DEVELOPMENT OF A NOVEL CAT LITTER FROM OLIVE OIL WASTE PRODUCTS

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Abstract

The aim of this study was to develop and test a novel cat litter (O-Litter) from the waste products of olive oil production. In order to do this, the waste products had to be tested to see if they had the characteristics of marketable cat litters. This was accomplished through three main experiments: cat preference for the litter, litter absorption capacity, and litter odour control.

To date, there is very little published research on cat litter and cat preferences. Therefore, much of the background information for the experiments described in this thesis came from patents, websites, or cat owners unsatisfied with litters and providing suggestions. Before starting any experiments on the development of a new cat litter from a waste product, determining which characteristics of a cat litter were of most importance was needed. A review of the available information did identify the main characteristics required, and subsequent experiments were performed to test them.

A pilot study was performed to determine an appropriate amount of time to leave the litter boxes in the cage with the cats in order to determine a preference between litters. Ten cats were given two types of litter in two litter boxes, with the positions interchanged daily for ten days. From this pilot study, we were able to determine that cats only needed exposure to the litter for two days to properly determine which litter they preferred.

The preference experiment was conducted with ten cats from the Centre for Feline Nutrition at Massey University. The cats were tested using a pair-wise preference test. Ten combinations of five different cat litter types (Vitapet Cat Litter, Natural O-Litter, Pellet A O-Litter, Pellet B O-Litter, and a control (empty litter box)) were used for a total of twenty days. Records of litter weight change and amount of excrement produced were used to determine which litter type the cats preferred. The results determined that based on the measurement criteria, the cats preferred the commercial brand, followed by Natural O-Litter.

For the absorption and odour control experiments, eight litter types were compared (VitaPet Purrfection, Breeder Celect, Excellence Ultra-Hygienic, VitaPet Cat Litter, Pellet A O-Litter, Pellet B O-Litter, and two versions of Natural O-Litter: old and new). In the absorption experiment a specified volume of water was added to the eight litter types (above). After 30 minutes the litter was strained, and it was determined that VitaPet Purrfection (which is a clumping variety of litter) was the best litter at absorbing water, followed by the two pelleted varieties of O-Litter.

For the odour control experiment, an ammonia-based cleaner was added to the litter which was contained in a preserving jar to prevent the ammonia from escaping. A filter paper soaked with hydrogen sulphate was used to absorb the ammonia that was not absorbed by the cat litter. An auto analyser was then used to determine the amount of ammonia absorbed by the filter paper, thus not absorbed by the cat litter. The results from this study showed that the two pellet varieties of O-Litter were the best at odour control for each time interval tested. In fact, all four O-Litters tested performed better than the commercial brands at absorbing the ammonia.

From these experiments, we are able to show that O-litter has the potential to make a marketable cat litter. When the O-litter products were compared to commercial brands, they either had qualities that were equal to or better than the commercial products they were compared with. Due to time constraints, not all of the characteristics of the litter were fully tested and some of these characteristics (e.g. dust control, clumping, and tracking) should be tested before the product goes on to the market. However, even without these additional tests, O-litter shows great potential as a cat litter.

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Chapter 1: General Introduction

Domestic cats (*Felis domesticus)* are kept by humans for many reasons: for rodent control, as a research animal, as a catalyst and facilitator in human relationships, for psychotherapy, and most commonly, for simple companionship (Molteno et al., 1998). New Zealand has one of the highest rates of cat ownership in the world. Over 53% of New Zealand households own at least one cat, and 18% own two or more cats (New Zealand Pet Food Manufacturer's Association).

In New Zealand, many cats are kept indoors, at least part of the time (Molteno et al., 1998), therefore, these cats need somewhere they can eliminate faecal and urine material. Domestic cats prefer to hide physical and olfactory evidence of their presence by burying their excrement (Beaver, 2003). This behaviour has led to the development of kitty litter, a product designed to allow faecal and urine elimination indoors.

The granular nature of litters allows animals to express their natural burying behaviour. When kittens are three weeks old, they begin to show elimination behaviour without the assistance of their mother. It is around this time that kittens begin to show "earth-rake" behaviour while in the dirt or litter box. Within a few days, the kitten will consistently use a litter box, eliminating faeces and urine in this litter and then covering up their excrements (Beaver, 2003).

Burying excrements is carried out to hide the physical and olfactory evidence of the cat from other cats. In the wild, cats such as tigers, cheetahs, and lions will not bury their excrements because they use them to mark their territories (Molteno et al., 1998). Territorial marking can also be achieved by spraying, whereby the cat marks something like a tree to show its territory. In domestic cats, this usually is only seen with a dominant feral cat. It is believed that domestic cats bury their excrements because they view their owner as dominant (Molteno et al.,1998).

The overall aim of this thesis was to evaluate the suitability of the waste products of the olive oil industry as a novel biodegradable cat litter (O-litter). The desirable characteristics of cat litters currently on the market are known and are outlined in Chapter two, along with a review of the literature on the cat-human relationship, cat behaviour, measurement of animal preferences and product development. Due to the small number of published scientific studies in this subject area, patents and websites are used as additional sources of information. The process of making a waste product of the olive oil industry into a biodegradable cat litter with the desirable components was novel and was the subject of this research project. With cat ownership and therefore cat litter usage increasing in New Zealand, a biodegradable cat litter is likely to be highly marketable.

To determine whether olive oil waste products could make a marketable cat litter, it was first necessary to evaluate whether cats would use it. Before starting a study to examine this, a pilot study (chapter three) was performed to determine the best methodology for the main experiment on cat preferences. After completing the pilot study, the main experiment formally testing the preferences of cats for various litter types was performed (chapter four).

Once it was determined that cats would, in fact, use the O-litter, laboratory testing was undertaken. Chapters five and six describe laboratory tests on several types of O-litter and four commercial litters. In chapter five, the ability of the litters to absorb liquid was measured. In chapter six, odour control properties of each of the litters were assessed. Chapter 7 provides a general discussion of the results of the three main experiments and discusses the suitability of olive oil waste products as a kitty litter.

Chapter 2: Literature Review and Research Question

2.1 Introduction

The domestication of the cat (*Felis domesticus)* was a gradual process and the precise origin and date of domestication cannot be precisely established. Bokonyi (1969) believed that the domestication process occurred in two phases:

1) Animal keeping: capturing and keeping of animals without deliberately attempting to modify the animals behaviour or alter breeding
2) Animal breeding: controlling the breeding of the animal and modification of its behaviour

If domestication was based on these phases, then the cat was not truly domesticated until the late 19th or early 20th century (Turner et al.,2000; Vigne et al., 2004). However, many believe that the cat, along with other carnivores belonging to the Felidae family, were domesticated by the Egyptians around 3000 BC (Figure 2.1; MacDonald & Rogers, 1984).

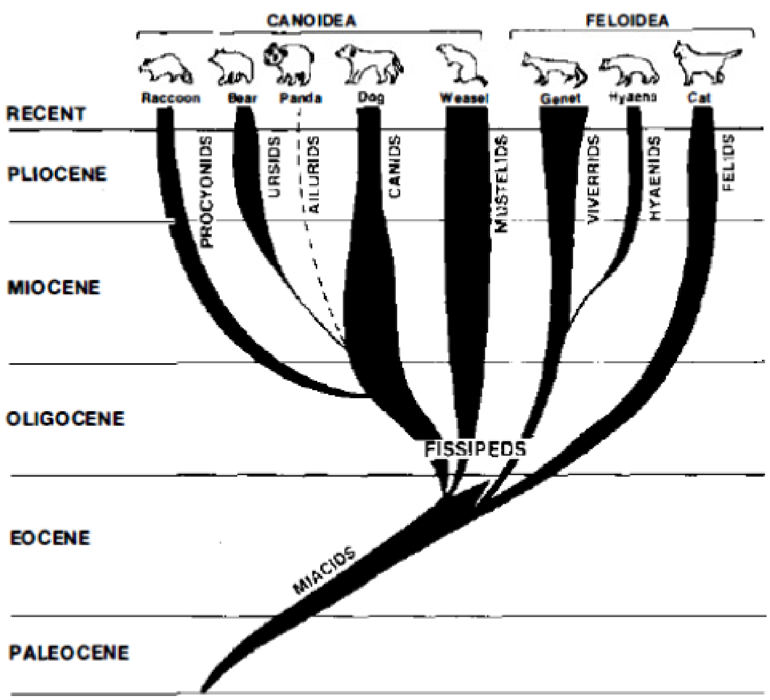


Figure 2.1: The evolution of the cat and its relationship to other animals in the order Carnivora (MacDonald & Rogers, 1984).

It is believed that the domestic cat of today was derived from the interbreeding of the European wild cat (*F. silvestrus*) and the African wild cat (*F. lybica*) (MacDonald & Rogers, 1984; Driscoll, et al., 2007). When classifying the cat, Carl Linnaeus named it *F. catus*, which he described as having blotched tabby markings. It wasn't until shortly after the mackerel coat type appeared (*F. torquata)* that the all-inclusive name *F. domesticus* appeared to represent all domestic cats.

2.2 Cat ownership and the kitty litter market

As mentioned above, the Egyptians saw their cats as sacred animals which they fed with bread, milk, and Nile fish, and even had them mummified with them when they died (MacDonald & Rogers, 1984). Even today, cats are considered cherished companions and are spoiled by their owners (Case, 2003). New Zealand has one of the highest levels of pet ownership in the world, with cats being the most common companion species (The New Zealand Companion Animal Council Inc, 2009). In 2008, there were approximately 1.1 million pet cats in New Zealand, with this number increasing by 0.5% annually (Euromonitor International, 2008).

Cat owners in New Zealand have traditionally used litter to toilet train kittens, but allowed their adult cats to toilet outside. However, owners are increasingly taking greater responsibility for keeping their cats inside at night to protect bird life. In addition, many owners in urban areas are opting to keep their cats indoors at all times due to increasing traffic levels. Therefore, the demand for cat litter is increasing in New Zealand (Euromonitor International, 2008).

The global kitty litter market is worth $350 million per annum and there are over 75 different commercial brands sold worldwide (Moser, 1988). New Zealand kitty litter sales were estimated to be $8.4 million in 2008 and growing annually in value by 9% (Euromonitor International, 2008). It has been estimated that for every dollar of cat food bought, about $0.25 worth of cat litter is purchased (Godley, 2003).

The increase in the New Zealand cat population will continue to drive the need and use of cat litter in New Zealand. Despite the 9% increase in overall sales over the last year, sales of clay and silica cat litter have been declining (Godley, 2003). Instead, sales of clumping and alternative forms of cat litter have increased. The reason may be that owners are becoming more educated about the types and performance of different cat litters today than they were in the past, and understand the benefits of the more expensive litters (Godley, 2003).

When purchasing a cat litter, consumers traditionally considered characteristics such as dust, litter tracking by the cat, and effectiveness at masking odours. However, consumers are becoming increasingly concerned about the effect of litter use on the environment. Most existing cat litters are clay-based and the raw material must be mined. The United States Geological Society estimates that 85% of the 2.54 million tons of clay used in the United States every year is used for pet absorbent waste products (Virta, 2001).

There are two ways in which cat litter affects the environment. Firstly, there is an environmental impact of the mining operations used to extract the clay. Secondly, clay-based cat litters are inorganic and do not break down in landfills. Therefore, use of these types of litters contributes to the increasing amount of space required for landfills globally (Virta, 2001). Therefore, clay-based cat litters affect the environment both through manufacturing and disposal processes.

2.3 Characteristics of marketable kitty litter

Kitty litters currently on the market have a number of characteristics that make them desirable to consumers. These characteristics are outlined below.

2.3.1 Animal preference

A key issue in the development of a new animal product is whether the animals will use it. Therefore, the first step in developing a kitty litter from the waste products of the olive oil industry must be to evaluate whether cats will use it as a litter. One way to achieve this is to conduct preference tests comparing the new O-litters to existing commercial litters.

Preference testing is a common tool used in the study of animal welfare, which is based on the assumption that animals will make the choice that is in their own best interest (Fraser & Matthews, 1997). The first formal preference tests were performed by Hughes and Black (1973). They allowed hens simultaneous access to two different types of flooring and monitored their behaviour to determine which type the hens preferred.

An alternative method is the Y maze preference test (e.g. Rushen, 1986; Pajor et al., 2003). In this method, two different objects or situations are placed at each end of a Y shaped maze. The animal has previously been subjected to both treatment options so they know what is at the end of each arm. During testing, animals are then sent down the centre of the Y maze and choose which side they want to go to, thereby demonstrating a preference.

A common method used for testing an animal’s preference for food is the two bowl free choice preference test (Griffin, 2000). This is where two diets are offered simultaneously to the animal with the bowls placed side by side. The animal is given an allotted period of time to eat the food, after which it is removed and the remaining amount of food is recorded by weight. Statistical analysis is then performed to determine which food was the most preferred. Although for the following experiments we will be using cat litter instead of food, the same principle will be applied. Instead of measuring the remaining amount of food, we will be measuring the amount of urine and faeces deposited in the litter boxes.

A preference test with cats and litter was used by Cottam and Dodman (2007)to determine if cats had a preference for litter sprayed with an odour eliminator. In this study, two litter boxes, containing the same type of litter, were placed next to each other in the cat owners house. The cat owner was asked to spray one of the litter boxes with the odour eliminator twice daily and record the cats interactions with the two litters. The litter boxes remained in the house for a four day period, with the boxes switched in their position on day two to ensure a side preference was not established. Preferences were determined based on a list of criteria which was established before the experiment was performed. Cottam's and Dodman's (2007) experiment aided in methodology procedures for chapter 3 and 4.

2.3.2 Odour Control

The cat's olfactory system is much more sensitive than that of humans (Bennet, 2002), with the surface area of olfactory epithelium being almost seven times larger (LeMagnen, 1951). Mammals have two olfactory organs: the main olfactory epithelium and the vomeronasal organ (Figure 2.2) (Keverne, 1999). The main olfactory system recognises most odours and sends the signals to a higher sensory centre in the cortex. This allows the animal to recognise and respond to the odour. The vomeronasal organ detects pheromones and sends the information through a different set of odorant receptors and neuronal projections are sent to different parts of the brain (Klein, 2000).

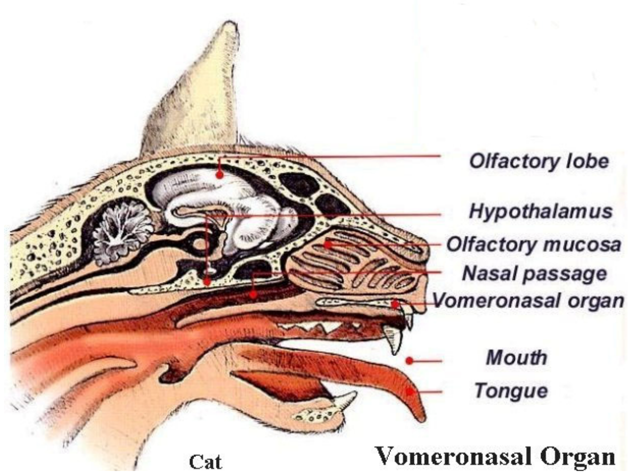


Figure 2.2: The cat's vomeronasal organs (Keverne, 1999).

The mammalian olfactory systems can distinguish thousands of different molecules (Klein, 2000). Odours that contact the nasal mucosa are sensed by olfactory neurons and stimulate the olfactory receptors. When an odour enters the nostril during inhalation, it travels from the back of the nasopharynx to the roof of the nasal cavity where the odour excites the neuron (Mennella & Beauchamp, 1998). The chemo-sensitive olfactory neuron and its central process passes to the olfactory bulb which is located in the central nervous system of the animal. The signals are then sent to the higher cortex an limbic system where they are decoded and a response to the odour occurs (Leffingwell, 2002). Second order neurons then pass to various areas of the cerebral hemisphere: amygdalae, piriform cortex, and entorrhinal cortex and the orbitofrontal cortex. The orbitofrontal cortex contains the secondary and teriary olfactory cortical areas which are important for the identification and discrimination of the odour (Rolls, 1996).

The sense of smell can be used in two ways: on its own in isolation, or together with the sense of taste. It is believed that if cats find the odour of one food more attractive than the other they will exclusively eat the more attractive smelling food (Hullar, et al. 2001). However, if neither of the diets have an attractive smell, the animal will taste both foods and make a decision. In our study however, we are not expecting the cat to taste the litter, we are hoping that when the cats is given two choices of litter, O-Litter will have a more attractive smell, or at least not an off-putting odour, therefore, the cat will only use it.

Odour control is an important characteristic of cat litter since the cat's olfactory receptors are very sensitive. An adult cat will use the litter box on average five times a day, producing between 20 to 44 ml/kg of urine and faeces, which in turn produces an odour (Neilson, 2004). The particularly strong smell of cat urine is due to the high protein diet which is a requirement of the species (Lewis & Morris, 1983; Zoran, 2002). Ammonia, urea levels, and enzymes cause the strong odour (Cliff & Heymann, 1991; Laurie, 2009), and the more concentrated these substances, the more pungent the odour becomes (Laurie, 2009). Table 2.1 gives the components of cat urine from a healthy cat.

