Investigations into Feline (*Felis catus*) Palatability

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Shay Rebekah Rutherford

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Abstract

Due to the finicky nature of the domestic cats' feeding habits, palatability is a highly complex area of feline nutrition, but one which is vitally important, with pet owners today selecting a brand based on palatability rather than nutritional value (Trivedi and Benning 1999).

To date there is little published literature about the specific compounds and ingredients responsible for the palatability of cat foods, however, some animal by-products, particularly fish offals have been reported as being highly palatable to the cat (MacDonald *et al.* 1984). There is much interest in the use of synthetic diets for palatability testing because these diets can be easily manipulated to control specific properties of the diet.

The aims of this study, using the domestic cat as the test species were to: (1) test the efficacy of using synthetic diets in palatability trials, (2) determine the palatability of various fish by-products when included in synthetic and commercially canned diets, (3) develop a method suitable for fractionating fish by-products and fractionate selected by-products, (4) determine the palatability of the fish by-product fractions obtained and (5) determine the palatability of various pure compounds when dissolved in pet milk.

All palatability trials carried out used the two bowl free choice testing method. The freeze dried forms of hoki, mussel, salmon and jack mackerel by-products were included in a synthetic diet and compared to an un-supplemented synthetic diet. Hoki, mussel, salmon and barracouta by-products and the three fish by-product fractions (salmon oil, salmon water and barracouta water) were included fresh in commercially made canned diets and compared to a common commercial diet (control). Finally various pure compounds (amino acids, malic acid, salts, urea, creatinine, creatine, etc.) were dissolved in pet milk and compared to un-supplemented pet milk (control). Feed intakes were recorded daily for all of the trials.

The test animals used were domestic short haired cats obtained from the Centre for Feline Nutrition (Massey University, Palmerston North, New Zealand) and all were in excellent health before testing began. The panels of cats used were either all castrated males aged three to nine years old, or a mixture of castrated males, entire females and entire males aged two to eleven years old.

The feed intake data collected during the trials were analysed using t tests, and repeat measures ANOVA. Feed intakes were low overall during the synthetic trial due to the

un-supplemented control diet itself not being very palatable. These low feed intakes resulted in weight losses in the cats and the study has to be abandoned prematurely. Therefore, the results are only based on a sample size of two and are of limited value as such. The other three trials were highly successful, with all cats remaining healthy, apart from two which were taken from the milk trial due to health reasons unrelated to these trials. Salmon and jack mackerel by-products were both significantly ($P<0.01$ and $P<0.001$ respectively) more palatable than the control diet, hoki by-product was significantly ($P<0.001$) less palatable than the control and mussel by-product was not significantly different ($P>0.05$) from the control when they were included in synthetic diets. When the fish by-products were included in commercial diets, the salmon and mussel by-products were the most palatable of the test diets, however, the salmon, mussel and barracouta by-products were not significantly different ($P>0.05$) from the control in terms of palatability. The hoki by-product was significantly ($P<0.001$) less palatable than the control. The trials using the fish by-product fractions illustrated that salmon oil containing diet was significantly ($P<0.05$) more palatable than the control diet. The salmon water and barracouta water containing diets were not significantly ($P>0.05$) different from the control in terms of palatability. Lastly, the trials involving the testing of pure compounds highlighted that at the 0.3% dose proline, lysine (lysine hydrochloride), histidine, cysteine (cysteine hydrochloride), glycine and sodium dihydrogen phosphate were all significantly ($P<0.001$) more palatable than the un-supplemented pet milk. At the 0.3% dose, other compounds investigated were no more palatable than the un-supplemented control milk. Proline and lysine (lysine hydrochloride) were also significantly ($P<0.001$) more palatable than the control at the 0.6% dose, however, histidine was no more palatable ($P>0.05$) than the control at this inclusion level.

In conclusion, salmon by-product was liked and hoki by-product was disliked by the cats when included in both synthetic and commercial diets. Mussel by-product was palatable when included in the commercial diets, but was no more palatable than the control when tested in the synthetic diet. Jack mackerel by-product was palatable in the synthetic diet and barracouta by-product was no more palatable than the control in the commercial diet. Salmon oil and the compounds proline, lysine hydrochloride, histidine, glycine, cysteine hydrochloride and sodium dihydrogen phosphate were highly palatable to the cat.

If future work with synthetic diets occurs it needs to be aimed at pre-weaned kittens as it would be easier to wean young inexperienced kittens onto a synthetic diet than older cats which are used to receiving a commercially made diet. The fish by-products tested here, along with other New Zealand fish species need to be further investigated. It may also be of use to test the fish by-products and their fractions in a different type of base diet in order to determine how this affects their palatability. The dose dependency of the compounds found palatable in the milk trials also needs to be established, in order to find an optimum dose for them to be used as palatability enhancers.

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General Introduction

The domestic cat (*Felis catus*) belongs to the Felidae family, with its original ancestor believed to be the African wildcat (*Felis sylvestris libyca*). The cat was domesticated approximately 8000 years ago, making it one of the most recently domesticated species of all the mammals and, therefore, the least altered in terms of morphology and behaviour (Hart 1985). The domestic cat is an obligate carnivore, as it is believed to have evolved on a diet consisting entirely of animal tissues.

Nowadays the international pet food industry is large, very competitive and rapidly growing, with a large proportion of people owning at least one cat. Cats have very distinctive feeding preferences and are well known for their fussy and finicky eating habits (Bradshaw *et al.* 1996). They are in fact highly sensitive to minute changes in the sensory properties of their food (Hirsch *et al.* 1978) and can therefore be very difficult to feed.

Palatability can be defined as the relative attractiveness, acceptability or preference for a particular food. Alternatively, palatability can simply mean "pleasant to the taste" (Tarttelin 1997). Cats like humans have very different individual food likes and dislikes. The concept of palatability as it relates to pet food is therefore very complex, but one which is vitally important, because a nutritionally "complete and balanced" diet is useless to the animal if they will not ingest it. However, despite this, little has been written on palatability in cat foods and there is a dearth of information about specific compounds and ingredients cats find palatable.

Previous studies have reported that cats find protein hydrolysates, meat extracts, fats and some free amino acids palatable (MacDonald *et al.* 1984). It is also a commonly held belief that cats prefer fish over other meats (Hegsted *et al.* 1956, Houpt and Smith 1981). Fish by-products offer a relatively cheap source of protein if found to be palatable to the majority of cats. New Zealand fish species have not yet been tested for their palatability enhancing properties and therefore, if found to be palatable they could provide a unique product for the New Zealand pet food industry.

The objectives of this study were two-fold. The first part aimed to determine the palatability of various fish by-products using the cat as the test animal species, to test the efficacy of using synthetic diets containing fish by-products in palatability trials, to develop a fractionation method for fish by-products and to determine the palatability of

fish by-product fractions. A second aim of this thesis was to establish the palatability of various synthetic compounds when dissolved in pet milk.

Chapter 1

Review of literature

1.1 Introduction

When dinosaurs became extinct, a range of ecological niches became empty. These were later occupied by various mammals; including the two main groups of carnivorous mammals, which later evolved into the Canids and Felids. The Canid family contained the domestic dog (*Canis familiaris*) and the Felid family the domestic cat (*Felis catus*). Despite the focus here being on palatability in cats, both cats and dogs will be discussed in order to provide a comparative approach. The relationship between these two families is illustrated below in Figure 1.

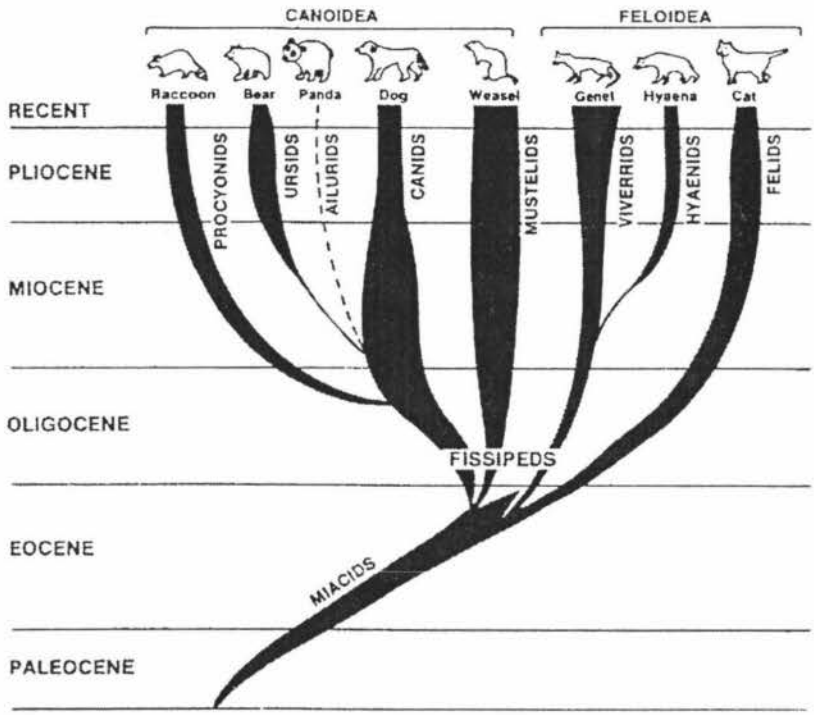

Figure 1: The relationships between the members of the order Carnivora (MacDonald *et al.* 1984).

It is believed that the domestic cat descended from the small African wild cat (*Felis sylvestris libyca*). Domestication of the cat is believed to have occurred approximately 8,000 years ago (MacDonald *et al.* 1984). It is, therefore, the most recently domesticated species of all the mammals and is the least altered species in terms of morphology and behaviour (Hart 1985). The cat was probably domesticated for rodent control, explaining why its domestication occurred along with the development of barns and grain stores. Humans and cats developed a symbiotic relationship, where the benefit to the cat was a large food supply and the benefit to man was rodent and pest

control. Cats, unlike dogs, are still very independent, solitary animals and their domestication is believed to be attributed to the ancient Egyptians, who later actually worshipped the cat, believing it was the living form of Bastet; a goddess symbolizing maternity and fertility (Wills and Robinson 2000).

Dogs were one of the first species to be domesticated, approximately 12,000 years ago. It is thought their most likely ancestor is the wolf, with the wolf being found to be 20 times more closely related to the dog than to the coyote, the wolves closest wild relative although, there is still much debate surrounding this. The relationship that subsequently developed between the dog and man was probably beneficial to both parties, with the dog receiving food and shelter, and humans possibly being supplied with a hunting partner, a security guard and a play toy for their children.

1.2 Palatability

Diodorous, a Roman historian, wrote that the Egyptians worshipped their cats and fed them on bread, milk and Nile fish (MacDonald *et al.* 1984). Today cats and dogs, through out the world are still being spoilt by their owners (Bennet 2002). Both cats and dogs have very distinctive feeding preferences, some of which are due to species evolution and are physiological, and others that can be linked to the individual animals' feeding history. Cats especially are known for their fussy and finicky eating habits and are highly sensitive to minute changes in the sensory properties of their food (Hirsch *et al.* 1978). In fact, some cats will refuse a food they do not consider to be palatable almost until the point of starvation.

Although individual animals have very different food likes and dislikes, unlike humans, cats and dogs have no choice in the foods their owners offer them. Pet owners decide which pet food to purchase. However, if able to, cats often supplement this diet by hunting prey such as mice and birds. The foods offered that cats and dogs like are referred to as being 'palatable', and those they dislike as being 'less palatable' or in the extreme, 'unpalatable'. Therefore, pet food companies need to formulate foods that are not only palatable, but are also nutritionally complete. As Young said in 1948 "an animal accepts what he likes as well as what he needs and it is an open question as to how far what he likes agrees with what he needs." Keeping your cat happy and offering him or her palatable foods can, therefore, be very difficult and frustrating, as each pet will have very different preferences and unlike humans, they can not simply tell us if

they liked a food, and if so why. Therefore, the concept of palatability as it relates to pet food is very complex, but one which is extremely important in the case of pet food manufacturers. However, despite this importance, relatively little has been written on palatability in cat and dog foods (Bradshaw *et al.* 1996, Houpt and Smith 1981, Trivedi and Benning 1999).

Yet another factor to consider concerning palatability of pet foods is the fine balance between providing a palatable ration and an unpalatable ration. A ration that is highly palatable can stimulate feed intake and cause obesity, especially in dogs, where obesity is often a problem. However, on the other hand anorexia is a problem in cats, resulting in their being a fine balance between unpalatable and too highly palatable foods (Houpt *et al.* 1978).

Domestic cats and dogs are often kept in an environment where they can't obtain their own food, as they would in the wild. It is, therefore, up to the owner to provide the animal an appropriate diet. This diet must supply all the required nutrients in the correct balance and also be one that is palatable to the animal. Both cats and dogs have certain dietary requirements, these being stricter in the cat compared with the dog. The cat has high dietary protein requirements due to its inability to regulate catabolic enzymes in the liver. As a result the adult dog has a lower dietary protein requirement compared to adult cats (8% vs. 14%). The specific amount of fat the cat and dog require in their diets is relatively unknown, although fat does have important functions, including providing a source of fat soluble vitamins and essential fatty acids and adding to the caloric density of the diet (NRC 1986). Cats unlike dogs can not synthesise niacin from tryptophan, can not convert carotene to vitamin A and linoleic acid to arachidonic acid and can not synthesise enough taurine to meet their daily needs. Therefore, cats need a dietary source of niacin, vitamin A, arachidonic acid and taurine, all of which can be found in the animal tissues they have evolved to consume exclusively. Dogs, however, can synthesise all of the above nutrients from other dietary components, which further illustrates that the dog is an omnivorous carnivore. Both dogs and cats are also sensitive to arginine deficiencies and therefore also require a dietary source of arginine. If cats are fed an arginine free diet they develop hyperammonaemia (ammonia toxicity). The reason for this need for dietary arginine is that in the cat, pyrroline-s-carboxylate and ornithine aminotransferase (enzymes involved in its synthesis) have a very low level of activity. Therefore if cats are fed an arginine-free diet there is not enough arginine

synthesised in vivo in order to maintain the urea cycle and therefore to detoxify any ammonia, meaning levels of ammonia increase, leading to hyperammonaemia.

1.3 The Importance of Palatability

Nutrient provision and palatability of diets are intricately linked. Palatability is vital because a nutritionally complete and balanced diet is un-nutritious to the animal if they will not ingest it. For this and a number of other reasons, palatability is a very important aspect to consider when developing pet foods. In fact, palatability is one of the key attributes of commercial pet foods that determine competitive advantage (Deffenbaugh 2000). The concept of palatability is complex, but can be defined as the relative attractiveness, acceptability or preference for a particular food. Alternatively, palatability can simply mean "pleasant to the taste" (Tarttelin 1997).

The palatability of a food is a function of a range of factors, for example taste, smell, texture and temperature. It is just one of the many factors that determine the response produced by the consumption of a food. An animal's food preference may be defined by either the amount of the food eaten when the animal is offered a choice of foods, or alternatively by the reward values of different foods in a learning situation. From this it can be expected that a food that is palatable to an animal, for example a cat, will be accepted by that animal and eaten to appetite, or simply, until they are full (Hullar *et al.* 2001).

Generally foods are assigned a ranking of palatability from high (very palatable) to low (not palatable at all). Therefore, palatability varies along one continuum, so that taste may be positive or negative or neutral, but may not be both positive and negative at the same time. However, this is only one model of palatability. This model has been criticized because the measures used to support it, such as preference tests, lick rates and intake tests all use responses which also vary along a single continuum. This means that even if the brain used a different mechanism to generate its responses, the impression given from the data would still be that taste responses vary along one continuum, which would not necessarily be true (Berridge and Grill 1983). Regardless of this criticism, it still seems to be the model that is most accepted and supported by research. The second proposed model of palatability is that it reflects two distinct analyses of positive and negative aspects of the taste, therefore a taste can be perceived

as both positive and negative, to varying extents, at the same time (Van den Bos *et al.* 2000).

Because of the adoption of domestic cats and dogs as pets in homes throughout the world, the pet food industry has grown significantly. The pet food industry has in the past aimed most of its research to formulating pet diets that meet the nutritional requirements of these animals. However, it seems focusing their attention and research on aspects of palatability is equally as important, as getting the animal to consume the food is obviously the first hurdle to overcome when formulating a new commercial pet food (Bradshaw *et al.* 1996).

