1, with intakes increasing on Day 2 ( $P < 0.05$ ). Intakes were also lowest on Day 1 of testing for lung and kidney, although not low enough compared to the rest of the week to indicate neophobia ( $P > 0.05$ ).

The first two days of heart testing showed intake being comparable with that of lung, kidney and tripe ( $P > 0.05$ ), with a significant difference only being observed between Day 3 kidney testing and Day 3 heart testing ( $P < 0.05$ ). However, Day 4, the first day of heart retesting, showed a much lower intake ( $P < 0.05$ ) of 81g compared to Day 2 at 136g. Days 5 and 6 showed the intake for heart increasing. These results indicate that the unknown loss of appetite not only caused a significant drop between food intake on Day 4 compared to Day 2 of heart testing ( $P < 0.05$ ), but also showed intakes beginning to recover by Day 6 ( $P < 0.05$ ).

In contrast, beef MDM intake was highest on the first day of testing (69g), with intake dropping to 21g on Day 2 and rising to 50g for the rest of the week, although no significance between the intake of MDM consumed each day was observed ( $P > 0.05$ ). Overall, the Day 1 intake of MDM, as shown in Figure 6.1, at 69g was much lower ( $P < 0.05$ ) than the beef heart and kidney intakes of 121g and 133g, respectively. However, the intake of MDM was not different ( $P > 0.05$ ) to that of lung and tripe on Day 1 at 109g and 93g, respectively. As the week progressed, the intake of MDM relative to kidney, lung and tripe on the equivalent days (i.e Day 2 MDM versus Day 2

kidney) was lower ( $P < 0.05$ ). Intake of heart was higher than MDM on Days 2, 3 and 5 ( $P < 0.05$ ), but not Day 4 ( $P > 0.05$ ).

As with the lamb MDM, the results indicated that cats tried beef MDM on Day 1 before intakes declined, as shown in Figure 6.2a and b. This low intake of beef MDM may again be a result of the relatively low protein content (15.6% - third lowest of all the beef ingredients) and high fat content (40.0%), compared to the other beef offals. These all had a higher protein content (12.5% to 25.9% as fed) and a lower fat content (1.7% to 6.8% as fed) as shown in Table 6.1. Although the protein content of MDM is within the range of the other beef offals, the decrease in palatability may be driven by the high fat percentage and greater likelihood of lipid oxidation taking place (Zaghini and Biagi, 2005).

*Table 6.1: As fed moisture, fat, protein and ash content in the six beef offal varieties*

Beef Offal	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Heart	78.1	5.0	16.2	1.0
Kidney	77.3	6.8	14.4	1.1
Liver	62.6	2.7	25.9	1.8
Lung	76.4	1.7	20.4	1.3
MDM	41.5	40.0	15.6	2.1
Tripe	82.2	6.0	12.5	0.5

For more information on the amino acid and fatty acid profiles of each offal used in this study on an as fed basis, please refer to Appendix G.

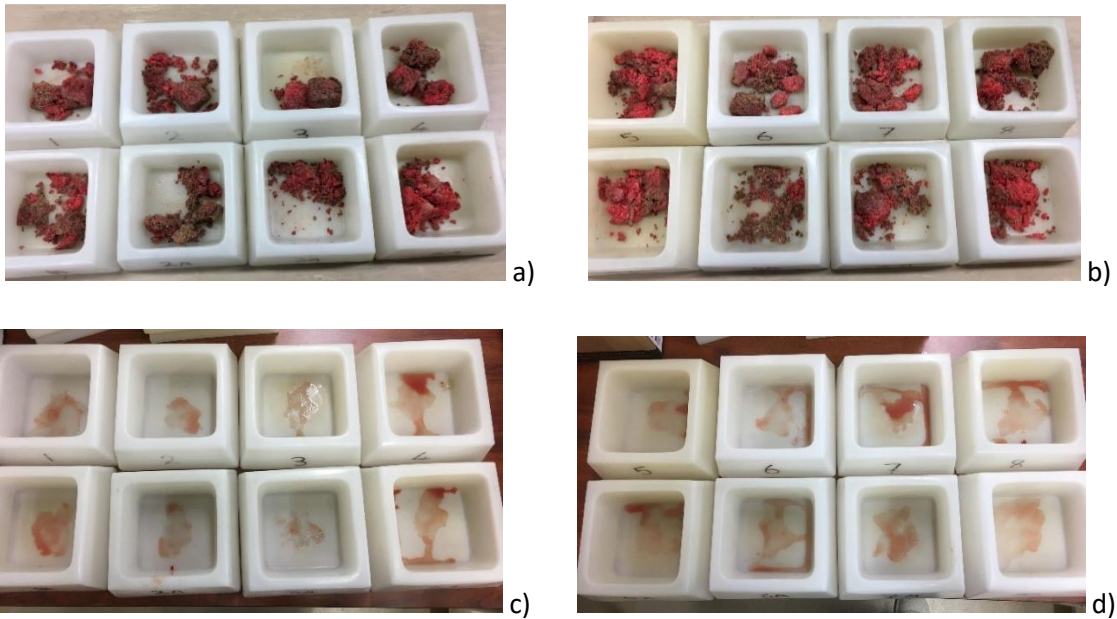


Figure 6.2: Top: Remaining MDM after Day 4 of beef acceptance testing.

Bottom: Remaining liver after Day 4 of beef acceptance testing

The intake of beef kidney remained consistent throughout the week ( $P > 0.05$ ). For liver, no difference between daily intakes were observed ( $P > 0.05$ ) as consistent near 100% consumption readings were recorded throughout the week, as shown in Figures 6.2c and d.

Overall, the fixed day effect and the interaction between the day and offal had no effect on the intake results ( $P > 0.05$ ). However, the fixed effect of offal had an effect on the intake results ( $P < 0.05$ ). This therefore indicated that cats demonstrated preferences between the different beef offals. In addition, neophobic behaviour was observed when feeding tripe on Day 1 compared to Day 2 ( $P < 0.05$ ).

### 6.2.2 Distribution of Food Intake

Intake patterns were investigated further by determining the proportion of food eaten each day as a percentage of the total intake over the week (see Figure 6.3). This corrected for the lower amounts of liver offered compared to the other offals.

It was clearly shown that lung, heart, kidney and tripe had lower percentage consumption on the first day at 15.2, 17.3, 17.7 and 15.0%, respectively, than the expected 20% if intake was consistent over the entire week (or 16.6% for heart over 6 days).

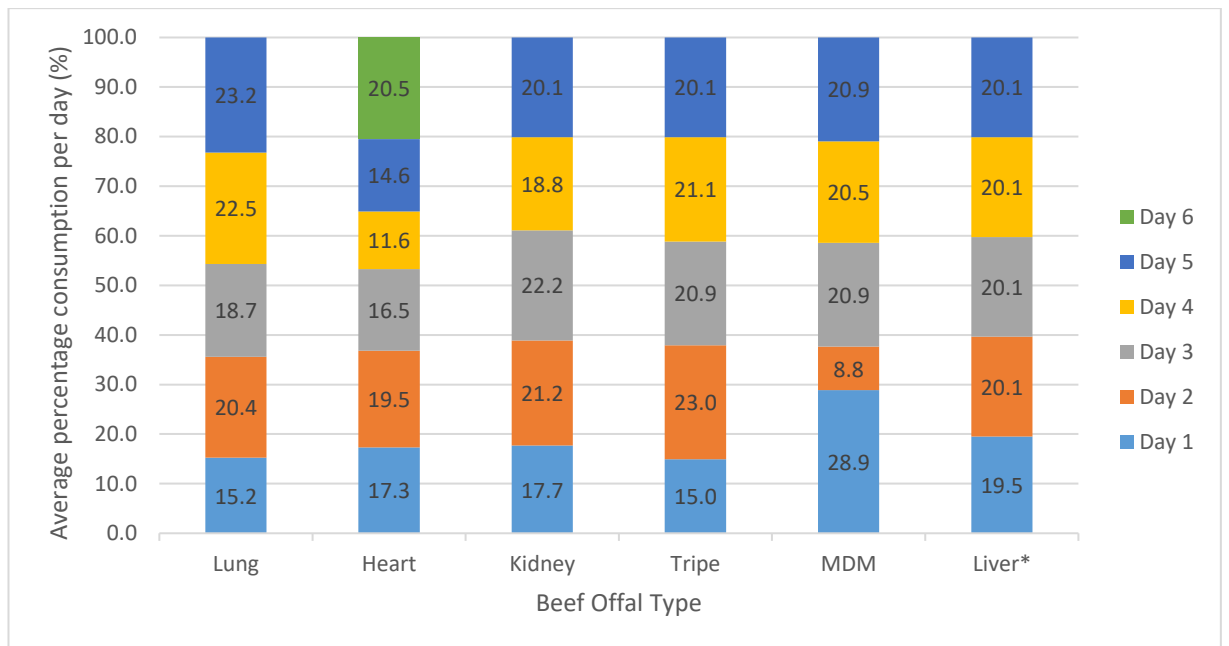


Figure 6.3: Average percentage consumption distribution of food consumed each day relative to the amount of food eaten throughout the testing week

The most consistent percentage intake was observed when feeding liver (Figure 6.2c and d). The percentage distribution of kidney was also fairly consistent with small fluctuations of 4.5% being displayed throughout the week. In both kidney and liver, no significant differences were observed between the intakes on each day of testing ( $P>0.05$ ).

MDM was the only ingredient that showed a substantial percentage decline between Day 1 and 2 (28.9% versus 8.8% of the weekly consumption), although no statistical significance was observed between each day of testing ( $P=0.06$ ) due to the low amounts being consumed (69g on Day 1 and 21g on Day 2). It should be noted that the total amount of MDM consumed over the week by the panel (239g) was much lower ( $P<0.05$ ) than that of lung, heart and kidney and tripe (which ranged from 622g to 751g).

This trend of a high relative percentage consumption of beef MDM on Day 1 followed by a substantial percentage decline on Day 2 of testing is similar to that observed for lamb MDM (28.0% versus 14.3% of the weekly consumption). The only difference being that lamb MDM showed statistical significance between Day 1 and 2 ( $P<0.05$ ), whereas no statistical significance was displayed in beef MDM ( $P=0.06$ ). The similar pattern of intake demonstrates that like lamb MDM, the cats tried beef MDM on Day 1 as it was a novel food to them, but intakes then declined throughout the week.

Lung and tripe both showed intake fluctuations throughout the week of 8%, with the intake of lung ranging from 15.2% on Day 1 to 23.2% on Day 5 and tripe showing the greatest percentage

intake on Day 2 at 23.0% and lowest on Day 1 at 15.0%. Significant differences in intake were only observed on Day 1 versus Day 4 and Day 1 versus Day 5 of lung testing, as well as Day 1 versus Day 2 of tripe testing ( $P<0.05$ ).

Finally, heart showed a percentage increase of 2.2% between Day 1 at 17.3% and Day 2 at 19.5% with intake then dropping to its lowest percentage of 11.6% on Day 4 before reaching its peak on Day 6 at 20.5%. Significant differences in intake within heart were observed on Day 2 versus Day 4, when colony intakes dropped, and Day 4 versus Day 6, when intakes began to recover ( $P<0.05$ ).

### 6.2.3 Average Percentage Consumption of Offal

In order to observe the difference in performance of individual cats in the panel, the weekly average intakes of each offal are shown in Table 6.2. For percentage consumption results on each day of testing, please refer to Appendix G.

*Table 6.2: Weekly percentage consumption results of the six beef offal varieties for each cat in the panel during acceptance testing (Note: superscripts are to be compared within a column)*

Cat	Beef Offal					
	Lung	Heart	Kidney	Tripe	MDM	Liver
1. Jetty	73.1 <sup>a,b,c</sup>	21.0 <sup>b</sup>	97.9 <sup>a</sup>	83.4 <sup>a</sup>	38.0 <sup>a</sup>	97.7
2. Kaia/Muse	59.0 <sup>a,b,c</sup>	55.0 <sup>a,b</sup>	38.0 <sup>b,c</sup>	36.3 <sup>b,c</sup>	23.1 <sup>a,b</sup>	98.3
3. Nyssa	56.1 <sup>b,c</sup>	54.8 <sup>a,b</sup>	89.7 <sup>a</sup>	78.1 <sup>a</sup>	22.1 <sup>a,b</sup>	98.9
4. Pango	37.9 <sup>c</sup>	24.4 <sup>b</sup>	19.8 <sup>c</sup>	2.8 <sup>c</sup>	0.8 <sup>b</sup>	99.4
5. Token	86.2 <sup>a,b</sup>	84.8 <sup>a</sup>	79.9 <sup>a</sup>	74.4 <sup>a</sup>	33.6 <sup>a</sup>	98.9
6. Fox	89.4 <sup>a,b</sup>	82.9 <sup>a</sup>	97.9 <sup>a</sup>	64.5 <sup>a,b</sup>	30.7 <sup>a,b</sup>	98.3
7. Orca	78.5 <sup>a,b</sup>	54.2 <sup>a,b</sup>	77.8 <sup>a,b</sup>	82.5 <sup>a</sup>	23.4 <sup>a,b</sup>	99.4
8. Gerrit	92.6 <sup>a</sup>	87.9 <sup>a</sup>	99.5 <sup>a</sup>	75.7 <sup>a</sup>	19.8 <sup>a,b</sup>	99.4
Average ± SEM	71.6±3.8	58.1±4.5	75.1±5.3	62.2±4.9	23.9±2.7	98.8±0.3

For lung, significant differences in consumption was observed between cat 8 and cats 4 and 3, as well as between cat 4 and cats 5, 6 and 7 ( $P<0.05$ ). Similar intakes were observed between cats 1, 2, 3, 5, 6, 7 and 8 (59.0% to 92.6%;  $P>0.05$ ), as well as between cats 1, 2, 3 and 4 (37.9% to 73.1%;  $P>0.05$ ). Overall, cats 1 and 2 showed no difference in intake between the other cats in the panel ( $P>0.05$ ).

For heart, cats 2, 3, 5, 6, 7 and 8 had similar intakes (54.2% to 87.9%;  $P>0.05$ ). Cats 1, 2, 3, 4 and 7 also showed similar intakes (21.0% to 55.0%;  $P>0.05$ ). Cats 1 and 4 showed the lowest intakes of 21.0% and 24.4%, respectively ( $P>0.05$ ), which were lower than that of cats 5, 6 and 8 ( $P<0.05$ ).

For kidney, cats 1, 3, 5, 6, 7 and 8 all had similar intakes (77.8% to 99.5%;  $P>0.05$ ). Cats 2 and 7 showed intakes of 38.0% and 77.8%, respectively and was seen as no difference to one another ( $P>0.05$ ). Cat 2 and 4 showed the lowest intakes of 38.0% and 19.8%, respectively ( $P>0.05$ ), which were lower than cats 1, 3, 5, 6 and 8 ( $P<0.05$ ).

For tripe, cats 1, 3, 5, 6, 7 and 8 all had similar intakes (74.4% to 83.4%;  $P>0.05$ ). Cats 2 and 6 showed intakes of 36.3% and 64.5%, respectively and was seen as no difference to one another ( $P>0.05$ ). Cats 2 and 4 showed the lowest intakes of 36.3% and 2.8%, respectively ( $P>0.05$ ), which were lower than cats 1, 3, 5, 7 and 8 ( $P<0.05$ ) and cat 4 had an intake lower than cat 6 ( $P<0.05$ ).

For MDM, cats 1 and 5 showed the highest intakes of 38.0% and 33.6%, respectively ( $P>0.05$ ), which were higher than cat 1 ( $P<0.05$ ). Cats 2, 3, 6, 7 and 8 all had similar intakes to cats 1 and 5 (19.8% to 38.0%;  $P>0.05$ ). Similarly, cats 2, 3, 6, 7 and 8 all had similar intakes to cat 4 (0.8% to 30.7%;  $P>0.05$ ). Overall, MDM was not highly consumed by all cats with intakes ranging from just 0.8% to 38.0%.

In contrast, all cats showed similar and consistently high intakes (97.7% to 99.4%;  $P>0.05$ ) when fed liver over the week.

Overall, the fixed effects of cat and offal and the interaction between cat and offal had an effect on the intake results ( $P<0.05$ ). This therefore indicates that the preferences of individual cats were different from one another as were their preferences for different offal. This was similar to the lamb acceptance testing (See Chapter 5).

To account for the lower amount of liver fed to cats (due to concerns regarding the high vitamin A content), the percentage consumption was calculated to determine an overall ranking of beef offal (as displayed in the bottom row of Table 6.2). Liver was the offal most accepted by cats with 98.8% consumption, ahead of all of the other offals. The second ranked offal was kidney with 75.1% consumption, which was followed closely by heart in third at 71.6% consumption. The fourth and fifth ranked offals were tripe and heart at 62.2% and 58.1%, respectively. Although not an offal variety, MDM was least accepted by cats with a percentage consumption of 23.9%.



When comparing the mean consumption of all possible beef offal pairings, all pairings except heart and lung, kidney and lung, tripe and lung, tripe and heart and tripe and kidney (highlighted in red) showed differences ( $P < 0.05$ ), as shown in Figure 6.4.

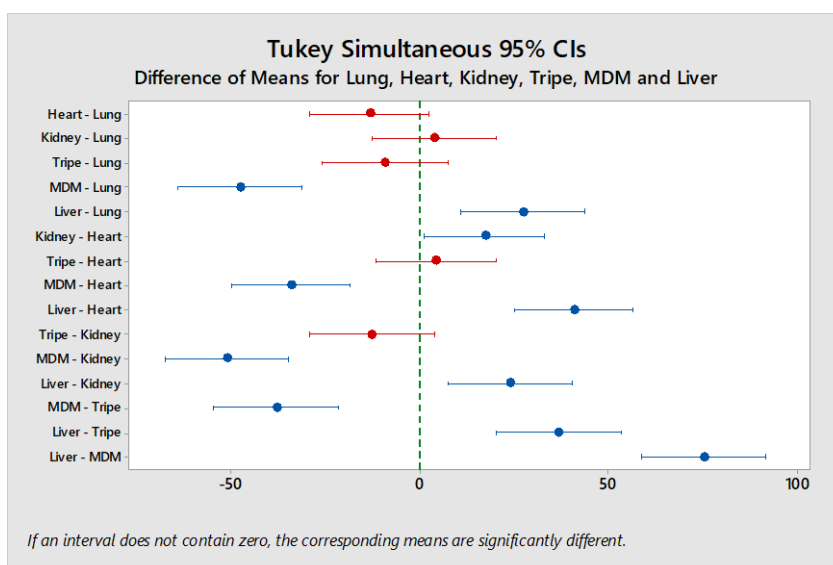


Figure 6.4: Tukey Simultaneous 95% Confidence Interval plot for all beef offal pairings

The grouping information from the Tukey analysis was used to develop a final ranking of offal acceptance which is shown in Table 6.3. In summary, liver was identified by the panel of cats as being the most palatable of all the beef offals ( $P < 0.05$ ). Kidney was the second most palatable ingredient along with lung and tripe ( $P > 0.05$ ). Lung and tripe were also ranked third alongside heart ( $P > 0.05$ ) however, heart was less palatable than kidney ( $P < 0.05$ ). Finally, as was also demonstrated in the lamb acceptance testing, MDM was ranked the least palatable of all the ingredients with intakes lower ( $P < 0.05$ ) than all of the other offals.

Table 6.3: Tukey Analysis and final ranking of beef offal

Offal	Mean Percentage Consumption (%)	Final Ranking
Liver	98.8 <sup>a</sup>	1
Kidney	75.1 <sup>b</sup>	2
Lung	71.6 <sup>b,c</sup>	2,3
Tripe	62.2 <sup>b,c</sup>	
Heart	58.1 <sup>c</sup>	3
MDM	23.9 <sup>d</sup>	4

The results from the series of beef acceptance tests shows a similar ranking to the lamb offal. The key difference was the movement of tripe up the ranking compared to the lamb acceptance results. Furthermore, the beef results showed the addition of a fourth rank of offal compared to three rankings in lamb, as well as the overlapping of lung and tripe between rankings two and three, something which did not arise in the lamb acceptance testing. This indicated that the cats were able to make clearer distinctions between the beef offals as rankings were spread over four levels. Overall, similarities were that liver was still the most palatable ingredient to the panel and MDM was the least palatable. Therefore, this also clearly demonstrates that the incorporation of more beef offal ingredients will improve the palatability of diets.

#### 6.2.4 Preference Test between Top and Bottom Ranked Beef Offal

A final two-bowl preference test was conducted to evaluate the intake patterns of the top and bottom ranked beef offals following acceptance testing. In this case, beef liver and MDM were evaluated side by side, as shown in Figure 6.5. Testing was again carried out using load cells which recorded the real time feeding pattern of each cat.



*Figure 6.5: Presentation of the bowls for the preference test (Note: this was the set up for Days 1, 3 and 5 of testing. For Days 2 and 4, offals were placed in the alternate bowls)*

Forty measurements for beef liver and MDM were obtained over a five-day testing period using the same panel of cats. All cats were presented with 35g of liver and 100g of MDM offal each a day (175g of liver and 500g of MDM offal over the week). Throughout the five-day testing period, liver was almost totally consumed on each day of testing for all cats ( $99.4\% \pm 0.1$ ), with the intake of MDM being significantly lower ( $12.6\% \pm 2.1$ ;  $P < 0.05$ ). Overall, the panel's average intake of liver and MDM over the week were  $174 \pm 0.3$ g and  $63 \pm 16.3$ g, respectively (see Figure 6.6), showing a clear preference for beef liver over MDM.

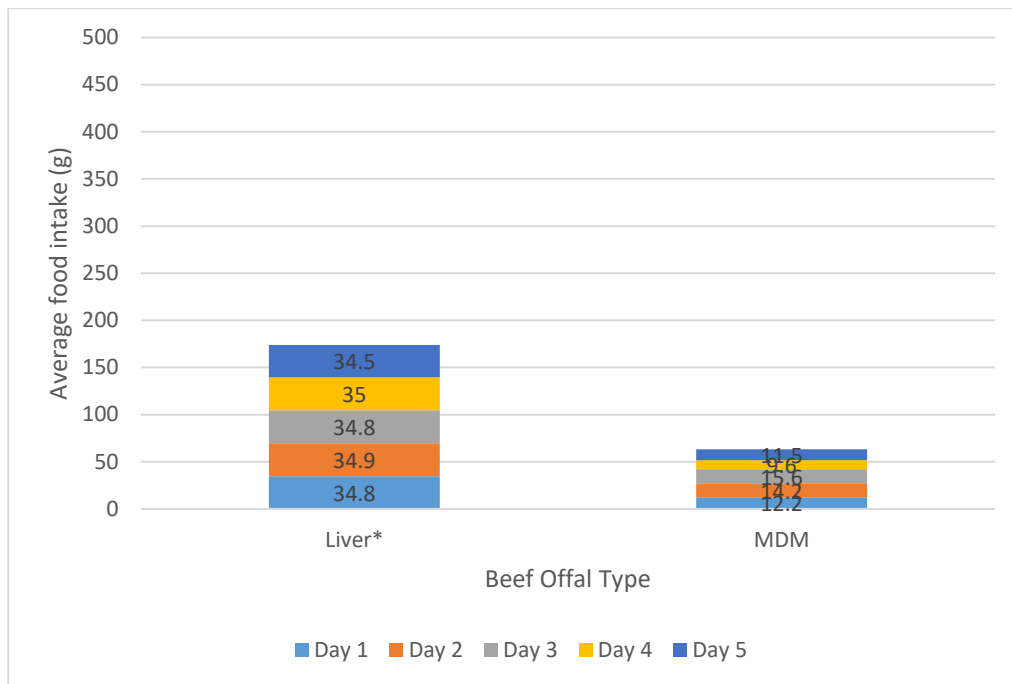


Figure 6.6: Average food intake of the top and bottom ranked beef offal, liver and MDM, on each day of preference testing (\*Liver had a maximum possible intake of 175g compared to 500g for MDM)

When evaluating the percentage distribution each day (see Figure 6.7), no difference was observed between the distribution within liver as near 100% consumption was observed on each day of preference testing and therefore resulting in near equal 20% distributions being observed. Similarly, no significant difference were observed within MDM daily intakes ( $P > 0.05$ ).

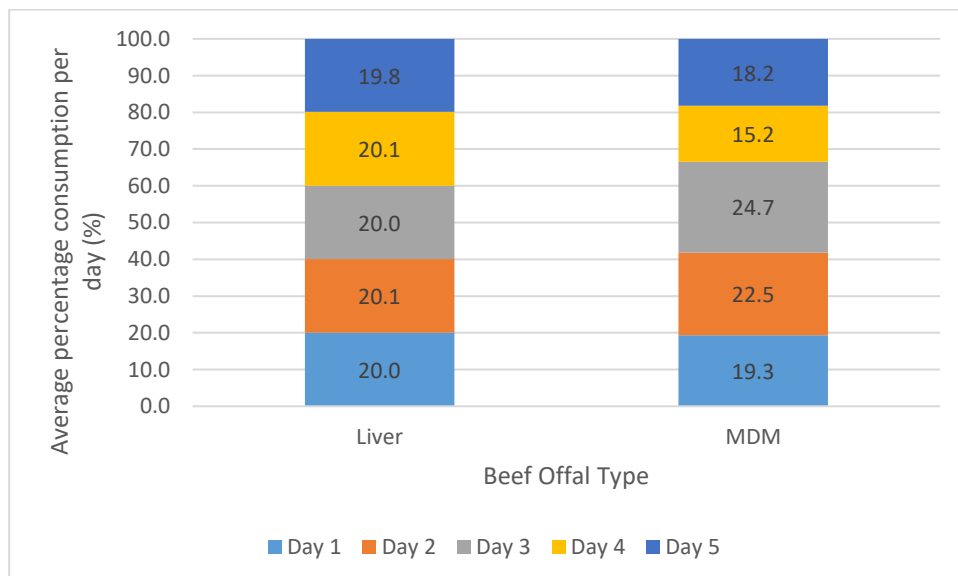


Figure 6.7: Average percentage consumption distribution of beef liver and MDM consumed each day over the preference testing week

The weekly average intake of both offal were also analysed to observe the difference in performance of individual cats in the panel. For percentage consumption results on each day of testing, please refer to Appendix G. All cats had the same intake of liver (98.8% to 99.8%;  $P>0.05$ ), the top ranked offal from acceptance testing. However, the intake of MDM varied amongst the cats in the panel from 0.1% to 30.9%. Cats 1, 3, 5, 7 and 8 showed intakes similar of 8.7% to 18.4% to that of cat 6 with the highest intake of 30.9% ( $P>0.05$ ), as well as with cats 2 and 4 with the lower intakes of 7.1% and 0.1%, respectively ( $P>0.05$ ). Cats 2 and 4 showed the lowest intakes of 7.1 % and 0.1%, respectively ( $P>0.05$ ), which were lower than that of cat 6 with an intake of 30.9% ( $P<0.05$ ).

Further analyses were carried out by evaluating the intake pattern of beef liver and MDM as determined from the load cell results. All graphical outputs for each day of testing and for each cat can be found in Appendix G.

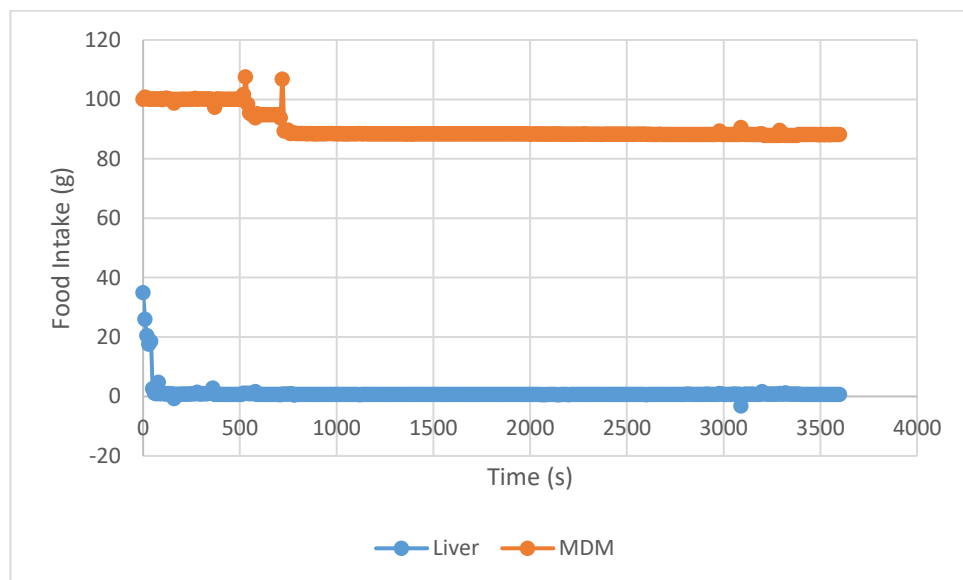


Figure 6.8: Example intake pattern of beef liver vs MDM (shown is the intake pattern of cat 7 intake on day 4 of testing)

The general intake pattern observed through the beef liver versus MDM preference test was similar to that of the lamb kidney versus MDM results. For beef, cats consumed all of the liver rapidly, before switching and subsequently consuming the MDM, as shown in Figure 6.8. Some cats however, did not consume any MDM indicating a strong preference for beef liver as an ingredient over MDM.

Upon analysis of the top and bottom ranked offals (see Table 6.1), MDM contained the highest fat content of 40.0% compared to all beef offals, but had a middle range protein content of 15.6%. Although the protein content was comparable to the remaining offals, the high fat to low

protein ratio composition may have been a likely driver in the ingredient being unfavourable to the panel. In contrast, liver presented the highest protein content of all the offals at 25.9% and the second lowest fat content of 2.7%, considerably lower than the 40.0% exhibited in MDM.

The results from the beef acceptance testing and final preference tests not only support the findings from Zaghini, & Biagi (2005) that pet food palatability and the amount of protein from animal origin is strongly correlated in cats, but again demonstrates that the choice of offal within a single protein can be an important element in improving palatability.

The findings from this series of tests are similar to that of the top versus bottom lamb offal testing. Although the top offal presented was kidney for lamb and liver for beef, both were compared to the same bottom offal, MDM, indicating an initial preference from the acceptance testing for organ meat over animal tissue/collagen. In addition, similar intake patterns between the top and bottom ranked beef and lamb offals were observed. For beef, liver was consumed rapidly before switching and subsequent consumption of MDM took place. The same was observed for lamb where kidney was consumed rapidly, followed by subsequent consumption of MDM. In both beef and lamb, some cats did not consume any MDM following the consumption of beef liver or lamb kidney which indicated a strong preference for the top ranked offals over MDM.

Incorporating more offal into pet food formulations may again be a better option in future high value pet foods which will result in increased diet palatability, as preference for offal meats over animal tissue/collagen were consistent in both beef and lamb ingredients.

## 7. Acceptance of Chicken Offal

### 7.1 Materials and Methods

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

#### 7.1.1 Test Animals

The same cat panel as used previously in Chapter 5 was used for chicken offal acceptance testing.

Testing was carried out from Monday 5<sup>th</sup> November until Friday 30<sup>th</sup> November 2018 at the Feline Nutrition Unit at Massey University, Palmerston North.

#### 7.1.2 Ingredient Used

Four chicken offal varieties were evaluated in this block of acceptance tests. These consisted of: heart, gizzard, mechanically deboned meat (MDM) and liver, and they were tested in this order. All ingredients were provided by MPI-accredited chicken processors through Zivi Ltd (Mount Maunganui, New Zealand), and were delivered in approximately 15kg frozen blocks.