Table 2.1: Components of normal cat urine (Cottam, Caley, Wamberg, & Hendriks, 2002).

Component	Percentage
Ammonia	0.05
Sulphate	0.18
Phosphate	0.12
Chloride	0.6
Sodium	0.1
Creatinine	0.1
Uric Acid	0.003
Urea	2.0
Water	95.0

Faecal odour is caused by the products of bacterial action on material in the large intestine (Hesta, et al., 2001). Bacteria aids the digestive system in breaking down nutrients for absorption. This process produces smelly sulphur-rich organic compounds such as indole, skatole, ethylphenol, phenol, p-Cresol, mercaptans, ammonia, and also inorganic gases such as hydrogen sulphide (Hesta, et al., 2001; Terada, et al., 1993). It is because of these odours that cats bury their faecal material. If their prey can detect that a cat may be near then they will avoid the area (Roth, et al., 2008)

Many products can be used to mask or eliminate faecal and urinary odours. The most common additive used to eliminate odours in cat litters today is activated carbon, but sodium bicarbonate (also known as baking soda), is growing in popularity and is also an organic product (Neilson, 2004; 2008a; 2008b). Some litter products have added perfumes that can actually deter the cat from the litter. For example, Neilson (2008) found that cats had a preference for odours of fish, bleach, and cedar, but that they rejected the smell of flowers and citrus. Some commercial litters currently use additives, such as a citrus fragrance to make them more pleasing to the owner, despite the fact that the opposite may be true for the cat (Neilson , 2008b).

2.3.3 Absorbency

Absorbency is the only characteristic that the Code of Welfare recognises as important for cat litters. It states that indoor cats must be provided with an absorbent material for them to deposit their faecal and urine material in to (The New Zealand Companion Animal Council Inc, 2009). Although this is an important characteristic, very little information could be found on absorbency of cat litters. Obtaining research that showed if cats preferred absorbent material over non-absorbent material could not be found. The only research found that was based on the litters ability to absorb liquids was provided by Richards (1991). He tested the absorbency of a novel cat litter by adding a specified amount of water to an equal amount of cat litter. This gave an indication of how the litter would perform for absorbing liquid, however, results may be different if cats urine was used.

2.3.4 Clumping

Another characteristic that consumers look for in kitty litter is whether it clumps (Hardin, 1993). Some of the clumping cat litters are made from montmorillonite, sodium bentonite, or smectite, which are all types of clay and thus contribute to the increasing volumes of litter entering landfills (Hardin, 1993). A clump is described as the particles firmly adhering to each other when wet to create a mass that has sufficient physical integrity to be removed from the remainder of the particles without crumbling or loss of surrounding material. When a clump is formed the liquid, in this case urine, is trapped within the clump and is removed with the clump. The litter material that surrounds the clump remains dry and can therefore be saved for later use. This characteristic reduces the frequency with which owners have to totally replenish the litter but does require additional litter be added more often (Hardin, 1993). This results in higher litter consumption and more frequent purchasing than non-clumping products, which in turn, contributes to the overall sales growth of the litter (Benkouider, 2003). Clumping litter is not recommended for kittens since they tend to eat the litter which has been reported to cause intestinal blockages (CBS, 2000).

2.3.5 Tracking and Dust

Low tracking is another characteristic that consumers look for in cat litter. Tracking is where the litter sticks to the paws of the cat when they leave the litter box and is "tracked" or spread around the house (Neilson, 2001). Tracking may not be a concern to cats, but is an irritant to owners because they have to continuously clean up after the cat.

Another characteristic of the litter which should be kept to a minimum is dust. As the cat uses the litter box, small particles of the litter material may be kicked up and spread in the air throughout the house. Neither the owner nor cat should be exposed to dust particles, particularly from litters made from silica (MacQuoid, 2003). Inhalation of silica dust may irritate the eyes and throat, especially for those who suffer from asthma or other allergies. Another problem associated with dust is the frequency with which cats lick their paws. Again, dust from litters containing silica may be ingested and is believed to be a potential carcinogen (MacQuoid, 2003).

2.4 Existing cat litters

About forty years ago, Ed Lowe developed the first cat litter from a "granulated dried mineral clay" (Ward, 1988). Today, there are over 75 brands of cat litter worldwide and the industry is growing in value by an average of 9% per year. Many of the existing cat litters are not biodegradable and concern has been expressed about the amount of litter, estimated to be more than 160,000 tonnes annually (Startup, 2009), being dumped in municipal solid-waste landfills worldwide. With the development of biodegradable cat litters, the amount of litter waste failing to break down in landfills is slowly declining. Other approaches designed to turn waste product streams into cat litter may decrease the amount of waste even further.

There are several different brands and forms of cat litters already available on the market in New Zealand. Table 2.2 lists the litters available in New Zealand along with the average price and claims made about the litter by the manufacturer. There are various clumping and non-clumping varieties with additives to control odour and odour-causing bacteria. Clay-based litters are still the most popular type in New Zealand (Types of Kitty Litter, 2006). These litters are made from a type of clay called sodium bentonite. It is an absorbent aluminium phyllosilicate (a type of rock-forming mineral) generally impure clay, which is formed by weathering of volcanic ash (Bentonite, 2010). This clay expands when wet, absorbing several times its dry weight, which makes it ideal as a cat litter. Clay litter is generally accepted by cats, but it does tend to track and need changing frequently. Some of the popular brands are Tidy Cats (Nestlé Purina® Petcare, Vevey, Switzerland), Fresh Step (The Clorox® Company, Oakland, California), and Excellence Ultra-Hygienic Litter (Virbac Ltd, Auckland, New Zealand).

Clumping litters are increasingly popular in the market because they are easy to clean up. These types of litters have a higher absorption capacity than most of the other types (House, 1993). When the cat urinates, the litter binds together to form a clump, which then can be easily scooped out. These litters require less changing but also come with a higher price. Another problem with clumping litters is they tend to track, where the finer granules stick to the cat's paw when it leaves the litter box, then are dispersed around the house (Hall, 2009). Examples of these clumping litters are Trouble & Trix Lavender Litter (Masterpet, Lower Hutt, New Zealand) and VitaPet Purrfection Clumping Litter (Masterpet, Lower Hutt, New Zealand).

Silica gel cat litter is a relatively new product on the market, made from silica dioxide sand, oxygen, and water (Types of Kitty Litter, 2006). The main advantage of this product is that the litter granules can absorb up to forty times their weight in moisture. Some disadvantages of this product are that it is the most expensive on the market, it is not safe for kittens because of the risk of choking, and the granules are tracked and roll throughout the house (Types of Kitty Litter, 2006; Paws & Claws Magazine, 2008). Litta-Beads (Rudducks Pty Ltd, Auckland, New Zealand) and Animates Litter Crystals (Animates, Auckland, New Zealand) are brands of this type of litter.

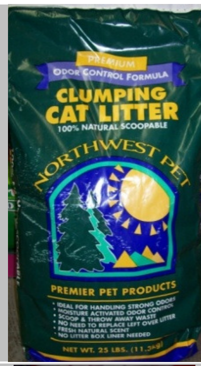
A number of biodegradable litters are already available overseas. These include whole kernel corn cat litter (LitterMaid: Applica© Consumer Products, Florida, and World's Best: GPC Pet Products, Muscatine, Iowa), ground wheat kernel cat litter (Swheat: Farmers Union Industries LLC, Redwood Falls, Minnesota), and citrus peel cat litter (Citra-Max: BDG, Pet Products, Inc, Van Nuys, California). In general, these biodegradable litters are usually more expensive and appear to be less effective in terms of either odour control, clumping abilities, or tracking. Many of these products are not available in New Zealand, so developing a new biodegradable product in New Zealand would provide a unique product for the local market.

Recycled paper is one biodegradable litter available in NZ (Types of Kitty Litter, 2006). This litter is a tightly compressed paper pellet which expands when wet. It is a little more expensive than clay litters and is not as effective in odour control, but is more environmentally friendly and tracks less. Yesterday's News (Nestlé Purina® Petcare, Vevey, Switzerland) and Breeder Celect (Fibrecycle Pty. Ltd, Queensland, Australia) are brands of recycled paper cat litter.

Sawdust can also be used as a cat litter. This product can be purchased in large quantities, is effective at masking odour because of the natural woody scent , but is not as absorbent as other litters and requires changing daily. This is not usually a product that can be purchased at your local supermarket or pet store, so convenience and access to the product may be a problem.

Table 2.2: A comparison of the commercial cat litters currently available in New Zealand based on claims and price.

Litter		Company	Claims	Size in kg (ℓ)	Price/kg*
Animates Clumping Cat Litter		Animates	Odour control Easy clean-up Economical 100% natural	15 kg	$2.00
Animates Litter Crystals		Animates	Reduced tracking Instant odour absorption Antibacterial Clump free	3 kg	$9.50
Breeder Celect Recycled Paper		Fibrecycle Pty. Ltd	Natural odour control Environmentally friendly Highly absorbent	15 ℓ**	$2.00
Catit Daily Scoops Litter Paper		Hagen	Highly absorbent Minimize odour No tracking Won't stick to tray	6 kg	$4.50
Excellence Ultra-Hygienic Litter		Virbac (NZ) Ltd.	Super absorbent Safer for cats & kittens & families 100% natural Superior odour control	7 ℓ **	$0.72

<table>
<tr><td>Northwest
Clumping Litter</td><td>
</td><td>Northwest Pet
Products Inc.</td><td>Eliminates odour
No need to replace left over litter
No chemicals or additives
No litter box liner needed</td><td>11.3 kg</td><td>$5.00</td></tr>
<tr><td>Trouble & Trix
Value Litter</td><td>
</td><td>Masterpet</td><td>All round effectiveness & value
Biodegradable</td><td>9 kg (15 ℓ)</td><td>$2.10</td></tr>
<tr><td>Trouble & Trix
Eco Litter (paper)</td><td>
</td><td>Masterpet</td><td>Light
Easy to use
Good absorbency
Biodegradable</td><td>6 kg (15 ℓ)</td><td>$4.15</td></tr>
<tr><td>Trouble & Trix
Lavender Litter</td><td>
</td><td>Masterpet</td><td>Clumping
Lavender scent assist odour control
Biodegradable</td><td>13 kg (15 ℓ)</td><td>$2.60</td></tr>
</table>

Trouble & Trix Angel Litter		Masterpet	Ultimate cat litter Extremely good absorbency Best odour control & dust tracking Longest lasting Biodegradable	6.4 kg (15 ℓ)	$5.50
VitaPet Cat Litter		Masterpet	100% Natural minerals Recyclable in soils Absorbent Neutralises urine odour	7 ℓ**	$0.80
VitaPet Purrfection Clumping Litter		Masterpet	Most advanced clumping litter available Last longer Fantastic odour control Maximum absorption 100% natural	5 kg	$2.10

* approximate price/kg – price may vary between stores
** litter measured in litres not kg

2.5 Development of a biodegradable cat litter from olive oil

2.5.1 Why develop a new cat litter?

One of the major issues associated with the increasing use of cat litters worldwide is the impact on the environment. The traditional cat litters sold in New Zealand are made from a type of clay called sodium bentonite. This is an absorbent aluminium phyllosilicate (a type of rock forming mineral), generally impure clay, which is usually formed after the weathering of volcanic ash (Bentonite, 2010). It expands when wet, absorbing several times its dry weight, which makes it ideal for cat litter. However this product is non-biodegradable and concern has been expressed about the amount of this litter, estimated to be more than 160,000 tonnes annually (Startup, 2009), being dumped in municipal solid-waste landfills worldwide. This has led to the development and marketing of alternative biodegradable sources of cat litter.

2.5.2 Marketing O-litter

Most of the existing biodegradable cat litters sell for 10% more than standard cat litters. It is anticipated that because the proposed product utilises a waste product stream from the production of olive oil, it can be sold for less than existing biodegradable litters, making it more affordable to the customers. We hope that by minimising cost, more customers will be able to purchase the "O-litter" (which is a biodegradable product), therefore limiting the volume of non-biodegradable cat litter waste in landfills.

Purchasers of this new cat litter are likely to be consumers who are concerned about the environment and are aware of how traditional cat litters affect it. Traditionally, purchasers of biodegradable cat litters have been cat breeders, cat fanciers, and the environmentally aware (Dempsey, 2010). The aim is to attract customers who currently purchase the traditional litter types, as well as those who currently purchase other biodegradable litters.   This may be achieved by The Village Press providing a clear understanding of the properties and advantages of a litter developed from olive oil by-products, followed by an effective marketing strategy, informing consumers of these advantages, while keeping cost to a minimum so it remains an affordable option.

2.5.3 The Olive Oil Industry

The Village Press was established in 1996 as a small family enterprise in Havelock North but has grown rapidly to become New Zealand's largest olive oil producer (Startup, 2009). In the 2008 season, The Village Press produced 120,000 litres of extra virgin olive oil, and 300,000 kilograms of waste (skin, pulp, stone, and husks). This production of olive oil, and therefore also biodegradable waste, is expected to continue to increase exponentially for at least the next 6 years to reach 400,000 litres of oil by 2014 with a corresponding increase in waste to 1,000,000 kilograms (Startup, 2009).

The production of olive oil involves a two stage process: 1. milling the olive fruit and 2. separating the oil from the water, which results in olive oil, solid waste (stones, skins, husks, and pulp), and waste water (Therios, 2009). There are two main systems used for the extraction of olive oil: a traditional discontinuous processing system, and a continuous-cycle centrifugation system. In the traditional system, milling is performed with a millstone or hammer stone and the extraction of the oil is done by hydraulic pressure. In the continuous-cycle system, the olives are ground by metal crushers, producing an olive paste, which is then centrifuged in a horizontal centrifugal decanter. The oil is then separated from the waste water by a vertical centrifuge. For extra virgin olive oil, the olive paste is cold pressed to influence the phenolic content (Therios, 2009). The Village Press uses a continuous-cycle centrifugation system (The Village Press, 2007).

The waste products from the olive oil production are ground to a small particle size during the production process. While it is still wet, this material is soft and mulch-like, but when dry, it has the consistency and texture of saw dust. Currently, no productive use for the waste products has been identified. The Village Press has started to investigate whether it would make for a suitable feed for horses, but testing has not been completed. The company has also used the waste on its grape vines as a fertiliser. However, again, there are no data on whether the waste product is suitable as a fertiliser. Therefore, up until now, the majority of the waste product has been sent to landfills.

2.6 Assessing suitability of olive oil waste products as a cat litter

In order to determine whether olive oil waste products will make a suitable cat litter, we must first determine if it has similar characteristics and performance to existing commercial cat litter. This thesis will only investigate some of these characteristics: cat preference, absorbency, and odour control. Determining whether cats will use the litter made from the waste products is the most important aspect of the research. If cats will not use it consistently, then there will be no point of continuing the research. However, the waste material does have the consistency of some other forms of cat litter, and anecdotal evidence suggests that cats will use it. Formal testing of cat preference, further processing options, moisture absorption, and odour controlling properties of the olive oil waste products will determine if "O-Litter" will make a suitable cat litter.

2.7 Research questions

The following questions will be investigated through a series of experiments presented in this thesis:

Will O-litters have the characteristics suitable for use as a cat litter?

Will the performance of O-litters match other commercial cat litters?

Chapter 3: Pilot Study for Litter Preference Experiment

3.1 Introduction

With indoor cats using the litter box an average of five times a day (Neilson, 2004), it is important to make sure that the selected litter is one the cats will use. Therefore, an important component of developing a novel cat litter is to determine whether cats will actually use the product. This requires testing of cat preferences among different litters.

There is very little published information on cat preferences, with most studies relating to cats based on testing of diet preferences. Published research on the development of cat litter is largely limited to patents. Therefore we started this process with little background knowledge to guide our experimental design. Performing a pilot study provided the information necessary to design an experiment to determine cats' litter preferences.

General preference protocols from Griffin (2000) were used to aid in designing an experiment to determine litter preferences. Griffin's protocols were chosen because his experimental design could be easily applied to cat litter preferences, with a few alterations. Griffin used a two bowl preference test to determine which type of food cats preferred. The cats were given two different types of food for an allotted period of time and preferences were determined by the amount of food the cat ate. Although Griffin tested food preference, we will apply the same principles to test for litter preferences.

In testing preference for food, the food trays do not need to remain in the cage for a long period of time, unlike testing litter preferences. Time is needed for the cats to be able to use the litter box since they may not need toilet directly after placing the litter boxes in. When testing food, preferences are usually determined by the amount of food eaten; this method will not work in this scenario. Therefore, determining which methods are appropriate for determining a preference is necessary. Also, determining if cats prefer to use the litter box on one side of the cage is necessary for accurately determining a litter preference, not a side preference.

In this pilot study, we will be concentrating on the following objectives:

- How long do the pairs of litters need to remain in the cage to establish a consistently expressed preference from an individual cat?
- What variables should be measured to evaluate litter preferences?