Palatability is not only important to pet food manufacturers and to the pet itself, but it is also important for pet owners. This makes the development of pet foods much more complex than that of human foods, where the purchaser is also usually the consumer (Hendriks 1999, Kemp 1999). Therefore, when a new pet food is developed, it must not only be nutritionally complete and balanced, and sufficiently palatable to the pet, but must also be seen to be acceptable to the owner, as it is ultimately the pet owner who will determine whether or not to repurchase the pet food in the future. This can be ensured if the food is highly palatable, so that a pet owner can see that their pet is enjoying the food they have provided, they will feel a sense of fulfilment and will be more likely to purchase that food again (Griffin 2000a).

1.4 Physiology of Taste and Smell

The domestic cat (*Felis catus*) is considered an obligate carnivore, as it is believed to have evolved on a diet consisting of animal tissues (Bennet 2002, Hendriks 1999). The closest relative of this family are the Canoidae, a family that includes the domestic dog (*Canis familiaris*). The dog is also classified as a carnivore, however, when we study its diet closely, we see the dog is more of an omnivorous carnivore (Hendriks 1999, Schanus 2000). The dog can ingest a far more varied diet than the cat, which has strict and numerous dietary guidelines. The differences in dentition between the dog and cat are shown in Figure 2.

In both the dog (omnivorous carnivore) and the cat (true carnivore), the canines are used to grip prey and rip and tear flesh, and the carnassials (the last upper premolar and the first lower molar) are used to slice and shear flesh into smaller pieces. The main differences in the dentition of dogs and cats, are that the dog has more premolars and

molars than the cat and dogs have 42 permanent teeth, whereas cats only have 30 permanent teeth. The eating patterns of these two species are also quite different, with the cat typically consuming small frequent meals throughout the day and night, in fact between 12 and 20 per day, if able to eat *ad libitum* (Bennet 2002, MacDonald *et al.* 1984). The dog on the other hand usually consumes two to three larger meals each day (Bennet 2002).

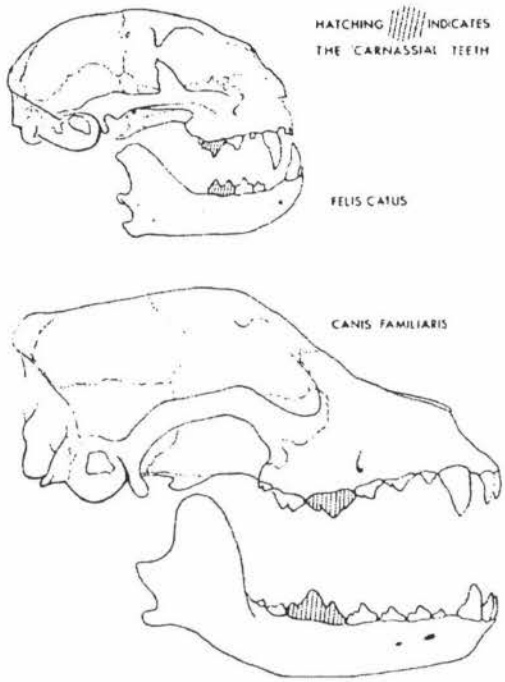

Figure 2: The skulls of the dog and cat (Boudreau and White 1978).

The areas of the body involved in palatability include the brain, central nervous system, mouth, nose and eyes. The major senses involved in palatability are gustation (taste) and olfaction (smell). Both cats and dogs use smell to detect and select food. In fact, cats and dogs rely heavily on olfaction to provide information about the objects and foods around them (O'Malley 1998). This sense is far more sensitive and therefore more relied on in these animals than it is in humans. However, despite its importance in these animals, it is the least well understood of their senses (Thorne 1998). Generally food is identified first by smell and then, provided the odour is acceptable, by taste, through a flick of the tongue (Kare and Mattes 1990).

Olfactory Physiology

Smell (olfaction) is one of the most ancient senses. However, there is far more published information on the taste systems of dogs and cats than there is on olfaction (Bradshaw 1991). The cat has a much more highly developed olfactory system than humans, but it is less developed than the dogs (Bennett 2002). The reason for this relates to differences in the number of olfactory cells and areas of olfactory epithelium between the three species, which are outlined below in Table 1. The relative surface area of the olfactory epithelium in these three species also illustrates the importance of smell to each of the species, with humans having 3-4 cm^2, cats 21 cm^2 and medium sized dogs 75 cm^2 surface area. Therefore, dogs and to a lesser extent, cats, rely on smell much more than humans do.

Table 1: Differences in olfactory physiology between the cat, dog and human (LeMagnen 1951).

Species	Olfactive Epithelium Area (per cm^2)	Olfactory Cells (x 10^6 per cm^2)
Human	10	10
Cat	21	67
Dog	200	200

Olfaction is considered a distance perception and is a function of the brain that is mediated by the olfactory neurons in the brain. Mammals have two olfactory organs; the main olfactory epithelium and the vomeronasal organ (Keverne 1999). The main olfactory system recognises most odours, and sends signals to higher sensory centres in the cortex. It allows the animal to recognise and respond to odourants. The vomeronasal organ (Jacobson organ) on the other hand detects pheromones, transmits this info by a different set of odorant receptors and neuronal projections are sent to different parts of the brain (Klein 2000). In the cat and dog this organ is an elongated and cartilaginous strip in the base of the nose. The vomeronasal organ consists of a pair of fluid filled sacs. It is thought that chemical stimuli are transferred to this organ through a pumping mechanism, with fluid expelled from the sacs into the canal and then drawn back into the organ carrying the chemical signals (Keverne 1999). The mucous membrane of the vomeronasal organ can catch volatile molecules and also non-volatile compounds, for example steroids (Bennet 2002). The vomeronasal organ therefore contributes to what the animal smells and also to the complexity of flavours of the ingested food (Boudreau

and Tsuchitani 1973). In contrast to the main olfactory system, the vomeronasal organ has a relatively small number of families of receptor genes (Keverne 1999).

Odours or olfactory stimuli consist of a large range of volatile molecules of different sizes and compositions (Kare and Mattes 1990), but all have some water solubility and lipid solubility, a low polarity and surface activity. In contrast to the limited tastes detected, the mammalian olfactory systems can distinguish hundreds, even thousands of different molecules (Klein 2000).

Odours impinging on the nasal mucosa are sensed by the olfactory neurons and stimulate olfactory receptors. There are millions of olfactory neurons in the olfactory epithelium and each receptor is likely to be capable of interacting with many odorants, which must be recognized by multiple receptors (Klein 2000). Odours enter the nostrils during inhalation and then travel from the back of the nasopharynx towards the roof of the nasal cavity where the odour excites the neuron (Mennella and Beauchamp 1998). The outer part of the olfactory neuron is chemo sensitive and its central process passes to the olfactory bulb, located in the animal's central nervous system (Lien *et al.* 1999). The anatomy of the olfactory bulb in dogs and cats can be regarded as one of the main contributing factors for their highly developed olfactory sense, with thousands of axons leaving the olfactory epithelium to connect with the olfactory glomerulae. This form of connection greatly amplifies signals coming in. These messages are then sent directly to higher levels of the central nervous system, such as the higher cortex and limbic system, where the signalling process is then decoded and a response occurs (Leffingwell 2002). Second order neurons (located in the olfactory bulb), pass to various areas of the cerebral hemisphere, including the amygdala, piriform cortex and entorrhinal cortex (Lien *et al.* 1999). There are a number of sensory areas in the cerebral cortex involved in smell, with the major (primary) olfactory area being the temporal lobe of the medial aspect (Sucan 2001). In the orbitofrontal cortex, there are the secondary and tertiary olfactory cortical areas (Rolls 1996). These areas are important for the identification and discrimination of the odour. These areas of the brain used in olfaction act as relay stations and integrate the sensory information provided and then initiate the appropriate response.

The sense of smell may be used in two ways; either on its own, or together with the sense of taste, providing the animal with a full picture of the flavour of the food (Thorne 1998). It is believed that if cats find the odour of one food is more attractive than that of the other food; they will eat it exclusively without even tasting the other less

attractive smelling food (Hullar *et al.* 2001). However, if neither of the two diets are very attractive smelling the animal will also taste the foods and then make a decision as to which one to eat based on both senses; smell and taste (Hullar *et al.* 2001). The dog is also attracted to the food that smells better initially although this attraction does not last and unless odour is paired with taste, then the food preference is not sustained. Therefore, Houpt and Smith (1981) hypothesised that smell influences more subtle food preferences, for example one type of meat over another.

Gustatory Physiology

Taste, unlike smell, is a contact sense and it includes receptors, the nervous system for the collection and transmission of information and the central nervous system for the analysis of the collected information (Boudreau and White 1978). The sense of taste in cats and dogs is also very different from humans; this can be seen by comparing the numbers of taste buds in these three species. The cat has approximately 473 taste buds, the dog has 1706 and humans have 9000 (Leibetseder 1978). Taste is especially important to the cat because they are sensitive enough to notice even the smallest changes in the food offered to them (Tortora and Grabowski 1996). This difference reflects the difference in the diets of humans and their animals. Certain tastes in humans are much more developed than in cats and dogs and other tastes are much less sensitive in humans (O'Malley 1998).

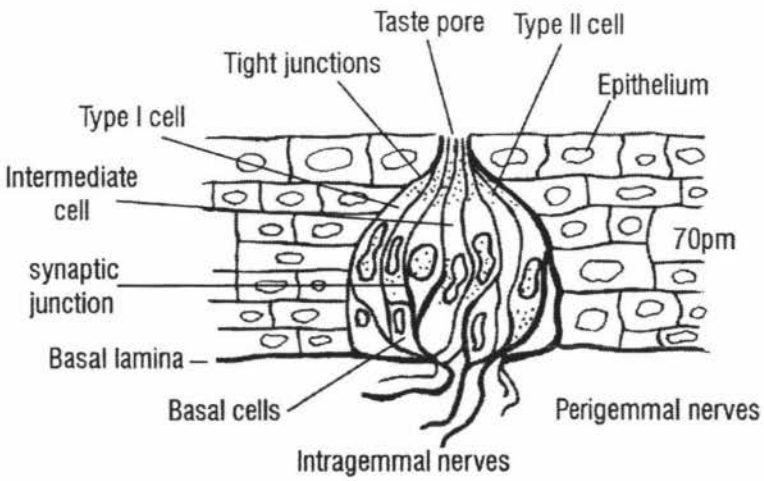

Figure 3: A taste bud (Klein 2000).

Taste buds are a globe like shape containing a collection of taste receptor cells which are long and spindle shaped, and a taste pore (Figure 3). The taste pore is lined

with cilia, where the tastants enter the taste bud and interact with taste receptors (Klein 2000). Taste buds consist of three types of epithelial cells; the supporting cells, the gustatory receptor cells and the basal cells (Tortora and Grabowski 1996). The majority of the taste buds in the cat and dog are small circular structures, which are grouped on fungiform papillae on the tongue's upper surface and also in the vallate papillae at the back of the tongue (Pfaffmann 1964). In the central region of the tongue are the filiform papillae, which apparently do not contain taste buds. On the back of the tongue, there are three types of papillae; vallate papillae, circumvallate papillae and foliate papillae. The taste buds are usually on the sides of these papillae in large numbers. The foliate papillae are found at the back lateral margin of the tongue, but may be missing on one or both sides of the cat tongue. The fungiform papillae are located on the front of the tongue. They are generally mushroom shaped but their sizes and shapes do depend on their exact location. The taste buds are found on the dorsal surface of the fungiform papillae and each fungiform papillae usually contains more than one taste bud (Boudreau and Tsuchitani 1973).

The taste buds transmit taste information to neurons using chemical transmitters that are secreted by the taste cells. These are then detected by neurons which react with an impulse that is then transmitted to the brain (Kinnamon *et al.* 1988). Three nerves transmit the taste information to the brain; the chorda tympani, the glossopharyngeal and the vagus nerve (Klein 2000).

Taste (gustation) involves the stimulation of gustatory receptors in the taste cells in the oral cavity by a stimulus (food). These receptors are sensitive to different ions and molecules, and receptors in certain parts of the tongue are known to react more strongly than others to the five different tastes (Pfaffmann 1964). These five tastes are sweet, sour, salty, bitter and umami (Kare and Mattes 1990). Tastants must be in solution in order to be perceived. There are two different types of receptors in the oral cavity; free nerve endings and taste buds. Stimulation of either of these two types of receptors activates neural fibers that lead to the central nervous system, where different areas of the brain again act as relay stations, integrating information and initiating the appropriate response to the stimuli (Teff 1996). The orbitofrontal cortex is the secondary taste cortex, where the reward value of taste is represented. It has a similar function in olfaction (Rolls 1996).

Taste is a unique sense in that unlike most senses, which are supplied with a single cranial nerve from the receptor to the brain; it has a multiple nerve supply

(Pfaffmann 1964). There are four cranial nerves that supply taste, specifically; the glossopharyngeal nerve, vagus nerve, trigeminal nerve and facial nerve. However, only the facial nerve has been studied in detail in the cat and dog, the roles of the other three are yet to be ascertained (Bradshaw 1991).

The areas of the brain involved in taste sensation include the tractus solitarius and the orbitofrontal cortex. The nucleus of the tractus solitarius is the primary site that receives information about taste. The secondary taste cortex is in the caudolateral part of the orbitofrontal cortex (Rolls 1996). This cortex receives its major projection from the primary taste cortex. The secondary cortex taste neurons are modulated by hunger and stop responding to the taste of a food with which the animal is fed to satiety. Therefore, the reward value of taste is represented here. However, the primary taste cortex is not modulated by hunger and therefore the identity of the taste and not the reward value of the taste is represented in this area (Rolls 1996).

1.5 Relationship between Taste, Smell and Palatability

The sense of taste is similar in the cat and dog, however, there are some important differences, especially in the spectra of compounds to which the different units respond (Bradshaw 1991). The most plentiful taste units on the cats' and dogs' tongues are those that are very sensitive to the amino acids that humans describe as having a 'sweet' taste (Thorne 1998). This is believed to be because of these animals' carnivorous dietary habits throughout evolution, particularly in the cat, which is one of the most committed animal tissue eaters of all the known carnivores (Stein 2001).

Despite the fact that the sensory qualities of foods are very important in terms of the sale of pet foods, the knowledge about taste and smell is relatively undeveloped (Kare and Mattes 1990). It is sometimes assumed animals, such as the cat and dog, share our taste preferences but in actual fact all species live in their own sensory world and, therefore, these worlds may or may not overlap with ours (Kare and Mattes 1990). In fact there are even within species differences in food preferences which are crucial, as there would be extreme competition for food if all individuals had the same taste and smell (Kare and Mattes 1990).

It is still unknown as to what specific compounds or ingredients in a palatable food are responsible for making it palatable or whether it is the combination of a few ingredients. This area is of great interest because if the compounds which different

animals find palatable could be determined, it may be possible to use these to make less palatable foods palatable.

1.6 Preferences of Cats and Dogs

Various compounds have been found to be palatable or not palatable to different species. However, the obvious focus here is palatability to cats and to a lesser extent dogs. The various food preferences of cats and dogs that have been identified and published have been based entirely on research results from laboratory cats and dogs. However, this information may be of limited value because pet cats and dogs live in a far more varied environment than laboratory animals.

Cats obviously prefer ingredients of an animal origin, especially proteins and fats (Bennet 2002). They prefer freshly killed prey to hung carrion and prefer their diet to be in a pellet form rather than a powder form (Bennet 2002). It has been suggested cats prefer fish, especially salmon. However, this seems to vary between individual animals (Houpt and Smith 1981) and therefore, as with all statements about food preferences, this is merely a generalisation (Bennet 2002). Dogs prefer cooked meat to raw, canned cooked meat to freshly cooked meat, ground meat to chunks, and moist foods to dry foods (Houpt and Hintz 1978). The latter authors also reported that dogs prefer beef and pork to lamb and horsemeat, lamb and chicken to horsemeat and have a preference for sweet and fatty foods. However, despite all these reported preferences for cats and dogs, realistically there are very few odours, tastes and flavours which can be defined as inherently palatable or unpalatable to these species, as every animal and every situation is different (Houpt *et al.* 1988).