#### 7.1.3 Ingredient Preparation

All the frozen blocks of chicken offal were prepared in the same manner as the lamb and beef offal (see Section 5.1.3).

#### 7.1.4 Testing Methods

The testing methods outlined in Section 5.1.4 were followed for the acceptance of chicken offal, with one amendment, the amount of chicken liver that was presented.

The recommended maximum daily intake of vitamin A for cats is given by AAFCO (2017) as 333,300 IU/kg of diet (equivalent to 99.99 µg/g as retinol). From here, the retinol value was divided by an estimated retinol level of vitamin A level found in raw chicken liver of 180.0 µg/g and multiplied by one hundred which gave a maximum intake of 56g/day. Given that the vitamin A level in chicken was an estimated value, 54g of liver was presented to each cat to remain below the estimated maximum intake.

#### 7.1.5 Data Collection and Statistical Analyses

Please refer to Section 5.1.5 for the data collection and statistical analyses.

## 7.2 Results and Discussion

Forty measurements for each of the four chicken offals were obtained over each five-day testing period using the eight cat panel to evaluate the acceptance of chicken heart, gizzard, MDM and liver.

### 7.2.1 Food Intake

All cats were offered 1kg of chicken offal each week (200g a day), except in the case of liver where 270g (54g a day) was presented. The total intake over the testing period for each offal ( $\pm$  SEM) was  $504.1 \pm 102.4$ g for heart,  $665.5 \pm 89.0$ g for gizzard,  $477.0 \pm 71.1$ g for MDM and  $258.0 \pm 6.9$ g for liver.

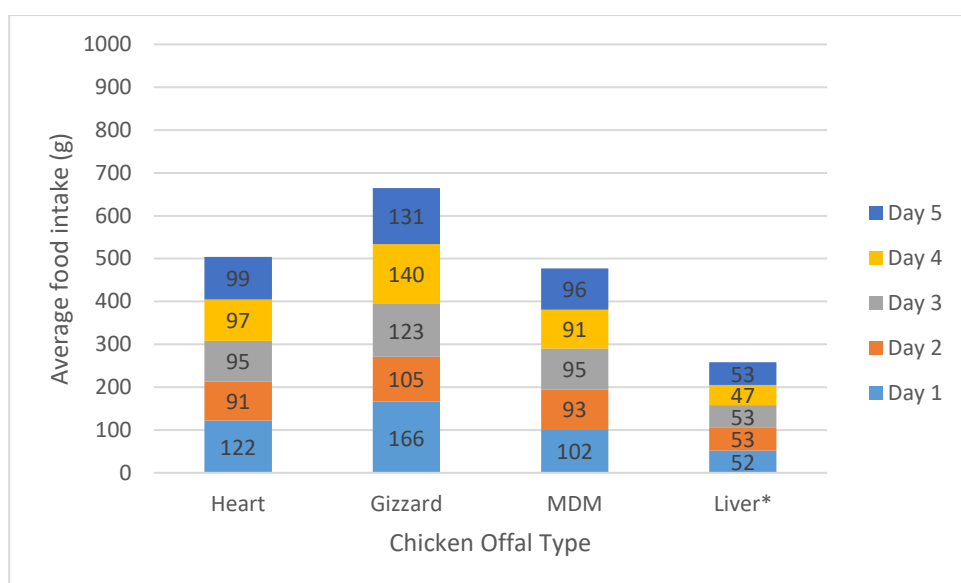


Figure 7.1: Average food intake of the four chicken offal out of the possible 1000g served throughout the week  
(\*maximum possible intake of liver was 270g compared to 1000g for the other offal varieties)

Figure 7.1 shows the average food intake on each day of acceptance testing. The cats displayed clear neophilic behaviour when fed gizzard on Day 1 compared to Day 2 ( $P < 0.05$ ). Intakes were also slightly higher on Day 1 of testing for heart and MDM, although not different enough from intakes during the rest of the week to suggest neophilia ( $P > 0.05$ ). Other than the Day 1 intake of gizzard being higher than the Day 2 intake ( $P < 0.05$ ), no differences between daily intakes were observed within each chicken offal variety, with intakes remaining consistent throughout the week ( $P > 0.05$ ).

The chicken offal with the lowest intake was again MDM, as shown in Figure 7.2a and b. However, unlike lamb and beef MDM which showed the worst ranking compared to the other offals within each species, intake of chicken MDM at 477g was comparable to that of chicken

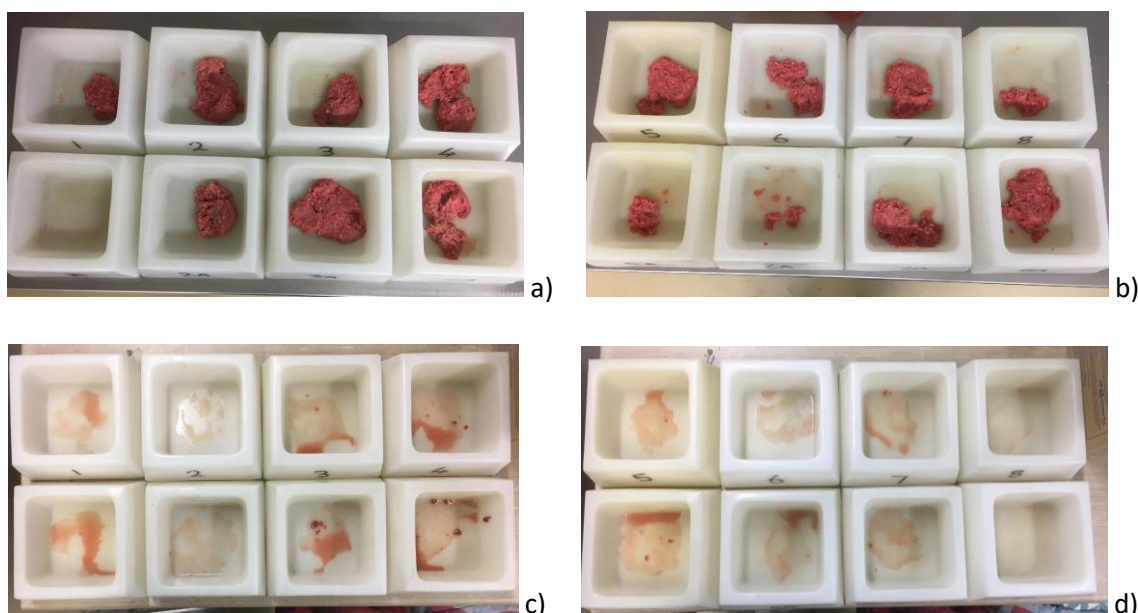
heart at 504g ( $P>0.05$ ). In contrast, the 258g intake out of a possible 270g of chicken liver over the week indicated a near 100% consumption rate, as shown in Figure 7.2c and d.

*Table 7.1: As fed moisture, fat, protein and ash content in the four chicken offal varieties*

Chicken Offal	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Gizzard	79.9	2.2	16.7	0.9
Heart	75.2	10.9	12.2	0.8
Liver	72.6	3.8	20.6	1.5
MDM	71.2	9.3	17.8	1.8

When evaluating the macronutrient content of the four chicken offals (as shown in Table 7.1), liver showed the highest protein and second lowest fat content of the ingredients (20.6% protein and 3.8% fat as fed). Chicken MDM contained the next highest amount of protein followed by gizzard and then heart at 17.8%, 16.7% and 12.2%, respectively. Although MDM had a higher protein content than gizzard and heart, it also contained a higher fat percentage of 9.3% along with heart at 10.9%. In comparison, the fat content in gizzard was much lower at 2.2%, which may indicate why gizzard was consumed in greater quantities than heart and MDM.

For more information on the macronutrient composition, amino acid and fatty acid profiles of each offal presented in this study, please refer to Appendix H.



*Figure 7.2a-d: Top: Remaining MDM after Day 3 of chicken acceptance testing.*

*Bottom: Remaining liver after Day 3 of chicken acceptance testing*



Overall, there was no effect of day or the interaction between the day and offal ( $P>0.05$ ), verifying that from day-to-day, intake was similar and the daily pattern of intake was consistent across the chicken offal. Cats had preferences for different chicken offal as indicated by differences in intake ( $P<0.05$ ).

### 7.2.2 Distribution of Food Intake

Intake patterns were investigated further by determining the proportion of food eaten each day as a percentage of the total intake over the week (see Figure 7.3). This corrected for the lower amounts of liver offered compared to the other offals.

It was clearly shown that heart, gizzard and MDM had a slightly higher percentage consumption on the first day at 24.2, 25.0 and 21.4%, respectively, than the expected 20% if intake was consistent over the entire week.

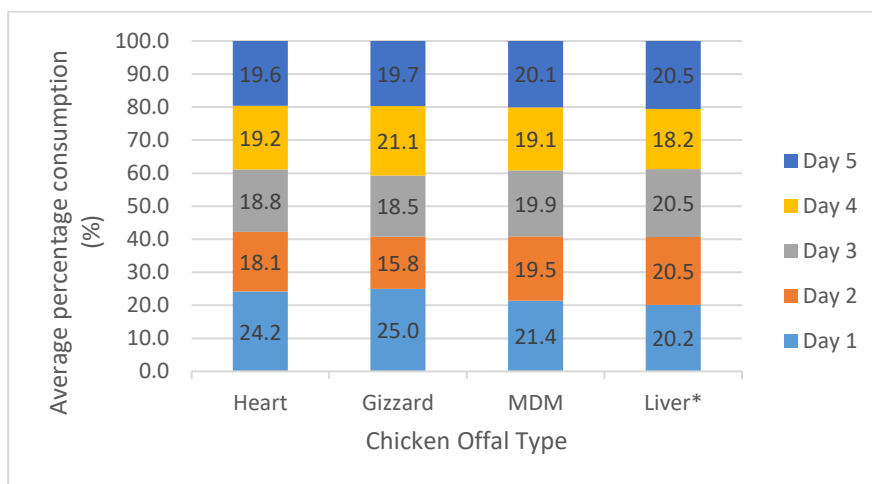


Figure 7.3: Average percentage consumption distribution of chicken offal consumed each day relative to the amount of food eaten throughout the testing week

Again, gizzard was the only ingredient that showed a decrease in intake between Days 1 and 2 ( $P<0.05$ ; 25.0% versus 15.8% of the weekly consumption), with daily intake patterns of heart, MDM and liver remaining consistent throughout the week ( $P>0.05$ ), which matches the observations of actual weighed amount of meat eaten.

### 7.2.3 Average Percentage Consumption of Offal

In order to investigate the performance of individual cats in the panel, the weekly average intakes of each offal are shown in Table 7.2. For percentage consumption results on each day of testing, please refer to Appendix H.

Table 7.2: Weekly percentage consumption results of the four chicken offal varieties for each cat in the panel during acceptance testing (Note: superscripts are to be compared within a column)

Cat	Chicken Offal			
	Heart	Gizzard	MDM	Liver
1. Jetty	57.2 <sup>b</sup>	83.9 <sup>a</sup>	69.5 <sup>a</sup>	78.1
2. Kaia	37.2 <sup>b,c,d</sup>	39.5 <sup>b</sup>	39.2 <sup>a,b,c</sup>	100
3. Nyssa	30.6 <sup>c,d</sup>	37.6 <sup>b</sup>	24.6 <sup>c</sup>	99.3
4. Pango	13.6 <sup>d</sup>	35.1 <sup>b</sup>	19.9 <sup>c</sup>	94.4
5. Token	32.5 <sup>c,d</sup>	71.8 <sup>a,b</sup>	65.6 <sup>a,b</sup>	97.8
6. Fox	98.0 <sup>a</sup>	96.7 <sup>a</sup>	66.2 <sup>a,b</sup>	97.8
7. Orca	47.6 <sup>b,c</sup>	79.5 <sup>a</sup>	35.4 <sup>b,c</sup>	98.9
8. Gerrit	86.6 <sup>a</sup>	88.3 <sup>a</sup>	60.4 <sup>a,b</sup>	98.9
Average ± SEM	50.4±4.7	66.6±4.6	47.6±3.8	95.6±2.4

Cat 4 showed the lowest percentage consumption of chicken heart, gizzard and MDM, with intakes ranging from 13.6% for heart to 35.1% for gizzard, with cat 1 showing the lowest percentage consumption of liver at 78.1%.

For heart, cats 6 and 8 showed similar intakes (98.0% and 86.6%;  $P>0.05$ ) for the week which were greater than the remaining cats ( $P<0.05$ ). Cats 1, 2 and 7 also showed similar intakes (37.2% to 57.2%;  $P>0.05$ ), as did cats 2, 3, 5 and 7 (30.6% to 47.6%,  $P>0.05$ ) and cats 2, 3, 4 and 5 (13.6% to 37.2%;  $P>0.05$ ). Differences in intake were observed between cat 1 versus cats 3, 4 and 5 ( $P<0.05$ ), as well as between cats 4 versus cats 1 and 7 ( $P<0.05$ ).

For gizzard, cats 1, 5, 6, 7 and 8 showed similar intakes (71.8% to 96.7%;  $P>0.05$ ), as did cats 2, 3, 4 and 5 (35.1% to 71.8%;  $P>0.05$ ). Differences in intake were observed between cats 1, 6, 7 and 8 versus cats 2, 3 and 4 ( $P<0.05$ ).

For MDM, cats 1, 2, 5, 6 and 8 showed similar intakes (39.2% to 69.5%;  $P>0.05$ ), as did cats 2, 5, 6, 7 and 8 (35.4% to 66.2%;  $P>0.05$ ) and cats 2, 3, 4 and 7 (19.9% to 39.2%;  $P>0.05$ ). Differences in intake were observed between cats 1 versus cats 3, 4 and 7 ( $P<0.05$ ), as well as between cats 1, 5, 6 and 8 versus cats 3 and 4 ( $P<0.05$ ).

Finally, all cats showed similar and high intakes (78.1% to 100%;  $P>0.05$ ) when fed liver over the week.

Overall, the fixed effects of cat and offal and the interaction between cat and offal had an effect on the intake results ( $P < 0.05$ ). This therefore indicates that individual cats showed differences between one another and also showed preferences for different offals.

To account for the lower amount of liver fed to cats, the percentage consumption was calculated to determine an overall ranking of chicken offal (as displayed in the bottom row of Table 7.2). Liver was the offal most accepted by cats with 95.6% consumption followed by gizzard at 66.6%. The third and fourth ranked offal were heart and MDM at 50.4% and 47.6%, respectively.

When comparing the means of all possible chicken offal pairings, all differences in means except for heart and MDM (highlighted in red) showed significant differences ( $P < 0.05$ ), as shown in Figure 7.4.

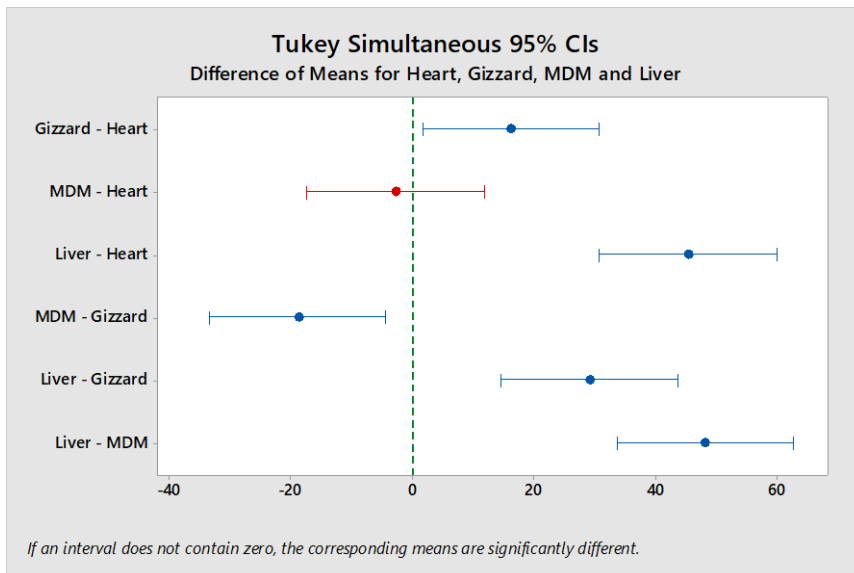


Figure 7.4: Tukey Simultaneous 95% Confidence Interval plot for all chicken offal pairings

The grouping information from the Tukey analysis was used to develop a final rank of offal acceptance and is shown in Table 7.3. In summary, liver was again identified by the panel of cats as being the most palatable offal ( $P < 0.05$ ). Gizzard was identified as the next most palatable offal, but less palatable than liver ( $P < 0.05$ ). Finally, heart and MDM were seen as the least palatable of the four offals tested ( $P < 0.05$ ), but equally palatable to one another ( $P > 0.05$ ).

Table 7.3: Tukey Analysis and final ranking of chicken offal

Offal	Mean Percentage Consumption (%)	Final Ranking
Liver	95.6 <sup>a</sup>	1
Gizzard	66.6 <sup>b</sup>	2
Heart	50.4 <sup>c</sup>	3
MDM	47.6 <sup>c</sup>	

The results from chicken acceptance testing revealed that like beef and lamb, liver was clearly the most palatable offal. Furthermore, the addition of gizzard in this series of acceptance testing showed greater palatability than heart and MDM. In contrast to the beef and lamb acceptance testing which saw MDM as the bottom ranked offal on its own, MDM was equally as palatable as heart. Compared with the total intakes of lamb and beef MDM of 343g and 239g, respectively, chicken MDM showed a much higher total intake of 476g. In addition, the intake of chicken MDM was equally palatable to that of chicken heart of 504g ( $P>0.05$ ), resulting in a shared third ranking rather than MDM being solely bottom ranked, as demonstrated in the lamb and beef acceptance testing. In summary, a clear ranking of the palatability of the four chicken offals was achieved. As a result, the conclusions drawn from the lamb and beef acceptance testing of including more offal with higher protein to fat ratios into future high value pet foods to increase diet palatability are supported by the chicken acceptance testing.

## 8. Preference between Equivalent Beef and Lamb Offal

After establishing a ranking of lamb and beef offals via acceptance testing in Chapters 5 and 6, a two bowl preference test between equivalent lamb and beef red meat offals was conducted to determine whether cats showed a preference for one species versus another.

To my knowledge, no studies in the scientific literature have evaluated the palatability of ingredients (predominately meat and meat offals) used in pet food, nor have they evaluated the palatability of equivalent offals from different species.

By comparing the acceptance testing results from lamb and beef, there are possible indications of the panel showing liking for one species of offal over the other. However, it is necessary to run a two-bowl preference test to substantiate such claims with confidence and provide statistical evidence. This series of tests therefore aims to determine if a preference for one species of offal over the other exists, or if intakes of equivalent beef and lamb offal are similar regardless of the species presented.

### 8.1 Materials and Methods

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

#### 8.1.1 Test Animals

The cat panel used previously in Chapter 5 was again used in this chapter to test the preference between six equivalent beef and lamb offals.

Testing was carried out from Monday 10<sup>th</sup> September until Friday 19<sup>th</sup> October 2018 at the Feline Nutrition Unit at Massey University, Palmerston North.

#### 8.1.2 Ingredient Used

Six beef and lamb offal varieties were evaluated alongside one another in this series of preference tests. These consisted of: lung, heart, kidney, tripe, mechanically deboned meat (MDM) and liver, and they were tested in this order. All ingredients were provided by MPI-accredited meat processors through Ziwi Ltd (Mount Maunganui, New Zealand), and were delivered in approximately 20kg frozen blocks.

#### 8.1.3 Ingredient Preparation

All the frozen blocks of beef and lamb offal were prepared in a similar manner as previously outlined (see Section 5.1.3), with the following amendments.

- 1) The size of vacuum packed portions

Rather than the 2kg used for acceptance tests, 1kg portions of each offal were vacuum packed for this series of preference tests.

### 2) Thawing of offal

On each day prior to testing, one bag of beef and one bag of the equivalent lamb offal were placed in a 7°C refrigerator to thaw overnight.

### 3) Switching of bowls on each day of testing

The positions of the two bowls were alternated each day to remove any possibility of cats showing a positional bias and preference for one bowl over the other (after Tartellin (1997) and Péron & Tobie (n.d.)).



*Figure 8.1: Presentation of the bowls showing 100g portions of beef and lamb lung on days 1, 3 and 5 of preference testing (offals were places in alternate bowls on days 2 and 4 to remove possible side bias effects)*

Finally, no additional samples were required for nutritional analyses as samples had already been collected during acceptance testing.

#### 8.1.4 Testing Methods

The testing was carried out in a similar manner as previously outlined for the acceptance of beef offal (see Section 6.1.4), with the following amendment.

### 1) Use of load cells

To record the real time intakes of equivalent beef and lamb offal, load cells were used in this series of preference tests. Before each week of testing, each set of load cells were calibrated

using two 500g weights before bowls were positioned onto the plates for testing, as shown in Figure 8.2.



Figure 8.2: Set up of the testing booths for the two bowl preference test (pictured is lamb tripe in bowl 7A and beef tripe in bowl 7)

## 2) The amount of liver that was presented

In order to present equal amounts of beef and lamb liver, the level of vitamin A level found in raw lamb and beef liver of 154.34  $\mu\text{g/g}$  and 283.19  $\mu\text{g/g}$ , respectively (after Purchas and Wilkinson, 2013) were multiplied by equal proportions of 0.225 each to give a maximum value of 98.44  $\mu\text{g/g}$ . This value was below that of the safe maximum daily intake of vitamin A as retinol for cats of 99.99 $\mu\text{g/g}$  (AAFCO, 2017). When converted to grams, 22.5g of beef and 22.5g of lamb liver was presented to each cat on each day of testing.

### 8.1.5 Data Collection and Statistical Analyses

On an individual cat basis, preference was determined as both the food intake (g) and converted to a percentage consumption (%), particularly for liver, using the following equations:

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

$$\text{Percentage consumption (\%)} = \frac{\text{food intake (g)}}{\text{initial weight of food (g)}} \times 100$$

Paired t-tests were carried out in Minitab 18 (Minitab Inc., State College, Pennsylvania, USA) and used to determine whether there were statistical differences between the overall intake of equivalent beef and lamb offals for the week, as well as on each day of testing.

Interactions between offal intake and days of testing were also analysed using a PROC mixed model in SAS 9.4 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Please refer to Appendix I for coding.

Load cell readings were also analysed to reveal the number of meals consumed, the length of meals (s) and the amount consumed (g) per cat and are summarised throughout the results. A meal was defined as the intake of offal greater than or equal to 10g consumed at single visit to a bowl, and a period of 80 seconds or longer was defined as the gap between one meal and the next, so any resumption of feeding with 80 seconds was defined as the continuation of the same meal (Thomas et al., 2018).

The rate of consumption of each offal was also analysed and determined using the following:

$$\text{Rate of consumption } \left( \frac{g}{min} \right) = \frac{\text{Total food intake (g)}}{\text{Point where food intake stops (min)}}$$

\*An example calculation using the rate of consumption equation is given in Appendix I

## 8.2 Results and Discussion

A five-day testing period using the eight cat panel (forty measurements) was used to evaluate the beef and lamb offal preference (lung, heart, kidney, tripe, MDM and liver).

### 8.2.1 Food Intake and Percentage Consumption

All cats were offered 500g each of the equivalent beef and lamb offal each week (100g a day), except in the case of liver where 113.5g (22.5g a day) was presented. The average food intake of lung, heart, kidney, tripe and MDM are given in Figure 8.3, and that of liver is given in Figure 8.5.



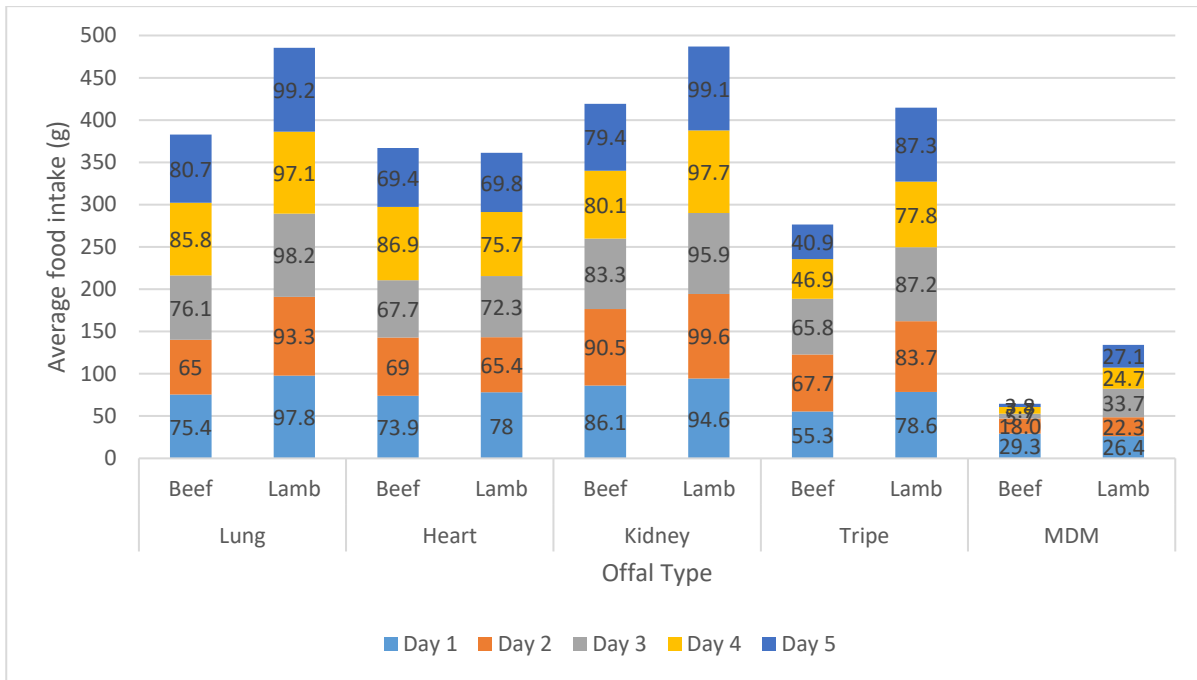


Figure 8.3: Average food intake of the five beef and lamb offals that had a maximum possible intake of 500g throughout the week

For lung, the total intakes of beef and lamb were  $383.0 \pm 54.7\text{g}$  and  $485.5 \pm 7.3\text{g}$ , respectively, giving percentage consumptions of  $76.3 \pm 5.2\%$  and  $97.0 \pm 1.3\%$  for the week. The total intake of beef lung versus lamb lung over the week was different ( $P < 0.05$ ), indicating a preference for lamb over beef.

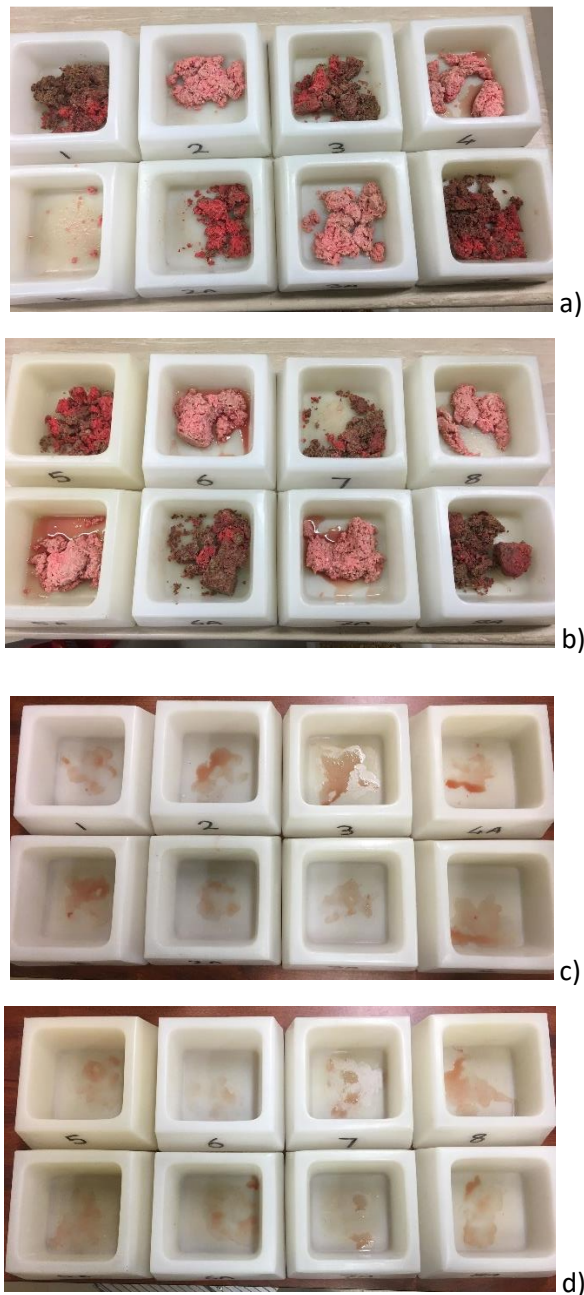
For heart, the total intakes of beef and lamb were  $366.8 \pm 48.4\text{g}$  and  $361.1 \pm 43.6\text{g}$ , respectively, giving percentage consumptions of  $73.3 \pm 5.2\%$  and  $72.2 \pm 4.9\%$  for the week. The total intake of beef heart versus lamb heart was similar over the week ( $P > 0.05$ ), indicating no preference for lamb or beef.

For kidney, total intakes of beef and lamb were  $419.4 \pm 58.1\text{g}$  and  $486.9 \pm 8.3\text{g}$ , respectively, giving percentage consumptions of  $83.6 \pm 5.1\%$  and  $97.0 \pm 1.2\%$  for the week. The total intake of beef kidney versus lamb kidney over the week was different ( $P < 0.05$ ), indicating a preference for lamb over beef.

For tripe, total intakes of beef and lamb were  $276.5 \pm 58.8\text{g}$  and  $414.7 \pm 58.3\text{g}$ , respectively, giving percentage consumptions of  $55.4 \pm 5.8\%$  and  $83.1 \pm 5.2\%$  for the week. The total intake of beef tripe versus lamb tripe over the week was different ( $P < 0.05$ ), indicating a preference for lamb over beef.

For MDM, the total intakes of beef and lamb were  $64.5 \pm 16.7\text{g}$  and  $134.2 \pm 63.6\text{g}$ , respectively, giving percentage consumptions of  $12.5 \pm 3.0\%$  and  $25.8 \pm 5.6\%$  for the week. The total intake

of beef MDM versus lamb MDM over the week was different ( $P < 0.05$ ), indicating a preference for lamb over beef, although intakes of both were considerably below 50% (shown in Figures 8.4a and b).



*Figure 8.4a-d: Top: Remaining MDM after Day 2 of beef vs lamb MDM preference testing  
Bottom: Remaining liver after Day 2 of beef vs lamb liver preference testing*

Liver was presented at a lower amount than the other five offals. The total intakes of beef and lamb were  $108.9 \pm 0.3\text{g}$  and  $109.6 \pm 0.3\text{g}$ , respectively (refer to Figure 8.5), giving percentage consumptions of  $99.2 \pm 0.2\%$  and  $99.4 \pm 0.1\%$  for the week (shown in Figures 8.4c and d). The

total intake of beef liver versus lamb liver over the week was similar ( $P>0.05$ ), indicating no preference for lamb or beef.