- What criteria can be used to indicate a significant individual preference?
- Do cats express a side preference rather than a litter preference?

From the results of this pilot study, we can determine how the main preference study should be performed.

3.2 Materials and Methods

3.2.1 Animals

Ten domestic short-haired cats from Massey University's Centre for Feline Nutrition were used for this pilot experiment. All of these cats were bred and raised at the Centre. The cats ranged from 1.5 to 11 years of age and there were five males and five females (Table 3.1). All cats were fed *ad libitum* Chef canned cat food and had access to water, as per standard Centre practice. Fresh food and water were offered each day. Before this experiment, cats were kept in colony pens consisting of up to 10 cats each and used sawdust for a cat litter; however, the cats were familiar with the individual cages used for the preference testing.

This study was carried out under Massey University Animal Ethics blanket policy through the Centre for Feline Nutrition: MUAEC protocol 09/62.

Table 3.1: The name, sex, birth date, age, and relatedness of the cats used for the pilot experiment.

Cat's name	Sex	Birth date	Age (years)	Sibling
Aura	male	12 November 2002	7.5	
Beeva	male	7 May 2001	9	
Billi	male	29 October 2008	1.5	Lea
Ra	male	20 January 2005	5	
Raven	male	11 March 2007	3	
Lea	female	29 October 2008	1.5	Billi
Jade	female	20 January 1999	11	
Tui	female	8 December 2004	5.5	
Rach	female	19 November 2002	7.5	
Esta	female	30 March 2007	3	

3.2.2 Cage and Litter Box Design

Cats were kept in individual cages for the duration of the pilot study: ten days. The period of ten days was chosen for sufficient time to allow the cats to familiarise themselves with the litter and also to determine if they would establish a preference within that allotted period of time. The litter boxes (25.5cm x 42.5cm) were placed in the back of each cage, raised approximately 9 cm above floor level because of a permanent plastic device for holding a single litter box used for the Centre's regular nutrition studies (Figure 3.1 and 3.2). The trays were raised using wood blocks placed underneath the front and back to stabilise them. The door of each cage contained a compartment to hold the food and water dishes. Food and water were placed in this compartment for all cats with the exception of one cat (Ra) that tipped his food and water dish over unless placed in the cage.

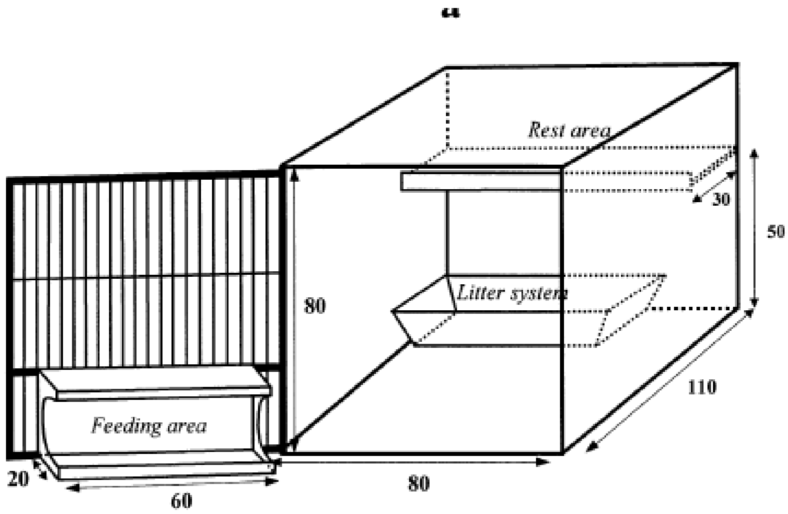


Figure 3.1: Design and dimensions of the single cages used to house the cats during the pilot study (modified to fit two litter boxes as shown in Figure 3.2) (Hendriks, et al., 1999).

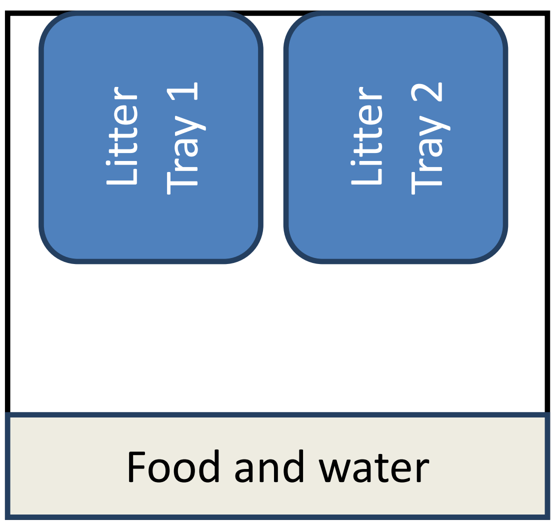


Figure 3.2: Plan of the single cage used for the pilot study. Instead of one litter tray in the back of the cage, the trays have been raised 9 cm on stable wooden blocks and rotated 90 degrees to allow the addition of another litter tray.

3.2.3 Litter Types and Processing

Five different types of litter were used for this experiment: a commercial brand, a negative control, the natural olive waste material, and two modified (pelleted) litters produced from the olive oil waste. The commercial litter was chosen because it is a commonly-used litter sold in supermarkets: Vitapet Cat Litter (Masterpet Ltd., Lower Hutt, NZ). This litter is a cheap, yet an efficient litter, that is used by many in their households. This litter was a basic mineral clay, non-clumping litter that is absorbent and neutralises the odour from urine. The negative control was an empty litter box which was used to determine whether the cats demonstrated a strong avoidance of any of the litters.

The natural raw material was the waste stream from olive oil production (pits, skins, and husks), obtained from The Village Press (Bridge Pa, Hawkes Bay, New Zealand). The raw material was obtained wet and had to be dried before it could be used as a litter (or processed, see below). For drying, the material was divided into small trays and placed in a large drying oven at 90°C for 48 hours. After this was completed, a proximate analysis was performed to determine the amount of protein, fat, ash, and carbohydrate in the raw material. From this analysis, we could tell how much of fat and moisture remained in the waste after the oil extraction process. This information was important because we were uncertain whether residual fat and moisture would affect further processing (pelleting), absorption of urine, and odour control in the finished cat litter.

For those samples to be processed further, the raw material was passed through a Hobart mixer (Hobart Manufacturing Co., Troy, Ohio) to break down the clumps of material. After mixing, pellet form A went through a hammer mill (Model DOL-16, Sprecher + Schuh, Houston, Texas) in which it was ground into a fine (<3mm) dust- like substance. Pellet form B was not hammer-milled before pelleting. An Orbit 15 pelleter (Richard Sizer Ltd., Kingston Upon Hall, United Kingdom) was used to produce 7mm pellets for both A and B samples (Figure 3.3).

Figure 3.3: Orbit pelleter.

3.2.4 Litter Combinations

One main purpose of this pilot study was to determine how long the litter combinations should be left in the cages to accurately determine the preferences of individual cats. Each cat was given one litter combination for a period of ten days (Table 3.2). During that ten-day period, the position of each litter tray was swapped from one side of the cage to the other on a daily basis. This re-positioning allowed us to differentiate between the cats having a side/location preference and a litter preference.

Table 3.2: Litter combinations offered to each cat for the ten day test period.

Cat	Litter Combination	
Raven	A	B
Tui	A	Natural
Ra	A	Commercial
Aura	A	Empty
Rach	B	Natural
Lea	B	Commercial
Beeva	B	Empty
Jade	Natural	Commercial
Esta	Natural	Empty
Billi	Commercial	Empty

3.2.5 Measurement of Cat Preferences

The preferences of individual cats were assessed by measuring objective parameters of litter box use.

3.2.5.1 Measurements

Day 0

On the first day of each combination (day 0), the litter boxes were cleaned with a disinfectant dishwashing liquid and allowed to air dry. A tare weight was taken of each empty litter box so we could determine the amount of litter added and daily weight change. Approximately 5.4 litres of litter (between 600g and 1200g depending on litter type) was added to the litter box. The weight of the box and litter was recorded to the nearest hundredth gram ("pre" value). The boxes were then placed into the cats' cages according to Table 3.2, taking note of which litter type was on each side. This was done between 0700 and 0900 hours.

Days 1 through 10

Each morning, the boxes were removed between 0700 and 0900 and weighed (box plus litter plus any excrements = ”post” value). Faecal excrements and urine clumps were removed after weighing using a pooper scooper. Urine clumps were defined by the area of the litter that was wet from urine. After removal of the excrements, the litter box and remaining litter were weighed again – this value was used as the “pre” value for calculating weight change from day 2 to day 3 and so on. The two litter boxes were replaced in the cage on the opposite side to previous day.

3.2.5.2 Data Analysis

In this pilot study there was only one cat per litter combination, therefore a statistical analysis was not performed on these data.

Individual cats were considered to have expressed a preference for one litter over the other if there was a weight change of 30 grams or greater in one litter over the other for each day or at least two excrements in the same litter per day. A difference of 30 grams was chosen because this value was higher than the average weight of litter tracked between boxes incidentally (tracking) but lower than the average weight of a single excrement (Table 3.3). Tracking was defined as litter being moved from litter box to another or around the cage, usually from being stuck to the paws. Some cats used both litter boxes in a given day. Using a minimum of two excrements in the same litter per day as the criterion for a preference allowed us to find the *most* preferred litter in such instances.

Table 3.3: Weight change (g) of single excrement and average weight due to tracking measured over all litter combinations and including data from all 10 cats. Standard deviation included for weight change and tracking. Tracking weight change could only be calculated when there was no excrement deposited in the litter box.

Average Weight of Single Excrement (g)	Standard Deviation	Average Tracking Weight (g)	Tracking Standard Deviation
58.4	61.3	5.3	19.1

3.3 Results

3.3.1 Proximate analysis

Table 3.4 shows the results of the proximate analysis performed on the raw olive oil waste material. After 48 hours of drying, the raw material had very little water remaining in it (less than 4%). Of the dry matter, almost all was carbohydrate, with the next highest component being fat. There was very little protein or ash in the raw waste material.

Table 3.4: Results from proximate analysis.

Component (g/100g DM)	Amount (g/100g)
Dry Matter (g/100g)	96.4
Crude Protein	3.3
Crude Fat	6.2
Crude Ash	0.5
Carbohydrate*	90

DM, Dry matter
*Calculated by difference (100-Crude Protein-Crude Fat-Crude Ash)

3.3.2 Litter Box Measurements

The daily weight measurements before use, after use, and the daily change in weight of each litter box, as well as the number of excrements (faeces plus urine clumps) for each cat are presented in Appendix 3.1.

Figures 3.4 to 3.11 show the daily weight change and number of excrements (faeces plus urine clumps) for each litter offered to each cat.

Regardless of the litter offered, all cats offered a litter and the empty box exclusively used the litter (Aura: pellet A (Figure 3.4); Beeva: pellet B (Figure 3.5); Esta: natural (Figure 3.6); Billi: commercial (Figure 3.7).

For those cats offered two different types of litter, the results varied. Two cats showed no clear preferences for the litters over the 10 day period. Raven showed no preference between pellet form A and B, sometimes using both types in the same 24 hour period (Figure 3.8). Likewise, Jade used both the natural olive oil waste material and the commercial litter during the ten day period (Figure 3.9).

In contrast, several cats showed clear preferences between litters. Ra used the commercial litter exclusively when the alternative was O-litter A (Figure 3.10). Tui predominantly used the natural waste material, with the exception of two days where she used pellet form A (Figure 3.11).

Two other cats showed a clear preference for one litter over the other in the first week of the study but then appeared to switch their preference. Rach used the natural waste material exclusively until day 8 when she switched to pellet form B (Figure 3.8). Likewise, Lea used the commercial litter exclusively until day 8, when she began to use pellet form B as well (Figure 3.9).

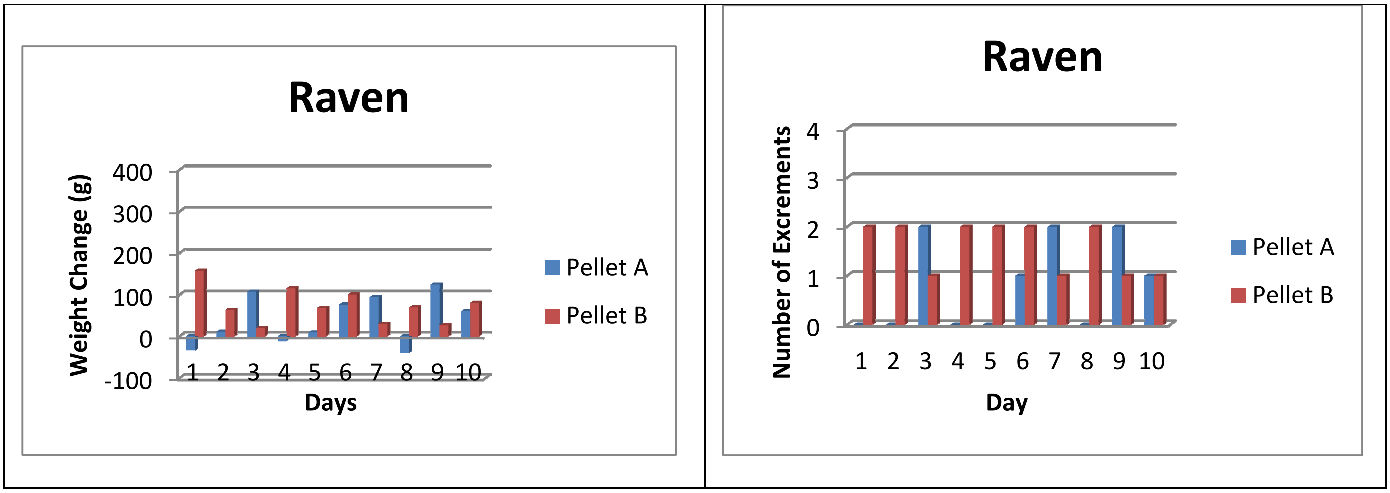


Figure 3.4: Weight change of litters and number of excrements each day for Raven who was offered O-litters A and B over the ten day period.

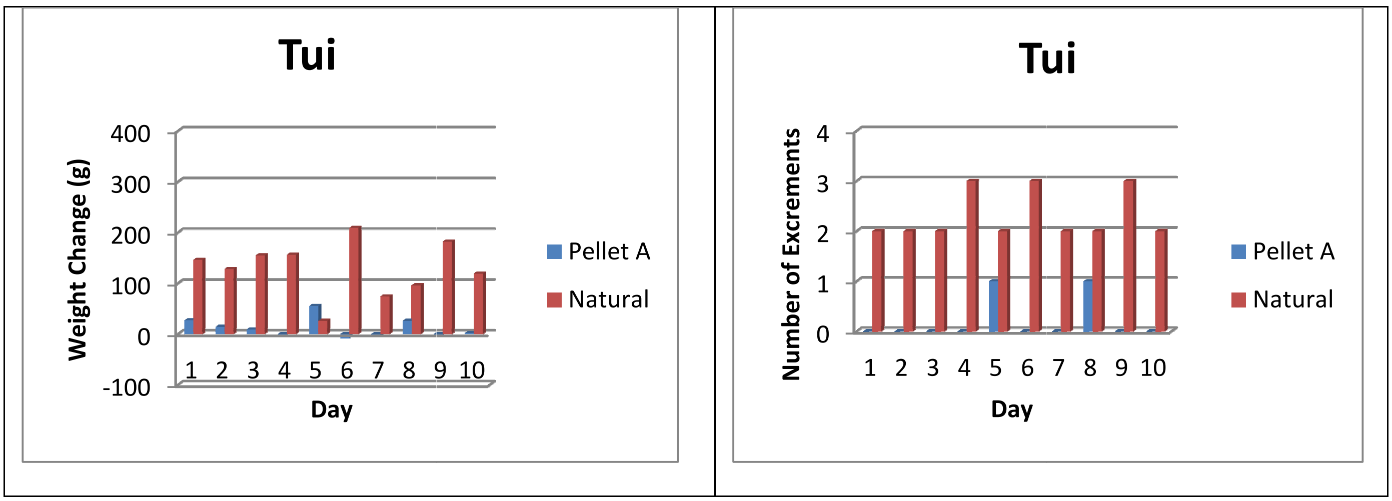


Figure 3.5: Weight change of litters and number of excrements each day for Tui who was offered O-litters A and Natural over the ten day period.