In both cats and dogs the amino acid taste system dominates. The amino acid system is a group of chemosensory neurons that respond to amino acids rather than to other compounds such as sugars. In the brain there are many different systems, or groups of neurons that respond to different groups of chemical compounds and in different species different systems will be dominant, depending mainly on the species diet. This system in the cat and dog is most sensitive to the amino acids described as 'sweet' in man; these include L-proline, L-cysteine, L-ornithine, L-lysine, L-histidine and L-alanine.

In cats there are three different groups of receptor units and each responds to different compounds. The first set responds to acids, the second to acids and salts and

the third set to acids and quinine (Pfaffmann 1964). These chemosensory neural groups in the cat are outlined in Table 2, and those for the dog are outlined in Table 3. In the cat, of the three cranial nerves that are known to convey information about taste, only the taste component of the facial nerve, the chorda tympani, has been studied in great detail. In this nerve, the amino acid system is predominant as expected and as mentioned, it is most sensitive to amino acids described as being 'sweet' in humans. In the cat, these amino acid units are inhibited by bitter amino acids such as L-tryptophan, L-isoleucine, L-arginine and L-phenylalanine (Bradshaw *et al.* 1996).

Table 2: Chemosensory neural groups in the geniculate ganglion of the cat (Boudreau and White 1978).

Group	Spontaneous activity	Latency to electrical stimulation	Responds to		Inhibited by
I	Low	Short	-Malic acid, NaH_2PO_4 -Di & tri phosphates -L-his, L-tau -Anserine, Carnosine -Thiamin, pyridine & imidazole compounds		-NaCl -KCl
II	High	Medium	-Di & tri phosphate -Nucleotides, Inorganic salts -L-pro, L-cys, L-lys, L-his, L-tau -$CaCl_2$, NaH_2PO_4 -Thiamin & imidazole compounds -L-tryp, L-isoleu		-Bases -Polyamines -Creatine -Creatinine
III	Low	Long	Subgroup A -Di & tri phosphates compounds -Nucleotides -NaH_2PO_4	Subgroup B -O_2	

It has been discovered that cats can not taste sweet sugars such as sucrose (Stein 2001). Also in a study conducted by Houpt and Smith in 1981, it was concluded that live prey is apparently the lowest food in the cat's taste hierarchy. This conclusion was made by these researchers because they found in their studies that cats preferred to eat cat food over a rat they had killed, and preferred to eat a cold dead rat over a freshly killed rat (Houpt and Smith 1981). In cats sodium appetite is not well developed either.

The dog also has a low sensitivity to sodium chloride (salt) and it is believed this low sensitivity to sodium chloride in these two species is related to the relatively high levels of sodium in much of their food, especially in the animal flesh they consume (Bradshaw 1991).

Table 3: Chemosensory neural groups in the geniculate ganglion of the dog (Boudreau and White 1978).

Group	Responds to
A	-L-pro, L-cys, ITP, ATP, IDP, NaCl
B	-L-malic acid, Quinine hydrochloride, ATP, HCl
C	-Nucleotides
D	-Phytic acid, Quinine hydrochloride, Butyryl choline chloride

On the other hand the tastes cats seem to prefer are associated with protein hydrolysates, meat extracts and certain free amino acids. Animal fats also seem to increase the level of palatability of a food, mainly because they affect the diet's texture (MacDonald *et al.* 1984). It has been noted that domestic cats generally prefer novel diets to familiar ones and generally prefer fish to commercial foods (beef, liver, chicken, etc) and commercial foods to prey such as rats (Houpt and Smith 1981). Cats and dogs are also believed to have a water taste (Bennet 2002, Bradshaw *et al.* 1996).

The dog's units are quite similar to the cat's, with both being highly responsive to amino acids. However, there is one important difference, this being that dogs are very responsive to a wide range of sugars, including mono- and disaccharides (Bradshaw 1991). The retention of these sweet receptors unlike in the cat is believed to be due to the fact that the dog consumes a more varied diet than the cat that includes plants as well as animals. The units that respond to sugars are more common in the glossopharyngeal nerve than in the other nerves involved in taste. Also in the dog, the amino acids that are inhibitory in the cat tend to be neutral or stimulating in the dog. Canine taste systems are also sensitive to umami substances, as are feline taste systems (Kurihara and Kashiwayanagi 2000). In the dog, there are four classes of neurons in the geniculate ganglion (A/B/C/D), two of these are similar to two groups in the cat, more specifically dog class A is similar to cat group two and class B is similar to cat group one.

It has also been discovered that heat processing in cat and dog diets can decrease the palatability of these foods; this may be due to the effect of this processing on the

protein part of the diet (Hendriks 1999). The texture of the food has also been identified as a factor that affects the palatability of cat and dog foods, with semi-moist food being most palatable for dogs and canned food for cats (Fazzina 1978).

Even with the knowledge of the ingredients and compounds that are more or less palatable to the domestic cat and dog, some further obstacles need to be overcome. Firstly by increasing the palatability of a food, generally the energy content of the food is also increased which may subsequently increase the risk of obesity in animals fed this diet, especially in certain breeds of dogs, as some are known to be obesity prone (Derua *et al.* 1999). Some animals are better at regulating their feed intake to meet their caloric needs than others, for example, in general the cat is better at this than the dog. Between breeds and within breeds of dogs there is a large variation in the efficiency with which feed intake is regulated (Kare and Mattes 1990). For example, Beagles have been shown to overeat by a factor of two to five (Mugford 1977). Support for the idea that cats can regulate their energy intake has been provided by Bradshaw and associates, who found in 1996, that domestic cats given purified and commercial diets, both of a good palatability, but different caloric contents, adjusted their intakes quickly, so as to maintain a nearly constant caloric intake (Bradshaw *et al.* 1996). Bradshaw and associates also found that when cats were fed diets containing different moisture contents, they maintained a nearly constant caloric intake despite the obvious differences in the composition of the diet (Bradshaw *et al.* 1996). MacDonald and colleagues also observed this ability in cats during their trials in 1984, when they fed cats two highly palatable diets with different caloric contents. The cats also adjusted the amounts they ate of each diet and maintained a relatively constant caloric intake (MacDonald *et al.* 1984). It is therefore believed that the ability of cats to maintain bodyweight may be why they have exhibited fewer problems with obesity in the past, compared with dogs, even when a very palatable diet is offered to them. However, it should be noted that not all cats manage to maintain this balance and therefore there are still overweight and even grossly obese cats. In fact obesity in cats is becoming a growing problem worldwide.

1.7 Intrinsic and Extrinsic Factors Affecting Palatability

Numerous factors other than the senses of taste and smell also contribute to the perceived palatability of a food or ingredient. These factors can be divided into two distinct categories; namely intrinsic factors and extrinsic factors.

Intrinsic Factors

Obviously as mentioned earlier, the animal's sense of taste and smell are examples of intrinsic factors that influence palatability. The individual species, breed, sex and age of the animal also are intrinsic factors. The inclusion of breed as an example of an intrinsic factor affecting palatability is somewhat controversial. It is believed that different breeds may have different food preferences. However, as concluded by Griffin (2000b), there is no known account in the literature that displays breed-specific differences in food preferences. Griffin and associates analysed for breed differences in 1984, but found no significant breed effect. The only account reported was of miniature breeds having different food preferences to all other breeds (Griffin 2000b). However, despite research showing there is in fact no breed effect on palatability, the pet food industry as a whole, is very reluctant to accept this as a possibility (Griffin 2000b).

In terms of sex being an intrinsic factor which affects palatability, it is not only male versus female that needs to be considered, but also de-sexed versus intact individuals. Age is also a very important intrinsic factor, as flavour perception changes with age and it has been shown that taste and smell can become less sensitive with increasing age (Rawson 2002). Generally animals, in particular cats, that are less than one year of age will eat nearly anything offered to them while cats over about five years of age are fussy and can't make decisions concerning whether or not they like the food in front of them. The animal's health is also an intrinsic factor as ill animals may exhibit a decreased ability to appreciate odour and taste, especially if the illness affects their nasal passage (Edney 1973).

The animal's appetite or level of hunger at the time of the food presentation also affects palatability. This is obviously an important factor to consider as cats that are hungry at the time of feeding will be trying to 'fill their gut' rather than to choose the most palatable food on offer, resulting in the relative intake of the two products being driven towards equality (Griffin 2000b). When cats are satiated at the time of feeding, the test outcome may also be driven towards equality, as they may ignore both of the

foods on offer, simply because they are already full, resulting in there being insufficient consumption data to be able to determine preferences reliably (Griffin 2000b). Other intrinsic factors are dietary aversions, the ingestive and general behaviour of the animal and past experience with food or the inborn tendencies (genetics) of the animal. The previous diet of the animals can affect the way the animal perceives palatability in a number of ways. For example, if an animal has had a bad experience in the past from a specific food then that animal may avoid any change in their diet at all. On the other hand, animals that have previously had experience with a wide range of foods are less likely to reject any new foods and more willing to accept changes in their diets (O'Malley 1998).

Previous food experience can have two contradictory effects on food selection; the novelty effect and the primacy effect. A number of studies have been conducted looking at these effects and the results are conflicting, with some support for each of the two effect. The primacy effect (neophobia) can be defined as the tendency to prefer the food that was first experienced in infancy. Studies carried out in 1967 by Kuo support this idea, in both cats and dogs. Kuo used chow puppies separated from their mothers at birth and hand reared. The pups were separated into three groups, with the first group receiving a diet containing soybeans, the second group a mixture of fruits and vegetables and the third group a large assortment of foods. Kuo found that most dogs in group one and two refused any new food offered to them, while in the third group, all the animals ate any new food offered to them, thus supporting the primacy effect. Kuo also carried out a similar experiment using newborn Chinese cats. These cats were also split into three groups and each group was fed different diets. The first group received soybeans, the second mackerel with rice while the third group received an assortment of different foods. The results produced were very similar to those from the chow puppy experiment. Any new food offered to groups one and two was refused by the majority of the cats in these groups, however, the cats in group three consumed any new food offered to them without hesitation. These results again support the primacy effect.

The novelty effect (neophilia) is the tendency of an animal to prefer novel foods. Studies conducted in 1977 by Mugford, support this effect. Mugford conducted various studies using dogs and cats in a way similar to Kuo's experiments in 1967, except that the animals were introduced to fixed diets at weaning not at birth. The results produced showed that the dogs and cats never developed a preference for their rearing diet and also that generally the dogs and cats used showed a preference consistently for the novel

diets offered. Hegsted and associates in 1956 and Waterhouse and Fritsch in 1967, found similar results to Mugford.

It has also been suggested that dogs and cats may like eating new foods unless they are in a new environment or are exposed to a stressful situation (Kare and Mattes 1990). There has been research carried out which supports this idea, for example a study was carried out where it was discovered that the preference of a group of cats for a familiar diet decreased with repeated exposure but testing the diet in a new environment led to a total rejection of the newer food, in favour of the familiar food (Thorne 1998). The cats were later returned to their normal environment and once this occurred, the cats' preference returned back to the novel diet thereby suggesting that "stress" may influence palatability.

Extrinsic Factors

The extrinsic factors that affect palatability include the qualities and characteristics of the food, environmental conditions and social influences. The characteristics of the food offered that can affect palatability are bulk density, appearance (including colour, size and shape), freshness of the food, temperature, and texture of the food. Food texture refers to how the food 'feels' in the mouth, for example its chewiness, crunchiness or hardness. Texture is of singular importance to the cat as they are extremely sensitive to changes in the sensory properties of the diet. For example Hirsch and colleagues observed this in 1978 when they changed the texture of their test diet from pellets to powder. It has also been shown that cats adapt easier to foods of semi-moist textures compared to sludgy, hard or dry textures (Sohail 1983). This was seen by Kane and associates in 1981 when they fed purified diets containing 10%, 25% and 50% yellow grease to two groups of cats. The cats preferred ($P<0.001$) the diet containing 25% yellow grease. The authors suggested the reason for this result was that the 10% yellow grease diet was quite powdery in texture and the 50% yellow grease diet was very greasy and, therefore, the cats obviously preferred a diet in between these two extremes (Kane *et al.* 1981).

Generally, in terms of shape and size of the diet, smooth edges and smaller size pieces are preferred. Shape, size and texture of the food are all very important, especially in the cat because they have no molars and therefore sharp edges can hurt their mouths and large un-wetted pieces can also hurt their stomachs, causing them to vomit (Schanus 2000). Freshly prepared food is obviously best to use and food offered

at room temperature or above is generally more accepted than that taken directly from the fridge or freezer. In fact food served cold or even sometimes at room temperature may be refused (Bradshaw *et al.* 1996). Other factors are the foods moisture content, ingredients, diet formulation and nutrient balance, form of the food and the methods of processing. Ingredient choice can be vital as low quality ingredients may be cheaper but may also put a negative palatability factor into the food (Hutton 2000).

The environmental extrinsic factors include the availability of water, presence of other animals, immediate surroundings, time of year and various other social influences (Lien *et al.* 1999). The aspects of the animals' immediate surroundings that may affect palatability are levels of light, sound, place of feeding, the presence of staff, whether the food is presented in a dish or on the floor and how clean the food dishes are.

1.8 Palatability Testing

The objectives of palatability testing can be to determine, the acceptability of a new diet to the animal, the effects of a new ingredient on pet food products or the status of a product relative to its nearest competitor. Other objectives of palatability testing may be to substantiate a marketing claim or to determine if an improvement in palatability can be seen by the purchasing consumer, the pet owner. It is also useful to use palatability tests to determine the acceptability of cheaper diets to pets, as ultimately the pet food manufacturer's goal is to prepare the cheapest diet possible that meets the requirements of being nutritionally balanced and highly palatable. Palatability is also used to compare diets prepared using ingredients from different suppliers to identify differences in the palatability of each supplier's ingredients, and to identify the best supplier to do business with.

Various methods have been proposed and used to test the palatability of foods and ingredients. However, there has been no general published protocol for palatability testing, and very little information is available on this topic for cats and dogs (Derua *et al.* 1999). It has been proposed that the industry needs to adopt common methods and analyses to remove some of the ambiguity from the test conclusions (Griffin 2000b). However, whether this will ever occur seems to be highly unlikely as this would require pet food manufacturers to agree on one protocol. The methods that have been implemented in the past can be divided into two categories; non-consumption tests and consumption tests. The non-consumption tests that have been used include instrumental

responses, for example Skinner's operant tests, and autonomic responses, for example Pavlov's salivation experiment. Skinner's operant conditioning methods involved training dogs to push a bar with their paws in order to get a small amount of food and then later teaching them to push two bars for two different foods. In this method dogs must make a choice between the two foods on offer every time they press a bar. It has the advantage of allowing several foods to be tested in one day (Houpt *et al.* 1978). No palatability data on the use of this method in cats seems to exist.

The consumption tests that have been used are a) number of licks or visits to the bowls, in which the number of times the animal licks the food or visits the bowl in the test period is recorded; b) rate of eating, where the amount of food consumed in a specified unit of time is measured; c) crossover trials, where one diet is tested for a certain number of days on some animals and the amount eaten each day recorded and then the next diet is tested using the same method on the same animals and d) the two bowl free choice preference test, in which two diets are offered simultaneously in bowls placed side by side and the amount of each eaten in the test period by each animal is recorded. The latter method is the most commonly used in the pet food industry (Griffin 2000b, Hendriks 1999, Sucan 2001).

The two bowl free choice method involves the researcher deciding on an appropriate number of animals to use of a certain gender, breed, age and size. The animals are then acclimatised to the conditions of the experiment and familiarized with the test procedure using a preliminary test (Bradshaw *et al.* 2000). The animals are usually deprived of food for approximately two to four hours before the testing is scheduled to begin. This food deprivation ensures the animals are not full but are not starving either (Berridge and Grill 1983). The animals, whether they are cats or dogs are then presented with two bowls placed side by side, each containing equal amounts of food, that is equivalent to one day's intake. One diet is put in one bowl and the other diet is put in the other bowl. The positioning of the bowls in relation to the other, i.e. whether it is on the left hand or right hand side is decided at random to remove the effects of side bias. This positioning is alternated across different test periods.