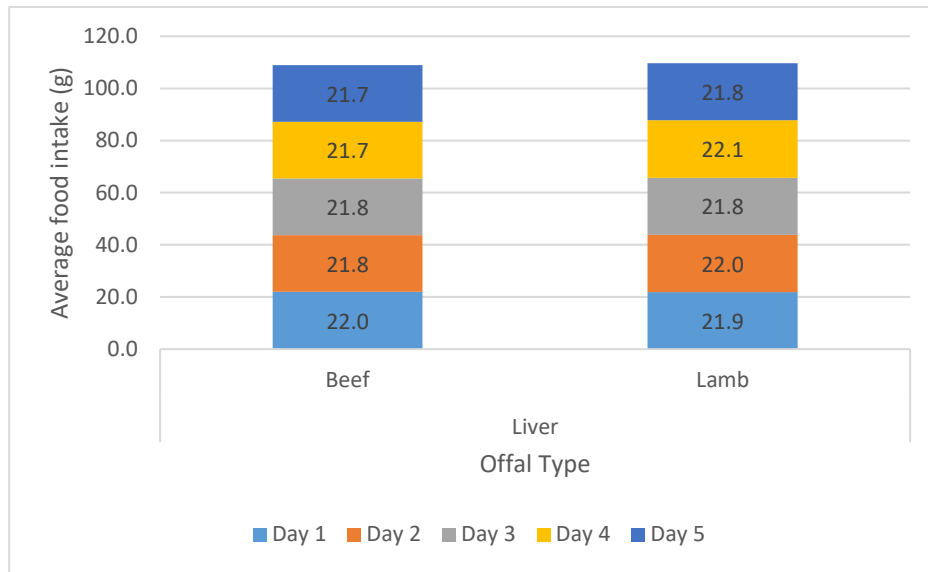


Figure 8.5: Average food intake of beef and lamb liver with a maximum possible intake of 113.5g throughout the week

The panel of cats showed a consistent preference for lamb over beef offals, with the exception of heart and liver which showed no difference in intake.

### 8.2.2 Distribution of Food Intake

Intake patterns were investigated further by determining the proportion of food eaten each day as a percentage of the total intake over the week (see Figure 8.6). This corrected for the lower amounts of liver offered compared to the other offals.

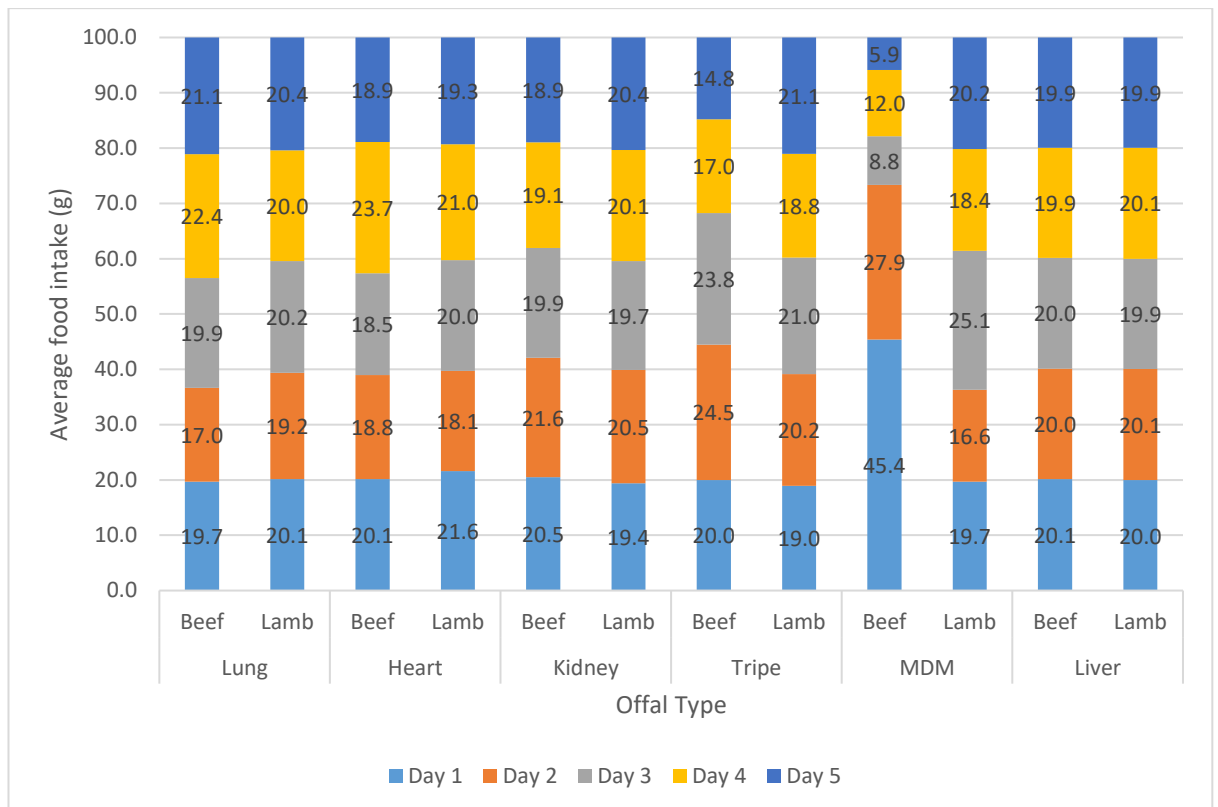


Figure 8.6: Average percentage consumption distribution of food consumed each day relative to the amount of food eaten throughout the testing week

Overall, the fixed effect of day and the interaction between the day and offal had no significance on the intake results ( $P > 0.05$ ). However, the fixed effect of offal had significance on the intake results ( $P < 0.05$ ). This therefore indicated that cats have preferences for different offal, as determined via previous acceptance testing. Rather than comparing across days of testing, only beef versus lamb intakes on equivalent days of testing were evaluated.

Differences between beef and lamb intake were only observed Day 2 of lung testing, as well as tripe testing on Days 1, 4 and 5 ( $P < 0.05$ ). All other daily beef versus lamb percentage distributions showed no difference ( $P > 0.05$ ). For lung on Day 2, the percentage consumption of beef at 17.0% was lower than that of lamb at 19.2% ( $P < 0.05$ ; 65g of the 383.0g and 93.3g of the 458.5g total intakes for beef and lamb lung, respectively).

For tripe, the percentage distribution on Day 1 of testing was greater for beef at 20.0% compared to lamb at 19.0%, which appears to be consistent with the expected 20% distribution throughout the week ( $P < 0.05$ ; 55.3g of the 276.5g and 78.6g of the 414.7g total intakes for beef and lamb tripe, respectively). However, Day 4 of tripe testing showed a higher intake of lamb at 18.8% compared to beef at 17.0% ( $P < 0.05$ ; 77.8g and 46.9g daily intakes for lamb and beef tripe, respectively). Similarly, Day 5 of lamb tripe testing also showed a higher intake at 21.1%

compared to beef at 14.8% ( $P < 0.05$ ; 87.3g and 40.9g daily intakes for lamb and beef tripe, respectively), indicating that the intake of beef tripe fell by the end of the week compared to the percentage consumption of lamb tripe on equivalent days of testing.

In addition, the MDM results in Figure 8.6 show a distorted distribution for beef MDM compared to lamb MDM, although no statistical significance was observed on each day of testing ( $P > 0.05$ ) due to the low overall intakes of both beef and lamb MDM throughout the week. Although there was a small overall intake of beef MDM, majority was consumed on the first day of testing, and 73.3% was consumed on Days 1 and 2 before dropping from Day 3 onwards.

### 8.2.3 Intake Patterns and Rate of Consumption

The preference between equivalent beef and lamb offals was investigated further by evaluating the meal size, frequency and intake patterns for each offal (shown in Table 8.1) and determining the rate of consumption (g/min) for the panel, shown in Table 8.2.

*Table 8.1: Average meal size and number of meals consumed for each beef and lamb offal over the preference testing week*

Offal	Lamb		Beef	
	Meal size (g)	Number of meals	Meal size (g)	Number of meals
Lung	78.3 ± 3.8	1.05	44.3 ± 5.8	1.65
Heart	45.3 ± 4.1	1.20	34.0 ± 1.9	2.00
Kidney	79.4 ± 6.7	1.13	47.0 ± 3.4	1.60
Tripe	57.0 ± 6.9	1.33	26.5 ± 2.1	1.60
MDM	12.7 ± 1.3	0.85	4.8 ± 2.5	0.40
Liver	21.2 ± 0.5	1.03	21.7 ± 0.1	1.00

Meal size has been defined as intake of offal greater than or equal to 10g consumed at single visit to a bowl and frequency as the number of visits to a bowl with a gap of 80 seconds or longer between one meal and the next.

Table 8.2: Average rate of consumption of equivalent beef and lamb offal averaged for the whole cat panel over the testing week

Offal	Rate of consumption (g/min)	
	Lamb	Beef
Lung	13.6 ± 1.1	8.1 ± 1.6
Heart	4.2 ± 0.8	3.4 ± 0.6
Kidney	13.9 ± 1.5	9.2 ± 1.1
Tripe	11.4 ± 1.4	3.8 ± 1.0
MDM	1.0 ± 0.2	0.4 ± 0.2
Liver	12.4 ± 1.1	11.4 ± 1.0

It should be noted that the low overall intakes of MDM compared to the other offal varieties made estimating the rate of consumption difficult due to greater variability in data points from the load cell graphs. As a result, calculating the rate of consumption using the method outlined in Section 7.1.5 is only accurate for offals which showed distinct meals being consumed as opposed to multiple stepwise decreases in intakes of 1 to 5g, which was common in the consumption of MDM.

### *Lung*

The cats consumed an average meal size of 78.3 ± 3.8 g for lamb lung (individual meal sizes ranging from 11.1g to 101.7g) which was larger than that of 44.3 ± 5.8 g for beef lung (individual meal sizes ranging from 10g to 99.8g) over the week (P<0.05). The meal sizes are consistent with the food intake results showing a preference for lamb over beef lung. On average, 1.05 meals of lamb lung and 1.65 meals of beef lung were consumed by the cats on each day of testing, with beef lung showing a gap of 494.9 ± 77.8 seconds between each meal.

For lung, the preference for lamb over beef in the initial food intake results (see section 7.2.1) was further demonstrated when evaluating the rate of consumption. The panel showed different rates of consumption (P<0.05) for lamb and beef lung of 13.6 ± 1.1 g/min and 8.1 ± 1.6 g/min, respectively. This showed that the preferred lamb lung was consumed in greater quantities and over a shorter period than beef lung which had a lower intake rate over a longer period. Relative to the food intake percentages, the panel spent 7.1 minutes and 9.4 minutes consuming lamb and beef lung, respectively.

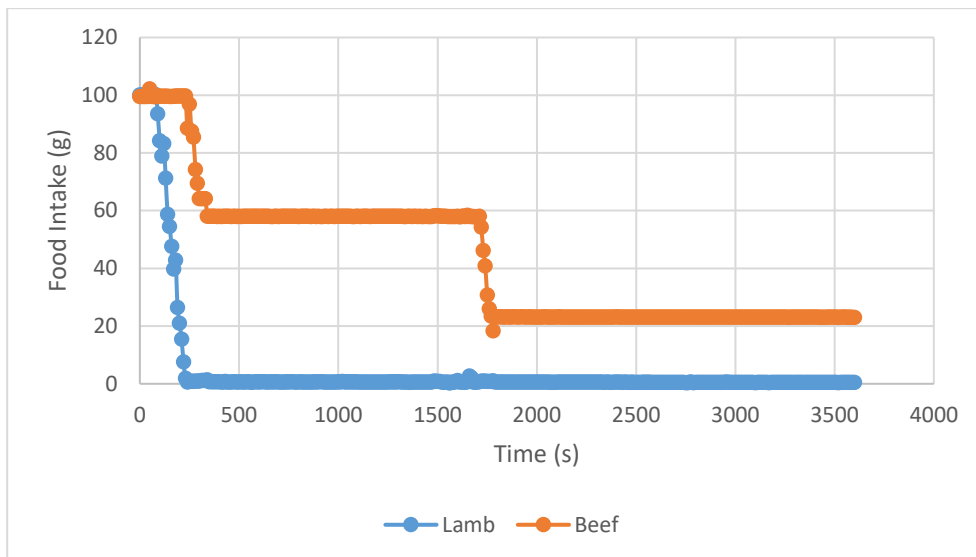


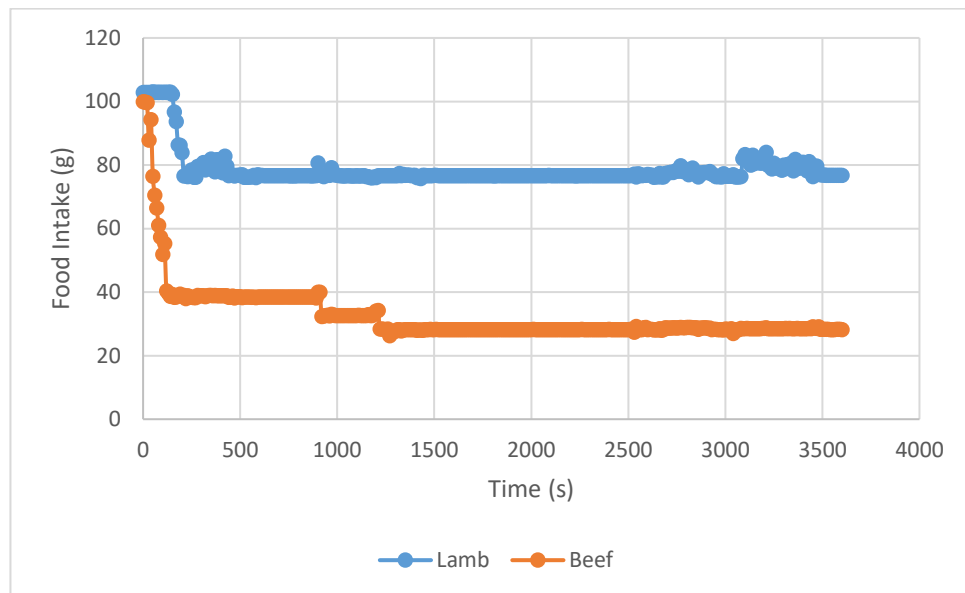
Figure 8.7: Example of the most common intake pattern observed in beef versus lamb lung preference testing (shown is the intake by cat 3 on day 5 of lung preference testing)

Food intake patterns given by the 40 load cell graphical outputs revealed that on 27 occasions, lamb lung was the first offal tried and completely consumed by cats from the panel before they either partially or fully consumed the beef lung, as shown in Figure 8.7. On only six occasions the opposite pattern was observed where beef lung was the first offal tried and completely consumed, followed by partial or full consumption of lamb lung. Finally, on seven occasions, beef lung was the first offal consumed by cats, but lamb lung was fully consumed first. Cats trying lamb lung but switching to beef and consuming it first was not observed during lung preference testing. Overall, a preference for lamb lung over beef lung was observed.

#### Heart

The cats consumed an average meal size of  $45.3 \pm 4.1$  g for lamb heart (individual meal sizes ranging from 10g to 98.6g) which was larger than  $34.0 \pm 1.9$  g for beef heart (individual meal sizes ranging from 10g to 98g) over the week ( $P < 0.05$ ). Although the average meal size for lamb was greater than beef heart, lamb heart was consumed in a smaller number of meals of 1.20 compared to two meals of beef heart, with beef heart showing a gap of  $719.7 \pm 166.0$  seconds between each meal. The results indicate that a total intake of 68g for beef heart was consumed by each cat on each day of testing which was greater than that of lamb ( $P < 0.05$ ). These inconsistencies are likely due to greater variability in intake data between cats with some consuming multiple (three to six) meals just above the 10g minimum required to be classified as a meal each day and other cats consuming much larger single meals. As a result, a lower average meal size and higher number of meals consumed each day was observed for heart.

For heart, no preference for lamb or beef in the initial food intake results (see section 7.2.1) was further demonstrated when evaluating the rate of consumption. The panel showed similar rates of consumption ( $P>0.05$ ) for lamb and beef heart of  $4.2 \pm 0.8$  g/min and  $3.4 \pm 0.6$  g/min, respectively. This showed that although lamb heart was consumed in greater quantities and over a shorter period than the beef heart, the difference was not large enough to show statistical significance. Relative to the food intake percentages, the panel spent 17.2 minutes and 21.6 minutes consuming lamb and beef heart, respectively.



*Figure 8.8: Example of the most common intake pattern observed in beef versus lamb heart preference testing (shown is the intake by cat 4 on day 2 of heart preference testing)*

Food intake patterns given by the 40 load cell graphical outputs revealed that on 14 occasions, lamb heart was the first offal tried and more was consumed compared to beef heart. On 11 occasions, the opposite pattern was observed where beef heart was tried first, and more was consumed compared to lamb heart. Collectively, these two intake patterns were most common over the heart preference testing week and are shown in Figure 8.8. In addition, the same amount of beef and lamb heart was consumed on 13 occasions, nine of which showed lamb being tried first and four showing beef being tried first. Finally, on two occasions, lamb heart was tried first, however, more of beef heart was consumed. On no occasion was beef heart tried first with more of lamb heart being consumed. Overall, no preference for lamb heart or beef heart was observed.

#### *Kidney*

The cats consumed an average meal size of  $79.4 \pm 6.7$  g for lamb kidney (individual meal sizes ranging from 10.4g to 100.5g) which was larger than  $47.0 \pm 3.4$  g for beef kidney (individual meal



sizes ranging from 10g to 100.3g) over the week ( $P < 0.05$ ). The meal sizes are consistent with the food intake results showing a preference for lamb over beef kidney. On average, 1.13 meal of lamb kidney and 1.60 meals of beef kidney were consumed by the cats on each day of testing, with beef kidney showing a gap of  $244.1 \pm 62.0$  seconds between each meal.

For kidney, the preference for lamb over beef in the initial food intake results (see section 7.2.1) was further demonstrated when evaluating the rate of consumption. The panel showed different rates of consumption ( $P < 0.05$ ) for lamb and beef kidney of  $13.9 \pm 1.5$  g/min and  $9.2 \pm 1.1$  g/min, respectively. This showed that the preferred lamb kidney was consumed in greater quantities and over a shorter period than beef kidney which had a lower intake rate over a longer period. Relative to the food intake percentages, the panel spent 7.0 minutes and 9.1 minutes consuming lamb and beef kidney, respectively.

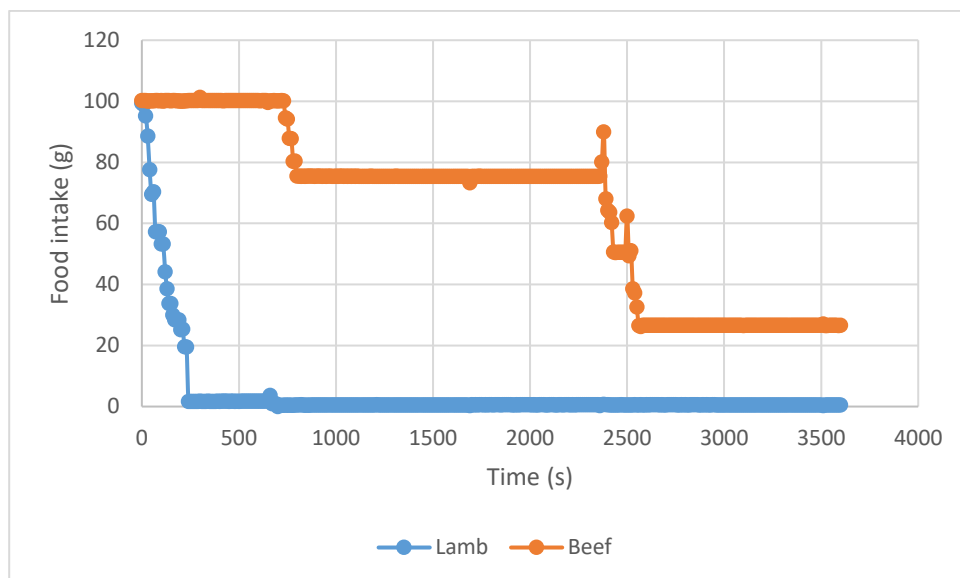


Figure 8.9: Example of the intake pattern in beef versus lamb kidney preference testing which resulted in an overall preference for lamb kidney (shown is the intake by cat 2 on day 3 of kidney preference testing)

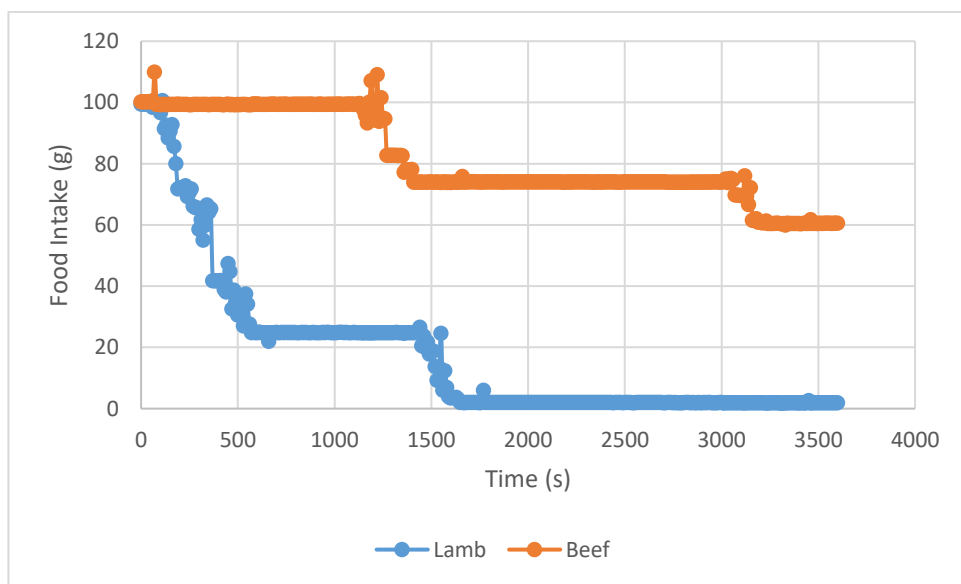
Food intake patterns given by the 40 load cell graphical outputs revealed that on 27 occasions, the same amount of beef and lamb kidney was consumed. Of these 27 occasions, 18 showed lamb kidney being the first tried and the remaining nine showed beef being the first offal tried. On seven occasions, lamb kidney was the first offal tried with more being consumed compared to beef kidney, shown in Figure 8.9. This was enough to drive the preference for lamb up as on just two occasions, beef kidney was the first offal tried with more being consumed compared to lamb kidney. Additionally, on two occasions, lamb kidney was the first offal tried however, more beef kidney was consumed overall. Finally, the reverse was demonstrated on two occasions where beef kidney was the first offal tried, however, more lamb kidney was consumed overall.

There were no occasions where lamb and beef kidney were not approached. Overall, a preference for lamb kidney over beef kidney was observed.

### *Tripe*

The cats consumed an average meal size of  $57.0 \pm 6.9$  g for lamb tripe (individual meal sizes ranging from 11g to 100.6g) which was larger than  $26.5 \pm 2.1$  g for beef tripe (meal sizes ranging from 10.3g to 97.6g) over the week ( $P < 0.05$ ). The meal sizes are consistent with the food intake results showing a preference for lamb over beef tripe. On average, 1.33 meals of lamb tripe and 1.60 meals of beef tripe were consumed by the cats on each day of testing, with beef tripe showing a gap of  $578.9 \pm 129.8$  seconds between each meal.

For tripe, the preference for lamb over beef in the initial food intake results (see section 7.2.1) was further demonstrated when evaluating the rate of consumption. The panel showed different rates of consumption ( $P < 0.05$ ) for lamb and beef tripe of  $11.4 \pm 1.4$  g/min and  $3.8 \pm 1.0$  g/min, respectively. This showed that the preferred lamb tripe was consumed in greater quantities and over a shorter period than beef tripe which had a lower intake rate over a longer period. Relative to the food intake percentages, the panel spent 7.3 minutes and 14.6 minutes consuming lamb and beef tripe, respectively.



*Figure 8.10: Example of the most common intake pattern observed in beef versus lamb tripe preference testing (shown is the intake by cat 5 on day 5 of tripe preference testing)*

Food intake patterns given by the 40 load cell graphical outputs revealed that on 23 occasions, lamb tripe was tried first and in greater amounts than beef tripe, shown in Figure 8.10. In contrast, there was only one occasion in which beef tripe was tried first and in greater amounts than lamb tripe. Furthermore, on eight occasions, the same amount of beef and lamb tripe was

consumed with six of these showing lamb being tried first and the remaining two showing beef being tried first. On five occasions, beef tripe was tried first but overall more lamb was consumed. The opposite was observed on one occasion in which lamb tripe was tried first but more beef was eaten. Finally, refusal to consume either beef or lamb tripe was observed on two occasions. Overall, a preference for lamb tripe over beef tripe was observed.

### MDM

The cats consumed an average meal size of  $12.7 \pm 1.3$  g for lamb MDM (meal sizes ranging from 10.1g to 42.7 g) which was larger than  $4.8 \pm 2.5$  g for beef MDM (meal sizes ranging from 10g to 50.3g) over the week ( $P < 0.05$ ). The meal sizes are consistent with the food intake results which showed a preference for lamb over beef MDM. On average, 0.85 meals of lamb MDM and 0.40 meals of beef MDM were consumed by the cats on each day of testing. In both cases, no complete meal was consumed.

For MDM, preference for lamb was displayed over beef through the initial food intake results (see section 7.2.1) however, slight discrepancies were demonstrated when evaluating the rate of consumption. The panel showed similar rates of consumption ( $P > 0.05$ ) for lamb and beef MDM of  $1.0 \pm 0.2$  g/min and  $0.4 \pm 0.2$  g/min, respectively. This showed that although lamb MDM was consumed in greater quantities and over a shorter period than the beef MDM, the difference was not large enough to show statistical significance. Relative to the food intake percentages, the panel spent 25.8 minutes and 31.3 minutes consuming lamb and beef MDM, respectively.

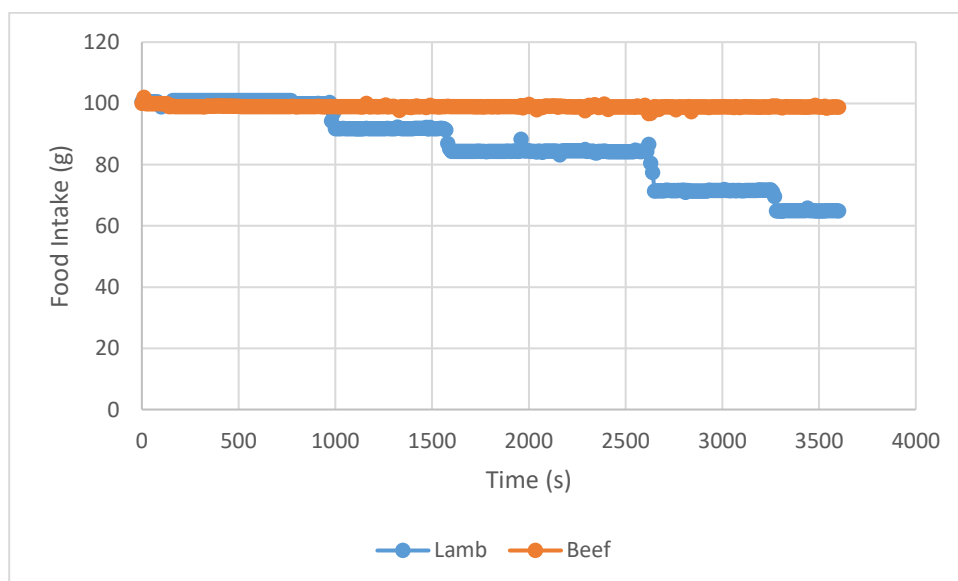


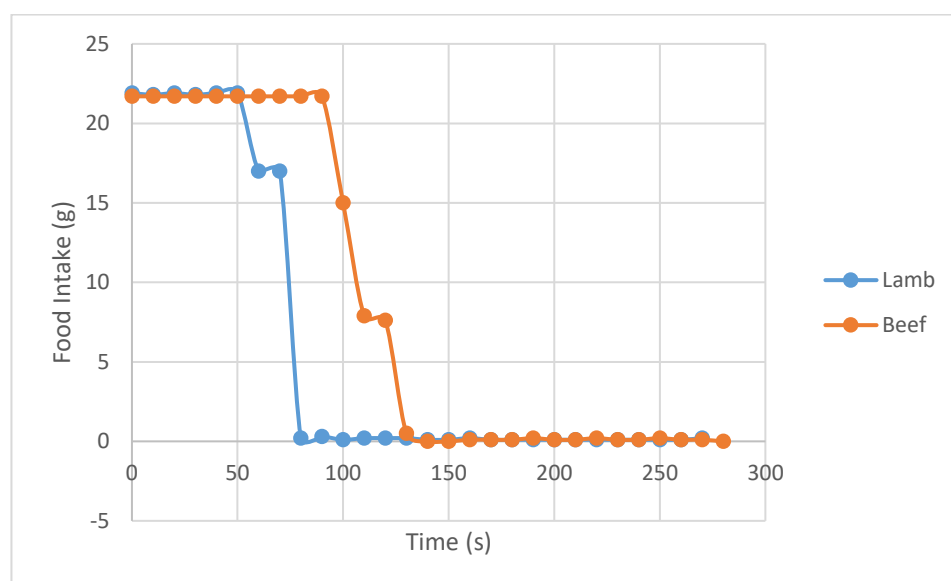
Figure 8.11: Example of the most common intake pattern observed in beef versus lamb MDM preference testing (shown is the intake by cat 8 on day 2 of MDM preference testing)

Food intake patterns given by the 40 load cell graphical outputs revealed that on 13 occasions, lamb MDM was tried first and in greater amounts than beef MDM, shown in Figure 8.11. On 11 occasion, the opposite was observed in which beef MDM was tried first and in greater amounts than lamb MDM. On six occasions similar amounts of MDM were consumed with three occasions showing lamb MDM being tried first and three showing beef MDM being tried first. Finally, on ten occasions, 25% of the observations, complete refusal for either MDM was observed by some cats in the panel. On no occasions were lamb and beef MDM both fully consumed. Overall, no preference for lamb MDM or beef MDM was observed.

### Liver

The cats consumed an average meal size of  $21.2 \pm 0.5$  g for lamb liver (meal sizes ranging from 10.5g to 23.1 g) which was similar to  $21.7 \pm 0.1$  g for beef liver (meal sizes ranging from 21.3g to 22.5 g) over the week ( $P > 0.05$ ). The meal sizes are consistent with the food intake results showing no preference for lamb or beef liver. On average, 1.03 meals of lamb liver and one meal of beef liver were consumed by the cats on each day of testing.

For liver, no preference for lamb and beef in the initial food intake results (see section 7.2.1) was further demonstrated when evaluating the rate of consumption. The panel showed similar rates of consumption ( $P > 0.05$ ) for lamb and beef liver of  $12.4 \pm 1.1$  g/min and  $11.4 \pm 1.0$  g/min, respectively. This showed that although lamb liver was consumed in greater quantities and over a shorter period than the beef liver, the difference was not large enough to show statistical significance. Relative to the food intake percentages, the panel spent 8.0 minutes and 8.7 minutes consuming lamb and beef liver, respectively.



*Figure 8.12: Example of the general intake pattern for beef versus lamb liver preference testing (shown is the intake by cat 1 on day 2 of liver preference testing)*

Food intake patterns given by the 40 load cell graphical outputs revealed that all liver presented was consumed by each cat. On 17 occasions, beef liver was tried and consumed first, followed by consumption of lamb liver. The same was also observed for lamb liver on 17 occasions. The remaining six occasions saw the intake of lamb liver being tried first but the beef liver being consumed first on three occasions, and vice versa for the remaining three. On no occasion did the cats leave any liver that was presented in the bowl. Overall, no preference for lamb liver or beef liver was observed.