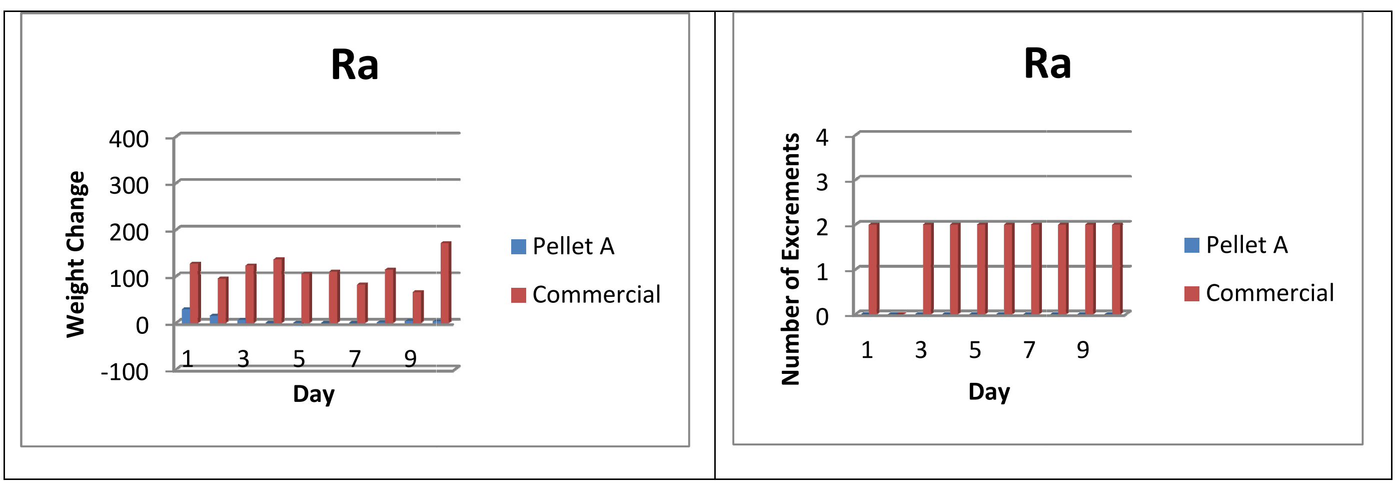


Figure 3.6: Weight change of litters and number of excrements each day for Ra who was offered O-litter A and commercial over the ten day period.

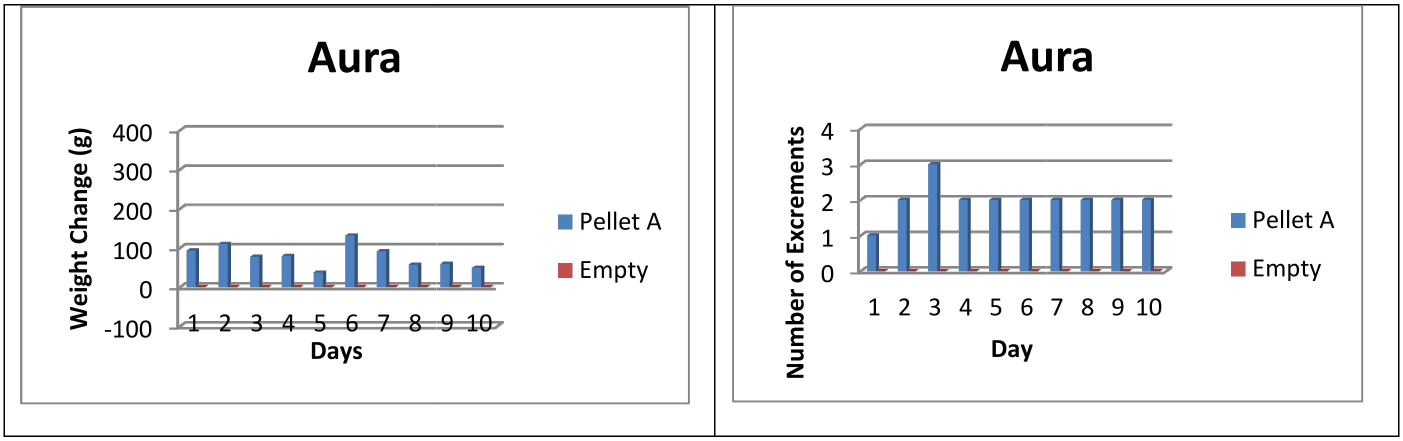


Figure 3.7: Weight change of litters and number of excrements each day for Aura who was offered O-litter A and empty over the ten day period.

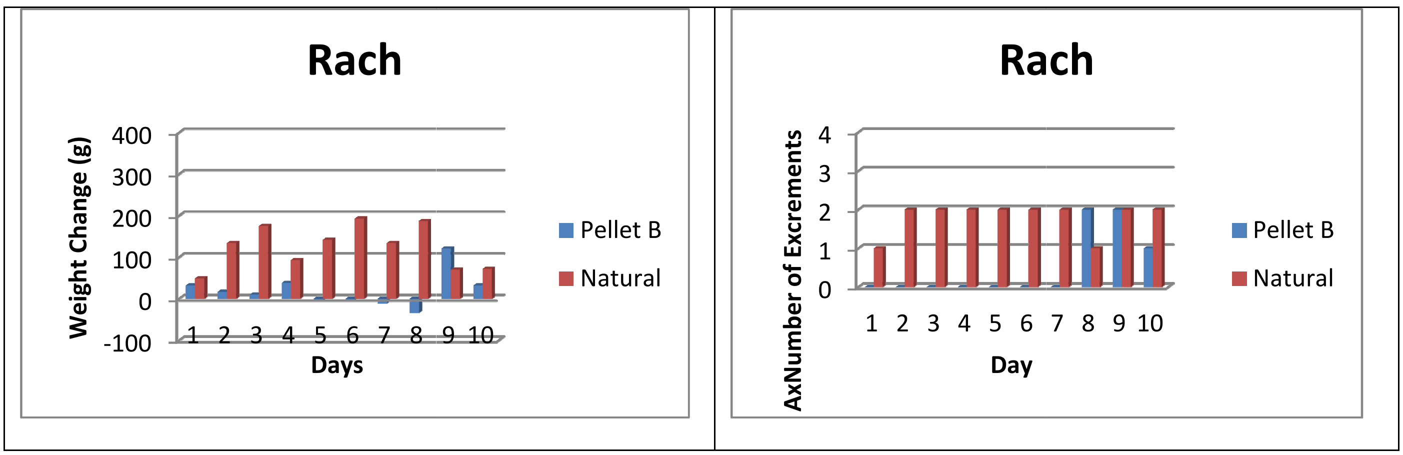


Figure 3.8: Weight change of litters and number of excrements each day for Rach who was offered O-litters B and Natural over the ten day period.

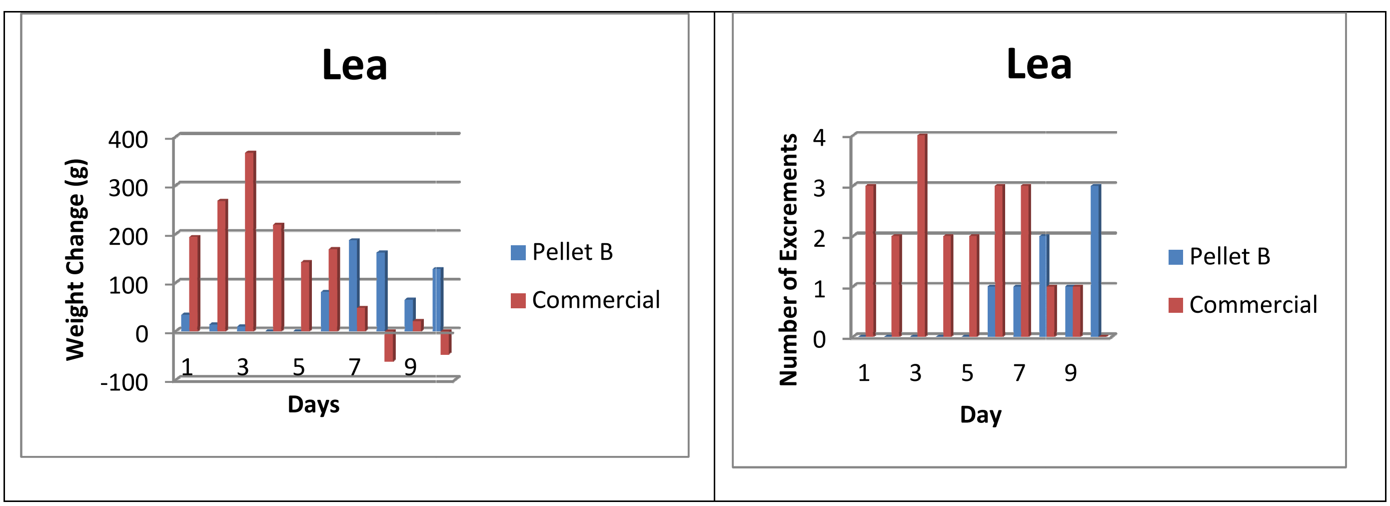


Figure 3.9: Weight change of litters and number of excrements each day for Lea who was offered O-litter B and commercial over the ten day period.

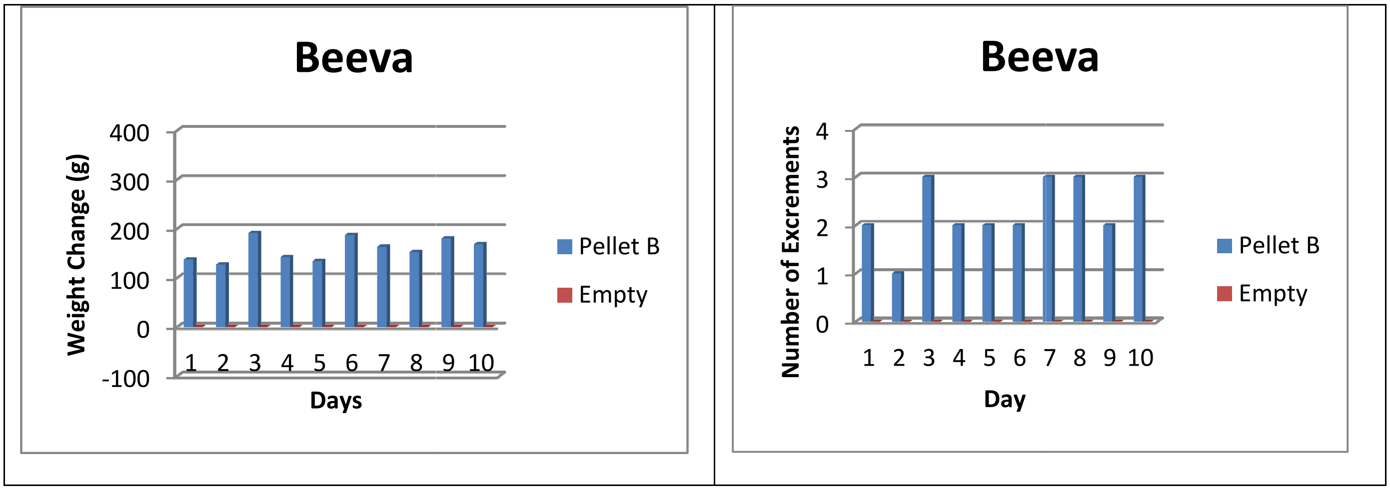


Figure 3.10: Weight change of litters and number of excrements each day for Beeva who was offered O-litter B and empty over the ten day period.

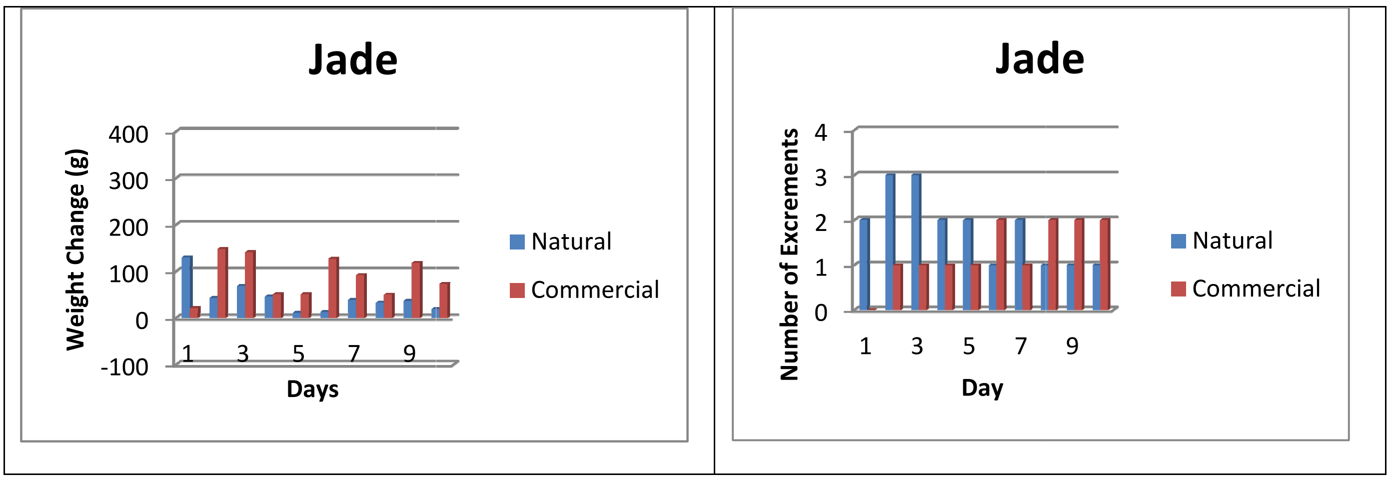


Figure 3.11: Weight change of litters and number of excrements each day for Jade who was offered O-litter natural and commercial over the ten day period.

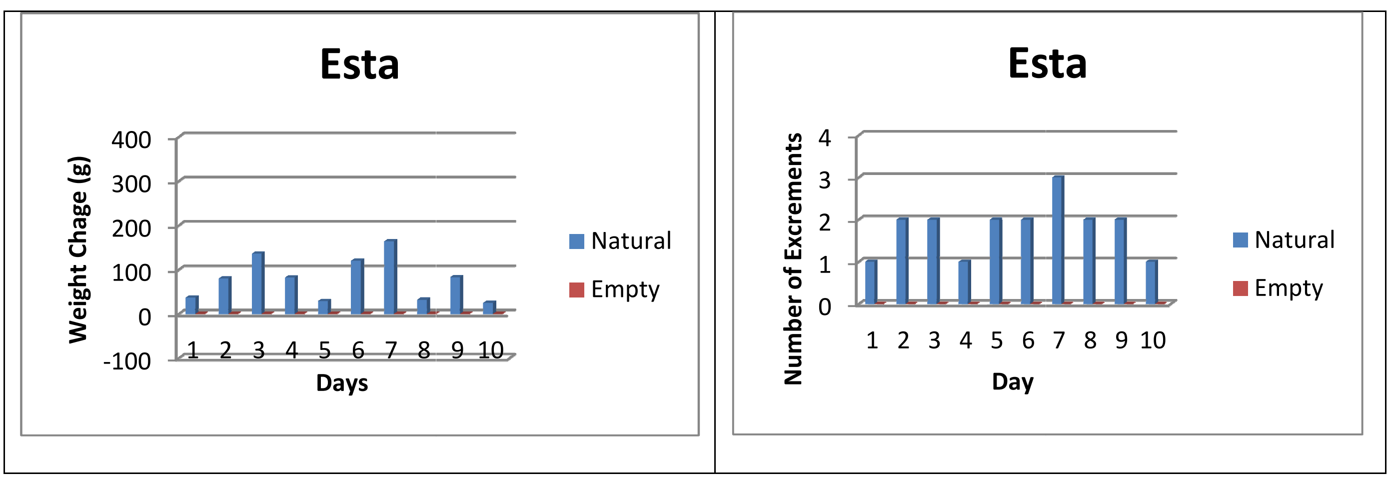


Figure 3.12: Weight change of litters and number of excrements each day for Esta who was offered O-litter natural and empty over the ten day period.

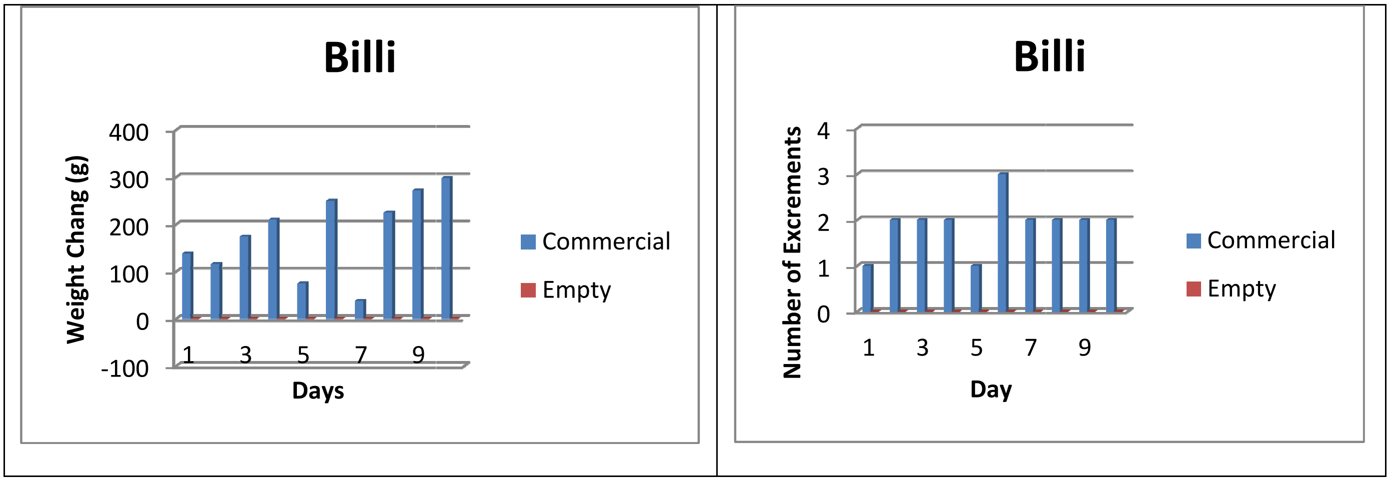


Figure 3.13: Weight change of litters and number of excrements each day for Billi who was offered litters commercial and empty over the ten day period.