The animals are then allowed a certain amount of time with the food, for example usually between 10 and 60 min. for dogs and 1 to 23 hours for cats, or alternatively until the food from one bowl has all been eaten. It has been recommended that the food is presented at or around the time the animal is normally fed. After the test period, the two bowls are removed and the remaining food, if any, is weighed. If less

than five grams has been eaten from both bowls, the test must be recorded as a refusal of both foods (Bradshaw *et al.* 2000). After this initial test the animals are fed a maintenance (control) diet. The two bowl free choice test has the advantages that many different diet types can be tested and it is relatively inexpensive and simple to run. Examples of two bowl preference tests have been published by Tarttelin (1997) and Waterhouse and Fritsch (1967).

After the results of the two bowl palatability test are recorded, they are analysed and various statistics are calculated. There are a number of ways in which the results can be presented. The data can be noted as the first choice diet (the one that has been eaten the most), as the consumption ratio (diet A/diet B), the percentage of each diet consumed, the average consumption ratio, as the comparative preference ratio (the larger preference divided by the smaller), and as the individual animal intake ratio (diet A/(diet A+diet B)). These methods of presenting the data should be evaluated in terms of objectivity, validity, reliability of measure to measurement sensitivity, freedom from extraneous biases, the extent to which each test subject has been weighted equally, their independence for nutritional and physiological effects and finally how amenable the measure is to statistical analyses (Griffin 1996). The individual animal intake ratio (relative consumption) is the only measure that meets all seven criteria (Griffin 1996). Despite this fact, the industry seems to prefer the diet A/diet B (consumption ratio) for expressing the data of two bowl palatability tests.

When designing and conducting a palatability trial, various factors need to be considered, including the animal's level of hunger because the level of hunger at the start of the trial will determine whether the animal will be eating to 'fill the gut' or to choose the most palatable food. On the other hand, if the animals are satiated at the start of the trial the test outcome may also lead to indifference as the animals may refuse both foods because they are already full (Griffin 2000b). The number of animals and the general characteristics of the animals to be used, for example their age, breed, origin, sex and live weight also need to be considered (Van den Bos *et al.* 2000). Generally the number of animals used is determined by economics, rather than by rationale. The number of test days and the test duration must also be decided on. Generally the more days the better, as with increasing days, comes increasing stability in the results. In the past test durations of 1 to 23 hours have been used for cats and 10 to 60 min. for dogs. Further factors to consider are the level of stress of the animals, previous housing and previous feeding history and the health of the test animals. The time interval between

tests, the time of the tests and the amount of food to place in each test bowl are also important factors to consider. The researcher must also ensure the diets used are nutritionally balanced and must decide on an appropriate control diet.

In general, the available time and resources often dictate the length of the palatability trial. Usually between one and eight test periods are used, but it has been noted that two test periods are as good as or better than three and requires less resources (Griffin 1996). As mentioned earlier for dogs, the test duration is usually 10 to 60 min. and for cats usually 1 to 23 hours. The reason for this difference lies in the fact that these two species have very different feeding patterns, as mentioned earlier (Griffin 1996). Certain environmental aspects must also be looked into and decided on such as, sound, temperature, lighting, water access, housing conditions and the location of the bowls in the test area (Ferrell 1984). Other aspects regarding the food and feeding also need to be considered, including diet preparation, cost, the storage temperature of the food and whether the food is to be fed in dishes or directly on the floor.

An example of a palatability test that has been carried out, is that run by a leading pet food manufacturer (Stasiak 2001). The aim of this trial was to determine if cats had a preference for tuna or beef, and if the level of variety in the diet early in life affected this preference. This trial started two hours after feeding with each test lasting three min. and being run in the cat's home cage. Each cat was offered two bowls; one bowl for each cat containing three portions (2.5 g each) of food B and the other bowl for each cat containing three portions of food T. The total amount of each food (food B and food T) eaten by the ten cats collectively was calculated. The results showed that 22 portions of beef were eaten and 25 portions of tuna, out of a possible 30 portions for each diet. The authors concluded from this test that there was no preference to beef or tuna in cats non-deprived of taste early in life (Stasiak 2001). A second example of a two bowl free choice palatability test carried out, was in Japan at Nihon University. The aim of this study was to observe how moistening pet food affected the palatability of the food to the pet. This trial used five male and five female Beagle dogs aged two to five years and weighing 8 to 14 kgs. The trial was run for 11 days in mid summer and it examined the preferences of the Beagles for different foods and for food that had been moistened over that that had not. The two foods (A and B) were placed in the two bowls and the dogs were then allowed free access to the foods for three min. After this time the amount of food left in each bowl was measured to determine how much of each diet had been consumed. The second part of the trial used diets A and C, which were

moistened with water for 10 min. before being offered. The trial was carried out identically to the first and the amounts of each diet eaten recorded. It was found in this experiment that dogs prefer dry food that has been moistened with water (Miyahara *et al.* 1995). A third example of a two bowl free choice palatability test was published by Tarttelin in 1997. This test used two groups of eight cats at a time. The cats had been trained to occupy an individual cage for two hours a day for five days of the week and to feed from small bowls without mess. Any lateral bias was removed from the test by alternating the positions of the bowls each day. The tests took place in an isolated room and each week four pairs of products could be tested. After the two hour study period, the cats were returned to their large colony cages and fed to appetite on a variety of canned and dry food, proven to be nutritionally complete. The test was then analysed using the weight of food consumed by the cats. Ultimately a method for measuring palatability should produce results that are objective, valid and reliable, free from bias, have independent nutritional and physiological effect and be equally representative of all the animals on the test. To date, it appears from the literature that the two bowl free choice method is the method proposed that best meets these requirements.

In conjunction with the two bowl preference tests, sometimes further tests are conducted using a panel of consumers; household cats, to confirm the results from the palatability tests. In these panel tests, the owners of the cats are asked to feed their animals a series of plain pack foods and then observe how much of each is eaten. These tests typically run for approximately one week and then a control diet is fed for a further week before the next panel test is run. The results from this test can then be compared with the more controlled two bowl preference tests (or other tests) to decide on each diet's palatability (Tarttelin 1997).

1.9 References

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Chapter 2

The palatability of fish by-products for domestic cats *(Felis catus)* when included in synthetic diets

2.1 Introduction

Palatability is a very complex area of animal nutrition, especially for the cat; a species notorious for their fussy eating habits (Bradshaw *et al.* 1996). Cats are actually so sensitive to slight changes in their food that they will refuse a food almost up to the point of starvation if they do not find it sufficiently palatable. Palatability tests are a useful tool in determining what compounds or food ingredients improve food intakes amongst cats.

The use of synthetic diets in palatability trials is topical. If a synthetic diet that is nutritionally complete and sufficiently palatable can be formulated, then it can be used as a base diet to study fundamental aspects of palatability. The majority of palatability trials have used commercially made canned and dry foods (Bradshaw *et al.* 2000, Hullar *et al.* 2001, Van den Bos *et al.* 2000). These types of diets are more difficult to manipulate and introduce the added complication of the effects of processing on palatability, making it difficult to study fundamental aspects. A synthetic diet, however, can be easily manipulated to control specific properties of the diet, such as levels of fat or energy, simply by adjusting quantities of certain ingredients.

Some animal by-products particularly fish offals are known to enhance the palatability of petfoods (MacDonald *et al.* 1984). Fish by-products, if palatable, offer a cheap source of protein for commercially made foods. However, this type of testing has yet to be carried out on New Zealand fish species. If a highly palatable fish by-product can be found that is native to New Zealand waters then this may lead to the development of a potentially unique product for the New Zealand industry.

There were two aims of the study; the first of which was to determine the relative palatability of selected fish by-products using the domestic cat as the test species. The by-products were added to a synthetic diet and compared to the un-supplemented synthetic diet (control diet). The second aim was to investigate the efficacy of using synthetic diets. The diets were made as palatable as possible, such that food intakes would be sufficient to maintain good health.

2.2 Materials and Methods

All animal procedures described in the study were approved by the Massey University Animal Ethics Committee (Anonymous 2003).

The fish by-products:

Seven fish by-products (salmon *Oncorhynchus tshawytscha*, mussel *Perna canaliculus*, jack mackerel *Trachurus novaezelandiae*, hoki *Macruronus novaezealandiae*, squid *Nototodarus sloanii*, ling *Geypterus blacodes*, and barracouta *Thyrsityes atun*) were sourced from Crop & Food Research, Auckland, New Zealand. They were made up of whole viscera, and were freeze dried and sterilised by gamma radiation before being used in the study.

The samples were analysed for dry matter, crude protein, total fat, amino acid content and fatty acids. Dry matter was determined using a convection oven at 105°C (AOAC 2000). Crude protein was determined using the Leco, total combustion method and crude fat was determined using a soxtec extraction (AOAC 2000). The amino acids were determined in duplicate samples. The samples were hydrolysed in 6M glass distilled HCl containing 0.1% phenol in evacuated sealed tubes for 24 hours at 110±2°C. The amino acids contained in the samples were then determined using a Waters ion-exchange HPLC system utilising post-column ninhydrin derivatisation and detection using absorbance at 570nm (440nm for proline). Cysteine and methionine were determined using the same method except that they underwent a preoxidation step in performic acid at 0°C for 16 hours to convert the acid labile cysteine and methionine to their more acid stable derivatives, cysteic acid and methionine sulphone. There was no correction made for the loss of amino acids during the acid hydrolysis. To calculate amino acid weights, free amino acid molecular weights were used. The fatty acids were analysed using the method of Sukhija and Palmquist, (1988). The fatty acids were extracted from the fat and converted to methyl esters by incubating them in methanolic HCl for 2 hours at 70°C. The methyl esters were then extracted in toluene and were then separated on a 50% cyanopropyl silicone fused silica GC column (ID 0.25mm, length 30m). A flame ionisation detector was then used to quantify the separated fatty acid methyl esters.

Of the seven "by-products", four were selected for use in the palatability trials. The selection criteria were based on the by-products availability, both in New Zealand and worldwide and their uniqueness to New Zealand. The four selected were salmon, mussel, hoki and jack mackerel.

Diet formulation:

The formulation of the synthetic diets was based on the work of Hendriks (1996). The formulation for the four test diets and control diet are outlined in Table 1. All diets contained approximately equal amounts of energy, protein and fat.

Test animals:

Eight adult, castrated male domestic short haired cats, aged three to nine years old were obtained for use from the Centre for Feline Nutrition, Massey University, Palmerston North, New Zealand, where they were housed for the duration of the study. All eight cats were in excellent health at the start of the study and ranged in bodyweight from approximately 3.36 to 4.18 kg. They all had their teeth scaled and polished before the trial and their weights were monitored on a weekly basis. The cats were kept outside in a colony cage during days one to seven of each experimental period. During days eight to fourteen of each experimental period the animals were housed in outdoor plastic metabolism cages (Hendriks *et al.* 1999). These cages were each fitted with a litter tray and water was available to the animals at all times.

Table 1: Diet formulation of the five experimental diets.

Ingredient	Control	Salmon	Hoki	Mussel	Jack Mackerel
			(%)		
Starch[1]	17.22	17.22	17.22	16.22	17.22
Cellulose[2]	0.69	0.69	0.69	0.69	0.69
Casein[3]	30.00	33.00	29.00	29.5	30.00
Vit-Min Mix[4]	0.20	0.20	0.20	0.20	0.20
Water	20.00	20.00	21.00	19.00	21.00
Tallow[5]	18.00	18.89	18.00	20.50	17.00
Soybean Oil[6]	3.89	0	3.89	3.89	3.89
Salmon[7]	2.50	10.00	0	0	0
Hoki[7]	2.50	0	10.00	0	0
Mussel[7]	2.50	0	0	10.00	0
Jack Mackerel[7]	2.50	0	0	0	10.00

[1] Primary Foods (Auckland, New Zealand)
[2] Pure Science Limited (Tawa, New Zealand)
[3] New Zealand Milk Products (Auckland, New Zealand)
[4] Unitech (Auckland, New Zealand)
[5] Tegel Foods Limited (Levin, New Zealand)
[6] Davis Trading (Palmerston North, New Zealand)
[7] Crop & Food Research (Auckland, New Zealand)

Testing methods:

The test animals were weaned off their normal canned food diet onto the synthetic control diet over a 10 week period. Initially the synthetic control diet was mixed with 30% of a commercial canned cat food, then the percentage of the canned food was slowly decreased over a period of three weeks until the cats were eating only the synthetic control diet. Once the cats were fully weaned off the canned diet, the experiment was started. For days one to seven the cats were fed the control diet and the accumulated feed intake of all eight cats for each day of this period was recorded. The control diet fed during this week was made fresh daily, fed at room temperature and presented in a pellet form.

From days 8 to 14 of the experimental period, the same eight cats were housed in individual metabolism cages. Each day during that week, each cat was offered two bowls, one containing the control diet and the other containing one of the four test diets. All of these diets were made fresh each day and were fed in a pelleted form at room temperature. Each bowl contained approximately 100 g of diet, which was deemed sufficient to last the cat one day. Each day, the two bowls were switched from the left hand side to the right hand side in order to eliminate any possible lateral bias. The two bowls were left in the cage for 24 hours, after which time they were removed, the amount eaten from each determined, leftovers discarded and bowls washed. Any spillages were collected and weighed and regarded as refusals. Fresh diet was then used the following day. This method was repeated each day for days 8 to 14 of the experimental period. The subsequent week the cats were again fed the control diet in their colony cage. This pattern was repeated until all four test diets had been tested on all eight cats. The planned order in which the diets were tested for each cat is shown in Table 2.

Table 2: Allocation of diets to cats for each test week.

Experimental	Cat							
Period	Puihi	Kaos	Rover	Hobo	Kohi	Kahn	Tama	Coppa
One	D1	D2	D4	D1	D2	D3	D4	D3
Two	D2	D4	D3	D2	D4	D1	D3	D1
Three	D3	D1	D2	D3	D1	D4	D2	D4
Four	D4	D3	D1	D4	D3	D2	D1	D2

D1=salmon, D2 =hoki, D3=mussel and D4 =jack mackerel

Data collation and statistical analyses:

Palatability was presented as the relative consumption of the test diets compared to the control diet, and was calculated as follows:

$$\text{Relative consumption} = \frac{\text{food intake (test diet)}}{\text{food intake (test diet)} + \text{food intake (control diet)}}$$

Paired t tests using the initial feed intakes from days 8 to 14 of the first experimental period were also performed to determine if the test diets were significantly different from the control diet.

2.3 Results

The seven fish by-products were analysed for dry matter, crude protein, and crude fat, the results of which are outlined in Table 3. The dry matters of the fish by-products ranged from 93.5 to 98.1, with the highest value being for salmon and the lowest for barracouta. These values were high, due to the fact that the samples had been freeze dried prior to analyses. In contrast the crude protein values were highest for barracouta and lowest for salmon, by more than two fold. Salmon had approximately seven times the amount of crude fat than mussel, which contained the lowest quantity of fat of the seven by-products.

Table 3: Dry matter, crude protein and crude fat contents of the seven fish by-product samples.

Sample	Dry Matter	Crude Protein (%)	Crude Fat
Jack Mackerel	96.8	45.3	44.4
Ling	95.8	58.5	27.9
Barracouta	93.5	65.0	18.8
Hoki	96.7	56.0	37.4
Squid	94.2	54.0	35.1
Mussel	97.7	52.5	9.1
Salmon	98.1	27.8	66.0

[1] Results are expressed on an as received basis

The results of the amino acid profile carried out on the seven by-products are shown in Table 4. Salmon had the smallest range of total amino acids, with between

0.30 and 2.59 mg while hoki had the largest range, with between 0.66 and 6.03 mg. The amino acids that were in the greatest and lowest abundance in all of the seven fish by-products analysed were glutamic acid and cysteine, respectively.

Table 4: Amino acid content of the fish by-product samples[1].