With the exception of heart in the meal size analysis and the rate of consumption results for MDM, the meal size analysis and rate of consumption results were consistent with the overall intake findings in section 7.2.1.

For lung, kidney, tripe and MDM, the meal sizes were greater for lamb over beef ( $P < 0.05$ ) and no difference was observed between the meal sizes consumed by the panel for lamb and beef liver ( $P > 0.05$ ). Differences were displayed between lamb and beef heart when analysing the meal sizes, showing preference for lamb over beef on an individual meal size basis ( $P < 0.05$ ), although the greater number of meals for beef heart resulted in a greater overall intake compared to lamb ( $P < 0.05$ ). This difference is likely due to beef heart showing greater variability in intake data between cats with some consuming multiple (three to six) meals just above the 10g minimum required to be classified as a meal and other cats consuming much larger single meals. As a result, a lower average meal size and higher number of meals consumed each day was observed for heart.

Consequently, the rate of consumption of lung, kidney, and tripe was greater for lamb over beef ( $P < 0.05$ ). Additionally, no difference between the rate of consumption of heart and liver from each species was displayed ( $P > 0.05$ ). Due to the overall low intake amounts of MDM, the methods used to determine the rate of consumption were inaccurate as the lower intake resulted in greater variability in data points. As a consequence, discrepancies were displayed between the total intake, which showed preference for lamb over beef MDM ( $P < 0.05$ ), and no difference being displayed when evaluating the rate of consumption ( $P > 0.05$ ).

## 9. Three-Bowl Preference Test

### 9.1 Materials and Methods

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

#### 9.1.1 Test Animals

The same cat panel as previously described (see Section 5.1.1) was used to test the preference of top and bottom ranked offals (liver and MDM), established in chapters 5, 6 and 7. Lamb, beef and chicken liver and MDM were assessed in a final three-bowl test.

Testing was carried out from Monday 3<sup>rd</sup> December until Friday 14<sup>th</sup> December at the Feline Nutrition Unit at Massey University, Palmerston North.

#### 9.1.2 Ingredient Used

Chicken, beef and lamb MDM and liver were tested in this block of preference tests and they were tested in this order. All ingredients were provided by MPI-accredited meat processors through Ziwi Ltd (Mount Maunganui, New Zealand), and were delivered in approximately 15kg (chicken) and 20kg (beef and lamb) frozen blocks.

#### 9.1.3 Ingredient Preparation

All the frozen blocks of offal were prepared in a similar manner as the beef and lamb offal (see Section 8.1.3), with the addition of thawing equivalent chicken offal. Previously in acceptance testing, chicken offals were vacuum packed into 2kg portions but were vacuum packed into 1kg portions for this series of three-bowl preference tests.

#### 9.1.4 Testing Methods

A three-bowl test, with the use of live video recordings, was carried out to reveal which MDM and liver the cat panel first approached, first consumed and first/most completed in the one hour testing periods over five days in order to obtain 40 measurements for each observation.



*Figure 9.1: Presentation of the individual testing booths on Day 1 of three-bowl MDM testing. Pictured is chicken MDM on the left, beef MDM in the centre and lamb MDM on the right (Note: the position of each MDM presented was changed each day of testing)*

All cats were presented with 100g of chicken, beef and lamb MDM (shown in Figure 9.1) and 30g of chicken, beef and lamb liver. Cats were removed before one hour had elapsed if they had consumed all of one of the MDM or liver treatments.

#### 9.1.5 Data Collection and Statistical Analyses

To determine the food intake, the equation given previously in Section 5.1.5 was used.

Following the three-bowl testing, a Chi-Square Goodness-of-Fit Test was carried out in Minitab 18 (Minitab Inc., State College, Pennsylvania, USA) and used to determine whether the observations of chicken, beef and lamb were equally distributed within each testing condition of first approached, first consumed and first/most completed.

## 9.2 Results and Discussion

Forty measurements of first sample approached, first consumed and first/most completed were obtained for the MDM and liver three-bowl preference test.

### 9.2.1 MDM Three-Bowl Analysis

Each cat was offered 100g each of chicken, beef and lamb MDM (300g a day) for five days. With three different MDM varieties being offered, if no preference was demonstrated, an expected value of 13.33 was predicted for the 40 observations for the first approached MDM.

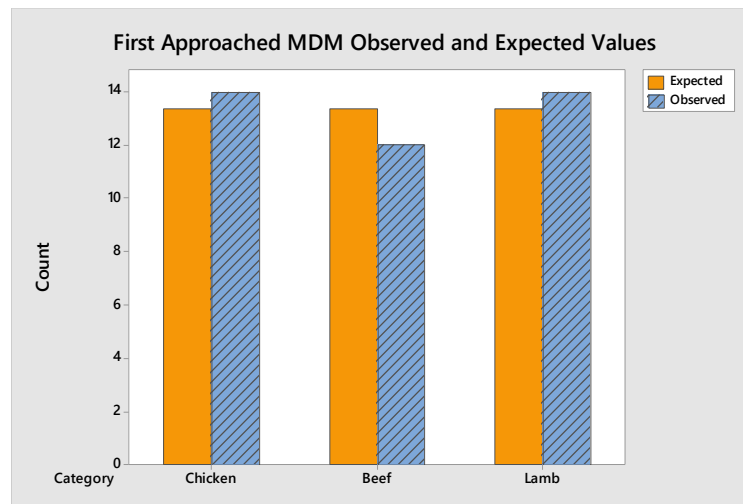


Figure 9.2: Expected versus observed values for the first approached MDM

The first approached MDM results revealed that the observed values were similar to the expected values ( $P > 0.05$ ; see Figure 9.2). The observed values were comparable between chicken, beef and lamb with 14, 12 and 14 observations, respectively.

Compared to the first approached MDM which had 40 observations, the first consumed and first/most completed MDM had 39 observations. This was due to cat 3 (Nyssa) showing refusal to consume any MDM on Day 3 of the three-bowl test. As a result, an expected value of 13 observations for each species of MDM was predicted.

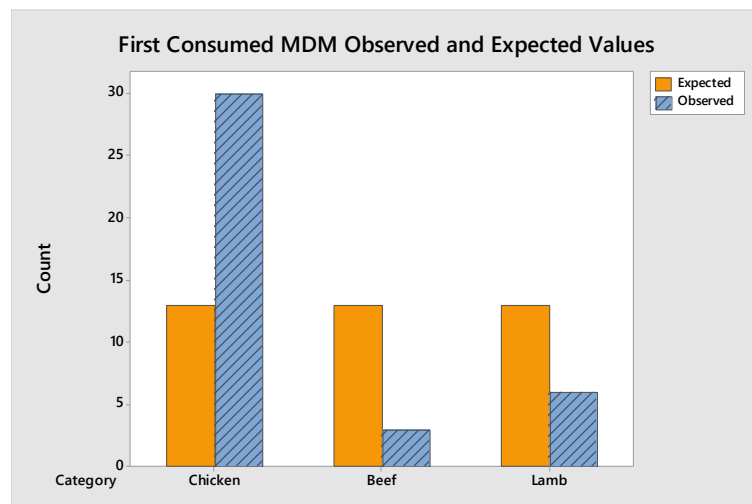


Figure 9.3: Expected versus observed values for the first consumed MDM

The first consumed MDM results revealed that the observed values were different to the expected values ( $P < 0.05$ ; see Figure 9.3). The observed values were very different between the three species. Chicken MDM was the first sample consumed in 30 of the 39 total observations, with beef MDM and lamb MDM consumed first three and six times,



respectively. This indicated that the cats consumed chicken MDM preferentially over beef and lamb MDM ( $P < 0.05$ ).

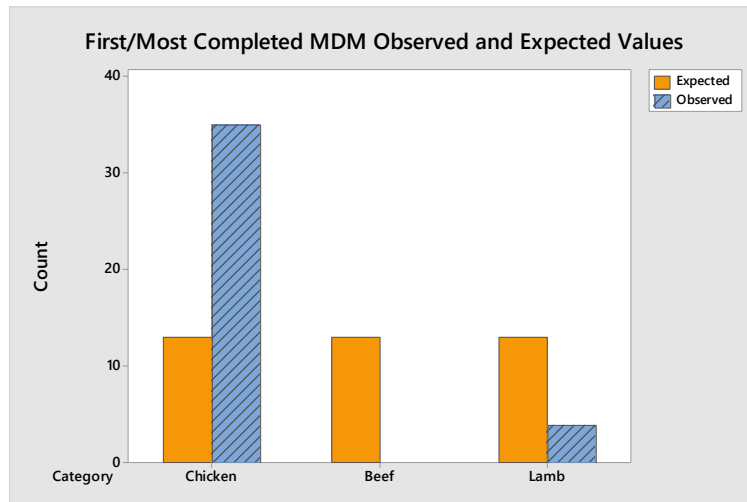


Figure 9.4: Expected versus observed values for the first/most completed MDM

The first finished MDM results were similar to the first consumed data. The observed values were again not consistent with the expected values ( $P < 0.05$ ; see Figure 9.4), and were again very different between the three species. Chicken MDM the first sample finished in 35 of the 39 total observations, with beef MDM and lamb MDM finished first zero and four times, respectively. This indicated that the cats not only first consumed chicken MDM almost exclusively and preferentially over beef and lamb MDM ( $P < 0.05$ ), but then continued to consume it and finish it before the other two samples ( $P < 0.05$ ).

Overall, these results showed that the cats consumed and completed chicken MDM preferentially over beef and lamb MDM, the lowest ranked offal for each species. This may be due to the cats finding the odour of chicken MDM more attractive than that of beef and lamb MDM, which is consistent with the previous findings of Hullár et al., (2001).

### 9.2.2 Liver Three-Bowl Analysis

Each cat was offered 30g each of chicken, beef and lamb liver (90g a day) for five days. With three different liver varieties being offered, again if no preference was demonstrated, an expected value of 13.33 was predicted for the 40 observations for the first approached, first consumed and first/most completed liver.

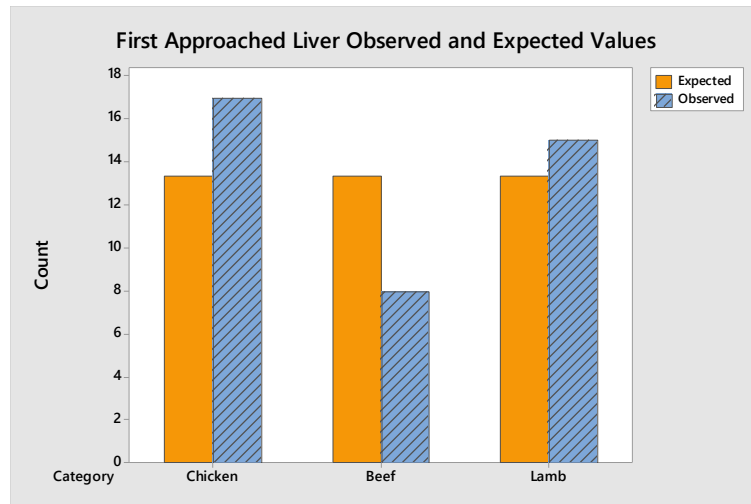


Figure 9.5: Expected versus observed values for the first approached liver

The first approached liver results revealed that the observed values were consistent with the expected values ( $P > 0.05$ ; see Figure 9.5), and were again comparable between chicken, beef and lamb with 17, 8 and 15 observations, respectively.

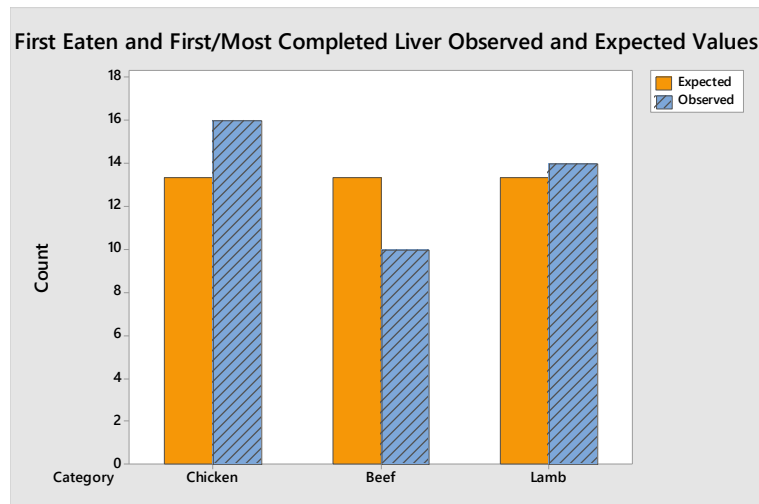


Figure 9.6: Expected versus observed values for the first eaten and the first/most completed liver

Similar to the first approached data, the first eaten and the first/most completed liver results also revealed no difference between the observed values and expected values ( $P > 0.05$ ; see Figure 9.6). The observed values were very similar between chicken, beef and lamb with 16, 10 and 14 observations, respectively.

Overall, the panel did not show a preference between chicken, beef and lamb liver in all three testing conditions (first approached, first consumed and first/most completed), during the final three-bowl test. These results indicate that liver, the top ranked offal from each species, is highly palatable to cats regardless of the species it is derived from.

## 10. General Discussion

This study investigated the palatability and acceptance of individual offal from lamb, beef and chicken sources used in the production of pet food for cats. To date, existing literature has focused heavily on the palatability of complete pet diets. However, no known studies have evaluated the acceptance and preference of ingredients, predominately meat and meat offals, to understand how individual components of the diet could be driving palatability. The results of the current study indicated that within lamb, beef and chicken there are clear differences in the acceptance of different offal types and that the palatability ranking appear to alter slightly depending on species from which the offal originated.

Pet food palatability and the amount of protein from animal origin has a strong correlation in cats (Zaghini & Biagi, 2005). Protein has been commonly cited throughout the literature as being a key driver for palatability in cats due to the species' high requirements for protein.

When the protein content of individual ingredients was compared to the palatability results for lamb, a general downward trend was observed between the least preferred offal types versus their protein content. There is an exception for tripe which has a higher protein content (as shown in Figure 10.1). Both liver and kidney had the highest amount of protein at 25.4% and 20.2%, respectively and had the highest palatability of all the lamb offal types investigated. In contrast, MDM was the least palatable of all the offals presented and had the lowest amount of protein, indicating some association between protein content and palatability.

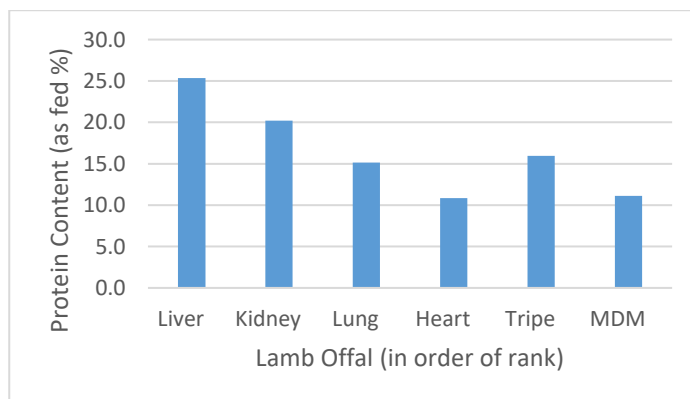


Figure 10.1: The final ranking of lamb offal and their respective protein contents

For beef, the distinctive downward trend as seen in the lamb, was not so clearly evident (shown in Figure 10.2). Overall, no evident trend was displayed between offal ranking and the respective protein contents. The key finding was that beef liver showed the highest

amount of protein of 25.9% and was the most palatable ingredient of the beef offals, which was consistent with the findings for lamb liver.

Although MDM was the lowest ranked ingredient of the beef offals, a middle range protein content of 15.6% was observed. This indicated that the panel's low acceptance for MDM was driven by other factors, possibly relating to the considerably high fat content of 40.0% and the greater likelihood of lipid oxidation taking place (Zaghini and Biagi, 2005). In addition, all of the beef offals are from older animals so may be more variable in their fat content and texture compared to lamb which is a younger fast-growing animal that is associated with more lean growth.

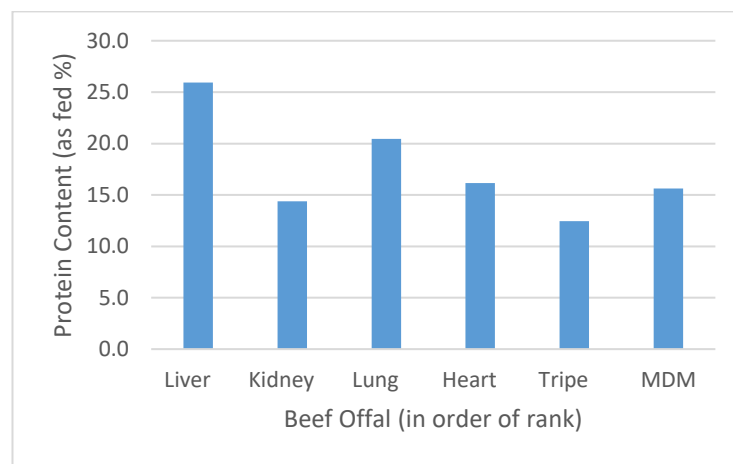


Figure 10.2: The final ranking of beef offal and their respective protein contents

For chicken, a downward trend similar to that in lamb was again observed, with the exception of MDM (shown in Figure 10.3). As demonstrated in the lamb and beef results, liver contained the highest amount of protein at 20.6% and was the most palatable chicken offal.

As shown in chapter 7, chicken MDM was consumed in greater quantities during acceptance testing of 476g compared to lamb and beef MDM at 343g and 239g, respectively. These results suggested that chicken MDM was the most palatable of the three MDM from different species.

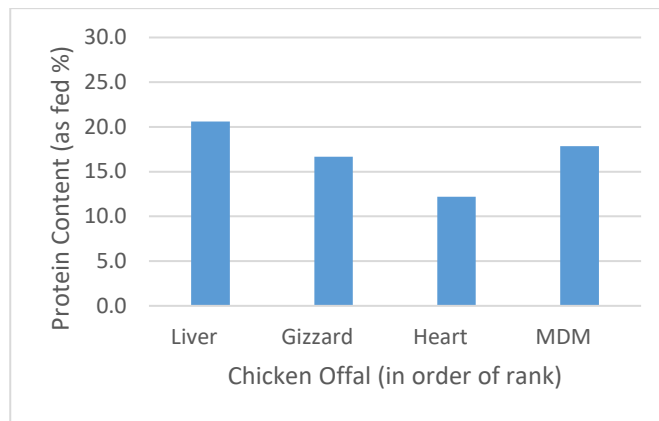


Figure 10.3 The final ranking of chicken offal and their respective protein contents

The final three-bowl test between lamb, beef and chicken MDM revealed that the cats did consume chicken preferentially and almost exclusively over beef and lamb MDM. Given the trends in protein content versus palatability, this may be attributable to chicken MDM having a slightly higher protein content of 17.8% compared to 11.1% for lamb and 15.6% for beef. However, other factors could be governing this response such as the age of animals at the time of slaughter used for ingredients in pet food may also have an influence on palatability. Chickens are 35-45 days old when they are processed for meat and organs compared to lambs which are typically 4 to 9 months at the time of slaughter while cull cows (the likely source of beef offal) are typically 5 to 8 years of age at slaughter. It is possible that off flavours may develop as the animals' age. It is also very likely that, in older animals, there is a greater presence of connective tissue which may influence the textural properties of the offal. This could be particularly relevant when considering beef MDM relative to chicken MDM, may have resulted in beef MDM being a less palatable ingredient.

The final three-bowl preference test was also carried out for liver, the top ranked offal from lamb, beef and chicken acceptance testing. The cats showed no preference for one species of liver over the other. Furthermore, the protein contents of 25.4% for lamb, 25.9% for beef and 20.6% for chicken liver were possibly all too high for cats to detect a difference and thus they all had an overall high palatability regardless of the animal it was derived from. These results suggest that protein may be driving the selection and that the palatability of liver may not change with the age of the animal, as chicken, lamb and beef are equally palatable. In addition, liver in both humans and animals has the ability of to regulate its growth until it is of normal mass and structure following injury whilst remaining in homeostasis during the regenerative process (Abercrombie et al., 1951; Fausto, 2000; Michalopoulos, 2007). It is

possible that the unique ability for liver to regenerate, unlike other organs, may also be a contributing factor in the selection of liver as well as its high protein content.

### 10.1 Limitations of the Research

This research provides a good initial evaluation into the palatability of offals within a single species and the difference between equivalent offals across species. However, there are still some limitations of this study.

The main limitation is that evaluating palatability in this study was restricted to comparing the nutritional composition of individual offals, particularly the protein contents, in order to determine overall palatability. However, other parameters such as texture, age of animals and ratio of collagen protein to muscle fibre protein should also be further explored to determine their influence on palatability.

Furthermore, offals were presented to the cats raw and unprocessed to limit variability in palatability due to the production of desirable compounds via Maillard reaction or the formation of lipid peroxides to give undesirable flavours as given by Hagen-Plantinga et al., (2017). It is possible that the outcomes for palatability of raw offal described in this study may differ to palatability results for single offals that have been processed. However, this study did not extend into comparing raw versus cooked offal.

### 10.2 Further Research

This study revealed that clear preferences between the different raw offals used in the production of pet food are displayed in the cat panel. However, more work is required to explore additional parameters which may influence palatability.

It is suggested that in future studies, other parameters such as the textural properties of offals be analysed to determine their influence on palatability. In addition, the age of the lamb, beef and chicken could be investigated further to determine how the age of the animal impacts palatability.

The results from this study also suggest that palatability of offals within a single species, particularly the top ranked offals, may be driven by protein content. Further research is required to validate these findings with confidence.

Extending on from section 10.1, it may be worthwhile to compare the palatability of offals that have been processed via methods such as air-drying or retorting to determine processing implications on the palatability of offal.

### 10.3 Conclusion and Implications for Industry

Overall, the cats were able to detect differences in palatability of offals within a species via two-bowl acceptance testing, as well as between equivalent offals from different sources via two and three-bowl preference testing.

This series of acceptance and final preference tests also supports the findings given by Stasiak (2002) and Zaghini & Biagi (2005) that cats have very high protein requirements which can be extended down onto an individual ingredient level. Therefore, selecting highly palatable ingredients whilst still meeting pet food manufacturing guidelines may also play a role in improving overall diet palatability.

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## Appendices

### Appendix A – AAFCO Nutrient Requirements for Cats (2017)

Table A1: AAFCO nutrient requirements for cats for both maintenance and during growth and lactation (expressed on a dry matter basis)

	Units	Maintenance		Growth/Lactation	
		minimum	maximum	minimum	maximum
<b>Crude Protein</b>	<b>%</b>	<b>26.0</b>	-	<b>30.0</b>	-
Taurine (canned)	%	0.2	-	0.2	-
Taurine (extruded)	%	0.1	-	0.1	-
Arginine	%	1.04	-	1.24	-
Histidine	%	0.31	-	0.33	-
Isoleucine	%	0.52	-	0.56	-
Leucine	%	1.24	-	1.28	-
Lysine	%	0.83	-	1.20	-
Methionine-cystine	%	0.40	-	1.10	-
Methionine	%	0.20	1.5	0.62	1.5
Phenylalanine-tyrosine	%	1.53	-	1.92	-
Phenylalanine	%	0.42	-	0.52	-
Threonine	%	0.73	-	0.73	-
Tryptophan	%	0.16	1.7	0.25	1.7
Valine	%	0.62	-	0.64	-
<b>Crude Fat</b>					
Linoleic acid	%	0.6	-	0.6	-
alpha-Linolenic Acid	%	ND		0.02	
Arachidonic acid	%	0.02	-	0.02	-
Eicosapentaenoic + Docosahexaenoic Acid	%	ND		0.012	
(Linoleic + Arachidonic):(alpha-Linolenic + Eicosapentaenoic + Docosahexaenoic Acid					
<b>Minerals</b>					
Calcium	%	0.6	-	1.0	-
Phosphorous	%	0.5	-	0.8	-
Ca:P ratio		-	-	-	-
Potassium	%	0.6	-	0.6	-
Sodium	%	0.2	-	0.2	-
Chloride	%	0.3	-	0.3	-
Magnesium	%	0.04	-	0.08	-
Iron	mg/kg	80	-	80	-
Copper (canned)	mg/kg	5	-	8.4	-
Copper (extruded)	mg/kg	5		15	
Manganese	mg/kg	7.6	-	7.6	-
Zinc	mg/kg	75	-	75	-

Iodine	mg/kg	0.6	9.0	1.8	9.0
Selenium	mg/kg	0.3	-	0.3	-
<b><i>Vitamins and others</i></b>					
Vitamin A	IU/kg	3332	333300	6668	333300
Vitamin D	IU/kg	280	30080	280	30080
Vitamin E	IU/kg	40	-	40	-
Vitamin K	IU/kg	0.1	-	0.1	-
Thiamine	mg/kg	5.6	-	5.6	-
Riboflavin	mg/kg	4.0	-	4.0	-
Pantothenic acid	mg/kg	5.75	-	5.75	-
Niacin	mg/kg	60	-	60	-
Pyridoxine	mg/kg	4.0	-	4.0	-
Folic acid	mg/kg	0.8	-	0.8	-
Biotin	mg/kg	0.07	-	0.07	-
Vitamin B12	mg/kg	0.02	-	0.02	-
Choline	mg/kg	2400	-	2400	-

Appendix B – Beef and Lamb Nutritional Composition as Derived from Purchas and Wilkinson (2013)

*Table B1: Muscle, fat, bone and waste percentage present in selected beef offal*

Offal Item	Muscle %		Fat %		Bone & Waste %	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Heart	86.0±5.4	82.7±8.9	13.1±5.5	16.2±9.1	0.9±0.6	1.1±0.4
Kidney	89.5±2.8	80.0±4.8	0	0	10.5±2.8	20.0±4.8
Liver	97.2±2.1	98.1±2.3	0	0	2.8±2.1	1.9±2.3
Tripe	89.0±7.4	91.4±5.6	11.0±7.4	8.6±5.6	0	0

Table B2: Nutrient items present in selected beef offal

Nutrient Item	Heart		Kidney		Liver		Tripe	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Water (%)	78.1	62.3	80.5	66.4	70.4	66.4	82.2	77.7
Energy (kJ/100 g)	436	747	361	653	494	564	322	429
Protein (%)	18.5	31.3	15.7	27.3	20.5	23.3	14.9	19.0
Fat (%)	3.4	6.0	2.6	5.3	4.1	4.7	2.0	3.0
Ash (%)	1.02	1.04	1.15	1.55	1.46	1.80	1.10	0.89
Vitamin B1 (mg/100 g) (Thiamine)	0.252	0.237	0.559	0.403	0.371	0.376	0.051	0.022
Vitamin B2 (mg/100 g) (Riboflavin)	0.68	0.98	2.12	2.95	2.35	3.04	0.19	0.10
Vitamin B3 (mg/100 g) (Niacin)	4.4	3.4	4.9	3.9	15.4	13.8	7.9	2.6
Vitamin B5 (mg/100 g) (Pantothenic acid)	1.8	1.2	4.0	3.1	10.3	9.8	0.7	0.2
Vitamin B6 (mg/100 g) (Pyridoxine)	0.160	0.122	0.316	0.254	0.428	0.452	0.035	0.013
Vitamin B12 (µg/100 g) (Cyanocobalamin)	10.8	6.7	27.7	21.3	84.5	96.0	7.1	2.3
Vitamin A (µg/100 g)	10.3	14.1	89.1	104.2	28319	21014	6.1	5.9
Vitamin D3 (µg/100 g)	0.15	0.17	0.15	0.83	0.03	0.11	0.20	0.26
25-OH Vitamin D3 (µg/100g)	0.270	0.357	0.321	0.284	0.174	0.149	0.282	0.085
Vitamin E (mg/100 g)	1.22	2.09	0.82	1.53	1.84	1.28	0.45	0.51
Cholesterol (mg/100 g)	123.7	200.7	404.2	1002.0	254.1	242.5	117.4	198.9
Calcium (mg/100 g)	4.06	5.58	9.20	13.88	3.80	4.20	112.21	157.63
Copper (mg/100 g)	0.37	0.65	0.41	0.56	5.30	5.73	0.09	0.11
Iodine (µg/100 g)	1.5	2.0	6.0	6.7	4.3	4.1	4.3	2.5
Iron (mg/100 g)	4.38	6.81	3.83	5.70	8.44	7.17	4.44	3.72
Magnesium (mg/100 g)	21.9	26.3	14.7	18.1	19.3	21.0	19.1	24.4
Manganese (µg/100 g)	33.9	41.5	108.7	154.8	299.0	328.0	4055.0	6066.0
Phosphorus (mg/100 g)	209	265	234	338	362	397	159	168
Potassium (mg/100 g)	275	184	225	144	327	336	217	102
Selenium (µg/100 g)	8.7	17.2	103.3	105.2	16.5	16.2	3.1	4.3
Sodium (mg/100 g)	86	59	175	123	53	55	81	40
Zinc (mg/100 g)	1.5	2.8	1.5	2.6	3.0	3.4	1.7	2.4

Table B3: Fatty acid composition of cooked and raw selected beef offal expressed as a percentage of total fatty acids

Fatty acid (% of total fatty acids except for Total FAs and the ratios)	Heart		Kidney		Liver		Tripe	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
C8:0 Caprylic	ND	ND	ND	ND	ND	ND	ND	0.39
C10:0 Capric	0.03	0.01	0.03	0.03	ND	0.06	0.04	ND
C11:0 Undecanoic	ND	ND	ND	ND	ND	ND	ND	ND
C12:0 Lauric	0.04	0.02	ND	ND	ND	ND	0.06	0.05
C13:0 Tridecanoic	ND	ND	ND	ND	ND	ND	ND	ND
C14:0 Myristic	0.95	1.24	0.44	0.41	0.67	0.69	2.47	2.43
C14:1n5 c9 Myristoleic	0.07	0.09	0.12	0.04	0.12	0.11	0.38	0.36
C15:1n5 c10 Pentadecenoic	0.37	0.28	0.31	0.35	0.11	0.11	0.10	ND
C16:0 Palmitic	16.23	17.32	18.21	17.88	13.19	14.17	22.61	22.5
C16:1n7 t9 Palmitelaidic	0.66	0.69	0.92	0.83	0.61	0.68	0.82	ND
C16:1n7 c9 Palmitoleic	1.42	1.30	0.84	0.81	1.24	1.33	1.95	3.11
C17:0 Margaric	1.71	1.87	1.54	1.46	1.74	1.94	2.80	2.80
C17:1n7 c10 Heptadecenoic	ND	ND	ND	ND	ND	ND	ND	ND
C18:0 Stearic	23.76	25.43	16.34	15.73	29.98	32.45	22.63	22.5
C18:1n9 t9 Elaidic	0.24	0.30	0.17	0.14	0.17	0.20	0.52	0.30
C18:1n7 t11 Vaccenic	1.86	2.17	0.99	0.84	2.23	2.33	3.75	3.57
C18:1n9 c9 Oleic	20.63	19.70	15.79	15.28	12.62	13.31	30.26	31.0
C18:1n7 c11 Vaccenic	1.42	1.34	1.99	2.02	0.86	0.87	0.99	1.07
C18:2n6 t Linolelaidic	ND	ND	0.11	ND	ND	ND	ND	ND
C18:2n6 c Linoleic	12.68	12.37	11.95	12.30	5.46	5.01	2.90	2.78
C20:0 Arachidic	0.21	0.24	0.53	0.54	0.12	0.13	0.32	0.26
C18:3n6 c Gamma linolenic	0.19	0.14	0.15	0.19	0.38	0.37	0.04	ND
C20:1n9 c11 Eicosenoic	0.15	0.15	0.33	0.37	0.11	0.09	0.17	0.18
C18:3n3 c Alpha linolenic	3.09	2.91	2.58	2.62	2.06	1.81	1.09	0.98
CLA C18:2-c9,t11	0.38	0.48	0.43	0.39	0.70	0.50	0.54	1.06
CLA C18:2-t10,c12	ND	ND	ND	ND	ND	ND	ND	ND
C21:0 Heneicosanoic	0.36	0.38	0.45	0.47	0.56	0.54	0.72	0.19
C20:2n6 c Eicosadienoic	0.14	0.14	0.26	0.27	0.28	0.24	0.11	ND
C22:0 Behenic	0.17	0.18	1.22	1.29	0.30	0.24	0.25	ND
C20:3n6 c Eicosatrienoic	1.31	1.20	1.79	1.88	3.83	3.37	0.46	0.57
C22:1n9 c13 Erucic	ND	ND	ND	ND	ND	ND	ND	0.13
C20:3n3 c Eicosatrienoic	0.07	0.10	0.60	0.61	0.10	0.10	0.11	ND
C20:4n6 c Arachidonic	5.58	4.82	10.81	11.98	7.43	6.47	1.40	1.36
C23:0 Tricosanoic	0.22	0.23	0.27	0.30	0.53	0.60	0.12	0.18
C22:2n6 c Docosadienoic	0.70	0.62	0.59	0.59	2.62	2.21	0.23	ND
C20:5n3 c EPA	3.23	2.59	4.92	4.95	4.39	3.67	0.61	0.64
C24:0 Lignoceric	0.17	0.18	0.67	0.68	0.35	0.41	0.19	ND
C24:1n9 c15 Nervonic	0.10	0.09	0.42	0.39	0.09	0.11	0.07	ND
C22:5n3 c DPA	1.62	1.28	3.24	3.35	5.64	4.66	1.15	1.23