Table 3.5: Summary of litter preferences for each cat and litter combination tested over 10 day period.

	Raven	Tui	Ra	Aura	Rach	Lea	Beeva	Jade	Esta	Billi
Tray 1	A	A	A	A	B	B	B	Natural	Natural	Comm.
Tray 2	B	Natural	Comm.	Empty	Natural	Comm.	Empty	Comm.	Empty	Empty
Litter Chosen By Weight Change	None	Natural	Comm.	A	Natural	Comm.	B	Comm.	Natural	Comm.
Litter Chosen By Number Excrements	B	Natural	Comm.	A	Natural	Comm.	B	Natural	Natural	Comm.
Overall Preference	B	Natural	Comm.	A	Natural	Comm.	B	None	Natural	Comm.

Based on the weight change criterion, eight of the ten cats showed a clear preference for one litter over the other while two cats used both litters and did not show a preference (Table 3.5). There was no preference shown between litters A and B nor between the natural and commercial litter. There were also two cats which started with a strong preference for one litter (Lea preferred commercial, Rach preferred natural), then at around eight days of the study, they switched to the other litter type (both switched to pellet B).

Similar results were obtained for preferences based on the number of excrements. Each cat clearly showed which litter they preferred based on the number of excrements, with the results the same as preferences based on the weight change with few exceptions. Raven had no preference for litters based on weight change but preferred pellet choice B based on the number of excrements. Jade preferred the commercial litter based on the weight change but preferred the natural form of waste material based on the number of excrements.

Overall, it appears that the most preferred litters were natural and commercial, followed by pellet form A and B. The empty litter box (control) was the least preferred choice with no cats leaving any excrements in the litter box.

3.4 Discussion

The main focus was to determine how long to leave the litters in the cages so cats could demonstrate a stable preference for one litter, if such a preference in fact existed. The results suggest that most of the cats decided which litter was their preferred within the first 24 hours. Eight out of 10 cats reached the weight change and excrement criteria on the first day and continued to choose the same litter on subsequent days.

These results suggests that the litter boxes only need to remain in the cage for one day in the main study to get an accurate idea of litter preferences. However, it was decided that each pair of litters should remain in the cage for two days to allow us to differentiate between a litter preference and a side preference. In addition, as each cat will be offered all 10 litter combinations in the main trial, leaving each combination in for 2 days will allow the cats to get used to them.

Two cats (Rach and Lea) showed a preference for one litter over the other within the first 24 hours and continued to use this litter for about the first 8 days. However, at this point, both cats switched to predominantly using the other litter. Lea was offered pellet form B and the commercial litter, and used the commercial litter for the first six days before switching to pellet form B. Rach was offered pellet form B and natural olive oil waste material litter, and used the natural litter for the first eight days before also switching to pellet form B. At about day eight, an ammonia odour could be detected in the commercial and natural olive oil litters during weighing. Therefore, their change in litter choice may be due to litter not being able to absorb the urine well any longer or that the litters odour controlling properties were no longer performing as they were originally. These two characteristics will be tested in further experiments (chapter 5 and 6). However, this may not be of much concern since most owners will not leave the same litter in the litter box for that length of time.

Measurement of the daily weight change of each litter box was useful for determining which litter the cats preferred. This variable was based on feed preference studies which measure the amount of food eaten to indicate an individual cat's preference (Kitchell & Baker, 1972). The thought process behind this method was that the greater the change of weight, the more the cat used that litter boxes, therefore the more it preferred that litter. There were small discrepancies in measurement associated with cats stepping into one litter box but not using that litter, then tracking litter into the other box. This resulted in a small negative weight change in one box and a small positive change in the other. We attempted to reduce the influence of tracking on the results by taking a weight change to be significant only if it was greater than 30 grams.

We also determined preference according to the number of excrements deposited in the litter boxes. This was an important preference determination since the weight change can vary based on each excrement and cat. When determining a preference based on food, the change in weight is a more accurate measurement. The weight of a cat's urinary bout or faecal excrement is dependent on the amount of food and water the cat had and the length of time they have been restraining themselves to use the box. Both of these methods for determining a preference will be used for the main study. This is to ensure an accurate determination of preference can be found.

One possible confounding factor in the process was that cats might demonstrate a side preference; i.e. a preference for the position of a litter box rather than the litter type contained within it. This was tested by switching the position of the litter boxes from side to side on a daily basis throughout the study. From the study, it appeared that most cats did not have a side preference. However there was one exception (Raven), who used the litter box on the right side of his cage more often than the litter box on the left side. This may indicate that Raven had a side preference. However, rather than having a side preference, it may also have been that Raven did not have a preference between the two litter choices. This is quite possible as Raven was offered pellet form A and B. The only difference between these litters was that pellet A went through a hammer mill before pelleting occurred. This may have made little difference to the cat and resulted in no preference.

Importantly, this pilot study also showed that cats, when given a choice of a litter box with any type of litter in it and an empty box, always chose a litter box that contains a litter. This suggests that the cats did not have an aversion to the O-litter products. This study also showed that eight of the ten cats had a preference for one litter over another litter or an empty tray. In contrast, two of the cats did not show a preference and used both litters provided equally. The next experiment will offer each of the ten cats each litter combination on a rotational basis so that statistical analyses can be performed to determine preferences at a population level (chapter 4).

One note of interest is that no cat produced more than 4 excrements per day, with most cats producing much less, This is in contrast to Neilson's average of 5 uses of the box per day. This may be due to different diets being fed to the cats in this study compared with Neilson's study. Environmental factors may also have played a role in the amount of times they used the litter box.

These preliminary results suggest that the cats preferred either the natural (unpelleted) olive oil waste material or the commercial cat litter over the two pelleted forms of waste material. This may have been due to the type of litter to which the cats were previously exposed. Sawdust is used as a cat litter at the Centre for Feline Nutrition, and the natural and commercial litters appear more similar to sawdust than do the pelleted forms. The one cat offered the two pelleted forms appeared to show an equal preference for pelleted litters A and B, which suggest that they either cannot distinguish the differences between them or that they did not have a preference between the two.

3.5 Conclusion

This pilot study provided information that will be used in the design of the main preference study. It appears that most cats show clear preferences for a particular litter within the first 24 hours, and in most cases, they did not show signs of a side preference. Therefore, in the main study, each litter combination will be offered to each cat for a period of 2 days. Litter preferences will be determined by measuring the weight change and the number of excrements each cat has each day.

The pilot study also found that the cats did not have an aversion to the O-litters. Overall, the cats tested appeared to prefer the commercial brand and natural waste material over the pelleted forms. Any litter was preferred to an empty box.

Chapter 4: Cat Preferences for Litter Type

4.1 Introduction

The purpose of this experiment was to determine what characteristics in cat litters cats prefer. This was performed by determining what cat litter the cats preferred when provided with multiple types of litter.

Animal preference is an important factor in the development of a new cat product. The type of litter provided can make a difference to some cats, with the wrong litter choice being the cause of many litter box problems in the household (Hotchner, 2007). Certain features, like texture of the litter and the way it smells, may alter a cat's behaviour towards the litter and determine whether the cat will use it.

For many cat owners, choosing a litter can be confusing since there are many options, but it is really what the cat wants that matters most. Hotchner (2007) states that the best way to choose a cat litter is to let the cat choose it: purchase a few different types of litter and see which one the cat uses. This was the basis for this experiment: we gave cats multiple choices of litter to determine which they preferred.

To determine which litter the cats prefer, we must first determine what a preference is. Kirkden and Pajor (2006) define a preference as behaviour that "denotes a difference between the strength of motivation to obtain or avoid one resource or stimulus and the strength of motivation to obtain or avoid another." Preference tests focus on the choices animals make between alternative resources and on ranking those resources (Kirkden & Pajor, 2006).

The benefits of using preference tests to evaluate an animal's motivation, needs or desires include:

1. Preference tests allow animals to express their own priorities, giving the researcher the best insight into what is important to the animal;

2. Preference tests can measure differences between items;

3. Preference tests are easy to interpret (Kirkden & Pajor, 2006).

The most commonly used preference test asks animals to choose between two objects/items. In these two choice tests, a preference can be determined by comparing the animal's use of one resource relative to the other, e.g. amount of food eaten or time spent at an object (Kirkden & Pajor, 2006). In cats, two choice preference tests are often used to evaluate the palatability of different types of foods.

A ‘two bowl’ test has been commonly used to determine which food a cat prefers (Kitchell & Baker, 1972). The two bowl method involves presenting individual cats with two containers of different food with known volume and weight and giving the cat a certain amount of time to eat as much food as it wants. The volume or weight of remaining food is measured and a preference determined as the food most consumed. This two bowl method of preference testing has been used by many researchers (Young, 1945; 1948; Wetzel, 1959; Christensen, 1961; Pfaffman et al 1967; Shaber, et al., 1970), and is an accepted procedure for measuring cat food preferences (Griffin, 2000).

Only one previous preference study relating to litter box use has been found. This study was aimed at determining whether cats’ litter box use is affected by the size of the litter box (Guy & Hopson 2006). The cats were provided with two different sized litter boxes, and a preference was defined according to the number of excrements in each box. The box with the most excrements was the most preferred. Using this criterion allowed the researchers to conclude that cats preferred the larger litter box (Guy & Hopson, 2006).

For the current experiment, the cats were given a series of choices between two litters, and preferences were evaluated based on the number of excrements deposited and the change in litter weight. Since there is little published data on the methods for testing preferences for cat litters, protocols for measuring cat litter preferences were derived from the two bowl methods of Kitchell and Baker (1972), the one published litter box experiment (Guy & Hopson, 2006) and the pilot study (chapter 3). Data were analysed using several different methods to ascertain the most reliable criterion for evaluating cat preferences for litter. Based on results from the pilot study, we hypothesised that cats would choose O-Litters as often as commercial litter and more often than the empty litter box.

4.2 Materials and Methods

4.2.1 Animals

The same ten cats and food was used for this experiment as the pilot study. There were five males and five females, ranging in age from 1.5 to 11 years (Table 3.1).

4.2.2 Cage and Litter Box Design

The cats were kept in the same cages as the ones described in the pilot study, with the litter box design remaining the same as well. The cats were kept in the cages for a period of 20 days continuously.

4.2.3 Litter Combinations

The same litters were used for this experiment as for the pilot study; however, all ten litter combinations were offered to each cat. Table 4.1 shows the order in which the litter combinations were offered to the cats. The first litter combination each cat received was the one to which they were exposed in the pilot study. Therefore, no two cats had the same litter combination at the same time, but all were offered the combinations in the same order. Each cat had each litter combination for a two-day period.

Table 4.1: Litter combinations used for preference experiment in the order in which they were presented to each cat. Each combination was presented to each cat for a two day period. Litter types explained in chapter 3.

Litter Combinations
A + Commercial
B + Natural
Natural + Empty
B + Empty
Commercial + Empty
B + Commercial
Natural + Commercial
A + Empty
A + B
A + Natural

4.2.4 Measuring Techniques

The following procedures were followed for each of the ten litter combinations.

Day 1

On the first day of each combination, the litter boxes were cleaned with a disinfectant dishwashing liquid and allowed to air dry. Approximately 5.4 litres of litter (between 600g and 1200g depending on litter type) was added to the litter box. The weight of the box and litter was recorded to the nearest hundredth gram ("pre" value). The boxes were then placed in the cage, taking note of which litter type was on each side. This was done between 0700 and 0900.

Day 2

The following morning, the boxes were removed between 0700 and 0900 and weighed (box plus litter plus any excrements = "post" value 1). Faecal excrements and urine clumps were removed after weighing using a pooper scooper. After removal of the excrements, the litter box and remaining litter were weighed again – this value was used as the “pre” value for calculating weight change from day 2 to day 3. The two litter boxes were replaced in the cage on the opposite side to day 1.

Day 3

Again, the weight of the box, litter and any excrements was measured for each litter (“post” value 2). Excrements were removed and the boxes reweighed. The boxes and litter were weighed again so that an accurate weight could be determined for the excrements. This weight was used in the analysis for determining which litter was the most preferred. Both boxes were then emptied, disinfected and the next combination was presented.

Weight change from day 1 to day 2 (change 1) and day 2 to day 3 (change 2) was calculated. Weight change 1 and 2 were averaged for each cat to be used in the analysis of preference. In addition, the number of excrements (faecal and urine clumps) were calculated for each day and the average number over the two days was used in the analyses.

4.2.5 Determination of Preference

Preferences between litters were determined by four methods. The results using each method were compared to see whether the overall ranks of litters differed. At the level of the individual cat, preferences were determined using weight change or number of excrements (as in the pilot study – chapter 3). At the population level, preferences were determined using pooled values (for ten cats) of weight change or number of excrements for each litter in each combination.

4.2.5.1 Individual Level Preferences

The first two methods used to determine which litter the cats preferred were the same as in the pilot study.

a. Weight Change

An individual cat was considered to have shown a preference for one litter over the other when the average weight change for that litter was at least 30 grams higher than for the other litter. A minimum difference of 30 grams was chosen as this represented the lowest measured weight for a single excrement (faeces or urinary event) yet was higher than the weight of litter tracked incidentally between boxes (Table 4.2). The average weight of a single excrement was calculated by adding the total amount of excrements for that litter and the total weight change and dividing them. This was performed for each litter. The values provided for tracking weight could only be calculated when the cat did not deposit an excrement in the litter box. Values given for low tracking rate show the instances where the cat may have tracked litter out of the litter box, therefore giving a negative value, or a small amount of litter was tracked into that litter box. The high tracking rate is the highest weight change that could be calculated due to tracking. For each litter combination, a binomial test was used to determine whether the number of individuals choosing the most preferred litter (highest number of cats reaching preference criterion) was significantly different from chance (2 tailed test $p<0.05$). When performing the binomial tests, the total number of cats varied because individuals showing no preference were excluded from the analyses.

Table 4.2: Average weight change (g) of single excrement and average weight of tracking measured over all litter combinations for all 10 cats. Standard deviation provided for average weight change and tracking. Tracking weight change could only be calculated when there was no excrement deposited in the litter box.

Litter	Average Weight of single excrement (g)	Standard Deviation	Average Tracking Weight (g)	Standard Deviation
A	60.4	11.7	7.78	12.00
B	60.6	16.7	3.71	10.31
Natural	69.5	27.4	10	8.55
Commercial	73.0	13.8	-	-
Empty	24.0	12.1	-	-

b. Number of Excrements

An individual was considered to have shown a preference for the litter having the higher average number of excrements (faecal and urinary). As above, binomial tests were used to test whether the number of cats choosing the most preferred litter in the combination differed significantly from chance.

4.2.5.2 Population Level Preferences

a. Weight Change

The average change in weight over 2 days for each litter in each combination was calculated for each of the ten cats. For each litter combination, a paired t-test was used to evaluate the significance of the difference in weight change between the two litters ($p<0.05$).

In addition, for each cat in each litter combination, a preference ratio was calculated for average weight change over 2 days. The ratio was calculated as $\frac{X-Y}{X+Y}$ where X equalled the grams of weight change of litter X and Y equalled the grams of weight change of litter Y. A value below zero (negative number) indicated that the weight change was higher for litter Y, while a positive value indicated that the weight gain was higher for litter X. A sign test was used to determine whether the median ratio for each litter combination was significantly different from zero. The benefit of this analytical method is that each cat contributes equally to the analysis regardless of the actual magnitude of the weight change.

b. Number of Excrements

The same analyses were performed using the average number of excrements deposited in each litter in each combination over two days.

4.2.6 Comparison of methods for determining preference

The rankings of the five litters developed using each data analysis technique were compared.

4.3 Results

4.3.1 Individual Level Preferences

a. Weight Change

Appendix 4.1 gives the daily weight measurements before use, after use, the change in weight of each litter for each cat each day, and the number of excrements. Table 4.3 shows the number of individual cats expressing a preference for one litter or the other in each combination, based on the weight change criterion. Most cats showed a preference for one litter over the other in each litter combination; however, litter preference varied between individuals. The number of cats preferring any litter (A, B, Natural, or Commercial) over an empty box was significantly different from chance (p=0.01). In addition, the number of cats preferring the commercial litter was higher than chance when the alternative was O-Litter B (p=0.04). In all other litter combinations, the number of cats showing preferences for one or other litter was not significantly different from chance (p≥0.11).