Amino Acids	Jack Mackerel	Mussel	Salmon	Hoki	Squid	Ling	Barracouta
				(g/100g)			
Aspartic Acid	3.08	4.77	1.97	4.02	4.51	4.09	4.55
Threonine	1.60	2.01	0.96	2.15	1.93	2.14	2.41
Serine	1.48	1.88	1.00	2.22	1.79	2.12	2.29
Glutamic Acid	4.40	5.64	2.59	6.03	5.40	5.56	6.23
Proline	1.65	1.58	1.21	2.12	1.98	2.80	2.36
Glycine	2.33	3.79	2.02	2.15	2.42	4.30	3.44
Alanine	2.17	2.06	1.39	3.11	2.29	3.18	3.20
Valine	1.72	2.00	1.08	2.54	2.04	2.45	2.73
Isoleucine	1.45	1.93	0.85	2.27	2.11	1.94	2.21
Leucine	2.53	2.76	1.45	3.76	3.19	3.46	3.77
Tyrosine	1.18	1.48	0.72	1.72	1.68	1.48	1.86
Phenylalanine	1.40	1.72	0.82	1.97	1.97	1.77	2.10
Histidine	0.96	0.94	0.61	1.18	1.09	1.26	1.46
Lysine	2.43	3.28	1.39	3.58	3.09	3.03	3.49
Arginine	2.37	3.30	1.47	2.99	2.96	3.25	2.80
Cysteine [2]	0.49	0.71	0.30	0.66	0.90	0.70	0.89
Methionine [2]	0.89	1.06	0.64	1.48	1.40	1.36	1.46

[1] Expressed on a dry matter basis

[2] Methionine and cysteine were determined using performic acid oxidation prior to analysis.

There was a noticeable difference between the quantities of total nitrogen and total amino acid nitrogen for the seven fish by-product samples. For all seven by-products the total nitrogen value was higher than the total amino acid nitrogen value. The total nitrogen values varied from 4.4 mg/100g for salmon up to 10.4 mg/100g for barracouta, whereas the total amino acid nitrogen values were much lower, ranging from 3.3 mg for salmon, up to 7.3 mg for barracouta. The percentage of total nitrogen made up by amino acid nitrogen varied between the seven by-products from 70.2% for barracouta to 79.8% for mussel.

Table 5: Nitrogen content of the fish by-product samples.

Sample	Total Nitrogen	Amino Acid Nitrogen[1]
	(mg/100mg)	
Jack Mackerel	7.2	5.1
Ling	9.4	7.2
Barracouta	10.4	7.3
Hoki	9.0	6.9
Squid	8.6	6.4
Mussel	8.4	6.7
Salmon	4.4	3.3

[1] Calculated using:

$$\frac{14 \times \sum (\text{N content of amino acid (mol/mol)} * \text{Quantity of amino acid (g/100g)})}{\text{Free molecular weight of amino acid (g/mol)}}$$

Fatty acid analysis was also conducted on the fish by-products, the results of which are shown in Table 6. Total fatty acids were by far the highest in salmon at approximately 32g per 100g, compared with only 9g/100g for barracouta and a mere 2g/100g in mussel by-product. The crude fat content in the fish by-products was much higher, with values ranging from approximately 9g/100g for mussel up to 66g/100g for the salmon by-product. The fatty acids that were found in the highest amounts in the fish samples were palmitic acid (C16:0) and cis-oleic acid (C18:1). The fatty acid in the lowest abundance in the samples varied greatly between the different fish types. The omega 3 fatty acid linolenic acid (C18:3) was present in all seven by-products except mussel. The levels of this fatty acid in the other six by-products ranged from 0.025g/100g in jack mackerel to 0.732g/100g in salmon.

Due to low food intakes, the cats did not satisfactorily maintain their weights during the palatability trial, with the eight test cats losing, on average, 220 g each over the initial experimental period. The entire study was therefore abandoned after the first test week and as a result the Latin square design was not completed, resulting in a sample size of only two. Two cats had low feed intakes and consequently did not meet their daily energy requirements throughout the trial, ranging from approximately 7 g per day to 46 g per day, which explains the observed weight losses in these cats of between 286 g and 588 g. These two cats were both being fed the diet containing the mussel by-product. One cat had a large drop in feed intake and subsequently a sudden and rapid weight loss. Initially this cat was easily meeting its required daily energy intake and consuming approximately 90g per day but this decreased rapidly to 20 g, prior to the cat

being removed from the study and the trial being stopped. This cat was on the jack mackerel diet. The other cat on the jack mackerel diet did extremely well, eating enough to meet its daily requirement for energy for the entire week. The cats being fed the hoki diet and salmon diet met their daily energy requirement some of the week, with the cats being offered the hoki diet consuming mainly the control diet, and the cats on the salmon diet predominantly eating the salmon diet. However, despite these intakes seeming reasonable overall, these cats were still not satisfactorily maintaining their bodyweights.

Table 6: Fatty acid content of the seven fish by-product samples.

Fatty Acids	Barracouta	Hoki	Jack Mackerel	Ling	Mussel	Salmon	Squid
				(g/100g)			
C 8:0 caprylic	0.07	0.06	0.07	0.05	0.03	0.07	0.06
C 10:0 capric	0.06	0.05	0.05	0.04	0.02	0.06	0.05
C 12:0 lauric	0.02	0.02	0.09	0.02	0.04	0.09	0.04
C 14:0 myristic	0.97	1.44	2.29	0.86	0.64	3.79	2.83
C 16:0 palmitic	2.85	4.13	4.70	2.91	1.02	7.53	6.96
C 16:1 trans palmitelaidic	0.03	0.10	0.05	0.06	0.01	0.05	0.07
C 16:1 cis palmitoleic	0.27	0.80	0.56	0.49	0.26	1.92	0.85
C 17:0 margaric	0.27	0.23	0.42	0.19	0.08	0.42	1.32
C 18:0 stearic	0.78	0.59	1.61	0.78	0.22	2.01	1.53
C 18:1 trans elaidic	0.02	0	0.19	0.02	0.02	0.04	0.04
C 18:1 cis oleic	2.26	5.95	5.46	5.02	0.06	8.28	4.13
C 18:2 trans linolaidic	0	0	0	0.02	0	0.03	0.03
C 18:2 cis linoleic	0.22	0.31	0.07	0.26	0.01	1.98	0.40
C 18:3 linolenic	0.14	0.34	0.03	0.16	0	0.73	0.38
C 20:0 arachidic	0.02	0	0.03	0.01	0.03	0.09	0.08
C 20:1 cis eicosenoate	0.19	1.44	0.15	0.83	0.03	0.60	1.43
C 22:0 behenate	0.17	0.63	0.04	0.47	0.03	1.29	2.70
C 22:1 cis erucic	0.12	0	0	0.44	0	0.49	0.75
C 24:0 lignoceric	0	0	0	0	0	0.03	0.01
C 24:1 nervonic	0.15	1.77	0	1.51	0.01	2.79	5.15
Total	8.58	17.85	15.78	14.11	2.48	32.30	28.80

Paired t tests using the feed intake data collected during the trial were conducted. The results of the statistical analyses carried out on the data are shown in Table 8. Mussel was found to have a similar palatability to the control diet ($P>0.05$), with the mean difference between the intakes of control diet and intakes of mussel diet being only 1.1 g. Salmon was significantly ($P<0.01$) more palatable than the control diet, with the mean difference being 45.1 g in favour of the salmon diet. Jack mackerel was also significantly ($P<0.001$) more palatable than the control diet, with a mean difference of 48.2 g. The control diet was found to be significantly ($P<0.001$) more palatable than the hoki diet, with the mean difference of 39.8 g.

Table 7: Results of the statistical analyses for each fish by-product.

Sample	Mean(d) ± Se(d)[1]	Significant Difference[2]
Mussel	1.1g ± 3.0	ns
Hoki	39.8g ± 4.8	***
Jack Mackerel	-48.2g ± 8.0	***
Salmon	-45.1g ± 10.1	**

[1] d= intake control diet (g) - intake test diet (g), Se = standard error

[2] ns=not significant, **=P<0.01, ***=P<0.001

2.4 Discussion

The aim of the trial was firstly to determine the palatability of the four fish by-products, when these were included as part of a synthetic diet and secondly to investigate the efficacy of using synthetic diets for palatability trials.

The protein levels varied greatly between the seven fish by-products. Salmon contained the lowest amount and barracouta contained the most. Based on the amino acid analysis, hoki contains the largest range of amino acids and salmon the smallest. Whether the total level of amino acids or the quantities of specific amino acids is responsible for palatability is not known. Of the amino acids measured, glutamic acid was found in the greatest abundance in all seven by-products and cysteine was found in the lowest abundance, again in all seven fish types.

There was a large difference between the total nitrogen and the total amino acid nitrogen content of the seven fish by-products. The total nitrogen values ranged from 4.4mg in salmon to 10.4mg in barracouta. The amino acid nitrogen values had a lower range of 3.3mg to 7.3mg. The total nitrogen was higher in all cases since non-protein nitrogen can be found in compounds such as amines, amides and nitrates, as well as

amino acids. It would be expected that these would be found mostly in the water fractions of the by-products. The reason total nitrogen and amino acid nitrogen values were compared rather than crude protein and free amino acid totals is that free amino acids are heavier than their protein bound counterparts, due to the extra water molecule added to each amino acid during hydrolysis.

There was a large variation in the levels of crude fat in the fish by-products. Salmon contained the most fat and mussel contained the least. The fatty acid analysis revealed that salmon contained by far the greatest amount of total fatty acids, followed by hoki and jack mackerel, which contained similar amounts to each other. Mussel contained the lowest quantities of total fatty acids. The fatty acids that were in the greatest abundance in the fish by-products were palmitic acid (C16:0) and cis-oleic acid (C18:1). Past studies have reported that animal fats improve the palatability of feline diets (MacDonald *et al.* 1984). MacDonald and associates investigated the palatability of bleached tallow versus six other fats, including butter oil, chicken fat, yellow grease and unbleached tallow. They found that the cats preferred the bleached tallow diets to those diets made with butter oil and chicken fat and that the cats tended to prefer the unbleached tallow and the yellow grease to the bleached tallow. MacDonald and colleagues also reported that the level of fat is important as it affects the texture of the food. Our results did not reflect the findings of MacDonald *et al.* (1984) since hoki, which contains moderate amounts of fatty acids, was relatively unpalatable to the cat. On the other hand, the results for the salmon did reflect the findings of MacDonald *et al.* (1984) as it contained high quantities of fat and was also palatable to the cat.

When the crude fat values were compared to the total fatty acid content, it was discovered that the crude fat was much higher. The likely explanation for this is that fat contains a glycerol backbone and fatty acids. In the measurement of crude fat, the triglycerol is included and as a result the weight of crude fat is higher. Also the crude fat values may also include phospholipids and other non-triglyceride lipids, as well as fatty acids. Omega 3 fatty acids in fish are of particular interest, because of their potential contribution to cardiovascular health. The omega 3 fatty acid linolenic acid (C18:3) was found in all the by-products except mussel. However, the omega 3 fatty acids EPA (C20:5) and DHA (C22:6) were not measured.

The palatability testing method used was the two-bowl, free-choice testing method. This method is the most suitable as it is objective, valid, reliable, free from extraneous bias, allows the test subjects to be weighted equally, is independent for

nutritional and physiological effects and is amenable to statistical analysis (Griffin 2000b). By conducting the paired t tests, it was shown that of the four fish by-products, jack mackerel and salmon appeared to be the most palatable, as both were preferred over the control diet. This was reflected by the fact that the mean difference in intakes between each of these by-products and the control diet were negative values. Both were significantly more palatable than the control. However, jack mackerel and salmon had very different compositions in terms of fatty acid and amino acid profiles. Salmon contained high levels of fatty acids and low amounts of amino acids, whereas jack mackerel had high amounts of amino acids and moderate amounts of fatty acids. The mussel by-product was no more palatable than the control and hoki was strongly disliked by the test cats significantly ($P<0.001$). Interestingly hoki contained similar quantities of fatty acids and amino acids as jack mackerel, yet these two by-products are very different in terms of their palatability.

It must be noted that due to the low food intakes, and the inability of the diets to maintain the bodyweights of the cats, the study had to be abandoned prematurely and as a result the Latin square design was not completed. It is possible therefore that the palatability differences observed are not representative of all cats since the sample size ($n=2$) was so small. Further testing is therefore needed. It has been shown that despite reports that cats have a preference for fish, this preference does vary widely between individual animals (Houpt and Smith 1981).

This study was abandoned following the first control week and first test week due to low food intakes and decreased bodyweights of the cats. Clearly the cats did not like the synthetic diet. The cats characteristically fussy eating habits makes it extremely difficult to change the cat's diet from a commercial wet diet to a synthetic diet, as was found in this study. An animals' previous diet plays a large role in an individual animal's perception of palatability. In future, work with synthetic diets the test subjects need to be more familiar with the diets and it is recommended that familiarisation occurs in the pre-weaned kitten.

In conclusion, despite the low sample size for the trial ($n=2$), there were some reasonably clear trends regarding palatability. Jack mackerel and salmon were highly palatable to the cats, mussel was similar to the control diet in terms of palatability and hoki was disliked by the test cats. Large differences were observed in the composition of the fish by-products.

Chapter 3

The palatability of fish by-products for domestic cats (*Felis catus)* when included in commercial diets

3.1 Introduction

Despite reports of the high level of perceived palatability of fish to cats (Hegsted *et al.* 1956), it is well known that not only are cats 'finicky eaters' (Chapter 2), they also have very individual preferences for foods (Bradshaw *et al.* 1996, Houpt and Smith 1981, Hullar *et al.* 2001). Therefore, although some cats may find fish to be highly palatable, other cats, raised on identical diets, may have a strong dislike for fish (Bradshaw *et al.* 1996). Palatability appears to be a very complex area of feline nutrition and it is difficult to identify compounds or ingredients that are palatable to all, or at least the majority, of domestic cats.

Palatability plays an important role in food preferences, especially when a choice between different commercial cat foods is offered to domestic cats. Despite this, very little information is published by pet food manufacturers in this area due to the highly competitive nature of the pet food industry (Bradshaw *et al.* 1996). All of the data generated from palatability tests are therefore kept confidential. The palatability of commercial diets can be affected by numerous factors other than diet formulation, including meat freshness, the nutritional status of the animal at slaughter and the specific processing methods employed by the company (Bradshaw *et al.* 1996).

The palatability of fish by-products is of interest because of the potentially cheap source of protein and fat they offer. Testing by-products from different fish species could potentially uncover a potent palatability enhancer. By-products from green shell mussels are of specific interest, as New Zealand is the only country worldwide that produces green shell mussels. Therefore, if palatable, this by-product could give our pet food industry a unique advantage. New Zealand salmon is of interest due to its unique composition, as outlined in Chapter 2, and the hoki and barracouta species in New Zealand are only found in two other countries worldwide.

The purpose of this trial was to determine the palatability of four fish by-products in domestic cats when they are included in commercial canned diets. It was also hoped that a better understanding of palatability testing methods would be achieved. Three of the four fish by-products to be tested in this trial had been incompletely tested as part of the synthetic diets in an earlier unsuccessful trial (refer to Chapter 2). These were salmon, hoki and mussel. The fourth by-product to be tested, barracouta, replaced jack mackerel as the latter by-product could not be obtained as whole viscera and therefore would not be economically viable to use in diets for pets.

3.2 Materials and Methods

The procedures described below were approved by the Massey University Animal Ethics Committee (Anonymous 2003).

The fish by-products:

Four of the seven available fish by-products (Chapter 2) were selected for use in this trial; three of which (salmon, hoki and mussel) had previously been unsuccessfully tested as part of synthetic diets and the fourth was barracouta. Jack mackerel was not retested as it was found not to be economically viable, due to the fact it could not be purchased as whole viscera. The fresh by-products were obtained by Crop & Food Research (Auckland, New Zealand) homogenized and then frozen. They were made up of whole viscera and were analysed for dry matter, crude protein and crude fat (Chapter 2).

Diet formulation:

The diets tested in this study were commercial canned wet diets, which were produced by a large well-known pet food manufacturer for the purposes of this trial. The control diet was based on a chicken recipe, with an extra 9.09% (w/w) of chicken waste added to the base recipe. The four test diets were identical to the control diet except that the additional 9.09% (w/w) chicken waste was replaced with 9.09% (w/w) of the appropriate fish by-product. Each diet was canned (700g) and heat sterilized in a laboratory autoclave.

Test animals:

Sixteen adult domestic short haired cats, aged 3 to 11 years of age were used in the trial. They were a mix of 13 castrated males and 3 entire females and were all from the Centre for Feline Nutrition (Massey University, Palmerston North, New Zealand). All test cats were healthy at the start of the trial and ranged in bodyweight from approximately 2.90 to 4.80 kg. The test cats were housed in two outside, semi-enclosed colony cages between trials and during the weekends, where they were fed to appetite from 5:30pm to 11:00am on a commercial canned moist food. All cats had been

previously trained to occupy small individual metabolism cages for the duration of the testing and had been familiarised with testing procedures.