C22:6n3 c DHA	0.22	0.16	0.95	1.00	1.53	1.24	0.14	0.20
SFA	43.86	47.10	39.74	38.80	47.43	51.20	52.22	51.40
MUFA	26.92	26.11	21.88	21.06	18.15	19.16	39.01	39.79
PUFA	29.22	26.79	38.37	40.13	34.41	29.64	8.78	8.81
P/S ratio	0.67	0.57	0.97	1.03	0.73	0.58	0.17	0.17
n-6/n-3 ratio	2.50	2.74	2.09	2.17	1.46	1.54	1.65	1.54
LCn3FA	5.14	4.12	9.71	9.91	11.66	9.66	2.01	2.07
Total FAs (g/100 g)	1.98	3.92	1.57	3.07	2.56	2.78	2.20	1.81

Table B4: Fatty acid composition of raw selected beef offal expressed as a g/100g of the lean tissue (except in the case of the ratios of P/S and n-6/n-3)

Offal Item	SFA	MUFA	Trans MUFA	PUFA	P/S	n-6 PUFA	n-3 PUFA	n-6/n-3	LCn3FA	Total FAs (g/100 g)
Heart	0.87	0.53	0.05	0.579	0.666	0.408	0.163	2.502	0.102	1.98
Kidney	0.63	0.34	0.03	0.606	0.966	0.405	0.194	2.087	0.154	1.57
Liver	1.21	0.46	0.08	0.880	0.726	0.511	0.351	1.457	0.298	2.56
Tripe	1.15	0.85	0.11	0.193	0.168	0.113	0.068	1.654	0.044	2.20

Table B5: Fatty acid composition of cooked selected beef offal expressed as a g/100g of the lean tissue (except in the case of the ratios of P/S and n-6/n-3)

Offal Item	SFA	MUFA	Trans MUFA	PUFA	P/S	n-6 PUFA	n-3 PUFA	n-6/n-3	LCn3FA	Total FAs (g/100 g)
Heart	1.85	1.02	0.12	1.052	0.569	0.757	0.276	2.744	0.162	3.92
Kidney	1.20	0.64	0.06	1.239	1.034	0.840	0.387	2.174	0.306	3.07
Liver	1.42	0.53	0.09	0.824	0.579	0.491	0.319	1.540	0.269	2.78
Tripe	0.93	0.72	0.07	0.159	0.171	0.085	0.055	1.544	0.037	1.81

Table B6: Muscle, fat, bone and waste percentage present in selected lamb offal

Offal Item	Muscle %		Fat %		Bone & Waste %	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Heart	75.8±3.0	72.3±3.5	14.0±2.0	16.9±2.5	10.2±2.6	10.8±2.6
Kidney	96.4±0.4	95.2±0.4	0	0	3.6±0.4	4.8±0.4
Liver	99.6±0.2	99.0±0.6	0	0	0.4±0.2	1.0±0.6

Table B7: Nutrient items present in selected lamb offal

Nutrient Item	Heart		Kidney		Liver	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Water (%)	77.8	66.6	81.0	75.1	70.8	64.6
Energy (kJ/100 g)	440	671	349	464	529	678
Protein (%)	18.1	26.3	15.2	19.8	20.7	25.8
Fat (%)	3.7	6.2	2.5	3.6	4.9	6.6
Ash (%)	1.13	0.94	1.19	1.41	1.36	1.56
Vitamin B1 (mg/100 g) (Thiamine)	0.519	0.229	0.413	0.462	1.210	1.570
Vitamin B2 (mg/100 g) (Riboflavin)	0.54	0.84	1.28	1.53	4.21	5.27
Vitamin B3 (mg/100 g) (Niacin)	5.8	4.2	8.4	9.1	13.7	12.8
Vitamin B5 (mg/100 g) (Pantothenic acid)	2.2	1.8	3.2	4.6	5.6	5.2
Vitamin B6 (mg/100 g) (Pyridoxine)	0.144	0.125	0.173	0.230	0.218	0.187
Vitamin B12 (µg/100 g) (Cyanocobalamin)	8.4	9.2	50.4	55.6	590	57.5
Vitamin A (µg/100 g)	5.4	3.5	61.3	85.2	15434	19872
Vitamin D3 (µg/100 g)	0.03	0.05	0.13	0.21	BDL	BDL
25-OH Vitamin D3 (µg/100g)	0.182	0.229	0.276	0.275	0.497	0.525
Vitamin E (mg/100 g)	0.65	0.63	0.42	0.57	0.86	1.12
Cholesterol (mg/100 g)	119.4	186.4	369.1	507.5	386.0	566.0
Calcium (mg/100 g)	4.65	5.48	7.91	9.47	4.20	5.00
Copper (mg/100 g)	0.41	0.64	0.36	0.42	11.40	13.40
Iodine (µg/100 g)	1.4	1.7	4.6	5.9	5.8	4.7
Iron (mg/100 g)	3.3	4.9	6.6	14.7	4.6	5.4
Magnesium (mg/100 g)	20.2	21.9	15.6	19.1	17.9	20.9
Manganese (µg/100 g)	22.2	27.7	84.1	104.5	330.0	370.0
Phosphorus (mg/100 g)	204	237	245	312	381	459
Potassium (mg/100 g)	277	187	231	271	285	287
Selenium (µg/100 g)	10.9	20.3	93.6	105.0	11.1	19.0
Sodium (mg/100 g)	94	67	168	199	59	59
Zinc (mg/100 g)	1.7	2.7	1.8	2.4	3.4	5.1

Table B8: Fatty acid composition of cooked and raw selected lamb offal expressed as a percentage of total fatty acids

Fatty acid (% of total fatty acids except for Total FAs and the ratios)	Heart		Kidney		Liver	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
C8:0 Caprylic	ND	ND	ND	ND	ND	ND
C10:0 Capric	0.17	0.27	0.51	<0.01	ND	ND
C11:0 Undecanoic	ND	ND	ND	ND	ND	ND
C12:0 Lauric	0.31	0.31	0.17	<0.01	<0.01	<0.01
C13:0 Tridecanoic	ND	ND	ND	ND	ND	ND
C14:0 Myristic	2.36	2.84	1.24	0.40	0.58	0.52
C14:1n5 c9 Myristoleic	0.17	0.10	0.26	0.25	<0.01	ND
C15:1n5 c10 Pentadecenoic	ND	ND	ND	ND	ND	ND
C16:0 Palmitic	15.40	16.79	16.33	15.37	15.16	15.11
C16:1n7 t9 Palmitelaidic	0.30	0.16	0.48	0.43	0.53	0.57
C16:1n7 c9 Palmitoleic	0.71	0.66	0.45	0.41	1.07	1.07
C17:0 Margaric	1.80	1.91	1.68	1.66	2.11	2.18
C17:1n7 c10 Heptadecenoic	ND	ND	ND	ND	ND	ND
C18:0 Stearic	24.47	27.34	22.26	20.50	27.61	28.47
C18:1n9 t9 Elaidic	0.29	0.31	0.29	0.23	0.44	0.44
C18:1n7 t11 Vaccenic	2.97	3.60	1.97	1.33	3.57	3.66
C18:1n9 c9 Oleic	17.87	19.27	17.81	16.05	18.55	17.96
C18:1n7 c11 Vaccenic	1.34	1.14	1.01	1.07	0.51	0.64
C18:2n6 t Linolelaidic	ND	ND	ND	ND	ND	ND
C18:2n6 c Linoleic	14.05	11.59	8.75	11.05	4.77	4.55
C20:0 Arachidic	0.18	0.16	ND	0.25	<0.01	0.13
C18:3n6 c Gamma linolenic	ND	ND	ND	ND	<0.01	<0.01
C20:1n9 c11 Eicosenoic	ND	ND	ND	<0.01	<0.01	<0.01
C18:3n3 c Alpha linolenic	4.36	3.77	2.88	3.49	4.27	4.16
CLA C18:2-c9,t11	1.51	1.46	0.86	0.87	1.59	1.45
CLA C18:2-t10,c12	ND	ND	ND	ND	ND	ND
C21:0 Heneicosanoic	1.02	1.02	0.92	0.84	ND	ND
C20:2n6 c Eicosadienoic	ND	ND	ND	ND	<0.01	<0.01
C22:0 Behenic	0.76	0.56	1.21	1.83	0.86	0.61
C20:3n6 c Eicosatrienoic	0.31	0.15	0.51	0.53	0.42	0.41
C22:1n9 c13 Erucic	ND	ND	ND	ND	<0.01	<0.01
C20:3n3 c Eicosatrienoic	ND	ND	ND	ND	<0.01	<0.01
C20:4n6 c Arachidonic	4.15	3.02	8.28	8.86	3.86	3.80
C23:0 Tricosanoic	0.4	0.16	0.62	0.31	0.508	0.46
C22:2n6 c Docosadienoic	ND	ND	ND	ND	<0.01	<0.01
C24:0 Lignoceric	ND	ND	1.07	1.31	0.35	0.39
C20:5n3 c EPA	2.71	1.67	5.40	6.84	4.00	3.99
C24:1n9 c15 Nervonic	ND	ND	0.48	0.56	<0.01	<0.01
C22:5n3 c DPA	1.46	1.08	2.55	2.98	4.19	4.35

C22:6n3 c DHA	0.94	0.67	2.08	2.60	5.08	5.07
SFA	46.87	51.36	46.03	42.46	47.17	47.87
MUFA	23.63	25.24	22.66	20.33	24.67	24.35
PUFA	29.49	23.40	31.31	37.21	28.16	27.78
P/S ratio	0.63	0.46	0.68	0.88	0.60	0.58
n-6/n-3 ratio	1.95	2.05	1.36	1.29	0.52	0.50
LCN3FA	5.11	3.42	10.03	12.41	13.26	13.41
Total FAs (g/100 g)	2.19	3.99	1.73	2.22	3.22	4.11

Table B9: Fatty acid composition of raw selected lamb offal expressed as a g/100g of the lean tissue (except in the case of the ratios of P/S and n-6/n-3)

Offal Item	SFA	MUFA	Trans MUFA	PUFA	P/S	n-6 PUFA	n-3 PUFA	n-6/n-3	LCn3FA	Total FAs (g/100 g)
Heart	1.03	0.52	0.078	0.646	0.629	0.405	0.208	1.953	0.112	2.19
Kidney	0.80	0.39	0.046	0.541	0.680	0.303	0.233	1.359	0.173	1.73
Liver	1.52	0.79	0.242	0.906	0.597	0.291	0.564	0.516	0.427	3.22

Table B10: Fatty acid composition of cooked selected lamb offal expressed as a g/100g of the lean tissue (except in the case of the ratios of P/S and n-6/n-3)

Offal Item	SFA	MUFA	Trans MUFA	PUFA	P/S	n-6 PUFA	n-3 PUFA	n-6/n-3	LCn3FA	Total FAs (g/100 g)
Heart	2.05	1.01	0.162	0.933	0.456	0.588	0.286	2.054	0.136	3.99
Kidney	0.94	0.45	0.044	0.826	0.876	0.454	0.353	1.286	0.275	2.22
Liver	1.97	1.00	0.290	1.143	0.580	0.360	0.723	0.498	0.552	4.11

Appendix C – Essential Amino Acids in Beef and Lamb Offal as given by Ockerman and Hansen (2000)

Table C1: Essential amino acid content (g/100 g protein) of beef by-products and FAO/WHO/UNU standard

Amino acid	Beef by-product				FAO/WHO/UNU
	Heart	Kidney	Liver	Lung	
Leucine	8.8	8.0	9.4	7.3	6.6
Isoleucine	4.4	4.1	4.6	4.8	2.8
Lysine	8.2	6.6	6.9	7.1	5.8
Methionine	2.6	2.1	2.5	2.0	2.5 <sup>b</sup>
Cysteine <sup>a</sup>	1.3	0.8	1.5	1.5	
Phenylalanine	4.5	4.8	5.3	4.1	6.3 <sup>c</sup>
Tyrosine <sup>a</sup>	3.6	3.8	4.0	2.2	
Tryptophan	1.1	1.4	1.4	0.9	1.1
Threonine	4.7	4.8	4.6	3.7	3.4
Valine	5.2	6.2	6.2	4.9	3.5
Histidine	2.7	2.6	2.7	3.0	1.9
Total	47.1	45.2	49.1	41.5	33.9

Table C2: Essential amino acid content (g/100 g protein) of lamb by-products and FAO/WHO/UNU standard

Amino acid	Lamb by-product				FAO/WHO/UNU
	Heart	Kidney	Liver	Lung	
Leucine	8.5	7.5	8.2	8	6.6
Isoleucine	4.3	4	4.3	3.2	2.8
Lysine	7.5	6.5	5.4	6.5	5.8
Methionine	2.2	2	2.1	1.8	2.5 <sup>b</sup>
Cysteine <sup>a</sup>	0.8	1.1	1	1.6	
Phenylalanine	4.3	4.7	4.5	4.1	6.3 <sup>c</sup>
Tyrosine <sup>a</sup>	3.1	3.5	3.6	2.8	
Tryptophan	1.1	1.4	1.2	0.9	1.1
Threonine	4.7	4.7	4.5	3.7	3.4
Valine	5	5.9	5.5	5.5	3.5
Histidine	2.3	2.6	2.4	2.5	1.9
Total	43.8	43.9	42.7	40.6	33.9

<sup>a</sup> Cysteine and Tyrosine are not essential but have sparing effects on Methionine and Phenylalanine

<sup>b</sup> Methionine + Cysteine

<sup>c</sup> Phenylalanine + Tyrosine

## Appendix D – Raw Data from Developing the Palatability Testing Protocol

Table D1: Raw data showing the amount of solid kidney and purge expressed in both grams and as a percentage

Parameters		Day 1	Day 2	Day 3	Day 4	Ave
Minced	Product weight (g)	1072.9	956.7	1039.8	957.6	1006.8
	Solid amount (g)	301.2	259.2	207.6	232.8	250.2
	Percentage solid (%)	28.1	27.1	20.0	24.3	24.9
	Liquid and losses (g)	772.7	699.8	832.2	725.6	757.6
	Percentage purge (%)	72.0	73.1	80.0	75.8	75.2
Cubed	Product weight (g)	1046.8	968.9	1014.9	1004.1	1008.7
	Solid amount (g)	734.6	752.8	761.0	722.8	742.8
	Percentage solid (%)	70.2	77.5	75.0	71.8	73.5
	Liquid and losses (g)	312.2	217.9	254.2	283.2	266.9
	Percentage purge (%)	29.8	22.5	25.0	28.2	26.5

Table D2: Information on the gender and date of birth of each cat used in the palatability test

Cat	Name	Gender	Neutered	Date of Birth	Age* (years)
1	Jetty	Female	No	1 December 2013	4.39
2	Kaia	Female	No	17 October 2016	1.52
3	Nyssa	Female	No	2 February 2016	1.22
4	Heka	Male	Yes	25 November 2013	4.41
5	Token	Female	No	1 December 2013	4.39
6	Fox	Male	Yes	28 December 2011	6.32
7	Leo	Male	Yes	7 April 2005	13.05
8	Gerrit	Male	Yes	25 November 2013	4.41

\* The age of the cats as at 23<sup>rd</sup> April 2018

Table D3: Weight of the bowls before and after palatability testing

Bowl	Weight of bowls (g)							
	Day 1		Day 2		Day 3		Day 4	
	Before	After	Before	After	Before	After	Before	After
1C	354.2	308.3	357.4	266.6	353.4	259.5	353.3	264.6
1M	295.6	272.0	290.4	275.1	289.7	274.6	287.7	271.5
2C	389.4	388.2	393.1	298.9	361.5	266.9	388.5	298.9
2M	303.3	302.7	298.3	266.8	328.1	299.1	295.3	266.8
3C	378.3	377.7	381.3	287.8	378.8	284.7	377.5	287.8
3M	321.5	320.0	315.9	284.7	312.5	287.8	313.5	284.7
4C	371.6	370.4	374.2	367.4	371.3	339.9	370.9	281.3
4M	313.5	312.9	308.6	306.6	305.5	280.7	305.5	280.5
5C	355.7	265.4	358.8	265.2	380.1	286.7	355.1	265.6
5M	322.5	287.0	317.1	286.0	289.8	265.3	314.5	286.1
6C	388.8	388.1	391.8	298.4	357.3	263.1	388.4	298.4
6M	299.5	294.4	294.5	263.4	323.1	299.0	291.5	263.4
7C	375.5	322.9	360.7	338.5	361.4	356.5	356.5	349.6
7M	303.3	302.8	298.3	273.5	291.3	290.9	295.6	295.1

8C	361.3	271.8	361.7	271.9	384.2	289.7	360.8	270.8
8M	319.3	289.8	321.0	289.6	295.8	270.9	318.7	290.2

Table D4: The amount of food placed in each bowl before palatability testing

Bowl	Weight of food before (g)			
	Day 1	Day 2	Day 3	Day 4
1C	91.6	94.2	95.0	90.1
1M	37.5	32.3	25.4	28.8
2C	91.4	94.6	95.1	89.7
2M	37.4	32.1	29.0	28.7
3C	91.4	93.9	94.8	90.1
3M	37.6	32	25.4	29.1
4C	91.8	93.9	95.0	90.3
4M	37.6	32.1	25.4	28.8
5C	91.6	94.1	94.9	90.2
5M	37.6	32.1	25.5	29.1
6C	91.4	94.1	95.9	90.2
6M	37.7	32.2	25.7	29.1
7C	91.7	94.6	95.0	90.1
7M	37.5	32.0	25.5	29.1
8C	93.7	91.6	95.0	90.2
8M	37.3	32.1	25.7	29.3

Table D5: Daily observations for the various meat preparation options

	Day 1	Day 2	Day 3	Day 4	Total
Minced	2	1	1	0	4
Cubed	3	1	2	3	9
No preference	0	6	4	5	15
Dislike both	3	0	1	0	4

## Appendix E – Raw Data from Refinements to Cat Palatability Panel

*Table E1: Weight of the bowls before and after the palatability test to replace cat 7*

Bowls	Weight of bowls (g)	
	Before	After
1C	333.1	322.5
1M	282.3	264.3
2C	341.5	267.3
2M	317.4	299.1
3C	358.6	285.1
3M	306.1	288.1
4C	349	338.5
4M	299.9	299.5

*Table E2: Initial amount of food presented to each of the four cats*

Bowls	Weight of food before (g)
1C	74.4
1M	18.9
2C	74.8
2M	18.6
3C	74.1
3M	18.6
4C	72.3
4M	19.3



## Appendix F – Acceptance of Lamb Offal Raw Data and Images

### Lung Acceptance Testing Data:

Table F1: Food intake results for lamb lung testing

Cat	Total food intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	82	150	114	158	130	127±13.6	634
2	143	199	199	199	200	188±11.3	940
3	93	144	147	136	110	126±10.5	630
4	52	133	61	36	81	73±16.8	363
5	154	173	172	186	197	176±7.2	882
6	160	191	172	200	198	184±7.8	921
7	126	150	171	165	200	162±12.2	812
8	128	198	62	108	139	127±22.1	635
Average	117	167	137	149	157	145±7.3	727.1±69.9

Table F2: Total percentage consumption results for lamb lung testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	41	75	57	79	65	63.5±6.8
2	71.5	99.5	99.5	99.5	100	94.0±5.6
3	46.5	72	73.5	68	55	63.0±5.3
4	26	66.5	30.5	18	40.5	36.5±8.4
5	77	86.5	86	93	98.5	88.0±3.6
6	80	95.5	86	100	99	92.0±3.9
7	63	75	85.5	82.5	100	81.0±6.1
8	64	99	31	54	69.5	63.5±11.1
Average	59	84	69	74	78	72.7±3.7



Figure F1: Presentation of bowls after Day 4 of lamb lung acceptance testing (showing roughly the average percentage consumption of lung over the week)

Heart Acceptance Testing Data:

Table F3: Food intake results for lamb heart testing

Cat	Total food intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	101	142	198	115	92	129.6±19.1	648
2	192	42	99	101	164	119.6±26.5	598
3	141	195	199	108	178	164.2±17.4	821
4	112	47	47	11	37	50.8±16.6	254
5	199	100	198	172	134	160.6±19.2	803
6	121	139	191	160	191	160.4±13.9	802
7	169	174	199	198	159	179.8±8.0	899
8	198	138	160	177	137	162.0±11.7	855
Average	154	122	161	130	137	140.9±8.3	704.4±73.1

Table F4: Total percentage consumption results for lamb heart testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	50.5	71	99	57.5	46	64.8±9.5
2	96	21	49.5	50.5	82	59.8±13.2
3	70.5	97.5	99.5	54	89	82.1±8.7
4	56	23.5	23.5	5.5	18.5	25.4±8.3
5	99.5	50	99	86	67	80.3±9.6
6	60.5	69.5	95.5	80	95.5	80.2±7.0
7	84.5	87	99.5	99	79.5	89.9±4.0
8	99	91.5	80	88.5	68.5	85.5±5.2
Average	77	64	81	65	68	71.0±4.2



Figure F2: Presentation of bowls after Day 5 of lamb heart acceptance testing (showing roughly the average percentage consumption of heart over the week)

Kidney Acceptance Testing Data:

Table F5: Food intake results for lamb kidney testing

Cat	Total food intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	165	197	193	199	198	190.4±6.4	952
2	199	196	197	198	200	198.0±0.7	990
3	83	182	198	198	198	171.8±22.4	859
4	51	111	174	105	112	110.6±19.5	553
5	198	198	198	197	200	198.2±0.5	991
6	180	197	199	198	195	193.8±3.5	969
7	198	199	199	199	200	199.0±0.3	995
8	198	200	197	197	199	198.2±0.6	991
Average	159	185	194	186	188	182.5±5.7	912.5±53.8

Table F6: Total percentage consumption results for lamb kidney testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	82.5	98.5	96.5	99.5	99	95.2±3.2
2	99.5	98	98.5	99	100	99.0±0.3
3	41.5	91	99	99	99	85.9±11.2
4	25.5	55.5	87	52.5	56	55.3±9.7
5	99	99	99	98.5	100	99.1±0.2
6	90	98.5	99.5	99	97.5	96.9±1.7
7	99	99.5	99.5	99.5	100	99.5±0.1
8	99	100	98.5	98.5	99.5	99.1±0.2
Average	80	93	97	93	94	91.3±2.9



Figure F3: Presentation of bowls after Day 2 of lamb kidney acceptance testing (showing roughly the average percentage consumption of kidney over the week)

Tripe Acceptance Testing Data:

Table F7: Food intake results for lamb tripe testing

Cat	Total food intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	134	134	172	139	147	145±7.1	726
2	78	114	124	117	99	106.4±8.2	532
3	29	96	96	186	199	121.2±31.6	606
4	86	88	58	71	66	73.8±5.8	369
5	183	189	194	162	187	183.0±5.5	915
6	114	196	188	193	31	144.4±32.2	722
7	198	198	160	92	165	162.6±19.4	813
8	130	175	199	185	196	177.0±12.5	885
Average	119	149	149	143	136	139.2±8.1	696.0±65.6

Table F8: Total percentage consumption results for lamb tripe testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	67	67	86	69.5	73.5	72.6±3.6
2	39	57	62	58.5	49.5	53.2±4.1
3	14.5	48	48	93	99.5	60.6±15.8
4	43	44	29	35.5	33	36.9±2.9
5	91.5	94.5	97	81	93.5	91.5±2.8
6	57	98	94	96.5	15.5	72.2±16.1
7	99	99	80	46	82.5	81.3±9.7
8	65	87.5	99.5	92.5	98	88.5±6.2
Average	59.5	74.4	74.4	71.6	68.1	69.6±4.1



Figure F4: Presentation of bowls after Day 5 of lamb tripe acceptance testing (showing roughly the average percentage consumption of tripe over the week)

MDM Acceptance Testing Data:

Table F9: Food intake results for lamb MDM testing

Cat	Total food intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	77	125	129	64	93	97.6±12.9	488
2	81	36	67	105	69	71.6±11.2	358
3	53	62	87	172	10	76.8±26.8	384
4	5	8	8	4	8	6.6±0.9	33
5	8	33	25	59	87	42.4±13.9	212
6	185	71	6	26	121	81.8±32.5	409
7	200	30	87	30	118	93.0±31.7	465
8	158	29	70	52	84	78.6±21.9	393
Average	96	49	60	64	74	68.6±8.3	342.8±53.1

Table F10: Total percentage consumption results for lamb MDM testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	38.5	62.5	64.5	32	46.5	48.8±6.4
2	40.5	18	33.5	52.5	34.5	35.8±5.6
3	26.5	31	43.5	86	5	38.4±13.4
4	2.5	4	4	2	4	3.3±0.4
5	4	16.5	12.5	29.5	43.5	21.2±6.9
6	92.5	35.5	3	13	60.5	40.9±16.3
7	100	15	43.5	15	59	46.5±15.8
8	79	14.5	35	26	42	39.3±10.9
Average	48	25	30	32	37	34.3±4.1



Figure F5: Presentation of bowls after Day 4 of lamb MDM acceptance testing (showing roughly the average percentage consumption of MDM over the week)

Liver Acceptance Testing Data:

Table F11: Food intake results for lamb liver testing

Cat	Total food intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	63	65	65	65	64	64.4±0.4	322
2	33	64	64	63	55	55.8±5.9	279
3	65	64	64	64	63	63.00±0.3	320
4	34	47	64	56	50	50.2±5.0	251
5	65	65	65	65	63	63.6±0.4	323
6	65	65	65	65	64	64.8±0.2	324
7	63	64	64	65	65	65.2±0.4	321
8	65	64	65	64	64	64.4±0.2	322
Average	57	62	65	63	61	61.6±1.2	307.8±9.7

Table F12: Total percentage consumption results for lamb liver testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	96.9	100	100	100	98.5	99.1±0.6
2	50.8	98.5	98.5	96.9	84.6	85.8±9.1
3	100	98.5	98.5	98.5	96.9	98.5±0.5
4	52.3	72.3	98.5	86.2	76.9	77.2±7.7
5	100	100	100	100	96.9	99.4±0.6
6	100	100	100	100	98.5	99.7±0.3
7	96.9	98.5	98.5	100	100	98.8±0.6
8	100	98.5	100	98.5	98.5	99.1±0.4
Average	87.1	95.8	99.2	97.5	93.8	94.7±1.9

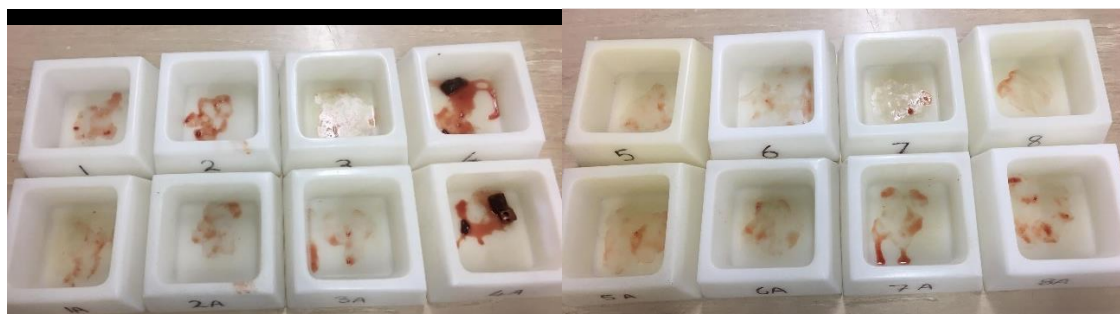


Figure F6: Presentation of bowls after Day 2 of lamb liver acceptance testing (showing roughly the average percentage consumption of liver over the week)

## SAS Statistical Outputs:

*Coding used to show interactions between offal intake and days of testing:*

```
ods html close;
ods html newfile=none;
dm 'odsresults; clear';
dm 'clear log';
dm 'clear out';
options ls=255 ps=6000 nocenter;
filename catpat dde 'Excel|H:\My Documents\Master 2018\2. Testing
Methodology\C. Lamb\SAS lamb\[Lamb interaction data
(101218).xlsx]Percentage consumption!R2C1:R241C4';
data catpat;
infile catpat lrecl=6000 dlm='09'x notab dsd missover;
input Day Cat$ Offal$ Intake
;
run;
proc mixed data=catpat;
class Day Cat Offal;
model Intake = Day Offal Day*Offal / solution ;
LSMeans Day Offal Day*Offal / pdiff ;
run;
quit;
```

*Coding used to show interactions between each cat and offal intake:*

```
ods html close;
ods html newfile=none;
dm 'odsresults; clear';
dm 'clear log';
dm 'clear out';
options ls=255 ps=6000 nocenter;
filename catpat dde 'Excel|H:\My Documents\Master 2018\2. Testing
Methodology\C. Lamb\SAS lamb\[Lamb interaction data
(101218).xlsx]Percentage consumption!R2C1:R241C4';
data catpat;
infile catpat lrecl=6000 dlm='09'x notab dsd missover;
input Day Cat$ Offal$ Intake
;
run;
proc mixed data=catpat;
class Day Cat Offal;
model Intake = Cat Offal Cat*Offal / solution ;
LSMeans Cat Offal Cat*Offal / pdiff ;
run;
quit;
```

## Kidney versus MDM Raw Data and Images:

Table F13: Food intake results for lamb kidney in the kidney versus MDM preference test

Cat	Kidney Intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM	
1	99.8	90.1	98.7	99.1	98.8	97.3 $\pm$ 1.8	487
2	99	99.4	98.9	98.1	99.2	98.9 $\pm$ 0.2	495
3	99.3	100.3	100.1	98.6	99.4	99.5 $\pm$ 0.3	498
4	95.5	99.9	99.0	99.3	99.0	98.5 $\pm$ 0.8	493
5	98.9	99.8	98.3	94.7	99.0	98.1 $\pm$ 0.9	491
6	99.4	99.8	99.6	99.7	99.8	99.7 $\pm$ 0.1	498
7	99.3	99.2	100.2	100.2	99.1	99.6 $\pm$ 0.2	498
8	99.3	100.1	100.0	100.0	100.7	100.0 $\pm$ 0.2	500
Average	98.8	98.6	99.4	98.7	99.4	99.0 $\pm$ 0.3	495.0 $\pm$ 1.6