Table 4.3: Each litter combination showing the number of cats with a preference between litters, cats with no preferences, the binomial statistics, and the preferred litter for each combination based on the results from the binomial test from average weight change over 2 days.

Combination	Number of cats showing preference for 1st (/10)	Number of cats showing preference for 2nd (/10)	Number of cats showing no preference	Binomial Statistic	Preferred litter
A + B	4	3	3	p=0.27	None
A + Commercial	5	5	0	p=0.25	None
A + Natural	4	5	1	p=0.25	None
A + Empty	10	0	0	p= 0.001	A
B + Commercial	2	8	0	p=0.04	Commercial
B + Natural	2	6	2	p=0.11	None
B + Empty	10	0	0	p=0.001	B
Natural + Commercial	2	6	2	p=0.11	None
Natural + Empty	10	0	0	p=0.001	Natural
Commercial + Empty	10	0	0	p=0.001	Commercial

b. Number of Excrements

Table 4.4 shows the number of individual cats expressing a preference for one litter or the other in each combination, based on the number of excrements criterion. As for the weight change analysis, the number of cats choosing litter over an empty box was always significantly different from chance, regardless of the type of litter (p=0.001). Significantly more cats than chance preferred Natural O-Litter when the alternative was O-Litter A (p=0.03) and there was a tendency for more cats than chance to prefer Natural over O-litter B (p=0.07). Finally, more cats than chance preferred the commercial litter when offered with the Natural O-Litter (p=0.02) and a tendency for more cats than chance to prefer commercial over O-litter B (p=0.07).

Table 4.4: Each litter combination showing the number of cats with a preference between litters, cats with no preferences, the binomial statistic, and the preferred litter for each combination based on the results from the binomial test from average excrements (faeces and urine) over 2 days.

Combination	Number of cats showing preference for 1st (/10)	Number of cats showing preference for 2nd (/10)	Number of cats showing no preference	Binomial Distribution	Preferred Litter
A + B	3	4	3	p=0.27	None
A + Commercial	3	7	0	p=0.12	None
A + Natural	1	7	2	p=0.03	Natural
A + Empty	10	0	0	p=0.001	A
B + Commercial	2	7	1	p=0.07	Tendency for Commercial
B + Natural	2	7	1	p=0.07	Tendency for Natural
B + Empty	10	0	0	p=0.001	B
Natural + Commercial	1	8	1	p=0.02	Commercial
Natural + Empty	10	0	0	p=0.001	Natural
Commercial + Empty	10	0	0	p=0.001	Commercial

4.3.2 Population Level Preferences

a. Weight Change

Figure 4.1 shows the average weight change (over 2 days) for each litter in each combination when data from all 10 cats were pooled. Table 4.5 shows the results of the paired t-tests on pooled average weight changes. When data from all 10 cats were pooled, the average weight change was significantly higher for all litters compared to the empty box. The weight change was significantly higher for the Natural O-Litter than for O-Litters A and B and significantly higher for the commercial litter than for the Natural O-Litter and O-Litter B.

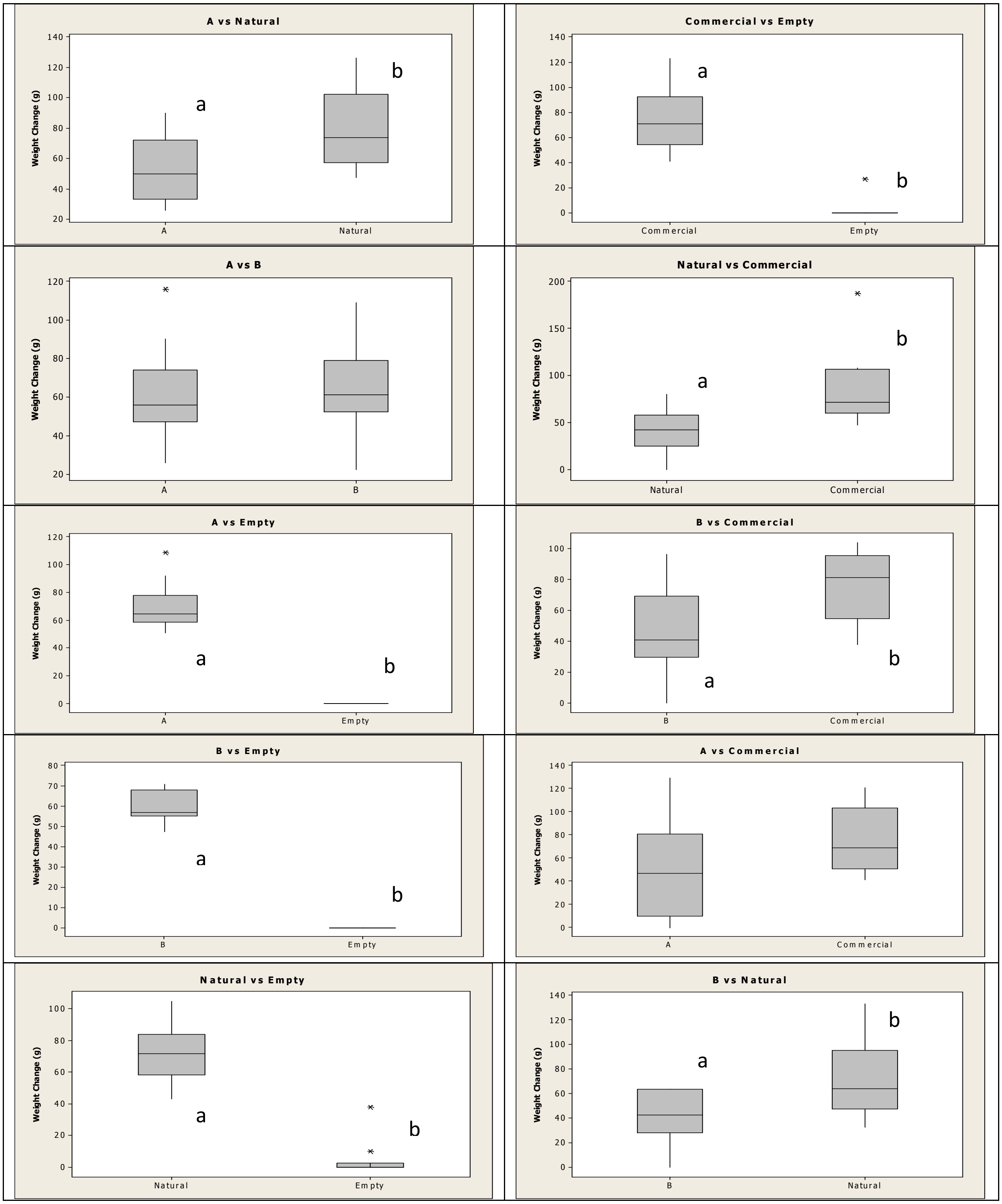
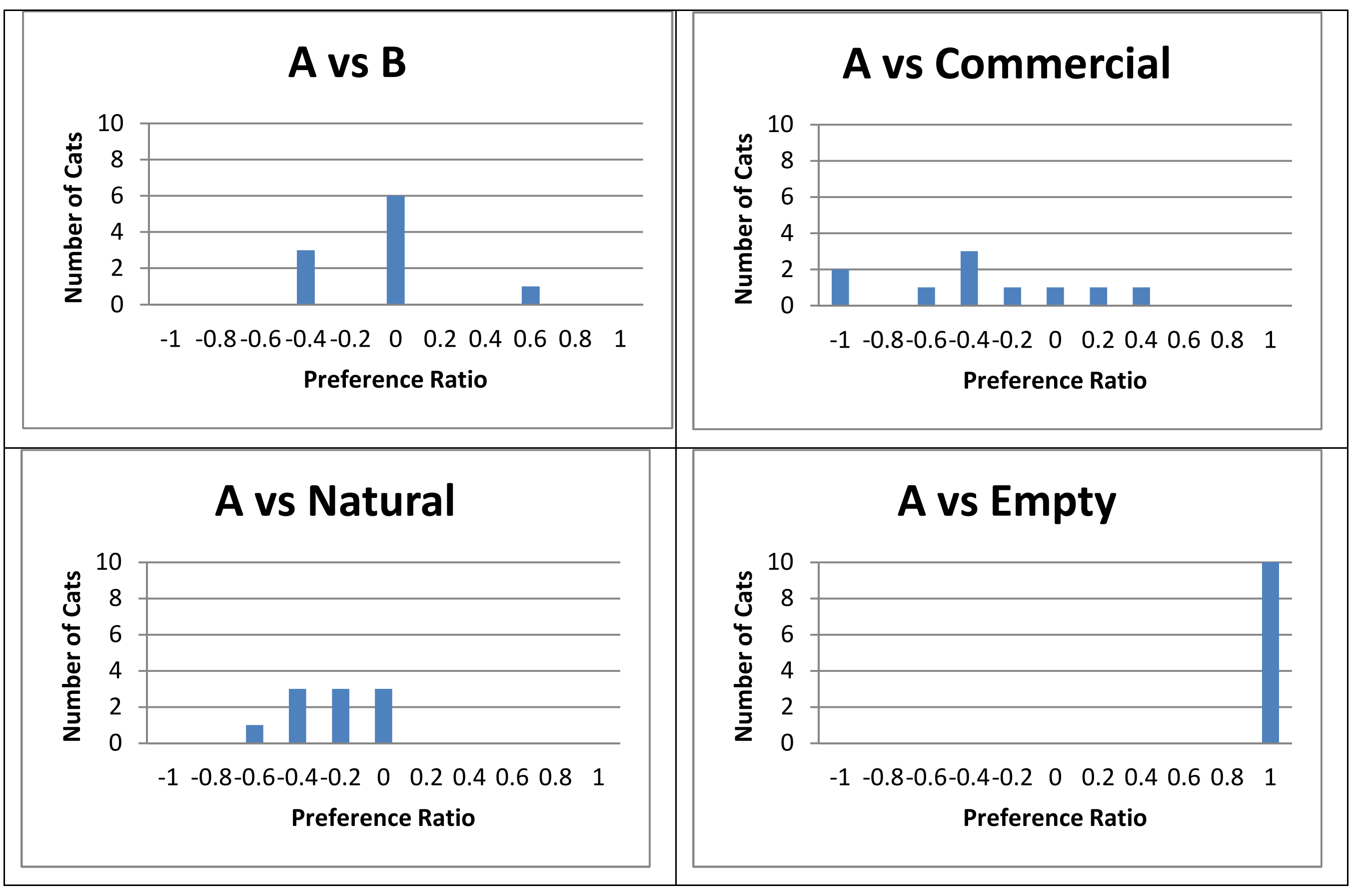


Figure 4.1: Average weight change for each litter in each combination using data from all 10 cats. The box represents values within the 95% confidence interval, the line within the box is the mean, and the lines above and below the box are values outside the 95% confidence interval. * represents outliers Letters signify significant difference between litters at p<0.05.

Table 4.5: Results of paired t-tests on average weight change for each litter combination based on data from all 10 cats.

	A	B	Natural	Commercial	Empty
A	- -	T = -0.22 P = 0.834	T = -2.43 P = 0.038	T = -1.81 P = 0.104	T = 12.30 P < 0.001
B		- -	T = -2.46 P = 0.036	T = -2.32 P = 0.045	T = 25.49 P < 0.001
Natural			- -	T = -3.20 P = 0.011	T = 8.94 P < 0.001
Commercial				- -	T = 8.05 P < 0.001

Figure 4.2 shows the distribution of preference ratios based on the weight change criterion for each litter combination. Table 4.6 shows the results of the sign test on the weight change preference ratios for each litter combination. In accordance with the results of the paired t-test, the median preference ratios for all litter combinations were significantly greater than zero when litters were compared to an empty box. However, the only other median preference ratio significantly different from zero was that calculated for Commercial vs Natural O-Litter: the weight gain was larger for commercial.



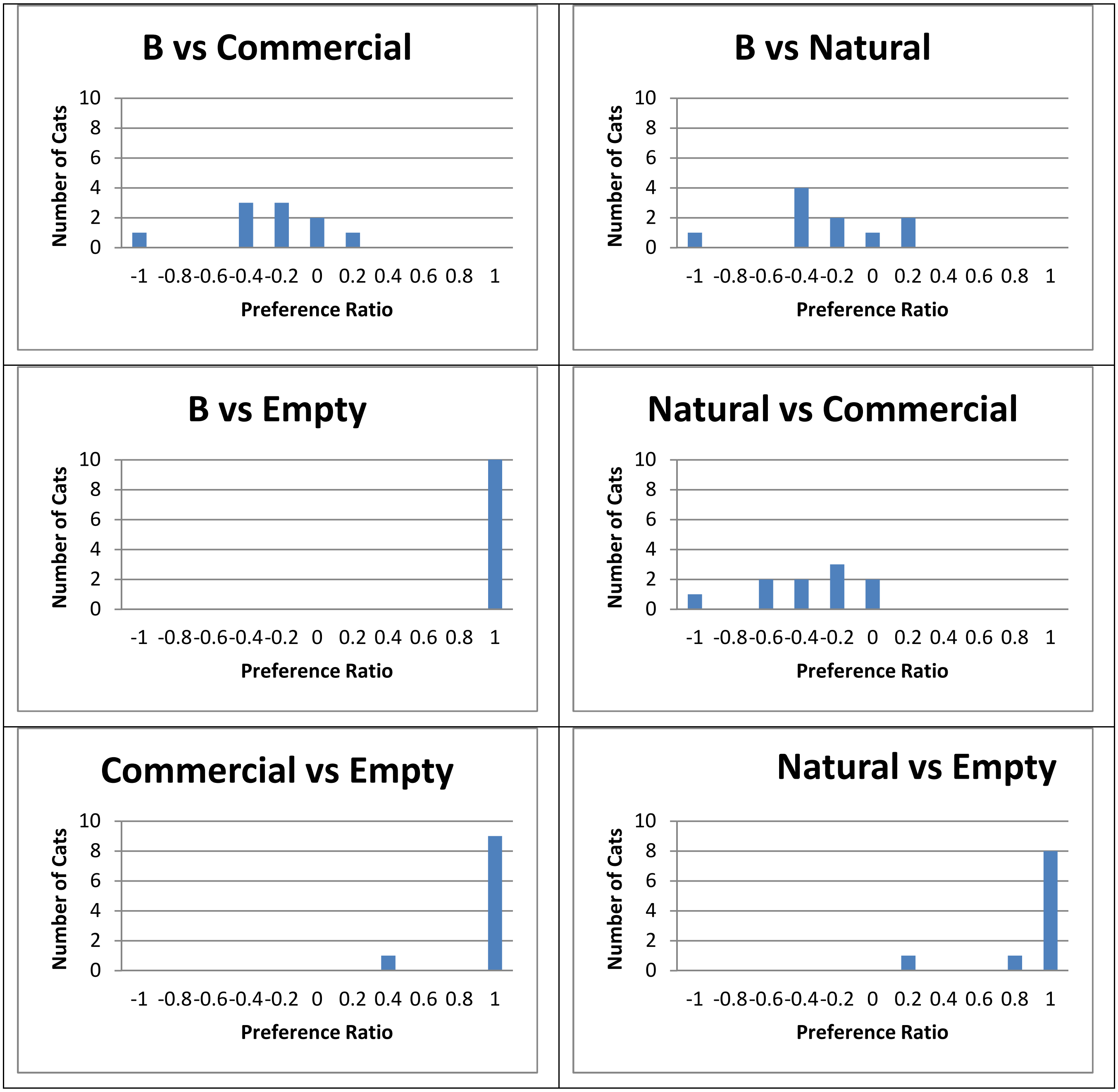


Figure 4.2: Distribution of preference ratios based on weight change criterion. Values greater than zero represent a higher weight gain for the first litter whereas values below zero represent a higher weight gain for the second litter.

Table 4.6: Results of sign tests on preference ratios based on weight change for each litter combination.

	A	B	Natural	Commercial	Empty
A	-	M=-1.0 *P=0.754*	M=-2 *P=0.344*	M=-2.0 *P=0.344*	M=5 ***P=0.002***
B		-	M=-2 *P=0.344*	M=-2 *P=0.344*	M=5 ***P=0.002***
Natural			-	M=-4 ***P=0.022***	M=5 ***P=0.002***
Commercial				-	M=5 ***P=0.002***

b. Number of Excrements

Figure 4.3 shows the average number of excrements (over 2 days) for each litter in each combination when data from all 10 cats were pooled. Table 4.7 shows the results of the paired t-test on pooled average number of excrements. Over the whole population, the average number of excrements was significantly higher for all litters compared to the empty box. However, there were no significant differences between litters.