Palatability trial:

Each day from Monday until Friday, the food was removed from the test cats' cages at approximately 11:00am. This was to semi-starve the animals since satiated animals consume too little of the test diet to allow comparisons between diets. Starving cats on the other hand would also be undesirable as the cats would be eating to 'fill the gut', rather than selecting a food on the basis of palatability.

At 3:30pm each afternoon the sixteen cats were taken from their colony cages and transferred inside to a quiet room, where testing occurred. Each cat was housed in an individual plastic metabolism cage, each fitted with a litter tray. The cats were each offered two bowls in their cage, one containing the control diet and the other a test diet. Each bowl contained a quarter of a 700 g can and the positions of the bowls were switched daily to remove any lateral bias. After two hours, the cats were returned to their colony cages and the amount eaten from each bowl was determined and recorded. Any spillages were collected and weighed. The bowls and cages were washed for the next day and the litter was changed. This was repeated each day from Monday until Friday and on each successive week a different test diet was tested against the control for a period of four weeks in total. Any effects of time on intakes were countered by using the same control diet every week.

Data collation and statistical analyses:

Once the trial was completed, a relative consumption ratio for each cat was calculated for each day of the trial. These were calculated by dividing the intake of test diet (in grams) for that day by the total intake (i.e. test and control diets) for that day. These ratios were then analysed using a repeat measures ANOVA procedure (SAS 1999) to determine if the test diets were more or less palatable to the cats than the control diet based on their mean relative consumption ratios. The variables time, diet, cat, and their interactions were also tested to see if they had any significant effect on the perceived palatability of the diets. A paired t test was also conducted for each test diet using the daily feed intakes for each cat on each day. The intake of control diet eaten was paired to the intake of the test diet eaten. The t test was used to determine whether or not the intakes of test diet were significantly different from the intakes of control diet.

Table 1: Dry matter, crude protein and crude fat content of the fish by-product samples[1].

Sample	Dry Matter	Crude Protein (%)	Crude Fat
Barracouta	26.4	15.0	10.0
Hoki	56.6	8.9	46.9
Mussel	24.9	18.2	2.20
Salmon	48.2	12.2	35.5

[1] Results are presented on an "as received" basis (fresh)

3.3 Results

The results of the analyses conducted on the fresh fish by-products are outlined above in Table 1. Of the four fish by-products, mussel contained the highest amount of protein, but contained by far the lowest amount of crude fat. Hoki on the other hand contained the lowest quantities of crude protein, yet had the largest amount of crude fat on a fresh basis. The predicted analytical results of the canned diet formulations are outlined in Table 2. The diets were formulated to have a predicted composition as equal as possible and were determined by the manufacturer responsible for making the diets using the compositions of their other commercial moist diets as a guide. The moisture, protein and ash values were very similar, however the fat and therefore metabolisable energy values varied somewhat, in particular for salmon and hoki, which contained higher crude fat than the other diets. Consequently, the calculated metabolisable energy of the hoki and salmon diets were also higher.

All 16 cats appeared healthy throughout the palatability trials and all satisfactorily maintained their bodyweights. At the end of the study the cats ranged in weight from 2.75 to 5.77 kg. The average daily intakes for the 16 cats over the five day test period for each diet are shown in Table 3. The average daily intake of the control diet was greater than the average daily intake of the hoki diet (Table 3) with approximately 73 g for the control and only 43 g for the hoki diet. The average daily intakes of both the mussel and salmon diets were higher than that of the control diet. The barracouta diet was eaten in similar amounts to the control diet. It was expected that the cats should eat at least 100g in total of the semi-moist diets in each two hour trial period for the intakes to be acceptable or 'normal' for this type of palatability test. For the hoki diet, of the 16 cats, only two of the cats ate 100g of food for each trial period

for the entire week and four of the cats ate less than 100g per trial period for the whole week. For the barracouta diet, six cats did not reach the target consumption of 100g at all during the test week and again only two reached this intake every day for the whole test week. Four cats out of the16 ate 100g or more every day for the mussel diet test week and only two did not reach this intake at all during the test week. Finally, during the week which tested the diet containing salmon, four cats consumed at least 100g every day of the week and three cats did not meet this target at all.

Table 2: Predicted composition of the five commercial diets.

Diet	Moisture	Crude Protein	Crude Fat	Ash	ME[1]
			(%)		
Control	81.7	9.2	5.3	2.2	820
Hoki	79.2	8.9	8.3	2.0	1069
Barracouta	81.9	9.5	5.0	2.1	804
Mussel	82.0	9.8	4.3	2.1	754
Salmon	79.9	9.2	7.3	2.1	992

[1] ME=Metabolisable Energy (kcal/kg) = ((protein % + carbohydrate %) x 3.5 + (fat % x 8.5)) x 10

Table 3: Average daily intakes of control and test diets for the sixteen test cats.

Test Diet	Average Intake			
	Control Diet		Test Diet	
	(g)	ME (kcal)	(g)	ME (kcal)
Hoki	73.4	60.2	43.0	46.0
Barracouta	43.5	35.7	44.1	35.5
Mussel	56.4	46.2	62.5	47.1
Salmon	50.5	41.4	59.7	59.2

The relative consumption values calculated for each diet, and these along with the repeat measures ANOVA analysis performed on the data and the results of the t test are shown in Table 4. The mean relative consumptions for the salmon, mussel and barracouta by-products when included in the chicken based diet show that these diets do not appear to be different to the control diet in terms of palatability. The t test showed that the feed intakes of these three by-products (salmon, mussel and barracouta), were not significantly ($P>0.05$) different from the intakes of the control diet. The mean

relative consumption of the hoki diet was significantly (P<0.001) different from the control diet.

The repeat measures analysis conducted also showed that the effect of day on the relative consumptions was not significant (P>0.05). The effect of the interaction between diet and day was also not significant (P>0.05). However, the effects of diet and the effect of the combination of cat and diet were significant (P<0.001) for the relative consumptions obtained.

Table 4: Mean relative consumption (±SE) of the fish by-products and their significance.

	Diets			
	Salmon	Hoki	Mussel	Barracouta
Mean RC[1]	0.54 (±0.030)	0.26 (±0.023)	0.52 (±0.036)	0.50 (±0.031)
Significance	ns	***	ns	ns

[1] Relative Consumption Ratio

[2] ns=not significant (P>0.05) and ***=significant (P<0.001)

3.4 Discussion

The purpose of this study was to establish the palatability of four different fish by-products (salmon, hoki, mussel and barracouta) when they were included in a commercially produced, canned diet and compared to an un-supplemented control diet.

The results of the initial analyses carried out on the fresh fish by-products showed that the by-products are all very different in terms of their compositions. Hoki contained the highest percentage of crude fat with 46.9%, but contained low amounts of crude protein, at only 8.9%. Mussel, however, contained a relatively high percentage of crude protein at 18.2% and the lowest amount of crude fat (2.2%). Barracouta and salmon both contained similar quantities of crude protein, with 15.0% and 12.2%, respectively, however, salmon contained higher amounts of crude fat with 35.5% compared to only 10.0% crude fat for barracouta. It may be expected that due to these very different compositions, the four fish by-products would also all have very different levels of palatability to the domestic cat.

For the palatability trials, the five diets; hoki, salmon, barracouta, mussel and control, were all formulated to have similar predicted compositions as possible. The purpose of doing this was to ensure the palatability of the fish by-products was being tested and not the palatability of other components of the diet, such as macronutrient

content. The quantities of protein, moisture and ash were very similar across all five diets, however, it was harder to keep the quantities of crude fat constant across the diets, due to the fact that hoki and salmon by-products naturally contain much higher amounts of fat than barracouta and mussel.

Based on relative consumptions, it appears that salmon, mussel and barracouta are not significantly ($P>0.05$) different to the control diet in terms of palatability with relative consumptions between 0.50 for barracouta and 0.54 for salmon. In contrast, in some previous studies salmon, in particular, was highly palatable and was preferred to other commercial cat foods (Houpt and Smith 1981). Hoki, however, appears to be significantly less palatable than all of the other diets with a relative consumption ratio of only 0.26. The difference in intake of the hoki diet and control diet was significant ($P<0.001$). Perhaps the hoki by-products combination of very high fat content and low protein content had a negative impact on its palatability. The low protein in the hoki by-product is of particular significance, as the cats' natural diet includes high levels of protein and moderately high levels of fat, although the optimum level of fat for cat diets is still uncertain (MacDonald *et al.* 1984). Hoki also live in a different habitat to salmon, mussel and barracouta and are therefore probably also caught and handled differently, which could possibly explain the different composition of these fish species. Hoki is a middle water fish, living at depths of between 300 and 600 metres and is caught by mid winter trawling, whereas salmon and mussels are farmed, and barracouta is a deep water fish which is caught by bottom trawling. Perhaps these differences may contribute partially to the unusual composition of hoki and its seemingly apparent lack of palatability in this study.

It has been previously stated that various factors influence the palatability of commercial cat foods, or more specifically the palatability of the meat in these foods, including its freshness, the status of the animal at slaughter and processing methods (Bradshaw *et al.* 1996). Whether these have affected the results obtained in this study is unknown. It perhaps would have been worthwhile to measure the peroxide levels using TBARS analysis to determine the level of rancidity in the fish by-products before including them in the commercial diets. It would also be of interest to see if there was an interaction between the chicken and fish components in the diets formulated, and if so how this affected the palatability of the diets.

Since only sixteen cats were available for the study, the trial was conducted over four weeks with each cat receiving a different diet each week. Consequently, any

possible time effects needed to be tested. The effect of time on the intake of the control diets was not significant ($P>0.05$), nor was the interaction between diet and time, so no time effects were observed. However, the test diet offered did have a significant effect on intake, as did the specific cat being used. This was not surprising because it is well known that individual cats have very different tastes and therefore perceive the palatability of diets differently. Therefore, the cat selection for such a trial can have a large and significant effect on the results. It is difficult to counter this other than to have more cats per treatment.

The results obtained from this study were similar to those from Chapter 2, with hoki being found to be significantly ($P<0.001$) less palatable than the control diets in both chapters. Salmon was found to be similar to the control diet in terms of palatability in this study however the salmon diet was significantly more palatable than the control diet in the study reported in Chapter 2. The palatability of the mussel diets in both chapters was not significantly ($P>0.05$) different from the palatability of the control diets.

In conclusion, the results of the statistical analyses illustrated that salmon, mussel and barracouta by-products were no different to the control diet in terms of palatability. Hoki, however, was strongly disliked by the test panel. Future work on the palatability of these fish by-products could be concentrated on retesting the by-products when included in a different type of base diet to see if a different base diet has an effect on palatability.

Chapter 4

The palatability of fish by-product fractions for domestic cats (*Felis catus)* when included in a commercial diet

4.1 Introduction

Today, palatability is the single most sought after characteristic of pet food (Trivedi and Benning 1999), with pet owners selecting a brand of food based on palatability rather than on nutritional value. Despite its importance, however, there is little information published on the palatability of different types of cat food. Furthermore, the specific compounds present in the food that are responsible for palatability are still unclear.

Previous studies have reported that the cat finds protein hydrolysates, meat extracts and some free amino acids palatable (MacDonald *et al.* 1984). While there are no data published from commercial sources relating to the effect of different ingredients on the palatability of diets for cats, it is known that ingredients such as fat are used extensively in some commercial feline diets (Kane *et al.* 1981). It is well-known that fats increase the palatability of cat foods (Bennet 2002, Kane *et al.* 1981, MacDonald *et al.* 1984). Fat is said to affect the texture of the food, and by this means, improve its palatability (MacDonald *et al.* 1984). Cats do prefer certain types of fat, however, with MacDonald *et al.* (1984) concluding that the cat finds medium chain triglycerides (8:0) unpalatable but finds short chain triglycerides highly palatable. Consequently it was hypothesised that the oils would be more palatable than solid fats.

There were two main aims of this trial. The first was to fractionate the fish by-products of interest into their three fractions, solid, water and oil. The second aim was to determine the palatability of the fractions, using the domestic cat, when included in a commercially produced, canned diet.

4.2 Materials and Methods

All procedures reported below were approved by the Massey University Animal Ethics Committee (Anonymous 2003).

<u>The fish by-products:</u>

Based on previous results (Chapter 2 and 3) and the known composition of the by-products, it was decided to fractionate the salmon and barracouta by-products. Approximately 30kg of each of the fresh and frozen by-products were obtained from Crop & Food Research (Auckland, New Zealand). The by-products contained whole viscera as in previous trials and had been homogenised.

Fractionation method for the salmon:

Each by-product was squeezed through fine mesh netting by hand, in order to remove some of the large solid material. The mesh netting used was made of nylon and was approximately one metre wide by one metre long with one mm^2 holes. The material that passed through the mesh was collected separately and stored. The filtrate was then centrifuged using a high speed IEC B-22M programmable centrifuge at 15,000xg for 30 min. at 20°C and the supernatant containing the oil and water fractions removed. The solid precipitate and residual liquid left in the tube was remixed and re-centrifuged to remove as much residual oil and water as possible.

Fractionation method for the barracouta:

It was hoped that the above method would also be suitable to fractionate the barracouta by-product, however, after an initial run, it was discovered that this would not be suitable, due to the comparatively low water and fat contents of barracouta. Consequently a different fractionation process was used. The barracouta by-product was heated to 55°C for 30 min., after which, it was centrifuged for 30 min. at 17,000xg. The supernatant containing the water fraction was decanted off and re-centrifuged at 17,000xg for 30 min. to remove any particles. The amount of oil present was too small to allow removal from the solid precipitate.

Laboratory analyses:

Once both fractionations were obtained, small samples of the water fractions of the salmon and barracouta were analysed for amino acid profiles (free amino acids and total amino acids, i.e. free amino acids plus protein bound amino acids) and a sample of the salmon oil was analysed for its fatty acid profile to determine total fatty acids, i.e. the sum of any free fatty acids plus the fatty acids in TAG (Chapter 2). The salmon and barracouta water fractions were also analysed for crude protein and crude fat (Chapter 2). It was decided not to analyse the solid fraction of the two fish, as this fraction was not used. Previous studies have shown that the solid fraction does not increase palatability when included in commercial diets for cats (Hendriks unpublished).

Diet formulation:

The salmon water extract, salmon oil extract and barracouta water extract collected were included in commercially produced canned diets at an inclusion rate of 2%. A

standard control diet was also formulated and manufactured by the same pet food company as mentioned in Chapter 3. The control diet was based on one of the company's chicken recipes and produced in 700 g cans. The three test diets, i.e. salmon oil, salmon water and barracouta water, were similar to the control except these diets had 2% water removed and 2% of each of the fractions added.

The test animals:

Sixteen adult domestic short haired cats, a mix of 12 castrated males and 4 entire females were used in the trial. All cats appeared to be in excellent health at the start of the study. They were 3 to 11 years of age, ranging from 2.75 to 5.70 kg in bodyweight and were obtained from the Centre for Feline Nutrition (Massey University, Palmerston North, New Zealand). Between trials and in the weekends the animals were housed in outside, semi-enclosed colony cages. During these times they were fed to appetite a mixture of various commercial canned foods between 3:00pm and 9:00am. All cats had been previously trained to occupy small individual cages for the duration of the testing and had been familiarised with testing procedures.

Palatability trial:

The palatability trial lasted for three weeks, with the experimental period being five days. For five days of the week at approximately 9:00am on each of these days, the food was removed from the test cats' cages. This was required so the animals would be semi-starved at the time of testing. The reason that semi-starved cats were used are the same as those outlined in Chapter 3.

Daily at 1:00pm the 16 cats were taken from their colony cages and put into a quiet room, where testing occurred. The cats were housed in individual plastic metabolism cages, each fitted with a litter tray. The cats were offered two bowls each, one containing the control diet and the other, one of the test diets. The bowls each contained a quarter of a 700 g can. Each day the positions of the bowls were switched to remove any possible lateral bias. The test cats were returned to their colony cages after two hours. The amount of food eaten from each bowl was then determined and recorded. Any spillages were collected, weighed and counted as refusals. The bowls and cages were cleaned for the following day and the litter changed. A different test diet was tested each week.