Table F14: Total percentage consumption results for lamb kidney in the kidney versus MDM preference test

Cat	Kidney percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM
1	98.7	90.0	98.4	99.1	99.0	97.0 $\pm$ 1.8
2	99.3	99.5	99.4	98.1	99.0	99.1 $\pm$ 0.3
3	100.0	99.9	100.1	98.5	99.8	99.7 $\pm$ 0.3
4	95.7	99.9	98.4	99.1	98.5	98.3 $\pm$ 0.7
5	99.1	99.7	98.0	93.9	99.4	98.0 $\pm$ 1.1
6	99.8	99.2	99.9	99.2	99.9	99.6 $\pm$ 0.2
7	99.7	98.9	99.8	99.4	99.4	99.4 $\pm$ 0.2
8	99.4	100.0	100.0	100.0	99.9	99.9 $\pm$ 0.1
Average	99.0	98.4	99.3	98.4	99.4	98.9 $\pm$ 0.3

Table F15: Food intake results for lamb MDM in the kidney versus MDM preference test

Cat	MDM Intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM	
1	52	43.3	45.4	34.7	53.7	45.8 $\pm$ 3.4	229
2	36.2	48.6	20.5	4.3	69.5	35.8 $\pm$ 11.2	179
3	9.3	0.2	0.0	0.0	0.3	2.0 $\pm$ 1.8	10
4	0.1	0.0	0.4	0.0	0.0	0.1 $\pm$ 0.1	0
5	74.5	33.9	42.0	18.8	48.9	43.6 $\pm$ 9.2	218
6	4.4	0.1	0.1	45.0	40.0	17.9 $\pm$ 10.1	90
7	0.0	23.5	72.0	28.9	49.4	34.8 $\pm$ 12.2	174
8	52.4	37.3	79.7	72.0	46.6	57.6 $\pm$ 7.9	288
Average	28.6	23.4	32.5	25.5	38.6	29.7 $\pm$ 4.1	148.5 $\pm$ 37.1



Table F16: Total percentage consumption results for lamb MDM in the kidney versus MDM preference test

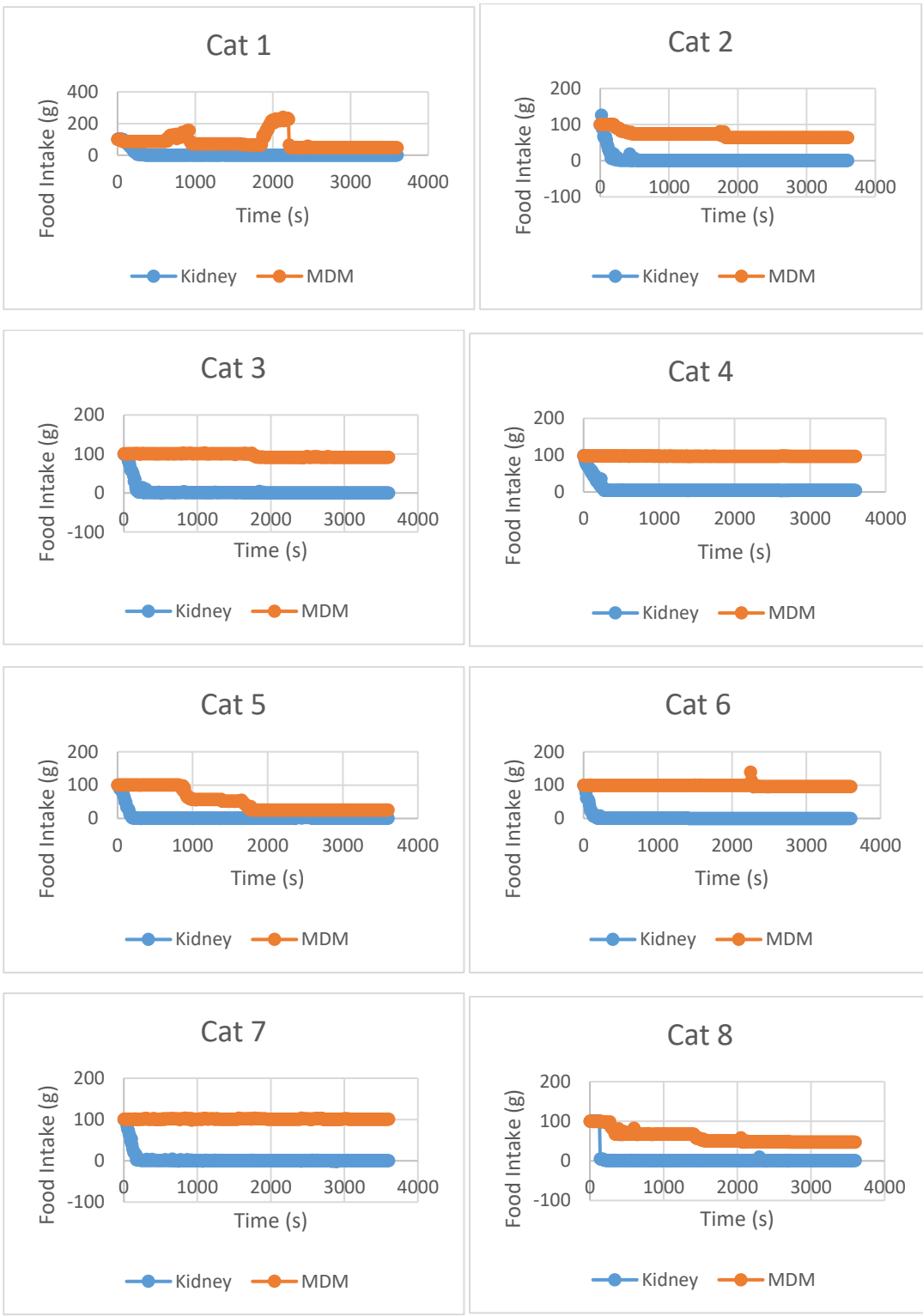
Cat	MDM percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM
1	51.7	43.3	45.5	34.5	53.4	45.7 $\pm$ 3.4
2	36.1	48.6	20.5	4.3	69.2	35.7 $\pm$ 11.2
3	9.2	0.2	0.0	0.0	0.3	1.9 $\pm$ 1.8
4	0.1	0.0	0.4	0.0	0.0	0.1 $\pm$ 0.1
5	74.2	34.0	41.8	18.8	48.9	43.5 $\pm$ 9.2
6	4.4	0.1	0.1	44.8	39.8	17.9 $\pm$ 10.1
7	0.0	23.5	71.5	29.0	49.3	34.7 $\pm$ 12.1
8	52.5	37.2	81.1	71.6	46.6	57.8 $\pm$ 8.1
Average	28.5	23.4	32.6	25.4	38.4	29.7 $\pm$ 4.1



Figure F7: Presentation of bowls after Day 1 of lamb kidney versus MDM preference testing (showing roughly the average percentage consumption of each offal over the week)

Table F17: Raw data from Day 1 of kidney versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

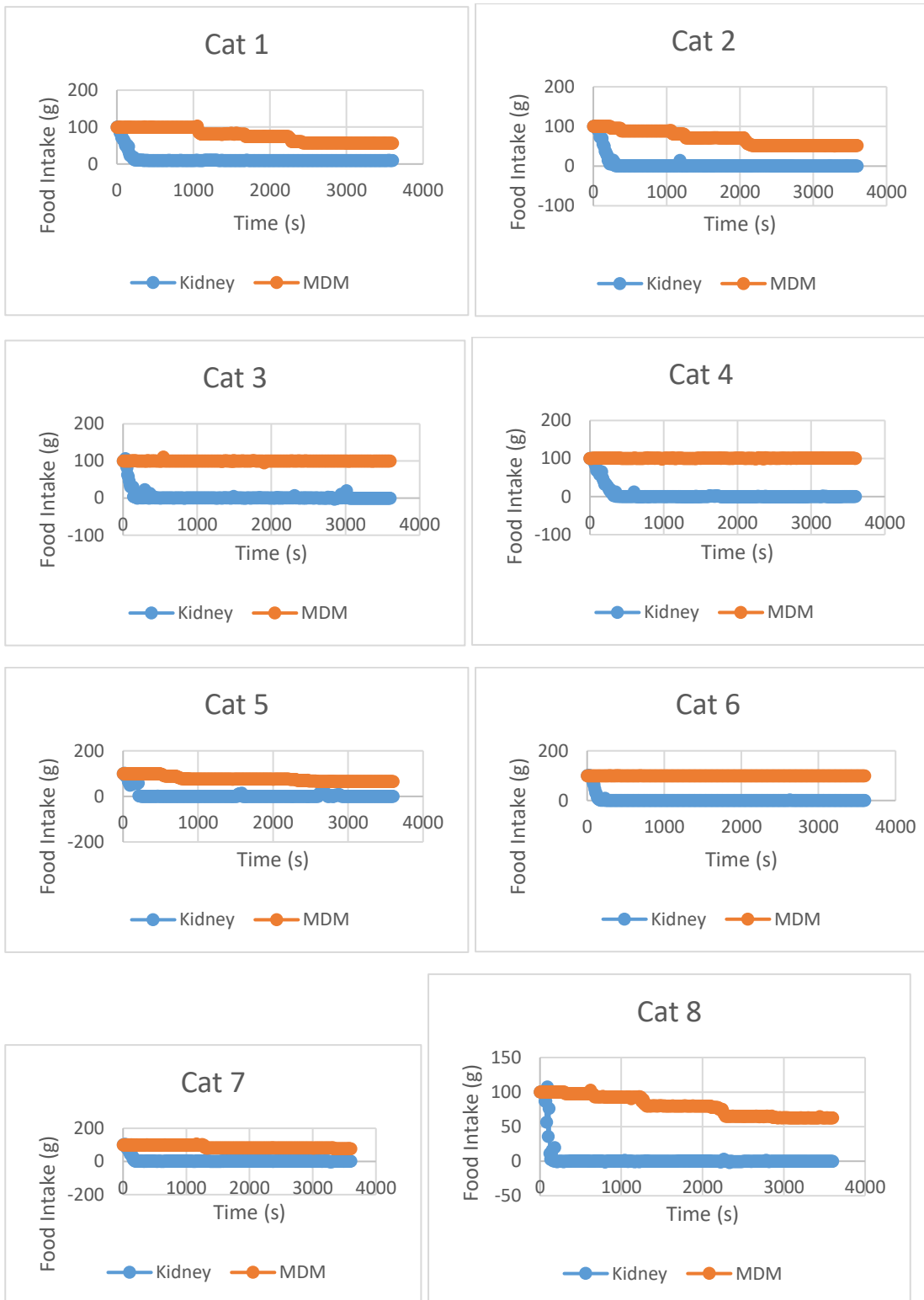
Cat	Lamb Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Kidney	1	310	99.6
	MDM	3	70	13.9
			350	16.2
			90	6.2
430	16.8			
2	Kidney	1	270	98.3
	MDM	2	30	0.6
			260	25.4
110	10.7			
3	Kidney	1	260	98.7
	MDM	0	170	9.2
4	Kidney	1	330	95.5
	MDM	0	-	-
5	Kidney	1	330	98.9
	MDM	2	220	43.7
			50	4.8
180	26			
6	Kidney	1	240	98.8
	MDM	0	30	0.6
7	Kidney	1	210	98.1
			120	1.1
8	Kidney	1	-	-
			220	99
	MDM	2	190	32.3
			140	16.3
			70	2.9
30	0.6			



Figures F8: Intake of lamb kidney and MDM for each cat on Day 1 of testing

Table F18: Raw data from Day 2 of kidney versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

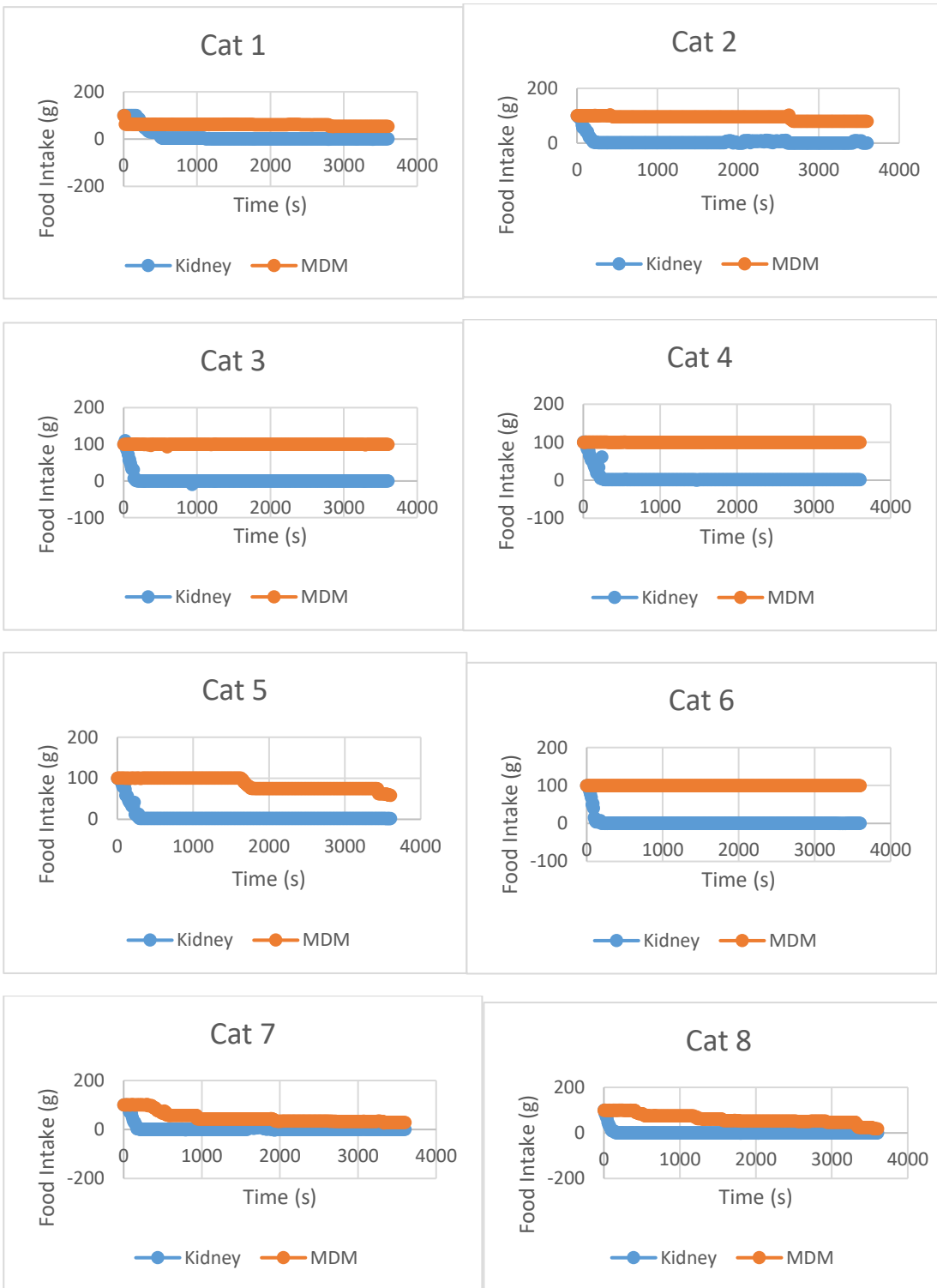
Cat	Lamb Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Kidney	1	320	90.1
	MDM	3	70	18.9
			20	5.9
			40	14
			50	3.9
			10	0.6
2	Kidney	1	270	99.1
	MDM	2	30	0.3
			30	5.2
			20	6.4
			70	7.4
			30	10.2
120	19.6			
3	Kidney	1	240	100
	MDM	0	-	-
4	Kidney	1	330	99.8
	MDM	0	-	-
5	Kidney	1	270	99.8
	MDM	2	70	10.5
			220	13.5
			140	5.8
			30	2.3
			40	1.8
6	Kidney	1	260	99.8
	MDM	0	-	-
7	Kidney	1	200	99.1
	MDM	1	120	18.7
			40	4.7
8	Kidney	1	150	99.9
	MDM	2	20	2.3
			190	5.1
			70	12.8
			190	14.8
			120	2.3



Figures F9: Intake of lamb kidney and MDM for each cat on Day 2 of testing

Table F19: Raw data from Day 3 of kidney versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

Cat	Lamb Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Kidney	1	360	95.6
			80	2.6
	MDM	1	130	37
			40	7.1
2	Kidney	1	250	98.3
	MDM	1	50	4.5
			70	16
3	Kidney	1	200	100
	MDM	0	-	-
4	Kidney	1	290	99
	MDM	0	-	-
5	Kidney	1	310	98.2
	MDM	2	170	25.5
			180	16.5
6	Kidney	1	220	99.7
	MDM	0	-	-
7	Kidney	1	200	100.2
	MDM	3	330	43.3
			30	14.3
			70	10.1
			70	4.8
8	Kidney	1	180	99.8
	MDM	3	250	24.6
			120	12.9
			30	6.7
			50	2.8
			10	4.3
			80	22.3
			70	6

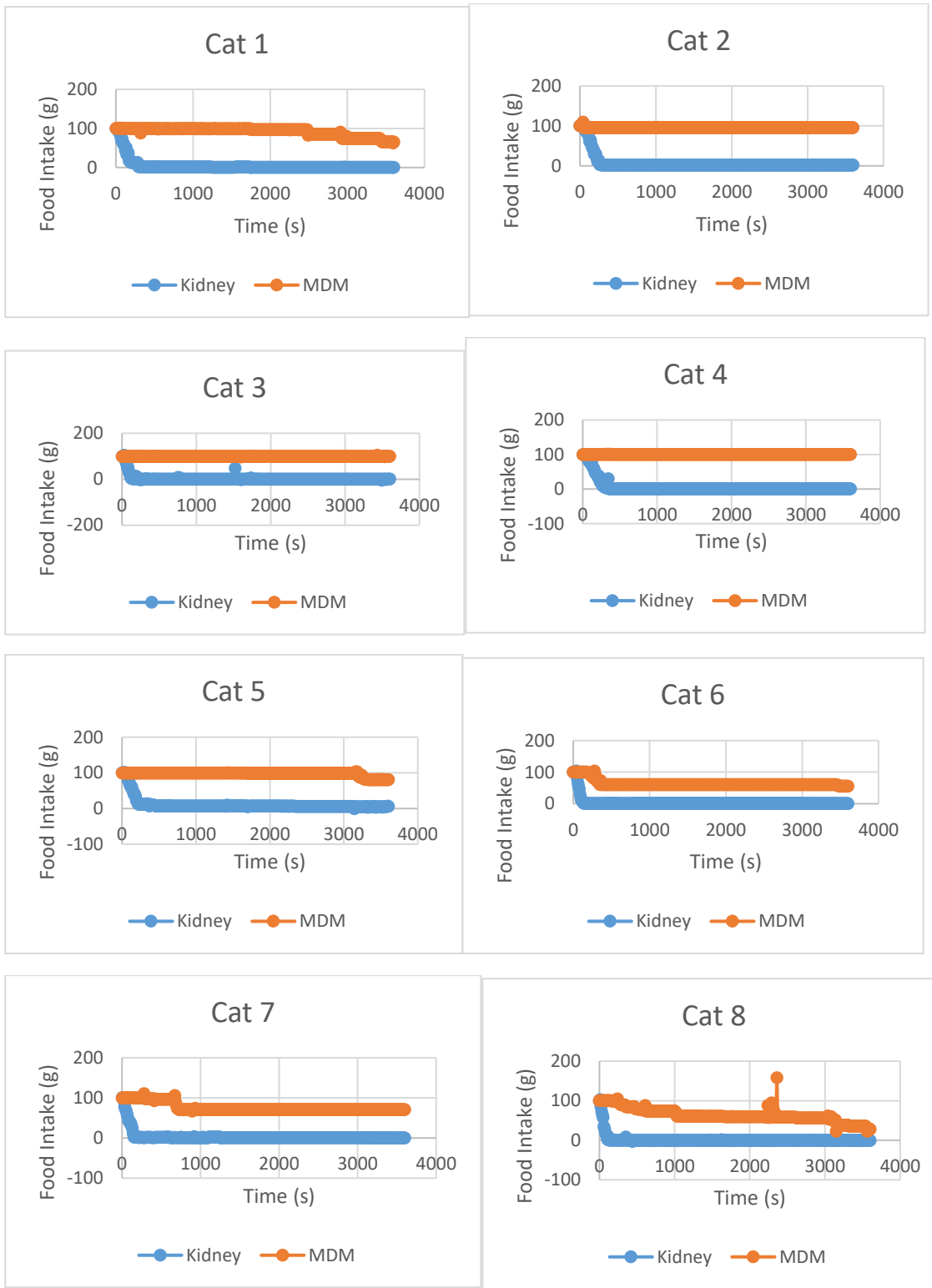


Figures F10: Intake of lamb kidney and MDM for each cat on Day 3 of testing

Table F20: Raw data from Day 4 of kidney versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

Cat	Lamb Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Kidney	1	330	98.4
	MDM	2	20	1
			10	2.6
			30	11.2
			40	11.1
30	8.8			
2	Kidney	1	240	98.1
	MDM	0	30	4.4
3	Kidney	1	250	98.9
	MDM	0	-	-
4	Kidney	1	330	99.3
	MDM	0	-	-
5	Kidney	1	430	93.5
			50	0.6
6	MDM	1	150	19.6
	Kidney	1	150	99.7
180			39.9	
7	MDM	1	70	5.1
			230	2.8
	Kidney	1	80	26
8	Kidney	1	170	99.7
	MDM	4	130	15.4
			190	11.1
			60	12.6
			50	0.4
			20	0.8
			10	1.7
			20	1.6
			150	18.2
			20	2.5
70	7.5			

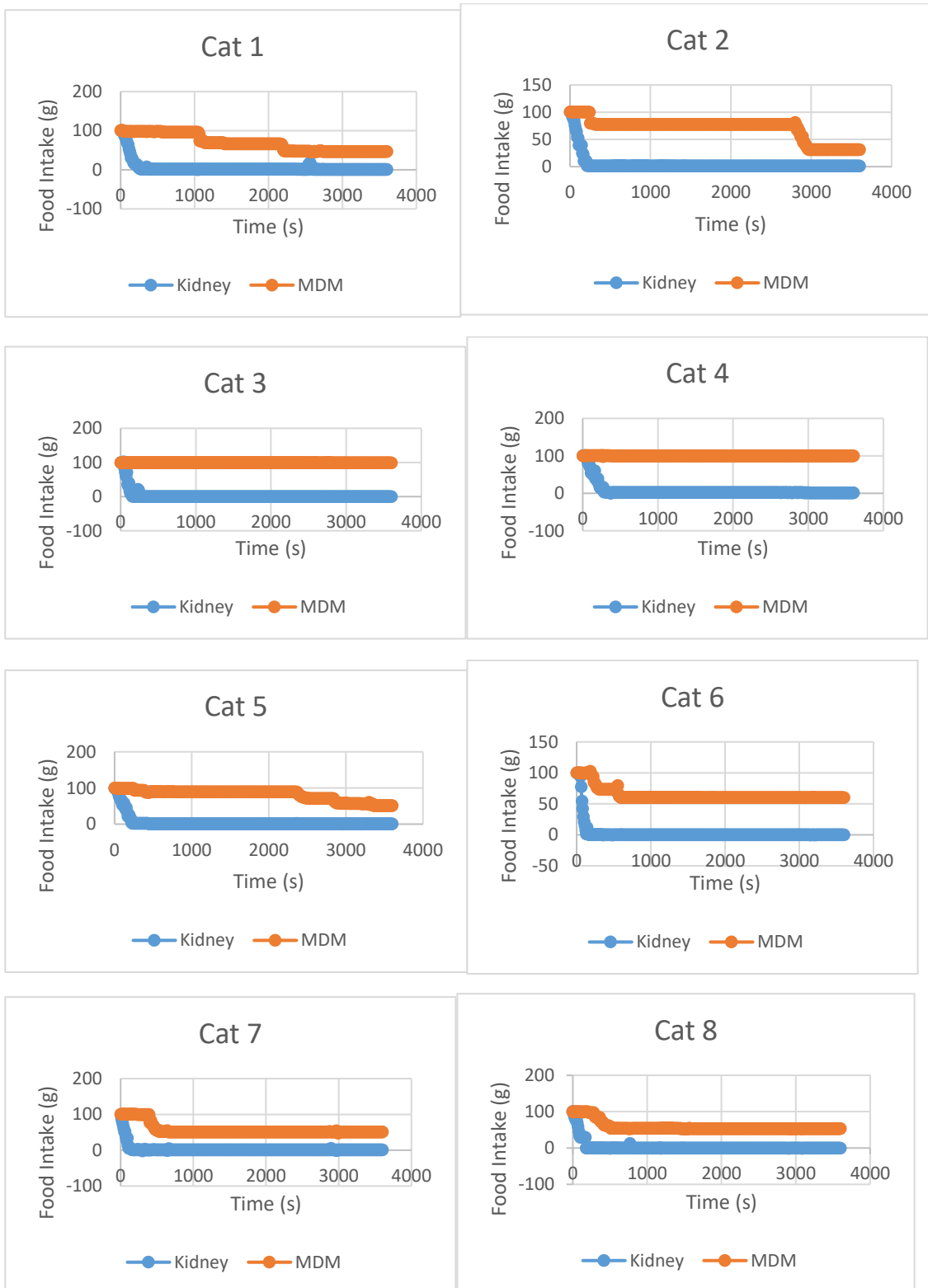




Figures F11: Intake of lamb kidney and MDM for each cat on Day 4 of testing

Table F21: Raw data from Day 5 of kidney versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

Cat	Lamb Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Kidney	1	350	98.1
	MDM	2	20	2.6
			50	2.1
			100	26.4
			20	2.8
50	18.7			
2	Kidney	1	270	99.1
	MDM	2	40	20.9
			180	46.7
3	Kidney	1	230	99.4
	MDM	0	-	-
4	Kidney	1	300	98.8
	MDM	0	-	-
5	Kidney	1	210	97.4
			30	1.5
	MDM	2	60	1.7
			60	4.7
			90	4.2
			170	18.2
			100	13.8
180	5.9			
6	Kidney	1	270	99.8
	MDM	2	150	26.5
			90	13.2
7	Kidney	1	180	99.1
	MDM	1	50	0.9
			260	48.2
8	Kidney	1	170	100.8
	MDM	1	310	45.4



Figures F12: Intake of lamb kidney and MDM for each cat on Day 5 of testing

## Nutritional Analyses of Lamb Offal:

*Table F22: As fed amino acid content of the six lamb offal varieties (units: mg/100mg)*

AMINO ACIDS	Heart	Kidney	Liver	Lung	MDM	Tripe
Aspartic Acid	0.78	1.50	2.01	1.23	0.85	1.30
Threonine	0.39	0.77	0.98	0.58	0.38	0.60
Serine	0.37	0.78	0.97	0.63	0.38	0.67
Glutamic Acid	1.24	2.04	2.59	1.63	1.36	2.11
Proline	0.45	0.86	1.18	0.85	0.83	0.99
Glycine	0.56	1.07	1.46	1.20	1.34	1.52
Alanine	0.58	0.95	1.29	0.91	0.86	0.96
Valine	0.49	0.97	1.36	0.79	0.55	0.70
Isoleucine	0.36	0.69	0.93	0.45	0.33	0.51
Leucine	0.77	1.48	2.02	1.15	0.71	1.06
Tyrosine	0.32	0.65	0.87	0.46	0.28	0.47
Phenylalanine	0.41	0.81	1.16	0.62	0.39	0.54
Histidine	0.22	0.44	0.60	0.36	0.22	0.30
Lysine	0.69	1.20	1.60	0.95	0.69	0.98
Arginine	0.58	1.10	1.38	0.93	0.72	1.08
Taurine	0.07	0.06	0.06	0.08	0.02	0.02
Cysteine	0.11	0.31	0.36	0.24	0.10	0.21
Methionine	0.22	0.42	0.50	0.34	0.19	0.32
Trpyptophan	0.13	0.30	0.42	0.18	0.09	0.16

Table F23: As fed fatty acid profile of the six lamb offal varieties (units: g/100g)

FATTY ACIDS	Heart	Kidney	Liver	Lung	MDM	Tripe
C6:0 Caproic	0.00	0.00	0.00	0.00	0.00	0.00
C8:0 Caprylic	0.00	ND	ND	ND	0.00	0.00
C10:0 Capric	0.04	0.01	0.02	0.01	0.03	0.01
C11:0 Undecanoic	0.00	ND	ND	ND	0.00	ND
C12:0 Lauric	0.06	0.01	0.01	0.00	0.04	0.01
C13:0 Tridecanoic	0.01	ND	ND	ND	0.01	0.00
C14:0 Myristic	0.57	0.05	0.04	0.05	0.55	0.15
C14:1n5 - cis-9-Myristoleic	0.01	<0.01	ND	<0.01	0.02	0.00
C15:1n5 - cis-10-Pentadecenoic	ND	ND	ND	ND	ND	ND
C16:0 Palmitic	2.68	0.60	0.97	0.58	3.87	1.15
C16:1n7 - cis-9-Palmitoleic	0.09	0.02	0.06	0.02	0.20	0.05
C17:0 Margaric	0.23	0.06	0.08	ND	0.34	0.14
C17:1n7 - cis-10-Heptadecenoic	<0.01	ND	ND	ND	ND	ND
C18:0 Stearic	3.78	0.84	1.13	0.58	4.00	1.53
C18:1n9t Elaidic	0.05	0.01	0.02	0.01	0.06	0.02
C18:1n7t Vaccenic	0.69	0.12	0.17	0.09	0.79	0.26
C18:1n9c Oleic	3.36	0.71	1.28	0.52	5.72	1.85
C18:1n7c Vaccenic	0.08	0.03	0.04	0.02	0.10	0.04
C18:2n6t Linolelaidic	ND	ND	ND	ND	ND	ND
C18:2n6c Linoleic	0.33	0.23	0.23	0.09	0.34	0.15
C20:0 Arachidic	0.03	0.01	0.00	0.01	0.03	0.01
C18:3n6 - cis-6,9,12-Gamma linolenic	0.00	ND	0.01	ND	0.00	ND
C20:1n9 - cis-11-Eicosenoic	0.01	0.00	0.01	0.00	0.01	0.00
C18:3n3 - cis-9,12,15-Alpha linolenic	0.24	0.08	0.13	0.05	0.36	0.12
C21:0 Heneicosanoic	<0.01	ND	ND	<0.01	<0.01	ND
C20:2n6 - cis-11,14-Eicosadienoic	0.00	<0.01	0.00	0.00	0.01	0.00
C22:0 Behenic	0.04	0.02	0.03	0.02	0.05	0.02
C20:3n6 - cis-8,11,14-Eicosatrienoic	0.01	0.01	0.02	0.01	0.00	0.00
C22:1n9 - cis-13-Erucic	0.00	ND	0.00	<0.01	0.00	0.00
C20:3n3 - cis-11,14,17-Eicosatrienoic	0.01	ND	0.01	0.00	0.01	0.01
C20:4n6 - cis-5,8,11,14-Arachidonic	0.07	0.27	0.22	0.10	0.02	0.04
C23:0 Tricosanoic	0.00	0.01	0.01	0.01	0.00	0.00
C22:2n6 - cis-13,16-Docosadienoic	0.01	ND	ND	0.00	0.00	0.00