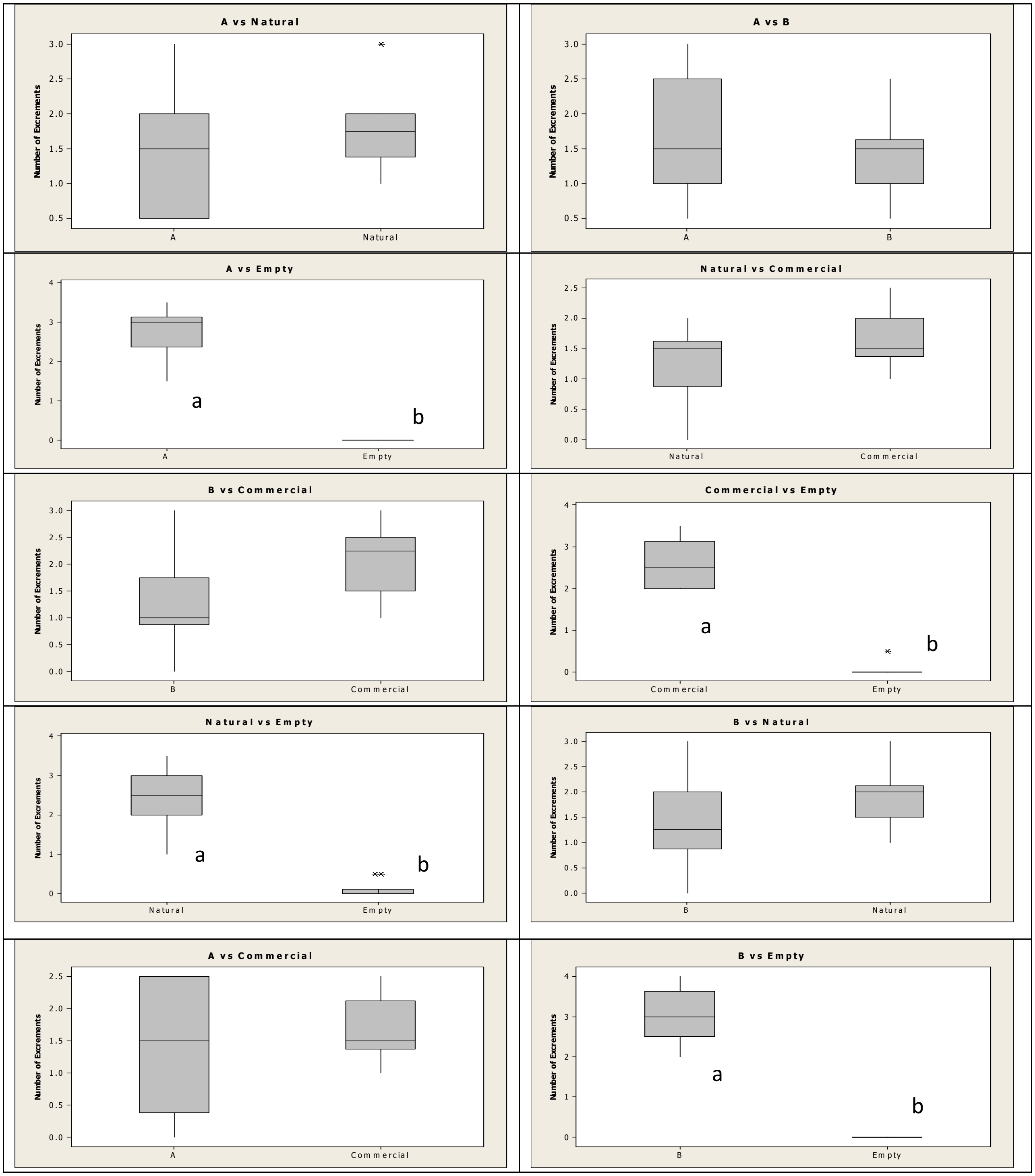
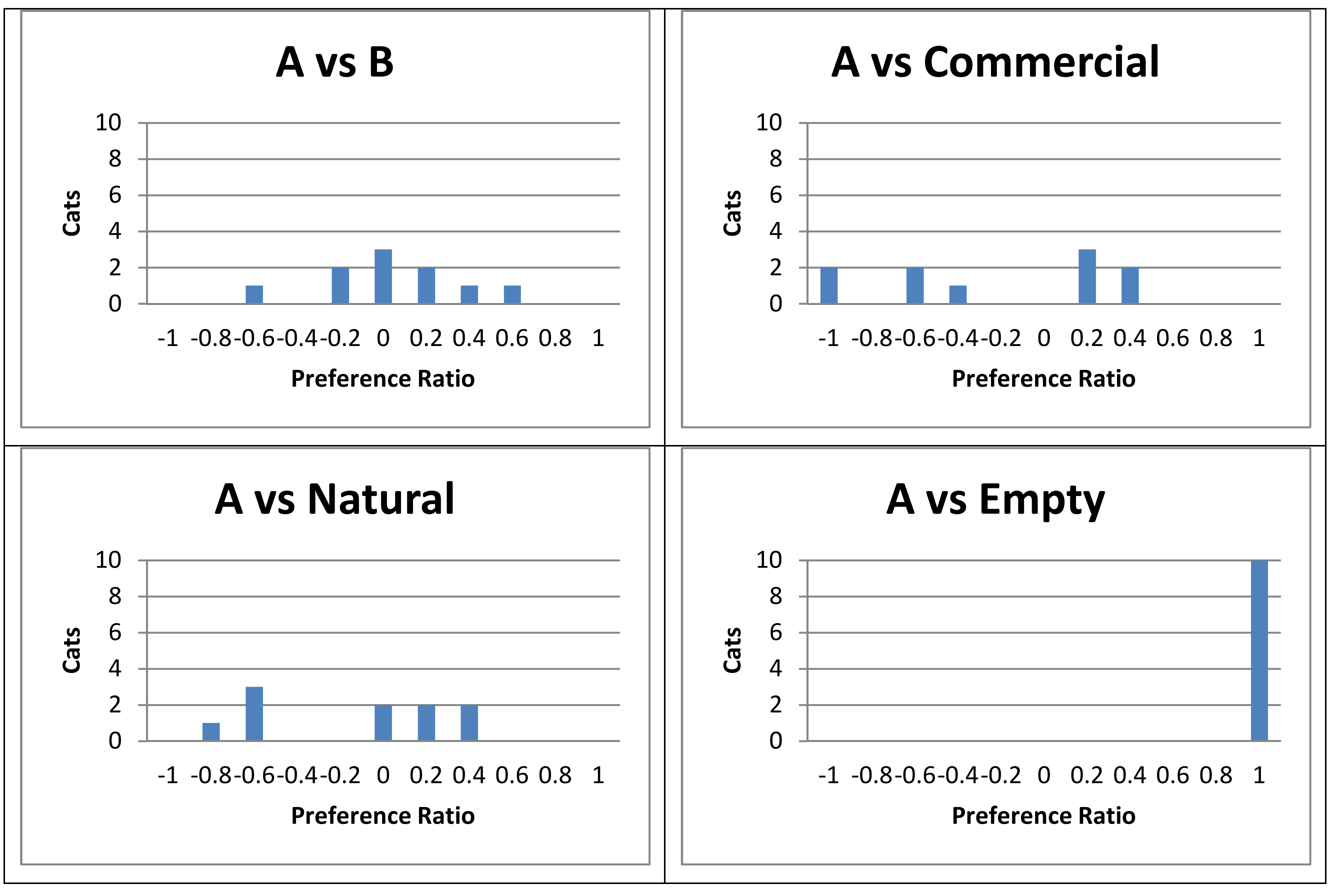


Figure 4.3: Average number of excrements for each litter in each combination using data from all 10 cats. The box represents values within the 95% confidence interval, the line within the box is the average, and the lines above and below the box are values outside the 95% confidence interval. * represents outliers. Letters signify significant difference between litters at $p<0.05$.

Table 4.7: Results of paired t-test based on average number of excrements for each litter combination.

	A	B	Natural	Commercial	Empty
A	- -	T=1.61 *P=0.126*	T=-0.84 *P=0.421*	T=-0.77 *P=0.460*	T=13.70 ***P<0.0001***
B		- -	T=-1.63 *P=0.137*	T=-1.80 *P=0.105*	T=14.89 ***P<0.0001***
Natural			- -	T=-1.50 *P=0.168*	T=10.48 ***P<0.0001***
Commercial				- -	T=15.00 ***P<0.0001***

Figure 4.4 shows the distribution of preference ratios based on the number of excrements for each litter combination. Table 4.8 shows the results of the sign test on the number of excrements preference ratios for each litter combination. As for the paired t-test, the number of excrements was significantly higher for all litters when the alternative was the empty litter box.



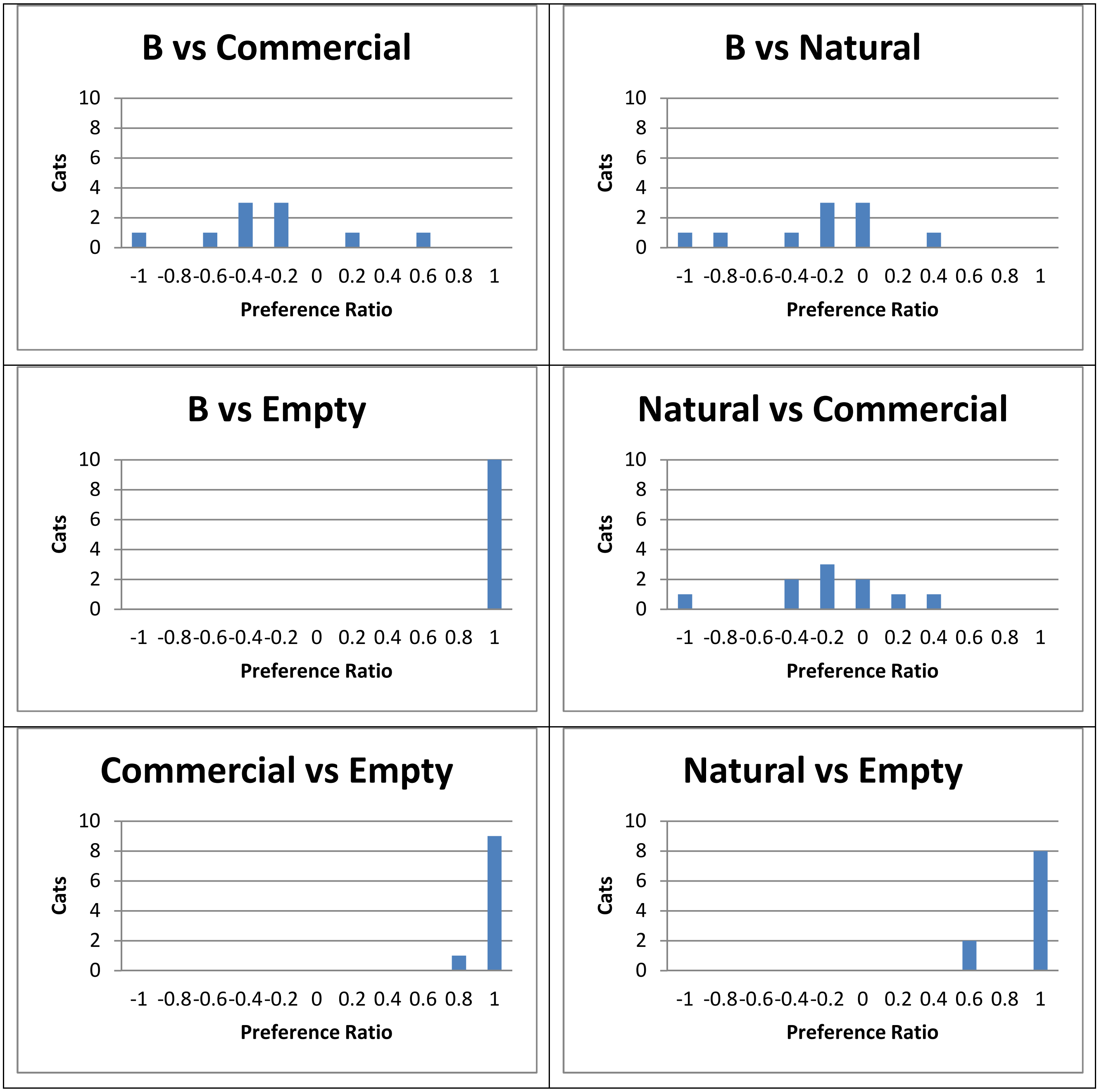


Figure 4.4: Distribution of preference ratios based on the number of excrements criterion. Values greater than zero represent a higher number of excrements for the first litter whereas values below zero represent a higher number of excrements for the second litter.

Table 4.8: Results of sign tests on preference ratios based on average number of excrements for each litter combination.

	A	B	Natural	Commercial	Empty
A	-	M=0.5 *P=1.0*	M=0 *P=1.0*	M=0 *P=1.0*	M=5 ***P=0.002***
B		-	M=-2 *P=0.289*	M=-3 *P=0.109*	M=5 ***P=0.002***
Natural			-	M=-2 *P=0.289*	M=5 ***P=0.002***
Commercial				-	M=5 ***P=0.002***

4.3.3 Comparison of rankings from various analytical methods

Table 4.9 shows the rankings of the five different litters determined by the various analytical methods. All methods found that any litter was preferable to an empty litter box. Two of the six methods suggest that the cats showed little preference between the litters presented: population level tests using the number of excrements. The results using the other methods suggest that the commercial litter was most preferred, followed by Natural O-Litter. O-Litter B appeared to be the least preferred litter offered.

Table 4.9: Approximate rankings of litters based on six analytical methods. Litters given a 1 are the most preferred litter choice, while 5 is the least preferred litter choice.

Method	Commercial	Natural	A	B	Empty
Individual Weight Change	1	1	3	3	5
Individual Number of Excrements	1	2	3	4	5
Population Weight Change t-test	1	2	3	3	5
Population Weight Change Sign test	1	2	2	2	5
Population Number of Excrements t-test	1	1	1	1	5
Population Number of Excrements Sign Test	1	1	1	1	5

4.4 Discussion

The purpose of this experiment was to determine which litter type the cats preferred from the waste products of the olive oil industry and how these litters compared with a commercial brand. Overall, we determined that cats had a preference for the commercial litter. These results were not surprising since this litter is commonly used so is obviously acceptable to cats. However, cats preferred to use any type of litter over the empty litter box, showing that they did not have an aversion to the O-Litter products and were quite happy to use them if no other litter choice was provided.

4.4.1 Criteria for Determining Litter Preference

4.4.1.1 Individual Level

Most individual cats showed a preference for one litter over another based on either weight change or excrement criteria, with only eight out of one-hundred trials resulting in no choice in each case. When a litter was paired with an empty litter box the cats chose the provided litter every time. This shows that the cats did not have an aversion to any type of litter and also that they prefer to bury their excrements in litter than to use an empty box where the excrements would be exposed. Overall, individual cats appeared to have a preference for the commercial litter. However, choices made by individuals were not always the same.

Based on weight change, the commercial litter and O-Litter Natural were the most preferred. No overall preference was found between the O-Litter pelleted products. Most individuals did show a preference between these litters but four cats preferred A and three preferred B. The remaining three cats showed no preference for either litter suggesting that these cats could not distinguish any difference between the two pelleted litters or that the difference was not important to them.

In contrast, the natural form of O-Litter has a different texture and was of a different particle size to the two pelleted forms. Therefore the cats should have been able to distinguish between the natural and pelleted forms. There were only one or two cats that showed no preference between the natural and pelleted litters, but because their choices as a group were split, there was no overall preference for that litter.

When examining the results of cat preferences at an individual level based on the number of excrements, the commercial brand of litter was the most preferred followed by the natural form of O-Litter. O-Litter Natural was preferred over pellet A and there was no difference between the two pelleted forms. Notice should be taken of litter combinations B vs commercial and B vs natural. Based on the statistical analysis, no overall preference could be determined; however, there was a tendency for more cats to choose commercial and natural over B.

4.4.1.2 Population level

At the population level, overall preferences were more difficult to establish. In particular, no preferences were found using the number of excrements.

Using the raw weight change, the results obtained were different at a population level than at the individual level, however the most preferred litter remained the same. The overall preference at the population level was the commercial brand. Natural O-Litter was the next preferred, followed by the two pelleted versions. As at the individual level, there was no preference between pelleted litters A and B at the population level. Again, the empty litter box (negative control) was the least preferred choice, rarely being used. The two combinations where there was no preference were A vs B and A vs commercial.

Using the preference ratio method provided information about the relative strength of the preference: the ratio reflects the weight change (or number of excrements) for one litter relative to the other. Almost all cats exclusively used boxes with litter in them when the alternative was an empty box (ratio of 1). In contrast, when two litters were offered, the strength of individual cat's preferences varied, with most cats using both litters to some degree (values between -0.6 and 0.6). This shows that the cats may have preferred one litter over another as individuals, but at the population level, often no preference could be determined because of the range of the individual cat preferences.