Data collation and statistical analysis:

Relative consumption ratios were calculated for each cat for each day of the trial. These were calculated by dividing the intake of test diet by the total intake (i.e. test and control diets). These ratios were analysed using a repeat measures ANOVA procedure to determine the palatability of the test diets in relation to the control diet. The effects of test day, diet and cat on the palatability of the test diets were also determined using this procedure. Using the daily feed intakes for each cat on each day, a paired t test was conducted for each test diet. The intake of control diet was paired to the intake of the test diet to determine whether or not the intakes of test diet were significantly different from the intakes of control diet.

4.3 Results

The crude protein and crude fat contents of the two by-product water fractions were determined and shown in Table 1. The crude protein content of the two by-products were similar, however the crude fat content of the salmon water was nearly double that of the barracouta water. The amino acid profiles were determined and are shown in Table 2. The quantities of free amino acids ranged from 0.047 for cysteine to 0.651 mg/100mg for glutamic acid in the salmon water and from 0.048 for cysteine to 0.702 mg/100mg for leucine in the barracouta water. The quantities of total amino acids varied between 0.011 and 1.468 for salmon water and between 0.031 and 1.613 for barracouta water. Free arginine was not detectable for the barracouta water.

Table 1: Crude protein and crude fat content for the water fractions[1].

Sample	Crude Protein (%)	Crude Fat
Salmon water	11.68	1.03
Barracouta water	14.84	0.56

[1] Results expressed on an as-is basis.

Table 2: Free and total amino acid content of the salmon and barracouta water fractions.

Amino Acid	Salmon Water Amino Acid Content		Barracouta water Amino Acid Content	
	Free	Total	Free	Total
	(mg/100mg)			
Aspartic acid	0.378	1.166	0.556	1.080
Threonine	0.356	0.635	0.337	0.612
Serine	0.575	0.594	0.571	0.549
Glutamic acid	0.651	1.468	0.616	1.613
Proline	0.252	0.559	0.220	0.643
Glycine	0.214	0.727	0.240	0.955
Alanine	0.434	0.717	0.547	0.847
Valine	0.518	0.729	0.547	0.678
Methionine	0.243	0.333	0.295	0.342
Isoleucine	0.349	0.558	0.431	0.559
Leucine	0.638	0.915	0.702	0.914
Tyrosine	0.102	0.172	0.225	0.284
Phenylalanine	0.299	0.441	0.356	0.450
Histidine	0.325	0.372	0.313	0.403
Lysine	0.554	0.516	0.566	0.786
Arginine	0.464	0.680	ND[1]	0.031
Cysteine	0.047	0.117	0.048	0.053
Taurine	0.330	0.428	0.132	0.352

[1] ND= Not detected.

Table 3: Fatty acid profile of the salmon oil.

Fatty acid		mg/100mg	Fatty acid		mg/100mg
C 8:0	caprylic	0.03	C 18:2	cis-linolaidic	2.76
C 10:0	capric	0.02	C 18:2	trans-linoleic	0.00
C 12:0	lauric	0.06	C 18:3	linolenic	2.06
C 14:0	myristic	3.17	C 20:0	arachidic	1.32
C 16:0	palmitic	6.65	C 20:1	cis-eicosenoate	0.10
C 16:1	cis-palmitelaidic	0.00	C 20:5	eicosapentaenoic	4.13
C 16:1	trans-palmitoleic	3.77	C 22:0	behenate	4.58
C 17:0	margaric	0.05	C 22:1	cis-erucic	0.04
C 18:0	stearic	0.72	C 24:0	lignoceric	0.04
C 18:1	cis-elaidic	0.79	C 24:1	nervonic	0.00
C 18:1	trans oleic	0.03			

The results of the fatty acid analyses carried out on the salmon oil are shown in Table 3 above. The quantities of fatty acids in the salmon oil varied from 0 mg for nervonic acid (C24:1) and cis-palmitelaidic (C16:1) up to 6.65 mg/100mg for palmitic acid (C16:0). The average intakes of the 16 cats over the study period for each diet are shown in Table 4. An intake of 100g or more during the two hour test period each day was considered a 'normal' intake. Based on this, for the diet containing salmon water none of the cats reached this target for the entire week, and four of the cats did not reach this target even once during the week. For the barracouta water containing diet, seven of the cats did not achieve the target intake of 100g at all during the week and only one ate at least 100g of food every day of the trial week. During the week testing the diet containing the salmon oil, two cats consumed 100g or more every day, however seven did not reach this target at all during the week. A summary of the results of the repeat measures analyses performed on the relative consumption ratios are outlined in Table 5 below. The diet containing the salmon water and barracouta water were not significantly ($P>0.05$) different from the control in terms of palatability, with mean relative consumptions of 0.51 and 0.47 respectively. The salmon oil containing diet was significantly ($P<0.05$) more palatable than the control diet. The repeat measures analysis showed that the effect of day on the intakes was not significant ($P>0.05$). Also the interaction between the specific diet being tested and the day it was being tested on was not significant ($P>0.05$). The cat being considered and the diet being tested on that cat exerted a significant ($P<0.001$) effect on the feed intakes. The effect of diet on the feed intakes was also significant ($P<0.01$).

Table 4: Average intakes of control and test diets for the sixteen test cats.

Fraction	Average Intake	
	Control Diet	Test Diet
	(g)	
Salmon water	38.8	39.8
Barracouta water	42.9	36.0
Salmon oil	28.1	39.9

Table 5: Mean relative consumption (±SE) and significant difference from control for the three fish by-product fractions.

	Diets		
	Salmon Water	Barracouta Water	Salmon Oil
Mean RC[1]	0.51 (±0.030)	0.47 (±0.031)	0.58 (±0.029)
Significance[2]	ns	ns	*

[1] RC = Relative consumption.

[2] ns=not significant (P>0.05) and *= significant at P<0.05.

4.4 Discussion

The purpose of this study was to determine the palatability of each of the fractions using the domestic cat when the fractions were included in commercially produced, canned diets.

The initial compositional analyses conducted on the three fractions illustrated that these fractions have very different compositions. The salmon water had a lower crude protein content than the barracouta water but had nearly double the content of crude fat. The amounts of specific amino acids in these two fractions (salmon water and barracouta water) were also quite different. The main differences appear to be that barracouta water contains much greater quantities of free and total tyrosine and alanine and total lysine than salmon water. However, salmon water contains far more taurine, arginine and total cysteine than barracouta water. Free arginine was in fact not detectable in barracouta water and the total arginine found in barracouta water was very low. Despite these compositional differences, these two fractions were equally palatable to the domestic cat. Unfortunately there was no other oil fraction with which to compare the salmon oil.

Of the three fish by-product fractions tested, the salmon oil was the most palatable to the test cats when added to the control diet with a relative consumption of 0.58. The salmon oil was found to be significantly (P<0.05) more palatable than the control diet. The salmon water and barracouta water fractions were found to be similar to the control diet in terms of palatability with relative consumptions of 0.51 and 0.47 respectively.

It was also discovered that the effects of diet and cat on the relative consumption ratios were significant, at P<0.01 for diet and P<0.001 for the cat effect. The effect of the cat on the relative consumption ratio was not unexpected with the very individual tastes

cats seem to have. Diets also would be expected to have an effect on the relative consumption. If the diet was liked, the relative consumption was high and if the diet was not liked, the relative consumption ratio was low. The effects of time and the interaction between day and diet on the feed intakes were not significant ($P>0.05$).

Despite the lack of published data from commercial sources relating to the palatability of cat foods, other studies have reported that protein hydrolysates, meat extracts and certain free amino acids are highly palatable to the domestic cat (MacDonald *et al.* 1984). It has also been reported in numerous publications that the cat finds fat palatable (Bennet 2002, Kane *et al.* 1981, MacDonald *et al.* 1984). Apparently cats do prefer some fats over others, however, with reports claiming that while the cat prefers short chain triglycerides, they find medium chain triglycerides unpalatable (MacDonald *et al.* 1984). Based on the studies of MacDonald *et al.* (1984), it was hypothesised that the cats would find the oil fraction more palatable compared to the water fractions. The solid fractions of the fish by-products were not tested for palatability. It was decided to use the oil and water fractions in the trials because it was believed they would be the most palatable (Boudreau and White 1978, MacDonald *et al.* 1984).

It was hoped that the mussel by-product could be fractionated along with the salmon and barracouta. However, as mentioned, it was not viable to carry out this fractionation due to the extremely low amounts of water and fat in this particular by-product. It was also hoped that barracouta oil could be extracted and subsequently tested for its palatability. However, due to barracouta containing only small quantities of oil, this proved prohibitive.

From completing this study, it was discovered that salmon oil was the most palatable of the fractions tested. Future research into this area could be aimed at further testing the salmon oil fraction at different dose rates to establish an optimum level of inclusion. It may also be of interest to test the palatability of the fractions when they are included in a different type of base diet and to investigate how cost effective it would be to use fish by-product fractions instead of using homogenised fish frames or viscera.

Chapter 5

The palatability of various pure compounds for domestic cats (*Felis catus*)

5.1 Introduction

To date, little is known about the specific ingredients or compounds responsible for the palatability of a cat food (Houpt and Smith 1981). However, there have been some reports claiming to have identified certain compounds as palatability enhancers for the cat (Boudreau and White 1978, Bradshaw 1991, Bradshaw *et al.* 1996, MacDonald *et al.* 1984, Stein 2001). It has been claimed that cats prefer ingredients of animal origin, in particular proteins or amino acids, and fats (Bennet 2002). This is consistent with the carnivorous eating habits of the cat.

Different species have different taste groups or taste systems that respond to different compounds (Bradshaw 1991). In the cat the dominant taste system is the amino acid system. The amino acid system in the cat is most sensitive to the amino acids humans find 'sweet', such as L-proline, L-cysteine, L-ornithine, L-lysine, L-histidine and L-alanine (Bradshaw 1991, White and Boudreau 1975). Bitter amino acids such as L-tryptophan, L-isoleucine, L-leucine, L-arginine, and L-phenylalanine inhibit the amino acid taste buds in the cat (Boudreau and White 1978, Bradshaw 1991).

Other compounds have also been described as having either positive or negative effects on the palatability of cat foods (Boudreau and White 1978, Bradshaw 1991, Bradshaw *et al.* 1996, MacDonald *et al.* 1984, Stein 2001). For example, cats have been found to be insensitive to salt (Bradshaw 1991), and it would, therefore, be expected that the cat would show little reaction to compounds such as sodium chloride and potassium chloride. Similarly the domestic cat, unlike the dog does not respond to sugars (Thorne 1998). However, despite all these reports little conclusive data exists in the literature to highlight what compounds or ingredients show palatable effects in the cat. Furthermore there are very few compounds which can be defined as being palatable to all cats, since testing is highly dependent on individual cats and environments (Houpt *et al.* 1988). The aim of this study was to determine the palatability of a range of compounds when dissolved in milk.

5.2 Materials and Methods

All procedures described below were approved by the Massey University Animal Ethics Committee (Anonymous 2003).

The milk:

Commercial "pet milk" was obtained from a local supermarket. This milk was specifically manufactured for both cats and dogs and was lactose free and taurine enriched. The composition of this milk is provided in Table 1. Each day two litres of milk was used; one litre as a control, with nothing added and the other litre as the test milk, with the selected test compound added at the desired inclusion rate, which had been decided upon after some discussion (Hendriks unpublished). The compound was added to the milk and mixed by stirring until it had all dissolved.

Table 1: Composition of the milk.

Component	Quantity (%)
Total Solids (min)	12.50
Protein	3.25
Total Fat (min)	4.00
Carbohydrate	4.70
Lactose	0
Energy (kJ/100 mL)	280

Test animals:

Eight adult domestic short haired cats, a mixture of three entire females and five castrated males were selected from the Centre for Feline Nutrition, (Massey University, Palmerston North). They were all in excellent health and were all known to have a preference for milk and therefore would be expected to drink sufficient to produce viable results in the trials. The cats used were between 2 and 10 years of age. Between trials the test animals were housed outside in semi-enclosed colony cages, where they were fed a mixture of canned moist food. Their weights were monitored on a weekly basis, as well as obvious signs of ill health. Their weights ranged from 2.62 to 5.36 kg at the start of the trial and ranged from 2.63 to 5.26 kg at the end of the trial.

Palatability test:

Every day at approximately 1:15pm the eight test cats were removed from their colony cages and relocated inside a quiet room where testing took place. Each cat was housed in an individual plastic metabolism cage in this room during the trial period. These cages were each fitted with plastic litter trays. The two bowl free choice palatability

testing method was used in this trial. The test cats were each given two bowls in their cages, one containing the control milk and the other the test milk, with each cat being offered approximately 125ml of each. After one hour the cats were returned to their colony cages and the amount of milk consumed (in grams) from each bowl by each cat was recorded. This was repeated each day from Monday until Friday, with a new compound being tested each week, except for weeks eight and twelve, where the test cats were offered two bowls of the un-supplemented control milk. These two weeks acted as control periods to ensure the cats were drinking the preferred milk rather than merely drinking from the preferred bowl. The trial was run for a period of 19 consecutive weeks. Each day the bowl positions in the cages were reversed to eliminate any lateral bias. During the weekends and between trials the cats were fed canned food in their colony cages. The testing schedule of test compounds and weeks is outlined below in Table 2.

Data collation and statistical analyses:

From the milk intakes, relative consumption ratios were calculated for each cat for each day of the trial, the calculation for which is outlined in Chapter 2. These relative consumptions were then analysed using a repeat measures ANOVA to establish the test diets palatability in relation to the palatability of the control diet. The effects of time, diet, cat and their interactions were also tested to determine their significance on the milk intakes. A paired t test using the milk intakes of the test diet paired to the milk intakes of the control diet was also conducted for each test week to determine whether the differences between intakes of test and control diet were significant ($P<0.05$).

Table 2: Schedule of test compounds, weeks and doses.

Week	Compound[1]	Dose (% wt/vol)
1	Proline	0.3
2	Lysine hydrochloride	0.3
3	Calcium chloride	0.3
4	Taurine	0.3
5	Sodium *di*hydrogen phosphate	0.3
6	Glycine	0.3
7	Arginine	0.3
8	Control	0.0
9	L-malic acid di-sodium salt	0.3
10	Histidine	0.3
11	Cysteine hydrochloride	0.3
12	Control	0.0
13	Methionine	0.3
14	Creatine	0.3
15	Creatinine	0.3
16	Urea	0.3
17	Proline	0.6
18	Histidine	0.6
19	Lysine hydrochloride	0.6

[1] Unless otherwise stated the compounds are "free base".

Table 3: Average intakes of control and test diets for the eight test cats for the week.

Compound (Dose, %)	Average Intake (g)		Compound (Dose %)	Average Intake (g)	
	Control	Test		Control	Test
Proline (0.3)	21.3	78.3	Cysteine.HCl (0.3)	28.1	55.3
Lysine.HCl (0.3)	19.5	53.5	Control (0)	34.8	28.8
$CaCl_2.2H_2O$ (0.3)	36.7	33.1	Methionine (0.3)	30.3	36.0
Taurine (0.3)	40.4	45.2	Creatine (0.3)	36.9	22.1
$NaH_2PO_4.2H_2O$ (0.3)	36.4	57.2	Creatinine (0.3)	42.7	27.0
Glycine (0.3)	24.3	48.5	Urea (0.3)	27.1	23.1
Arginine (0.3)	41.9	36.5	Proline (0.6)	23.3	55.6
Control (0)	23.5	28.0	Histidine (0.6)	31.0	33.3
L-malic acid.di-Na salt (0.3)	32.1	28.2	Lysine.HCl (0.6)	22.2	47.6
Histidine (0.3)	28.3	43.3			

5.3 Results

The majority of the cats remained healthy throughout the trial. However, two cats were removed during the trial; one female and one male, due to health reasons not related to this study. These two cats were removed from the trial after the 10th week of testing. They were replaced by two new adult cats, which were in excellent health and were also shown to drink sufficient quantities of the milk. The average intakes of the test and control diets are shown in Table 3 while the results of the statistical analyses are summarised in Table 4.

Table 4: Mean relative consumption (±SE) and significant difference from control for the test compounds.