C24:0 Lignoceric	0.00	0.02	0.01	0.01	0.00	0.00
C20:5n3 - cis-5,8,11,14,17-Epa	0.03	0.10	0.13	0.03	0.02	0.02
C24:1n9 - cis-15-Nervonic	<0.01	0.01	0.01	0.00	0.00	0.00
C22:5n3 - cis-7,10,13,16,19-DPA	0.04	0.07	0.20	0.05	0.03	0.04
C22:6n3 - cis-4,7,10,13,16,19-DHA	0.02	0.05	0.15	0.02	0.01	0.02

## Appendix G - Acceptance of Beef Offal Raw Data and Images

### Lung Acceptance Testing Data:

Table G1: Food intake results for beef lung testing

Cat	Total food intake (g)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM	Weekly total
1	85	143	152	182	169	146.2 $\pm$ 15.0	731
2	148	101	112	105	124	118.0 $\pm$ 7.6	590
3	32	98	115	141	175	112.2 $\pm$ 21.4	561
4	35	55	79	86	124	75.8 $\pm$ 13.5	379
5	147	188	168	182	177	172.4 $\pm$ 6.4	862
6	189	195	118	193	199	178.8 $\pm$ 13.7	894
7	98	196	132	200	159	157.0 $\pm$ 17.3	785
8	140	194	194	199	199	185.2 $\pm$ 10.2	926
Average	109	146	134	161	166	143.2 $\pm$ 7.6	716.0 $\pm$ 67.6

Table G2: Total percentage consumption results for beef lung testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM
1	42.5	71.5	76	91	84.5	73.1 $\pm$ 8.4
2	74	50.5	56	52.5	62	59.0 $\pm$ 4.2
3	16	49	57.5	70.5	87.5	56.1 $\pm$ 11.9
4	17.5	27.5	39.5	43	62	37.9 $\pm$ 7.5
5	73.5	94	84	91	88.5	86.2 $\pm$ 3.6
6	94.5	97.5	59	96.5	99.5	89.4 $\pm$ 7.6
7	49	98	66	100	79.5	78.5 $\pm$ 9.7
8	70	97	97	99.5	99.5	92.6 $\pm$ 5.7
Average	54.6	73.1	66.9	80.5	82.9	71.6 $\pm$ 3.8

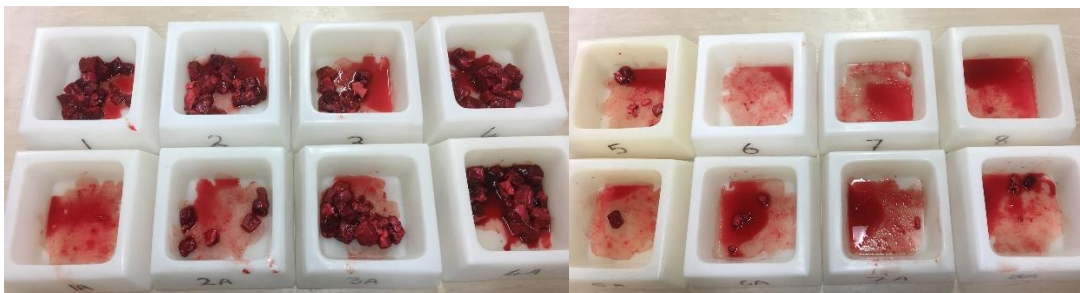


Figure G1: Presentation of bowls after Day 2 of beef lung acceptance testing (showing roughly the average percentage consumption of lung over the week)

## Heart Acceptance Testing Data:

Table G3: Food intake results for beef heart testing showing intake results on all days of testing (Note: average and weekly totals are based off Days 1, 2 and 3 of original testing and Days 1, 2 and 3 repeat testing)

Cat	Total intake (g)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1 repeat	Day 2 repeat	Day 3 repeat	Average ± SEM	Weekly total
1	39	79	120	0	0	0	0	14	42.0±19.8	252
2	91	136	114	0	0	91	73	155	110.0±12.7	660
3	136	175	113	0	14	26	105	102	109.5±20.0	657
4	27	69	52	108	34	15	26	104	48.8±13.7	293
5	199	196	128	15	30	155	159	180	169.5±11.1	1017
6	186	149	200	77	49	130	139	191	165.8±12.2	995
7	153	93	26	21	56	64	116	198	108.3±25.2	650
8	140	188	164	154	42	163	200	200	175.8±9.8	1055
Average	121	136	115	47	28	81	102	143	116.2±8.9	697.5±110.7

Table G4: Total percentage consumption results for beef heart testing on all days of testing (Note: average and weekly totals are based off Days 1, 2 and 3 of original testing and Days 1, 2 and 3 repeat testing)

Cat	Total percentage consumption (%)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1 repeat	Day 2 repeat	Day 3 repeat	Average ± SEM	
1	19.5	39.5	60	0	0	0	0	7	21.0±9.9	
2	45.5	68	57	0	0	45.5	36.5	77.5	55.0±6.3	
3	68	87.5	56.5	0	7	13	52.5	51	54.8±10.0	
4	13.5	34.5	26	54	17	7.5	13	52	24.4±6.8	
5	99.5	98	64	7.5	15	77.5	79.5	90	84.8±5.6	
6	93	74.5	100	38.5	24.5	65	69.5	95.5	82.9±6.1	
7	76.5	46.5	13	10.5	28	32	58	99	54.2±12.6	
8	70	94	82	77	21	81.5	100	100	87.9±4.9	
Average	61	68	57	23	14	40	51	72	58.1±4.5	



Figure G2: Presentation of bowls after Day 3 of beef heart acceptance testing (showing roughly the average percentage consumption of heart over the week)



Kidney Acceptance Testing Data:

Table G5: Food intake results for beef kidney testing

Cat	Total intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	183	199	199	198	200	195.8±3.2	979
2	55	83	105	81	56	76.0±9.4	380
3	106	198	197	198	198	179.4±18.4	897
4	21	9	38	47	83	39.6±12.7	198
5	126	193	199	197	84	159.8±23.4	799
6	198	195	198	198	190	195.8±1.6	979
7	174	199	200	5	200	155.6±38.0	778
8	198	199	199	200	199	199.0±0.3	995
Average	133	159	167	141	151	150.1±10.7	750.6±106.2

Table G6: Total percentage consumption results for beef kidney testing

Cat	Total percentage consumption (%)						Average ± SEM
	Day 1	Day 2	Day 3	Day 4	Day 5		
1	91.5	99.5	99.5	99	100	97.9±1.6	
2	27.5	41.5	52.5	40.5	28	38.0±4.7	
3	53	99	98.5	99	99	89.7±9.2	
4	10.5	4.5	19	23.5	41.5	19.8±6.3	
5	63	96.5	99.5	98.5	42	79.9±11.7	
6	99	97.5	99	99	95	97.9±0.8	
7	87	99.5	100	2.5	100	77.8±19.0	
8	99	99.5	99.5	100	99.5	99.5±0.2	
Average	66	80	83	70	76	75.1±5.3	



Figure G3: Presentation of bowls after Day 5 of beef kidney acceptance testing (showing roughly the average percentage consumption of kidney over the week)

Tripe Acceptance Testing Data:

Table G7: Food intake results for beef tripe testing

Cat	Total intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	133	189	196	172	144	166.8±12.3	834
2	98	113	19	75	58	72.6±16.4	363
3	118	178	187	131	167	156.2±13.5	781
4	2	1	15	8	2	5.6±2.7	28
5	106	193	148	136	161	148.8±14.3	744
6	22	112	173	181	157	129±29.3	645
7	131	189	183	191	131	165±13.9	825
8	137	167	121	153	179	151.4±10.3	757
Average	93	143	130	131	125	124.4±9.1	622.1±100.4

Table G8: Total percentage consumption results for beef tripe testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	66.5	94.5	98	86	72	83.4±6.2
2	49	56.5	9.5	37.5	29	36.3±8.2
3	59	89	93.5	65.5	83.5	78.1±6.7
4	1	0.5	7.5	4	1	2.8±1.3
5	53	96.5	74	68	80.5	74.4±7.2
6	11	56	86.5	90.5	78.5	64.5±14.6
7	65.5	94.5	91.5	95.5	65.5	82.5±7.0
8	68.5	83.5	60.5	76.5	89.5	75.7±5.2
Average	47	71	65	65	62	62.2±4.9



Figure G4: Presentation of bowls after Day 5 of beef tripe acceptance testing (showing roughly the average percentage consumption of tripe over the week)

MDM Acceptance Testing Data:

Table G9: Food intake results for beef MDM testing

Cat	Total intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	103	50	53	80	94	76.0±10.7	380
2	73	51	35	37	35	46.2±7.3	231
3	85	18	35	66	17	44.2±13.5	221
4	0	0	1	4	3	1.6±0.8	8
5	140	4	90	50	52	67.2±22.7	336
6	69	19	69	85	65	61.4±11.1	307
7	38	0	47	41	108	46.8±17.4	234
8	46	27	73	29	23	39.6±9.2	198
Average	69	21	50	49	50	47.9±5.4	239.4±40.0

Table G10: Total percentage consumption results for beef MDM testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	51.5	25	26.5	40	47	38.0±5.3
2	36.5	25.5	17.5	18.5	17.5	23.1±3.7
3	42.5	9	17.5	33	8.5	22.1±6.8
4	0	0	0.5	2	1.5	0.8±0.4
5	70	2	45	25	26	33.6±11.4
6	34.5	9.5	34.5	42.5	32.5	30.7±5.6
7	19	0	23.5	20.5	54	23.4±8.7
8	23	13.5	36.5	14.5	11.5	19.8±4.6
Average	35	11	25	25	25	23.9±2.7



Figure G5: Presentation of bowls after Day 3 of beef MDM acceptance testing (showing roughly the average percentage consumption of MDM over the week)

Liver Acceptance Testing Data:

Table G11: Food intake results for beef liver testing

Cat	Total intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	33	35	34	34	35	34.2±0.4	171
2	35	34	35	34	34	34.4±0.2	172
3	35	34	34	35	35	34.6±0.2	173
4	34	35	35	35	35	35.8±0.2	174
5	33	35	35	35	35	35.6±0.4	173
6	34	35	34	34	35	35.4±0.2	172
7	34	35	35	35	35	35.8±0.2	174
8	35	34	35	35	35	34.8±0.2	174
Average	34	35	35	35	35	34.6±0.1	172.9±0.4

Table G12: Total percentage consumption results for beef liver testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	94.3	100	97.1	97.1	100	97.7±1.1
2	100	97.1	100	97.1	97.1	98.3±0.7
3	100	97.1	97.1	100	100	98.9±0.7
4	97.1	100	100	100	100	99.4±0.6
5	94.3	100	100	100	100	98.9±1.1
6	97.1	100	97.1	97.1	100	98.3±0.7
7	97.1	100	100	100	100	99.4±0.6
8	100	97.1	100	100	100	99.4±0.6
Average	98	99	99	99	100	98.8±0.3

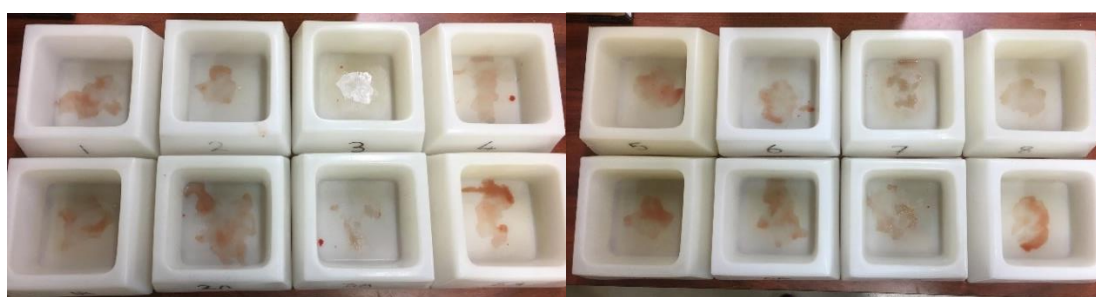


Figure G6: Presentation of bowls after Day 2 of beef liver acceptance testing (showing roughly the average percentage consumption of liver over the week)

## SAS Statistical Outputs:

*Coding used to show interactions between offal intake and days of testing:*

```
ods html close;
ods html newfile=none;
dm 'odsresults; clear';
dm 'clear log';
dm 'clear out';
options ls=255 ps=6000 nocenter;
filename catpat dde 'Excel|H:\My Documents\Master 2018\2. Testing
Methodology\D. Beef\Beef SAS\[Beef interaction data (111218).xlsx]All
beef offal!R2C1:R249C4';
data catpat;
infile catpat lrecl=6000 dlm='09'x notab dsd missover;
input Day Cat$ Offal$ Intake
;
run;
proc mixed data=catpat;
  class Day Cat Offal;
  model Intake = Day Offal Day*Offal / solution ;
  LSMeans Day Offal Day*Offal / pdiff ;
run;
quit;
```

*Coding used to show interactions between each cat and offal intake:*

```
ods html close;
ods html newfile=none;
dm 'odsresults; clear';
dm 'clear log';
dm 'clear out';
options ls=255 ps=6000 nocenter;
filename catpat dde 'Excel|H:\My Documents\Master 2018\2. Testing
Methodology\D. Beef\Beef SAS\[Beef interaction data (111218).xlsx]All
beef offal!R2C1:R249C4';
data catpat;
infile catpat lrecl=6000 dlm='09'x notab dsd missover;
input Day Cat$ Offal$ Intake
;
run;
proc mixed data=catpat;
  class Day Cat Offal;
  model Intake = Cat Offal Cat*Offal / solution ;
  LSMeans Cat Offal Cat*Offal / pdiff ;
run;
quit;
```

Liver versus MDM Raw Data and Images:

Table G13: Food intake results for beef liver in the liver versus MDM preference test

Cat	Liver Intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM	
1	34.5	34.6	35	34.5	34.6	34.6 $\pm$ 0.1	173
2	34.7	35.2	35.1	34.9	34.7	34.9 $\pm$ 0.1	175
3	35.1	34.5	35.4	34.1	34.7	34.8 $\pm$ 0.2	174
4	34.7	35.3	35.2	35.2	34.3	34.9 $\pm$ 0.2	175
5	35.4	34.5	34.7	35.2	33.6	34.7 $\pm$ 0.3	173
6	34.7	34.8	34.6	35.0	34.9	34.8 $\pm$ 0.1	174
7	34.3	35	34.2	35.2	34.3	34.6 $\pm$ 0.2	173
8	35.1	35.2	34.1	35.6	35.2	35.0 $\pm$ 0.3	175
Average	34.8	34.9	34.8	35.0	34.5	34.4 $\pm$ 0.1	174 $\pm$ 0.3

Table G14: Total percentage consumption results for beef liver in the liver versus MDM preference test

Cat	Liver percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM
1	98.9	100.0	99.4	99.7	98.6	99.3 $\pm$ 0.3
2	99.4	99.7	99.7	99.4	100.0	99.7 $\pm$ 0.1
3	100.0	99.7	100.0	98.3	100.0	99.6 $\pm$ 0.3
4	98.6	100.0	99.4	99.7	99.1	99.4 $\pm$ 0.2
5	100.0	99.4	99.7	99.7	97.4	99.2 $\pm$ 0.5
6	99.4	97.8	100.0	97.2	99.4	98.8 $\pm$ 0.5
7	100.0	99.4	100.0	99.7	98.0	99.4 $\pm$ 0.4
8	100.0	99.4	99.7	100.0	100.0	99.8 $\pm$ 0.1
Average	99.5	99.4	99.8	99.2	99.1	99.4 $\pm$ 0.1

Table G15: Food intake results for beef MDM in the liver versus MDM preference test

Cat	MDM Intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM	
1	1.5	21.1	0.3	0.0	30.2	10.6 $\pm$ 6.3	53
2	4.4	8.9	9.3	12.9	0.2	7.1 $\pm$ 2.2	36
3	21.4	6.7	1.8	16.7	0.3	9.4 $\pm$ 4.2	47
4	0	0.1	0.2	0.0	0	0.1 $\pm$ 0.0	0
5	23.6	1.5	8.9	0.1	9.4	8.7 $\pm$ 4.2	43
6	2.5	34.8	55.5	29.0	32.6	30.9 $\pm$ 8.5	154
7	34.9	18.4	12	1.2	11.9	15.7 $\pm$ 5.5	78
8	9	22.3	36.9	17.0	7.3	18.5 $\pm$ 5.3	92
Average	12.2	14.2	15.6	9.6	11.5	12.6 $\pm$ 2.1	63 $\pm$ 16.3

Table G16: Total percentage consumption results for beef MDM in the liver versus MDM preference test

Cat	MDM percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM
1	1.5	21.0	0.3	0.0	30.2	10.6 $\pm$ 6.3
2	4.4	8.9	9.3	12.8	0.2	7.1 $\pm$ 2.2
3	21.4	6.7	1.8	16.8	0.3	9.4 $\pm$ 4.2
4	0.0	0.1	0.2	0.0	0.0	0.1 $\pm$ 0.0
5	23.5	1.5	8.9	0.1	9.4	8.7 $\pm$ 4.2
6	2.5	34.7	55.9	28.9	32.3	30.9 $\pm$ 8.5
7	34.7	18.3	11.9	1.2	11.9	15.6 $\pm$ 5.5
8	9.0	22.3	36.8	16.9	7.3	18.4 $\pm$ 5.3
Average	12.1	14.2	15.6	9.6	11.5	12.6 $\pm$ 2.1

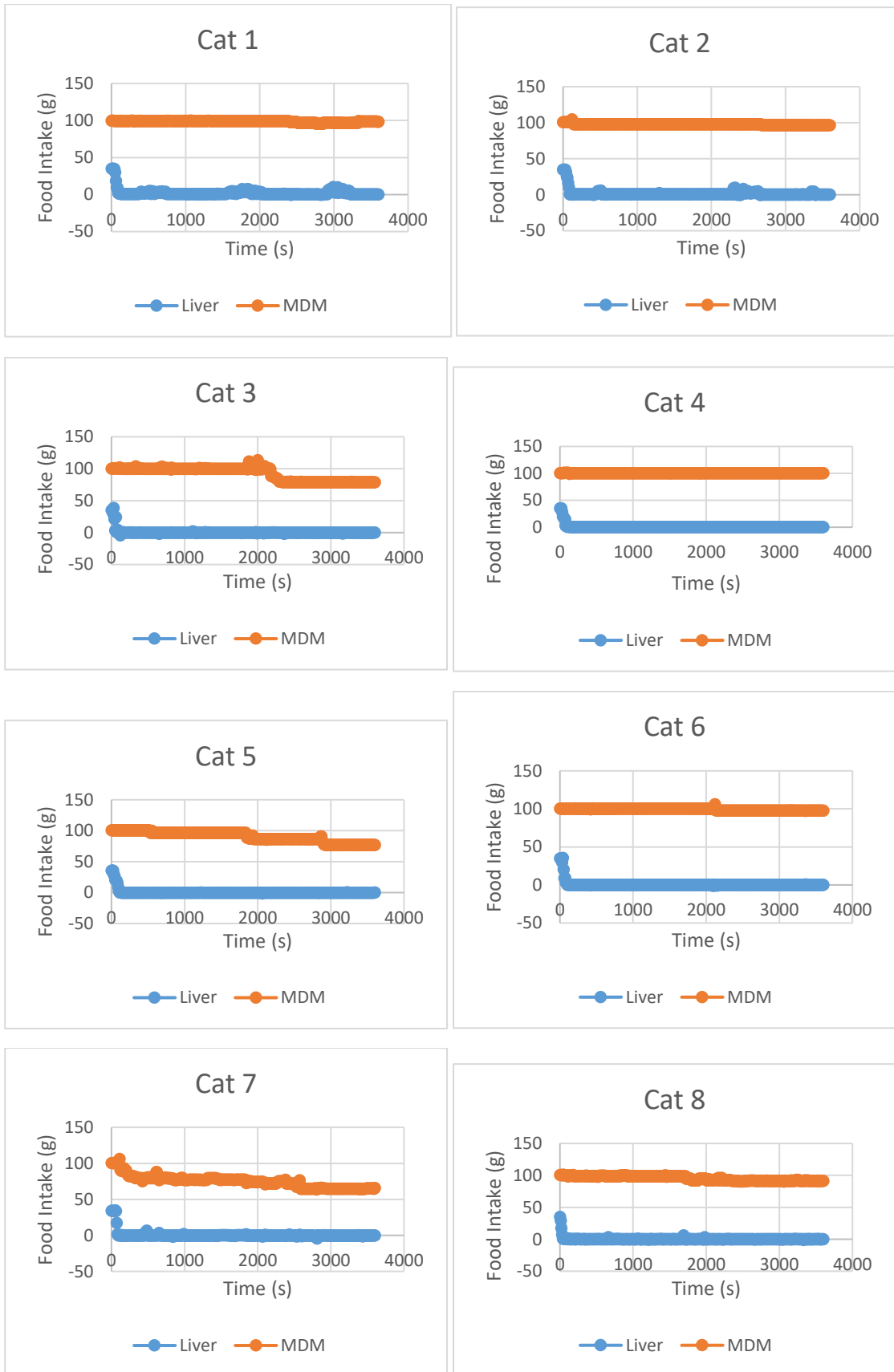


Figure G7: Presentation of bowls after Day 1 of beef liver versus MDM preference testing (showing roughly the average percentage consumption of each offal over the week)

Table G17: Raw data from Day 1 of liver versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

Cat	Beef Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Liver	1	90	33.9
			60	0.4
	MDM	0	130	2.9
2	Liver	1	80	34.7
			30	2.8
	MDM	0	30	1.5
3	Liver	1	90	35.1
	MDM	1	150	21.1
4	Liver	1	130	34.6
	MDM	0	-	-
5	Liver	1	100	35.4
			70	4.2
	MDM	0	120	9.9
			90	9.4
6	Liver	1	80	34.7
	MDM	0	80	2.5
7	Liver	1	110	34.3
			250	21.3
	MDM	2	80	2.4
			230	10.2
8	Liver	1	110	35.1
			50	2.2
	MDM	0	110	6.5

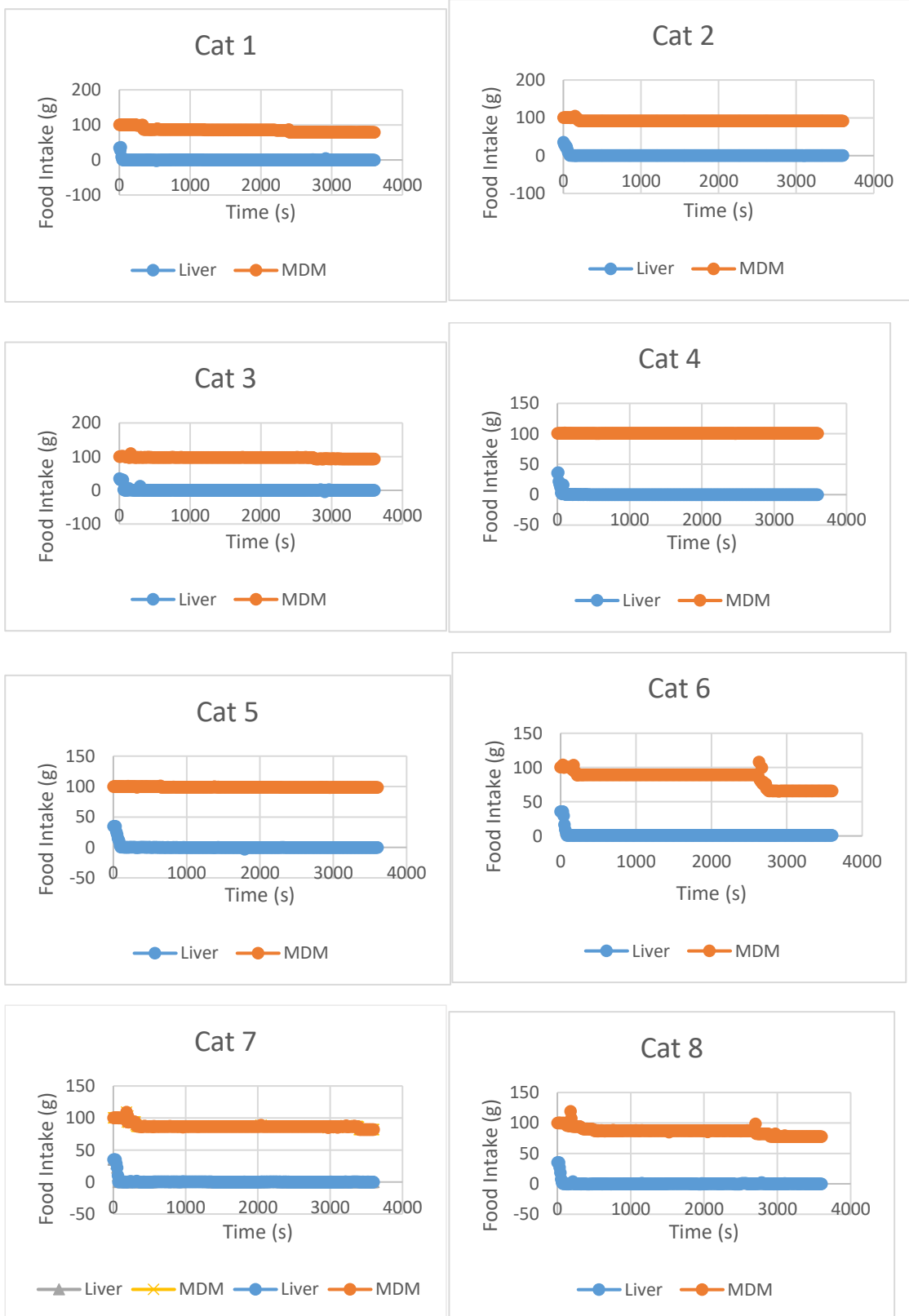




Figures G8: Intake of beef liver and MDM for each cat on Day 1 of testing

Table G18: Raw data from Day 2 of liver versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

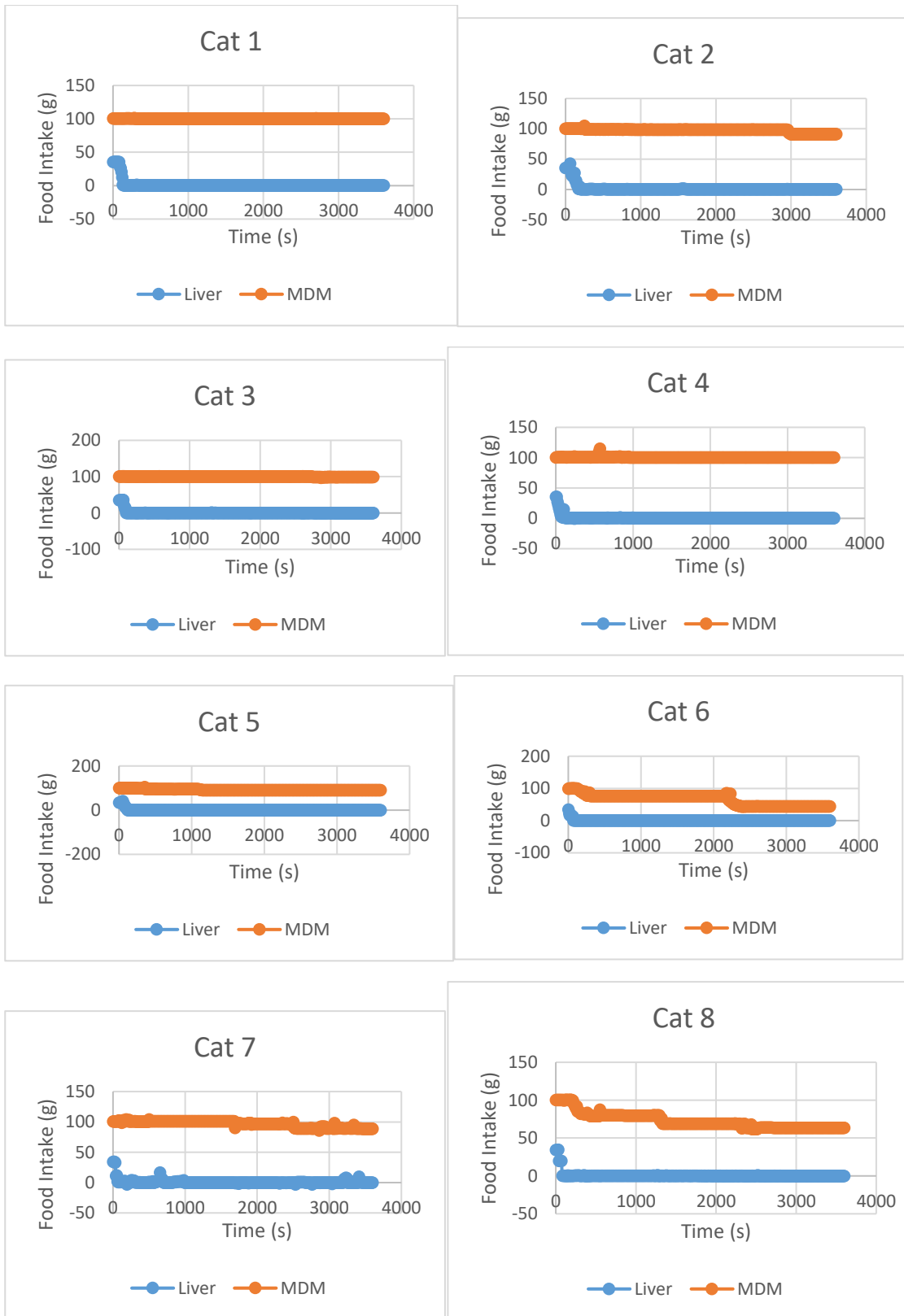
Cat	Beef Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Liver	1	100	34.6
	MDM	1	120	13.1
			40	1.3
			10	0.9
			50	5.5
2	Liver	1	100	35.2
	MDM	0	100	8.9
3	Liver	1	110	34.5
	MDM	0	180	2.2
			40	4.5
4	Liver	1	120	35.2
	MDM	0	-	-
5	Liver	1	130	34.5
	MDM	0	-	-
6	Liver	1	100	34.8
	MDM	2	140	10.9
			160	24
7	Liver	1	110	35
	MDM	1	180	13.5
			40	4.9
8	Liver	1	80	35.3
	MDM	0	100	5.4
			50	4.2
			50	2.9
			50	5.1
			120	4.6



Figures G9: Intake of beef liver and MDM for each cat on Day 2 of testing

Table G19: Raw data from Day 3 of liver versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

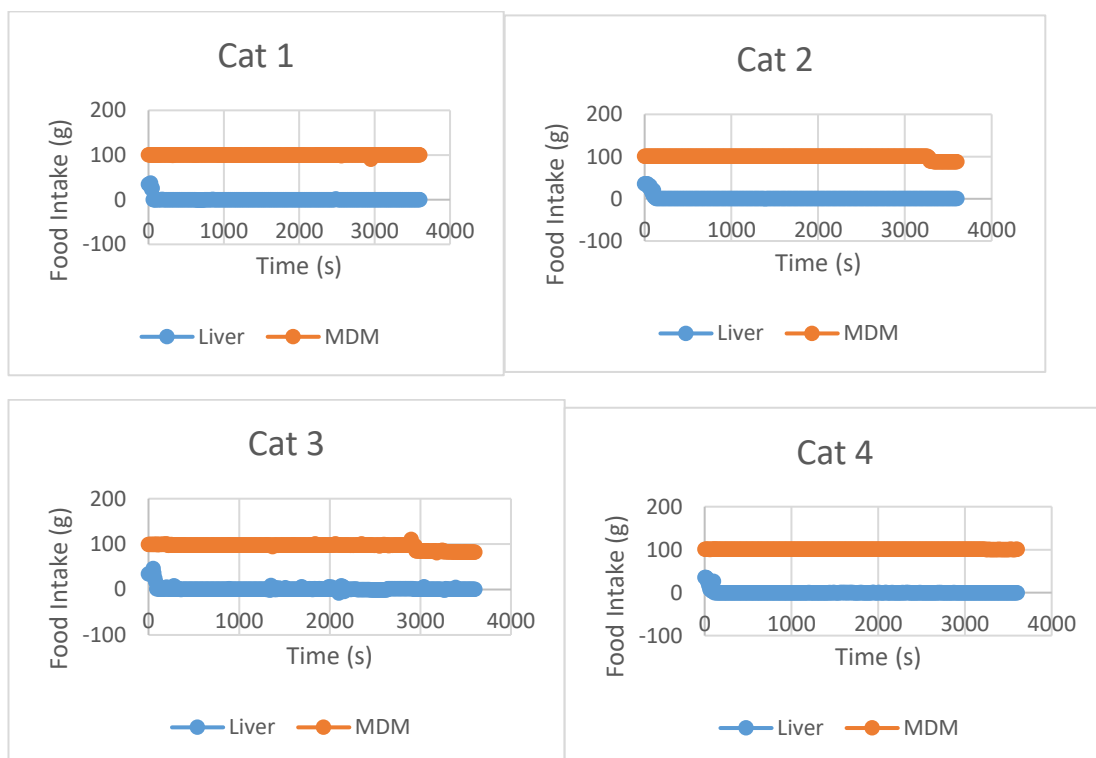
Cat	Beef Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Liver	1	80	34.9
	MDM	0	-	-
2	Liver	1	160	35.2
	MDM	0	40	1.6
			60	7.5
3	Liver	1	60	35.3
	MDM	0	20	1.7
4	Liver	1	140	35
	MDM	0	-	-
5	Liver	1	100	34.7
	MDM	0	120	4
			20	1.1
			70	3.8
6	Liver	1	80	34.5
	MDM	2	200	23.2
			210	32.4
7	Liver	1	180	34.1
	MDM	0	100	4.1
			60	7.9
8	Liver	1	100	34.1
	MDM	2	220	20.5
			40	10.9
			90	5.7

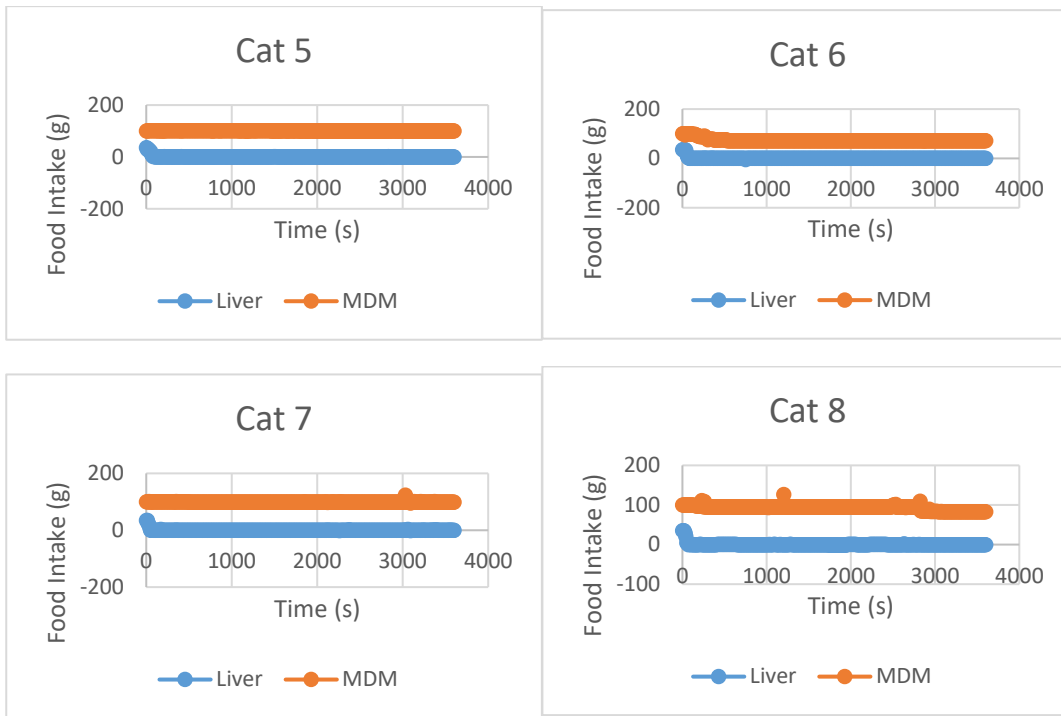


Figures G10: Intake of beef liver and MDM for each cat on Day 3 of testing

Table G20: Raw data from Day 4 of liver versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

Cat	Beef Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Liver	1	80	34.5
	MDM	0	-	-
2	Liver	1	100	34.9
	MDM	1	70	12.6
3	Liver	1	110	33.9
	MDM	1	30	1.4
			110	13
4	Liver	1	100	35.3
	MDM	0	-	-
5	Liver	1	100	34.8
	MDM	0	-	-
6	Liver	1	110	35
	MDM	1	310	25.2
			10	3.7
7	Liver	1	110	34.9
	MDM	0	10	1
8	Liver	1	90	35.5
	MDM	1	160	5.5
			240	11.3

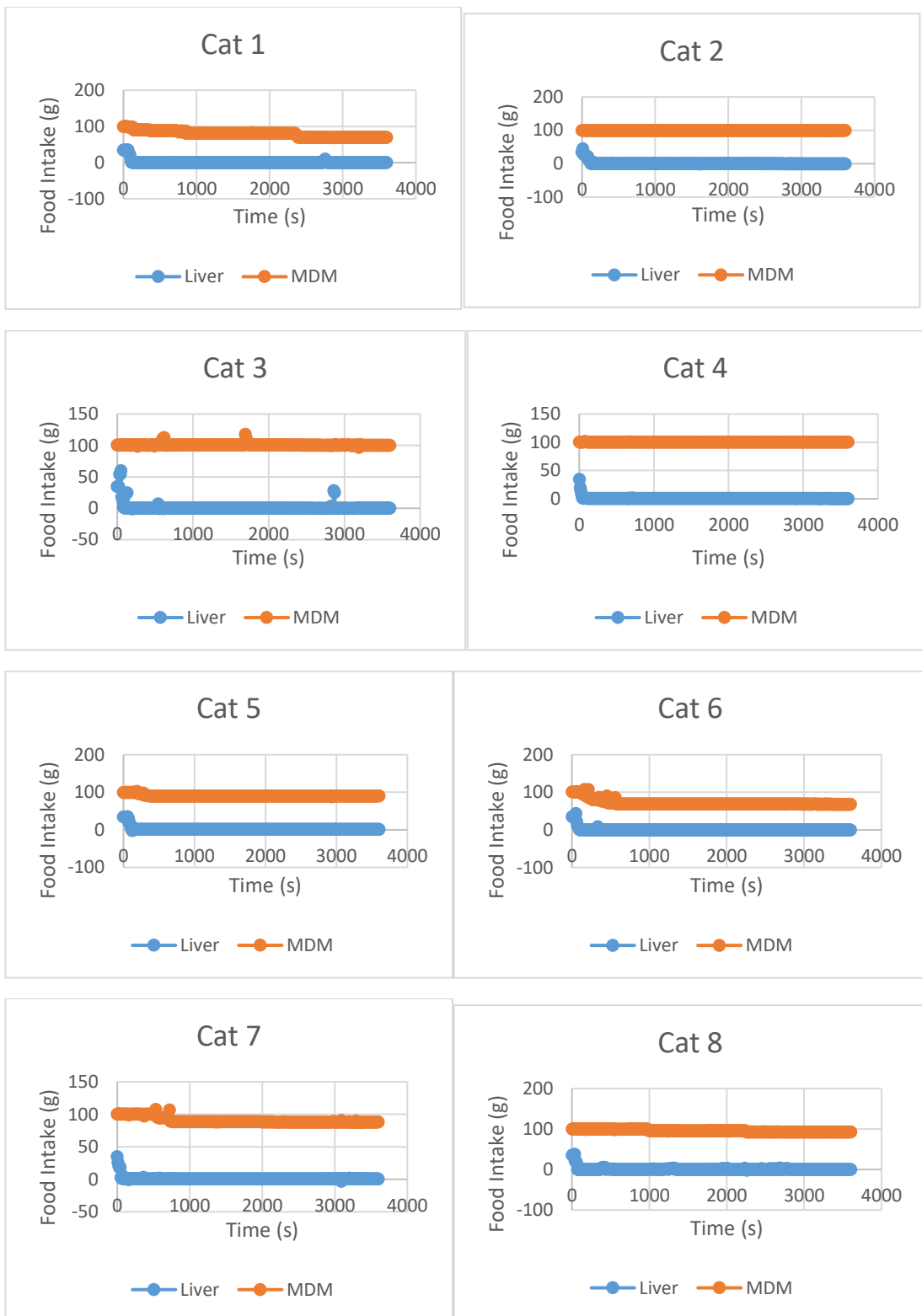




Figures G11: Intake of beef liver and MDM for each cat on Day 4 of testing

Table G21: Raw data from Day 5 of liver versus MDM preference testing (Note: size of a meal must be 10g or greater to be classified as a single meal)

Cat	Beef Offal	Number of meals	Length of meal (s)	Size of meal (g)
1	Liver	1	90	34.6
	MDM	1	120	8.9
			20	2.8
			140	7.1
50	11.6			
2	Liver	1	120	34.4
	MDM	0	-	-
3	Liver	1	140	34.7
	MDM	0	-	-
4	Liver	1	110	34.1
	MDM	0	-	-
5	Liver	1	90	33.6
	MDM	0	230	9.3
6	Liver	1	60	34.9
	MDM	1	490	32.6
7	Liver	1	120	34.2
	MDM	0	90	5.2
			60	6.4
8	Liver	1	80	35.2
	MDM	0	50	3.8
			40	3



Figures G12: Intake of beef liver and MDM for each cat on Day 5 of testing



Nutritional Analyses of Beef Offal:

*Table G22: As fed amino acid content of the six beef offal varieties (units: mg/100mg)*

AMINO ACIDS	Heart	Kidney	Liver	Lung	MDM	Tripe
Aspartic Acid	1.24	1.17	2.34	1.68	1.40	0.95
Threonine	0.60	0.58	1.12	0.77	0.67	0.41
Serine	0.56	0.61	1.14	0.82	0.62	0.48
Glutamic Acid	2.00	1.64	3.00	2.09	2.31	1.50
Proline	0.58	0.66	1.15	1.03	0.72	0.69
Glycine	0.70	0.89	1.40	1.52	0.90	1.08
Alanine	0.80	0.74	1.38	1.28	0.96	0.69
Valine	0.67	0.69	1.42	1.11	0.74	0.51
Isoleucine	0.52	0.48	1.01	0.49	0.55	0.37
Leucine	1.10	1.04	2.12	1.56	1.17	0.73
Tyrosine	0.46	0.47	0.91	0.57	0.48	0.33
Phenylalanine	0.57	0.57	1.21	0.86	0.61	0.38
Histidine	0.36	0.32	0.68	0.57	0.52	0.21
Lysine	1.10	0.91	1.83	1.37	1.33	0.69
Arginine	0.85	0.83	1.52	1.09	1.00	0.74
Taurine	0.04	0.03	0.03	0.08	0.01	0.02
Cysteine	0.19	0.22	0.44	0.28	0.18	0.15
Methionine	0.39	0.29	0.67	0.34	0.40	0.24
Tryptophan	0.21	0.20	0.47	0.24	0.22	0.12

Table G23: As fed fatty acid profile of the six beef offal varieties (units: g/100g)

FATTY ACIDS	Heart	Kidney	Liver	Lung	MDM	Tripe
C6:0 Caproic	0.00	0.00	0.00	0.00	0.01	0.00
C8:0 Caprylic	ND	0.00	ND	ND	0.00	ND
C10:0 Capric	0.00	0.01	0.00	0.00	0.02	0.01
C11:0 Undecanoic	ND	ND	ND	ND	ND	ND
C12:0 Lauric	0.01	0.01	0.00	0.00	0.03	0.01
C13:0 Tridecanoic	0.00	0.00	ND	ND	0.01	0.00
C14:0 Myristic	0.17	0.17	0.01	0.02	0.78	0.18
C14:1n5 - cis-9-Myristoleic	0.01	0.01	ND	0.00	0.07	0.01
C15:1n5 - cis-10-Pentadecenoic	ND	ND	ND	ND	ND	ND
C16:0 Palmitic	1.15	1.68	0.38	0.44	8.19	1.47
C16:1n7 - cis-9-Palmitoleic	0.06	0.07	0.02	0.02	0.54	0.07
C17:0 Margaric	0.07	0.14	0.04	0.02	0.69	0.11
C17:1n7 - cis-10-Heptadecenoic	ND	ND	ND	ND	0.00	ND
C18:0 Stearic	1.33	2.28	1.23	0.31	9.62	1.50
C18:1n9t Elaidic	0.01	0.02	0.00	0.00	0.08	0.01
C18:1n7t Vaccenic	0.10	0.27	0.05	0.02	1.33	0.25
C18:1n9c Oleic	0.95	1.74	0.50	0.42	10.41	1.41
C18:1n7c Vaccenic	0.03	0.06	0.02	0.01	0.24	0.04
C18:2n6t Linolelaidic	ND	ND	ND	ND	ND	ND
C18:2n6c Linoleic	0.24	0.21	0.45	0.06	0.63	0.08
C20:0 Arachidic	0.01	0.03	ND	0.01	0.08	0.01
C18:3n6 - cis-6,9,12-Gamma linolenic	ND	ND	ND	ND	ND	ND
C20:1n9 - cis-11-Eicosenoic	0.01	0.01	0.01	0.00	0.03	0.01
C18:3n3 - cis-9,12,15-Alpha linolenic	0.09	0.09	0.27	0.02	0.47	0.05
C21:0 Heneicosanoic	ND	0.00	ND	ND	0.01	0.00
C20:2n6 - cis-11,14-Eicosadienoic	0.00	0.01	0.01	0.00	0.02	0.00
C22:0 Behenic	0.01	0.03	0.01	0.02	0.04	0.01
C20:3n6 - cis-8,11,14-Eicosatrienoic	0.03	0.02	0.04	0.03	0.01	0.01
C22:1n9 - cis-13-Erucic	ND	ND	ND	0.00	0.01	ND
C20:3n3 - cis-11,14,17-Eicosatrienoic	ND	0.01	0.01	0.00	0.01	0.00
C20:4n6 - cis-5,8,11,14-Arachidonic	0.08	0.15	0.31	0.12	0.03	0.03
C23:0 Tricosanoic	0.00	0.01	0.02	0.00	0.01	0.00
C22:2n6 - cis-13,16-Docosadienoic	0.00	0.00	ND	ND	0.01	0.00
C24:0 Lignoceric	0.00	0.01	0.01	0.01	0.01	0.00
C20:5n3 - cis-5,8,11,14,17-Epa	0.05	0.05	0.07	0.03	0.01	0.01
C24:1n9 - cis-15- Nervonic	0.00	0.00	0.00	0.00	0.00	0.00

C22:5n3 - cis-7,10,13,16,19-DPA	0.03	0.04	0.18	0.06	0.02	0.02
C22:6n3 - cis-4,7,10,13,16,19-DHA	ND	0.01	0.05	0.01	ND	0.00

## Appendix H – Acceptance of Chicken Offal Raw Data and Images

### Heart Acceptance Testing Data:

Table H1: Food intake results for chicken heart testing

Cat	Total food intake (g)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	Weekly total
1	156	85	112	85	134	114.4±13.9	572
2	88	55	76	68	85	74.4±6.0	372
3	38	94	49	52	73	61.2±10.0	306
4	67	37	24	7	1	27.2±11.8	136
5	103	65	46	69	42	65.0±10.8	325
6	200	197	195	196	192	196.0±1.3	980
7	138	57	73	100	108	95.2±14.1	476
8	186	136	188	198	158	173.2±11.4	866
Average	122	91	95	97	99	100.8±9.3	504.1±102.4

Table H2: Total percentage consumption results for chicken heart testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	78	42.5	56	42.5	67	57.2±6.9
2	44	27.5	38	34	42.5	37.2±3.0
3	19	47	24.5	26	36.5	30.6±5.0
4	33.5	18.5	12	3.5	0.5	13.6±5.9
5	51.5	32.5	23	34.5	21	32.5±5.4
6	100	98.5	97.5	98	96	98.0±0.7
7	69	28.5	36.5	50	54	47.6±7.0
8	93	68	94	99	79	86.6±5.7
Average	61.0	45.4	47.7	48.4	49.6	50.4±4.7



Figure H1: Presentation of bowls after Day 5 of chicken heart acceptance testing (showing roughly the average percentage consumption of heart over the week)

Gizzard Acceptance Testing Data:

Table H3: Food intake results for chicken gizzard testing

Cat	Total food intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	188	94	181	198	178	167.8±18.8	839
2	169	48	60	73	45	79±23.0	395
3	101	47	62	60	106	75.2±11.9	376
4	99	71	61	72	48	70.2±8.4	351
5	180	115	68	177	178	143.6±22.5	718
6	195	197	187	196	192	193.4±1.8	967
7	200	104	170	199	122	159±19.7	795
8	199	163	197	148	176	176.6±9.8	883
Average	166	105	123	140	131	133.1±9.1	665.5±89.0

Table H4: Total percentage consumption results for chicken gizzard testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	94	47	90.5	99	89	83.9±9.4
2	84.5	24	30	36.5	22.5	39.5±11.5
3	50.5	23.5	31	30	53	37.6±5.9
4	49.5	35.5	30.5	36	24	35.1±4.2
5	90	57.5	34	88.5	89	71.8±11.3
6	97.5	98.5	93.5	98	96	96.7±0.9
7	100	52	85	99.5	61	79.5±9.9
8	99.5	81.5	98.5	74	88	88.3±4.9
Average	83.2	52.4	61.6	70.2	65.3	66.6±4.6



Figure H2: Presentation of bowls after Day 5 of chicken gizzard acceptance testing (showing roughly the average percentage consumption of gizzard over the week)

MDM Acceptance Testing Data:

Table H5: Food intake results for chicken MDM testing

Cat	Total food intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM	
1	185	134	154	139	83	139.0±16.6	695
2	119	64	69	95	45	78.4±12.9	392
3	69	43	38	33	63	49.2±7.1	246
4	28	8	27	30	106	39.8±17.0	199
5	97	126	128	152	153	131.2±10.3	656
6	83	197	145	102	135	132.4±19.6	662
7	48	92	89	41	84	70.8±10.9	354
8	183	81	106	136	98	120.8±17.9	604
Average	102	93	95	91	95.875	95.2±7.6	477.0±71.1

Table H6: Total percentage consumption results for chicken MDM testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average ± SEM
1	92.5	67	77	69.5	41.5	69.5±8.3
2	59.5	32	34.5	47.5	22.5	39.2±6.5
3	34.5	21.5	19	16.5	31.5	24.6±3.6
4	14	4	13.5	15	53	19.9±8.5
5	48.5	63	64	76	76.5	65.6±5.1
6	41.5	98.5	72.5	51	67.5	66.2±9.8
7	24	46	44.5	20.5	42	35.4±5.4
8	91.5	40.5	53	68	49	60.4±9.0
Average	50.8	46.6	47.3	45.5	47.9	47.6±3.8



Figure H3: Presentation of bowls after Day 3 of chicken MDM acceptance testing (showing roughly the average percentage consumption of MDM over the week)

Liver Acceptance Testing Data:

Table H7: Food intake results for chicken liver testing

Cat	Total intake (g)						Weekly total
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM	
1	52	51	54	2	52	42.2 $\pm$ 10.1	211
2	54	54	54	54	54	54.0 $\pm$ 0.0	270
3	53	54	53	54	54	53.6 $\pm$ 0.2	268
4	51	50	51	52	51	51.0 $\pm$ 0.3	255
5	50	53	53	54	54	52.8 $\pm$ 0.7	264
6	53	52	53	53	53	52.8 $\pm$ 0.2	264
7	52	53	54	54	54	53.4 $\pm$ 0.4	267
8	53	53	54	54	53	53.4 $\pm$ 0.2	267
Average	52	53	53	47	53	51.7 $\pm$ 1.3	258 $\pm$ 6.9

Table H8: Total percentage consumption results for chicken liver testing

Cat	Total percentage consumption (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Average $\pm$ SEM
1	96.3	94.4	100.0	3.7	96.3	78.1 $\pm$ 18.6
2	100.0	100.0	100.0	100.0	100.0	100.0 $\pm$ 0.0
3	98.1	100.0	98.1	100.0	100.0	99.3 $\pm$ 0.5
4	94.4	92.6	94.4	96.3	94.4	94.4 $\pm$ 0.6
5	92.6	98.1	98.1	100.0	100.0	97.8 $\pm$ 1.4
6	98.1	96.3	98.1	98.1	98.1	97.8 $\pm$ 0.4
7	96.3	98.1	100.0	100.0	100.0	98.9 $\pm$ 0.7
8	98.1	98.1	100.0	100.0	98.1	98.9 $\pm$ 0.5
Average	97	97	99	87	98	95.6 $\pm$ 2.4



Figure H4: Presentation of bowls after Day 3 of chicken liver acceptance testing (showing roughly the average percentage consumption of liver over the week)

## SAS Statistical Outputs:

*Coding used to show interactions between offal intake and days of testing:*

```
ods html close;
ods html newfile=none;
dm 'odsresults; clear';
dm 'clear log';
dm 'clear out';
options ls=255 ps=6000 nocenter;
filename catpat dde 'Excel|H:\My Documents\Master 2018\2. Testing
Methodology\I. Chicken\[Chicken acceptance.xlsx]Chicken
SAS!R2C1:R161C4';
data catpat;
infile catpat lrecl=6000 dlm='09'x notab dsd missover;
input Day Cat$ Offal$ Intake
;
run;
proc mixed data=catpat;
  class Day Cat Offal;
  model Intake = Day Offal Day*Offal / solution ;
  LSMeans Day Offal Day*Offal / pdiff ;
run;
quit;
```

*Coding used to show interactions between each cat and offal intake:*

```
ods html close;
ods html newfile=none;
dm 'odsresults; clear';
dm 'clear log';
dm 'clear out';
options ls=255 ps=6000 nocenter;
filename catpat dde 'Excel|H:\My Documents\Master 2018\2. Testing
Methodology\I. Chicken\[Chicken acceptance.xlsx]Chicken
SAS!R2C1:R161C4';
data catpat;
infile catpat lrecl=6000 dlm='09'x notab dsd missover;
input Day Cat$ Offal$ Intake
;
run;
proc mixed data=catpat;
  class Day Cat Offal;
  model Intake = Cat Offal Cat*Offal / solution ;
  LSMeans Cat Offal Cat*Offal / pdiff ;
run;
quit;
```



## Nutritional Analyses of Chicken Offal:

*Table H9: As fed amino acid content of the four chicken offal varieties (units: mg/100mg)*

AMINO ACIDS	Chicken gizzard	Chicken heart	Chicken liver	Chicken MDM
Aspartic Acid	1.39	0.95	1.64	1.42
Threonine	0.65	0.46	0.82	0.64
Serine	0.61	0.40	0.76	0.60
Glutamic Acid	2.14	1.41	2.12	2.12
Proline	0.88	0.54	0.83	0.77
Glycine	1.28	0.77	0.94	1.09
Alanine	0.91	0.67	1.06	1.01
Valine	0.74	0.69	1.09	0.77
Isoleucine	0.60	0.46	0.82	0.67
Leucine	1.10	0.89	1.58	1.15
Tyrosine	0.54	0.40	0.73	0.50
Phenylalanine	0.59	0.46	0.88	0.60
Histidine	0.30	0.25	0.48	0.42
Lysine	1.03	0.83	1.39	1.28
Arginine	1.17	0.69	1.27	1.09
Taurine	0.09	0.21	0.08	0.04
Cysteine	0.19	0.14	0.28	0.19
Methionine	0.38	0.27	0.47	0.43
Tryptophan	0.15	0.15	0.32	0.21

Table H11: As fed fatty acid profile of the four chicken offal varieties (units: g/100g)

Fatty ACIDS	Gizzard	Heart	Liver	MDM
C6:0 Caproic	0.00	ND	0.00	<0.01
C8:0 Caprylic	ND	ND	ND	<0.01
C10:0 Capric	0.00	0.00	0.01	0.00
C11:0 Undecanoic	ND	0.00	0.00	ND
C12:0 Lauric	0.00	0.00	0.00	0.00
C13:0 Tridecanoic	ND	<0.01	ND	ND
C14:0 Myristic	0.01	0.06	0.01	0.06
C14:1n5 - cis-9-Myristoleic	0.00	0.02	0.00	0.02
C15:1n5 - cis-10-Pentadecenoic	ND	ND	ND	ND
C16:0 Palmitic	0.47	2.36	0.73	2.20
C16:1n7 - cis-9-Palmitoleic	0.09	0.61	0.08	0.41
C17:0 Margaric	0.01	0.02	0.01	0.02
C17:1n7 - cis-10-Heptadecenoic	ND	ND	ND	ND
C18:0 Stearic	0.19	0.72	0.62	0.69
C18:1n9t Elaidic	0.00	0.02	0.01	0.02
C18:1n7t Vaccenic	0.00	0.02	0.00	0.00
C18:1n9c Oleic	0.71	4.51	0.97	4.17
C18:1n7c Vaccenic	0.04	0.21	0.07	0.19
C18:2n6t Linolelaidic	ND	ND	ND	ND
C18:2n6c Linoleic	0.35	2.12	0.62	1.48
C20:0 Arachidic	0.00	0.01	0.00	0.01
C18:3n6 - cis-6,9,12-Gamma linolenic	0.00	0.01	0.01	0.02
C20:1n9 - cis-11-Eicosenoic	0.01	0.05	0.02	0.04
C18:3n3 - cis-9,12,15-Alpha linolenic	0.03	0.18	0.03	0.21
C21:0 Heneicosanoic	ND	ND	ND	ND
C20:2n6 - cis-11,14-Eicosadienoic	0.01	0.02	0.01	0.02
C22:0 Behenic	0.00	0.00	0.01	0.00
C20:3n6 - cis-8,11,14-Eicosatrienoic	0.02	0.03	0.05	0.02
C22:1n9 - cis-13-Erucic	0.00	0.00	ND	0.00
C20:3n3 - cis-11,14,17-Eicosatrienoic	ND	0.00	ND	0.00
C20:4n6 - cis-5,8,11,14-Arachidonic	0.10	0.21	0.32	0.09
C23:0 Tricosanoic	ND	0.00	0.00	0.00
C22:2n6 - cis-13,16-Docosadienoic	ND	ND	ND	ND
C24:0 Lignoceric	0.00	0.00	0.00	0.00
C20:5n3 - cis-5,8,11,14,17-Epa	0.00	0.01	0.04	0.01
C24:1n9 - cis-15- Nervonic	0.01	0.00	0.01	0.00
C22:5n3 - cis-7,10,13,16,19-DPA	0.01	0.01	0.04	0.02
C22:6n3 - cis-4,7,10,13,16,19-DHA	0.01	0.01	0.07	0.01

## Appendix I – Beef versus Lamb SAS Outputs and Calculations

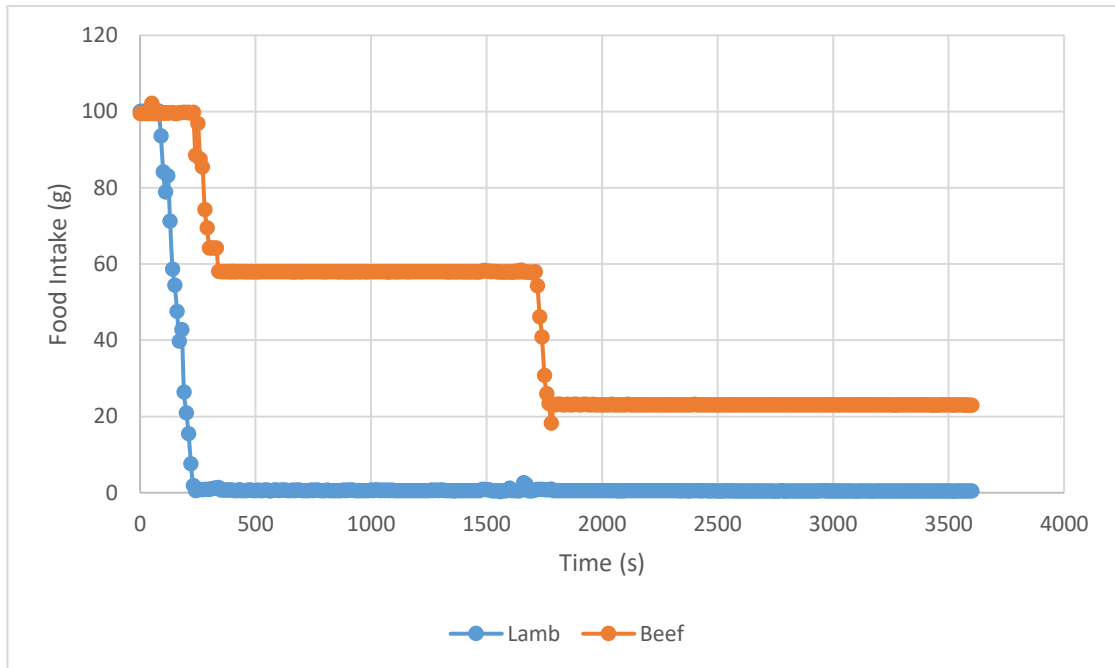
SAS Statistical Outputs:

Coding used to show interactions between offal intake and days of testing:

```
ods html close;
ods html newfile=none;
dm 'odsresults; clear';
dm 'clear log';
dm 'clear out';
options ls=255 ps=6000 nocenter;
filename catpat dde 'Excel|H:\My Documents\Master 2018\2. Testing
Methodology\F. Beef vs Lamb\[Rate of consumption.xlsx]B vs L
SAS!R1C1:R481C4';
data catpat;
infile catpat lrecl=6000 dlm='09'x notab dsd missover;
input Day Cat$ Offal$ Intake
;
run;
proc mixed data=catpat;
class Day Cat Offal;
model Intake = Day Offal Day*Offal / solution ;
LSMeans Day Offal Day*Offal / pdiff ;
run;
quit;
```

Example Rate of Consumption Calculation for Lamb Lung:

$$\text{Rate of consumption } \left( \frac{g}{min} \right) = \frac{\text{Total food intake } (g)}{\text{Point where food intake stops } (min)}$$



$$\begin{aligned} \text{Total food intake } (g) &= \text{weight of food before } (g) - \text{weight of food after } (g) \\ &= 100.1 - 0.6 \\ &= 99.5g \end{aligned}$$

$$\text{Point where food intake stops } (min) = \frac{240 \text{ s}}{60} = 4 \text{ minutes}$$

$$\begin{aligned} \text{Rate of consumption } \left( \frac{g}{min} \right) &= \frac{\text{Total food intake } (g)}{\text{Point where food intake stops } (min)} \\ &= \frac{99.5g}{4 \text{ minutes}} \end{aligned}$$

$$\therefore \text{Rate of consumption} = 24.9 \text{ g/min}$$