Week	Compound	Dose (%)	Mean RC[1]	SD[2]	Cat Effect
1	Proline	0.3	0.75 (± 0.046)	***	
2	Lysine hydrochloride	0.3	0.69 (± 0.047)	***	ns
3	$CaCl_2.2H_2O$	0.3	0.50 (± 0.056)	ns	
4	Taurine	0.3	0.54 (± 0.058)	ns	
5	$NaH_2PO_4.2H_2O$	0.3	0.59 (± 0.052)	*	Day effect
6	Glycine	0.3	0.63 (± 0.054)	**	
7	Arginine	0.3	0.51 (± 0.055)	ns	***
8	Control	0.0	0.55 (± 0.062)	ns	
9	L-malic acid di-sodium salt	0.3	0.49 (± 0.055)	ns	
10	Histidine	0.3	0.63 (± 0.047)	**	Diet effect
11	Cysteine hydrochloride	0.3	0.62 (± 0.054)	**	
12	Control	0.0	0.43 (± 0.057)	ns	***
13	Methionine	0.3	0.56 (± 0.057)	ns	
14	Creatine	0.3	0.46 (± 0.056)	ns	
15	Creatinine	0.3	0.47 (± 0.053)	ns	Diet*day effect
16	Urea	0.3	0.44 (± 0.057)	ns	
17	Proline	0.6	0.66 (± 0.056)	***	ns
18	Histidine	0.6	0.55 (± 0.056)	ns	
19	Lysine hydrochloride	0.6	0.67 (± 0.057)	***	

[1] RC= Relative consumption.

[2] Significant difference from control diet, where ns= not significant (P>0.05), * = P<0.05, ** = P<0.01, *** = P<0.001.

When 0.3% of the test compounds were included into the milk and the palatability compared to un-supplemented control milk, proline, lysine (lysine hydrochloride), glycine, sodium dihydrogen phosphate, histidine and cysteine (cysteine

hydrochloride) were significantly more palatable than the control diet. These diets all had mean relative consumptions between 0.59 and 0.75. At this same dose, calcium chloride, taurine, arginine, malic acid (L-malic acid di-sodium salt), methionine, creatine, creatinine and urea were not significantly ($P>0.05$) different from the control. The mean relative consumptions for these diets were between 0.43 and 0.56. In weeks eight and twelve, the cats were offered two bowls containing un-supplemented control milk only. The relative consumption value for these two bowls of control milk was not significantly ($P>0.05$) different.

Milk containing proline, histidine and lysine hydrochloride had the highest palatability of the compounds tested, therefore, it was decided to test these compounds at a higher inclusion rate (0.6%) to see if there was an effect of dose rate on palatability. The results of these palatability tests were varied. At this higher dose, as with the low dose, proline and lysine hydrochloride were found to be significantly ($P<0.001$) more palatable than the control with relative consumptions of 0.66 and 0.67 respectively. Histidine was not significantly ($P>0.05$) different from the control diet at this dose, with a relative consumption of 0.55.

5.4 Discussion

The aim of this trial was to determine the palatability of various compounds when they were dissolved in milk and compared to a common control, plain milk. The compounds chosen had all previously been studied, and were compounds that were readily available and not limited by cost. From the data collected throughout the trial, relative consumptions were calculated for each test animal for each day, and each test compound. A relative consumption of 0.5 means the control and test diets are equally liked. The statistical analyses conducted showed that of the compounds tested, the amino acids, proline, lysine (lysine hydrochloride), histidine, glycine and cysteine (cysteine hydrochloride), and the compound sodium dihydrogen phosphate when included in milk, are significantly more palatable to the domestic cat at the dose of 0.3% than the un-supplemented milk. Proline and lysine hydrochloride were also found to be more palatable than the control at the higher dose of 0.6%, however, the palatability of histidine was not significantly different from the milk when present at the higher inclusion rate. This, therefore, raises the important question as to what the optimum dose is for the use of these amino acids as palatability enhancers. The testing

carried out relating to the dose dependency in this study, only examined two dose rates and was therefore only a "snap shot". A greater number of inclusion rates needs to be tested in order to draw definite conclusions as to the optimum dose rate for enhancing palatability with these compounds.

The other compounds tested in this trial were found to have no significant effect on the palatability of the milk. To test whether the cats were drinking from a preferred bowl rather than preferred milk, an experiment was conducted where for two separated weeks, both bowls contained un-supplemented milk. During this time there was no significant difference in the relative consumption for each bowl indicating that the bowl was not having an effect on the cats' choice of bowl.

The effects of diet and day were both significant ($P<0.001$) on the relative consumption ratio. However, the interactions between diet and day and cat and diet were not significant ($P>0.05$). It was expected that the relative consumption would vary significantly with each cat since cats have very individual feeding preferences however this effect was not significant in this study. The effect of time on the food intakes is particularly important to consider in this case, due to the length of the trial (i.e.19 weeks). Because this trial was so long, it can not be guaranteed that all aspects of the trial were the same at the start and end of the testing. To overcome this problem the trial was designed such that the control milk was fed over the entire trial period and the intakes of the control were compared to the test milk. The relative consumption value was used so if the actual intakes changed throughout the trial it would change for test and control and the relative consumption would not change. In this manner the relative consumption for the test compounds could be compared directly. The findings in this study were consistent with Bradshaw (1991) and White and Boudreau (1975) who found proline, lysine (lysine hydrochloride), cysteine (cysteine hydrochloride) and histidine to be palatable to the cat at doses of 50mM. Whether the palatability enhancing effects of lysine hydrochloride and cysteine hydrochloride are due to the amino acids, HCl or a combination of the two remains to be determined. In contrast, however, Bradshaw (1991) and Boudreau and White (1978) reported arginine to have an inhibitory effect on the cats' amino acid taste system at a dose of 50mM, whereas in this study, arginine had a palatability similar to the un-supplemented milk (control). Taurine has also been reported to trigger the cats' taste systems, as has malic acid (Bradshaw 1991). However, the conclusions from this trial were that both taurine and malic acid (L-malic acid di-sodium salt) at an inclusion level of 0.3%, were no more

palatable than the control. The salts calcium chloride and sodium dihydrogen phosphate have been reported as being amongst the most excitatory compounds at dose rates of 50mM (Boudreau and White 1978). This conclusions from this trial were that calcium chloride did not increase the palatability of milk but that sodium dihydrogen phosphate when included in milk at 0.3% significantly ($P<0.05$) increased the palatability. The results showed that urea, creatine and creatinine were no more palatable than un-supplemented milk, which is consistent with previous reports (White and Boudreau 1975).

From this trial a better understanding of the concept of palatability was gained and so to an insight into some of the specific compounds that domestic cats find palatable. Future research into the palatability of specific compounds to domestic cats needs to be aimed at testing a wider range of compounds, as well as investigating the dose response for palatability. An optimum dose needs to be determined for compounds found to be palatable, such as proline, lysine hydrochloride and histidine, in order to optimize the palatability of these amino acids. It would also be of interest to ascertain the effect of the counter compound on the palatability of the compounds tested here, such as hydrochloride for lysine and cysteine.

Chapter 6

General discussion

This study certainly supports the statement that palatability is a complex area of feline nutrition, due to the finicky eating habits of the cat (Bradshaw *et al.* 1996). The cat is in fact highly sensitive to minute changes in the sensory properties of its food (Hirsch *et al.* 1978) and cats, like humans also have very different individual food preferences, a point which can also be illustrated by the results of this study. Unfortunately for pet food manufacturers, the concept of palatability is one which is vitally important because their pet food will not be eaten and therefore bought again unless it is highly palatable.

To date very little is known about the specific compounds and ingredients cats find palatable (Houpt *et al.* 1978), however, some animal by-products, particularly fish offals have been reported as being palatable to the cat (MacDonald *et al.* 1984). Fish by-products could offer a cheap source of protein if found to be palatable to the majority of cats, and the discovery of a palatable New Zealand fish species could result in the availability of a unique product to the pet food industry in New Zealand.

The trial using synthetic diets found that salmon by-product and jack mackerel by-product were palatable, and hoki by-product was unpalatable to the test cats. The mussel by-product and control diet were equally palatable. However, these results are only based on a very small sample size (n=2), due to the study being abandoned early on and therefore not a lot can be taken from them. In terms of composition, these four fish by-products were also very different. Although jack mackerel by-product and salmon by-product both seemed palatable to the cat from the limited amount of data collected, their compositions were quite different, with the salmon by-product containing high quantities of fat and low quantities of crude protein and the jack mackerel by-product containing high quantities of protein and only moderate fat levels. From the data the cats appeared not to like the hoki by-product, despite it containing high amounts of crude protein and amino acids and moderate amounts of fat and fatty acids. Overall the un-supplemented synthetic control diet was not very palatable; a problem which is commonly encountered with synthetic diets and therefore makes them difficult to use successfully in palatability trials. This resulted in low feed intakes for this study and meant the cats did not satisfactorily maintain their bodyweights. Therefore, the majority of the cats had to be removed from the study and consequently the trial was ended after the initial experimental period. The results for this trial using synthetic diets are therefore only based on the small sample size of two and realistically are of limited value. Also despite finding the jack mackerel by-product to be palatable to cats, this fish is of limited use commercially as jack mackerel is only available as

whole fish and therefore the viscera is not obtainable by itself. The aims of this study were achieved in respect to determining the efficacy of using synthetic diets in palatability trials for cats. It is clear from this study that unless the researcher is prepared to spend a large amount of time trying to make these diets highly palatable, they are more of a burden than they are worth. While they may have the benefits of being easy to manipulate and being free of any extra complications such as processing, they are obviously naturally unpalatable to cats and this problem means it is extremely difficult to obtain any worthwhile palatability results using these types of diets. The second aim which was to determine the palatability of the fish by-products when they were included in the synthetic diets was not satisfactorily achieved due to the incompleteness of the results and therefore their limited value. If future palatability research is carried out using synthetic diets I suggest it needs to be aimed at pre-weaned kittens, as it would be easier to wean these young, inexperienced kittens onto a synthetic diet than older cats used to receiving a palatable commercial diet. However, care would need to be taken to ensure the diets were complete and balanced for the growing kitten and energy dense enough so that the kittens used would be able to consume sufficient to meet their daily nutrient requirements in this crucial life stage.

Studies conducted in the past have reported that fats improve the palatability of cat diets (MacDonald *et al.* 1984), which reflects our finding that salmon by-product is palatable to the cat, as the salmon by-product contained large amounts of fat. However, the palatability of the hoki by-product does not reflect these findings of MacDonald *et al.* (1984), as it was unpalatable to the test cats, yet contained reasonable quantities of fat. It is also well known that the natural diet of the cat contains high protein levels, therefore, it may be expected the cats would like the fish by-products high in protein, such as hoki, however, this does not appear to be the case as the hoki by-product was unpalatable to the cats and the salmon by-product, which is low in protein, was liked by the cats. These compositional differences between the fish by-products and therefore the observed differences in palatability may be due to the differences in habitats of the fish species and also the way in which the fish are caught and handled. Salmon and mussels are farmed species, whereas hoki is a middle water fish which is caught by mid winter trawling. The ages of the fish used would also be of interest.

The quantities of omega 3 fatty acids in these fish by-products are of particular interest, because of their contribution to cardiovascular health. The omega 3 fatty acids EPA (C20:5) and DHA (C22:6) were not measured in these fish, however, the omega 3

fatty acid linolenic acid (C18:3) was found in varying amounts in all the by-products except mussel. The quantities of this fatty acid were particularly high in the salmon by-product.

The second trial testing the palatability of the fish by-products when they were included in commercial diets illustrated again that the salmon by-product was palatable to the cat and that in this case mussel was palatable to the test cats also. However, the salmon, mussel and barracouta by-products were not significantly different from the control in terms of palatability. Again the hoki by-product was found to be unpalatable. The fresh salmon by-product contained high levels of fat and moderate levels of protein, and the mussel by-product contained high protein levels and very low fat levels, yet both of these by-products were palatable to the cat. The hoki by-product, which was unpalatable to the cats, contained the lowest levels of crude protein and highest levels of fat. This composition obviously had a negative impact on palatability. It could perhaps be speculated that the hoki diet was less palatable to the cats than the salmon diet, despite it having similar levels of fat, because it did not contain the large quantities of omega 3 fatty acids that the salmon by-product did. These differences in composition and palatability may again be due to differences in habitats, ages and harvesting methods between the different species.

The trials where the fish by-products were added to commercial diets were highly successful and all test cats remained healthy throughout the trial. From these trials the palatability of the different fish by-products was successfully determined. Salmon and mussel by-products may be of benefit to use in pet food to increase palatability, however hoki by-product should not be used in commercial cat foods as this fish is unpalatable to cats. Mussel by-product may be of particular interest for future palatability research because New Zealand is the only country worldwide which produces the green shell mussel and it would therefore be a product unique to the New Zealand pet food industry.

The study aimed at fractionating fish by-products and testing their palatability when they were included in commercially canned diets was also successful, with both aims being achieved. When the salmon and barracouta fish by-products were fractionated and palatability tested, it was found that salmon oil was highly palatable and salmon water and barracouta water were only as palatable as the control diet used. Despite barracouta water and salmon water being equal in regards to palatability, they have quite different compositions. Barracouta water contains higher quantities of crude

protein than salmon water, but contains nearly half the amount of total fat than salmon water. As one may expect, the quantities of total fat in the salmon oil were high, however there was no other oil of which to compare this to. The salmon offal was obviously palatable due to the oil content. Fish oils, such as salmon oil may also be of benefit in the pet food industry, but further testing of different types of fish oils would be required to verify this. It may also be useful to use a different commercial base diet to test the whole fresh fish by-products and fractions in, to determine the effect this diet has on the level of palatability observed. Future work using fish by-product fractions could also be aimed at determining the optimum dose rate for including these fish by-product fractions in diets and establishing the cost effectiveness of using fish by-product fractions rather than homogenised fish frames.

Of the compounds tested in the pet milk, proline, lysine (lysine hydrochloride), histidine, glycine, cysteine (cysteine hydrochloride) and sodium *di*hydrogen phosphate were the most palatable at the 0.3% inclusion rate. The other compounds were no more palatable than the un-supplemented control milk. Proline and lysine hydrochloride were also palatable at the 0.6% dose, however histidine was no more palatable than the control at this inclusion rate. The results for proline, lysine hydrochloride, histidine and cysteine hydrochloride are consistent with the findings of Bradshaw (1991) and White and Boudreau (1975) that these four amino acids are palatable to the cat. In contrast, Boudreau and White (1978) and Bradshaw (1991) reported that arginine has negative effects on palatability, yet in this study, arginine had a similar level of palatability as the un-supplemented pet milk. Previously taurine and malic acid have been reported to trigger the cats' taste systems (Bradshaw 1991) however in this trial taurine and malic acid (L-malic acid di-sodium salt) were no more palatable than the control milk. Urea, creatine and creatinine were no more palatable than the un-supplemented milk in this study which is consistent with studies conducted previously (White and Boudreau 1975). Calcium chloride and sodium dihydrogen phosphate have been said to be among the most excitatory compounds to the cats' taste systems (Boudreau and White 1978). In this study, however, although sodium dihydrogen phosphate was palatable to the cats, calcium chloride was only as palatable as the plain control milk.

A much wider range of compounds, such as nucleotides and various salts and acids, needs to be tested in the pet milk, as the number tested here was limited by time and the availability of the compounds. It would also be beneficial to establish the effect that the counter compound involved has on palatability. Also the dose dependency of

the compounds needs to be established in order to determine the optimum dose for using the compounds as palatability enhancers in cat foods. The testing here relating to dose dependency was again limited by time and therefore only provides a 'snap shot' of this effect. A greater number of inclusion rates on a greater number of compounds need to be tested. However, overall this trial produced some excellent results while achieving the aim.

In conclusion, although the study here shows some clear trends in the food preferences of domestic cats, further research needs to be conducted using a wider range of by-products and compounds and different base and control diets. The trials illustrated the benefits of using commercially canned diets over synthetic diets for palatability testing. From these trials it seems salmon and mussel by-products may be of benefit for use in cat food, as may be the compounds proline, lysine hydrochloride, histidine, cysteine hydrochloride, glycine and sodium dihydrogen phosphate. Hoki by-product should not be used in feline diets if a high level of palatability is required.

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