Using preference ratios based on weight change, all litters were preferred over the empty box and commercial was preferred over natural. Using the number of excrements ratio, significant preferences were found only for litter over the empty box. Less significant differences were found using a sign test on the preference ratio data than using a paired t-test on the raw data. Paired t-test are parametric and assume data are normally distributed which is unlikely to be true for such a small dataset (Rybarczyk et al. 2001). On the other hand, the sign test is extremely conservative and may underestimate the significance of differences between litters (Zar, 1999). The advantage of using preference ratios is that datum from each cat is equally weighted in the analysis. The ratio also provides information on the magnitude of the differences between the litters.

Taking into account both statistical methods used, based on population level preferences, we can only determine that the commercial brand was the most preferred and the empty litter box was the least preferred.

4.4.2 Methods Used to Determine Preference

Several methods were used to evaluate cats' preferences for litters in this study. Preferences were determined using weight change and the number of excrements as well as at an individual cat level along with at a colony level. Each method gave slightly different results; however, overall there was preference for the commercial cat litter, with the natural O-litter product just behind in preference. In addition, all methods clearly showed that cats prefer to use any litter over an empty box.

Each of the four methods for determining preferences is useful in its own way. The standard method of testing food preferences is based on measurement of the change in the weight of food provided, and the weight of food eaten reflects a preference (Griffin, 2000). This criterion allowed clear differentiation between most of the litters at both the individual and population level. However, this criterion is not necessarily as accurate for determining litter preferences as it is for food preferences. Cats are likely to produce different numbers and weights of excrements, depending on factors such as the amount they have eaten or drunk throughout the day, the ambient temperature, and health conditions, which have nothing to do with their preference for a litter. Therefore, we suggest that the number of excrements would be a more accurate method for determining the most preferred litter. This method was also used by Guy and Hopson to determine if cats had a preference size of litter boxes. In the current study, using the number of excrements criterion indicated that most cats have a clear preference for a particular litter at the individual level.

As well as using two different variables, we also tested preferences at the individual and population level. From the point of view of the average cat owner, the preference of the individual cat is probably most valuable. In addition, evaluating preferences only at the population level can be misleading. Individual cats may have strong preferences for one litter over another, but when grouped as a population, these preferences may not show. On the other hand, if someone has a cattery, they may be interested in results from a population level analyses. This test method would show, on average, which litter type cats prefer the most and can therefore make their purchasing easier. The advantage of testing preferences at a population level is that an arbitrary criterion for 'preference' does not have to be applied.

4.4.3 Comparison of the three O-litters

The results of these studies provide information for The Village Press to use in making decisions about how to process the raw waste products from the olive oil industry. Based on the results of these preference tests, the most appropriate choice to begin manufacturing is the natural form of olive oil waste products. Regardless of which statistical test is used, the natural O-Litter was the most preferred of their products. Once completion of the other two test (absorption and odour control), the most appropriate litter to manufacture may change.

4.4.4 Limitations of Study

Some discrepancies were noted in the weight change of the litters. This was most likely due to tracking litter from one box to another or throughout the cage, and not actually using the litter box. This was also noticed in the pilot study: the cat stepped into a litter box, didn't use it, but tracked litter out, sometimes into the other litter box, altering the weight of both litter boxes. This was dealt with by calculating a preference of litters based on weight change only if there was at least a 30 gram difference.

One thing that may have influenced the cats' behaviour was the first litter combination offered which was the same one offered in the pilot trial. However, the cats did had a period of rest (approximately one month) during which they were returned to their colony pens and used their normal sawdust litter between the pilot study and the main trial. It was believed that this wash-out period would mean that all litters were novel to the cats during the main trial.

In some trials, the cats were given the same litter type in two consecutive trials e.g.: A vs B then A vs Commercial. This may have made one type of litter more familiar to the cats, thereby influencing their subsequent litter choice. Another limitation was that the cats were given the litter combinations at different times, but in the same order. However, there was no evidence of an order effect on preferences.

4.4.5 Future Study Directions

As noted above, the number and weight of faecal and urinary excrements can vary according to factors not related to preference for the litter. Another method for determining the cats' preferences among the different litters might be to monitor the cats' behaviour with the litter. This could be done through a similar method to that used by Neilsen (2004) in which video surveillance was used to monitor the cats' interactions with litters with different odour controlling properties. Behaviour was not monitored in the current study due to time constraints, but could yield different preference results. The time a cat spends in the litter box or burying its excrements could be two factors of importance for measuring cat preferences. These both can relate to the smell of the litter both before and after use which may alter the cat's use.

4.5 Conclusion

The findings from this preference experiment support the hypothesis that O-litter will make a marketable cat litter. Individual cats demonstrated that they did have a preference for litter type. Although the O-litter products were not the most preferred, the cats were willing to use all three types of litter when the alternative was an empty box, so did not appear to have an aversion to them. Cat preference is very important when designing a cat litter because if the cat will not use the litter, then alterations are needed or a new design should be considered. The next step from this experiment is to make sure that O-Litter has qualities that meet the owners' needs relating to absorption and odour control.

Chapter 5: Absorption

5.1 Introduction

The degree to which a litter absorbs water plays an important role in litter choice by the owner and use by the cat. It was noticed in the pilot study (chapter 3), that after about eight days, the cats switched litters from the one they originally preferred to the other litter choice provided. The exact reason for this is unknown, however it may have been due to the litter not being able to absorb the urine as well as it originally did. The Companion Cats Code of Welfare (The New Zealand Companion Animal Council Inc, 2009), states that indoor cats must have a litter tray that is filled with an absorbent material. This is the only litter characteristic that recognised by the Code of Welfare, therefore illustrating the importance of this characteristic. It is because of the welfare code that this experiment was the first lab tests to be performed for the development of O-Litter products. This experiment was based on the method of Richards (1991), who assessed the absorbency of litter by adding a known volume of water to a known volume of cat litter and allowing it to stand for 30 minutes. After this, the mixture was poured through a colander and the amount of water absorbed was determined.

5.2 Materials and Methods

5.2.1 Experimental Design

Eight litters were selected for the study: four of which were different forms of O-Litter and four that were commercial cat litters. The four commercial litters were chosen because they were common cat litters sold in the supermarket. The four litters from the olive oil waste product consisted of two in the natural (non-processed) form, and two that were further processed, Pellet A, and Pellet B (see section 3.2.3 for processing details). Of the two natural products, one was selected from older waste material from the 2009 season (NO) and the other was from fresh product from the 2010 season (NN). These two litters were compared to see if there was an effect of storage time on the performance of the raw material. A proximate analysis was performed on the fresh product for comparison (Table 5.1). Fresh (2010 season) waste product was further processed to produce the pellet A (NA) and pellet B (NB) litters. The commercial litters used included a clumping litter: VitaPet Purrfection (VP: Masterpet, Auckland, New Zealand); a recycled newspaper litter: Breeder Celect (BC: Fibrecycle Pty. Ltd, Queensland, Australia); and two clay type litters: Excellence Ultra-Hygienic (EU: Virbac Ltd, Auckland, New Zealand); and VitaPet Cat Litter (VC: Masterpet, Auckland, New Zealand).

Table 5.1: Results from proximate analysis of new olive oil waste products.

Component (g/100g DM)	Amount
Dry matter (g/100g as is)	98.0
Crude Protein	3.7
Crude fat	11.5
Crude ash	1.3
Carbohydrate*	80.1

DM, Dry matter
*Calculated by difference (100 - crude protein - crude fat - crude ash)

5.2.2 Experimental Procedure

Richards (1991) did not specify the dimensions of the container used for measuring the absorption capacity. Preliminary studies were performed prior to the main experiment to determine what type of container would work best to test our series of litters. Different size bowls were used, but problems associated with the clumping litter and the water not able to reach the bottom litter granules was a concern, since the litter was not able to demonstrate its full water absorbing potential. Finally, it was decided to use a litter box since that was how the litter is used naturally for the cats and the water was able to reach all litter granules. Another difference between the Richards protocol and the one we developed for this study was the different litter volume to water volume ratio. Richards (1991) used an equal volume of litter to water. In the preliminary study, it was determined that the clumping litter could absorb a greater volume of water than the volume of litter itself. Since this experiment was to determine the maximum absorption capacity of the litters, a greater amount of water needed to be added.

An equal volume (500ml) of each litter was measured out, weighed, and then spread out evenly in individual, standard size litter boxes (40 x 29 x 12 cm). One litre (1000 ml) of tap water was poured into each litter box containing the different litters. The water was left to absorb into the litter for 30 minutes, with a 5 second stir at the half way point (15 minutes). At the end of this period, the litter and water were poured into a colander with a #12 sieve to remove any excess water. The litters were allowed to drain for 30 minutes, weighed again, and the amount of water absorbed into the litter was determined. This experiment was repeated three times to generate an average absorption capacity for each litter.

5.2.3 Statistical Analysis

A general linear model ANOVA from Minitab 15 (Minitab Inc., United States) was used to determine if there was a difference between the eight tested litters in their ability to absorb water, with level of significance set at $p < 0.05$. Data are presented as mean (± SD) unless otherwise stated.

5.3 Results

The average percent water absorbency of each litter is shown in Table 5.1. VitaPet Purrfection Clumping Litter absorbed the most water of all the litter types with an average water absorption of 832 ml of the 1000 ml added (83.2%) and had a statistical significant difference of p=0.0001 when compared to all other litters. Pellet form A had the second highest absorption capacity of 294.67 ml (29.5%). The remaining commercial litters had average absorptions that ranged from 130 ml (13.0%) up to 276 ml (27.6%).

Table 5.2: Absorption capacity for each litter (mean ± SD) and percentage of the water added that was absorbed by each litter.

Litter Name	Absorption Capacity (ml*)	% Absorbency
Breeder Celect (BC)	269 ± 61	26.9
Excellence Ultra-Hygienic (EU)	130 ± 2	13.0
VitaPet Cat Litter (VC)	166 ± 13	16.6
VitaPet Purrfection (VP)	832 ± 49	83.2
New Natural O-Litter (NN)	122 ± 29	12.2
Old Natural O-Litter (NO)	150 ± 21	15.0
Pellet A O-Litter (NA)	295 ± 50	29.5
Pellet B O-Litter (NB)	277 ± 36	27.7

*1 ml of water = 1 g.

When comparing statistical significant difference between litters, we found that the pelleted varieties only had significant difference with VitaPet Purrfection and Excellence Ultra-Hygienic. The Old Natural waste product had no significant difference with the two pelleted varieties, along with VitaPet Cat Litter and Breeder Celect. The New Natural waste product was not significantly different from Excellence Ultra-Hygienic and VitaPet Cat litter. VitaPet Purrfection stood on its own showing significant difference from all the other litters.

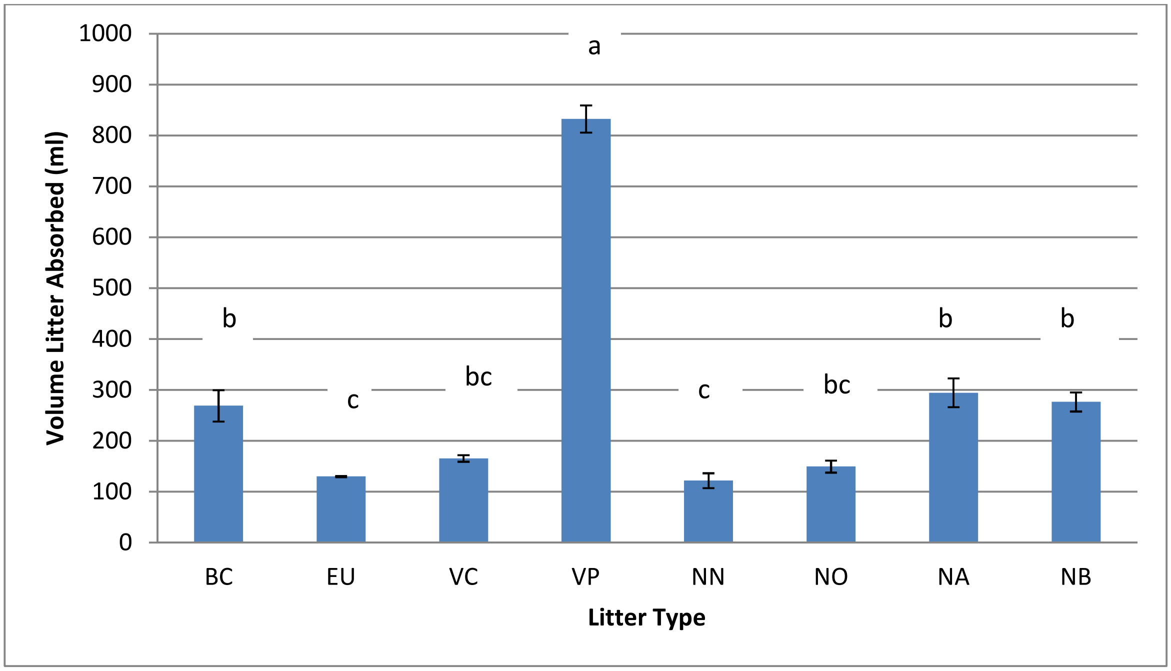


Figure 5.1: Absorption capacity for each litter (mean ± SD). Matching letters above columns represent no significant difference (p<0.05).

5.4 Discussion

5.4.1 Experiment

Absorption of urine is an important characteristic that many owners consider when purchasing cat litter. The results presented here show that the further processed forms of O-Litter are comparable to most of the commercial brands, absorbing significantly more moisture than Excellence Ultra-Hygienic and comparable to Breeder Celect and VitaPet Cat Litter (Table 5.2). The only litter that had a higher absorption capacity was the clumping variety. Clumping litters are claimed to have excellent absorption capacities due to unspecified additives contained within them. The two pelleted forms of the waste products, Pellet A and Pellet B, had the next highest absorption capacities, with Pellet A having a higher average than Pellet B, and having significant difference with the new natural waste product, Excellence Ulta-Hygienic, and the two VitaPet cat litters. The two natural O-Litters had absorption capacities similar to those of the two remaining commercial brands (Excellence Ultra-Hygienic and VitaPet Cat Litter).

The two pelleted versions of O-Litter had a high absorption capacity when compared to the other litters, with the exception of VitaPet Purrfection as mentioned above. This shows that further processing (pelleting) of the olive oil waste product, increased the absorption capacity significantly. The new and old natural waste material still had a similar absorption capacity to the other litters, however once pelleted, the absorption capacity nearly doubled.

Also, it was noticed that pellet A absorbed more water on average than pellet B, but did not have a statistical significant difference. The only difference between the two pellets is that pellet A went through a hammer mill which broke the waste products down to a smaller particle size (see section 3.2.3 for more details on processing). This shows that the finer the material is before being pelleted, the better it is at absorbing a liquid.

There was a difference in performance observed between the two natural products that may need to be considered in the commercialisation process. The older product had a higher absorption capacity than the newer product. The reason for this is unknown, since both products went through the same process from olive oil production to drying in the oven at Massey University. A proximate analysis was done on the newer product to determine if the nutrient profile was the same as the older product (Tables 3.3 and 5.1). These results show that there is little difference between the two.

Although the exact reason for why the O-Litter products absorbed more water than the commercial brands was not established, it is assumed that they were able to absorb a large amount of water because the olive oil waste product naturally holds a large amount of liquid. Through the oil production system, the waste products are removed of the liquid material, then they were further dried in a drying system. Therefore, this allowed for the O-Litter products to absorb a high amount of liquid.

5.4.2 Limitations and Future Direction

In this experiment tap water was used to determine the absorption capacity for each litter. This was the most suitable method to use for the time allotted, however a more accurate measurement would have involved actual cat urine. Since cat urine has a slightly acidic pH, typically 6.0 to 6.4 (Cottam, et al. 2002), and higher specific gravity (1.034) than water (Cottam, *et al.* 2003), it may take longer for the litter to absorb the urine than it did the water. Different results may or not have been obtained if the cat urine was used for the experiment but that is one testing alteration that could be performed in the future.

5.5 Conclusion

From this experiment it was determined that the processed form of O-Litter has a superior water absorption capacity to that of the majority of commercial brands. Since this an important characteristic for cat litters, it showed that O-Litter products could provide a marketable litter option, and the results could clearly support a high absorption capacity commercial claim.

Chapter 6: Odour Control

6.1 Introduction

Having a malodorous litter box is unacceptable to cat owners, or cats for that matter. Therefore, having a cat litter that minimises the odour of cat urine and faeces is beneficial. The pilot study (Chapter 3) showed that after approximately six to eight days, some cats changed litter preferences. This may have occurred due to a failure of the litter to continue to absorb urine (see Chapter 5), or it may have been due to a reduction in odour control properties.

Cats urine produces an ammonia odour while their faecal excrements produce a sulphur like odour. Domestic cats like to hide their presence, both physically and through olfactory evidence (Beaver, 2003). One method that they do this is through the burying of the excrements in a way that other animals cannot smell it. Since the cat's olfactory receptors are so sensitive, it is important that their litter controls their excrement odours, therefore hiding their presence to other animals.

The aim of this final experiment was to determine if the O-Litter products were comparable to commercial litters in their ability to control odour.

6.2 Materials and Methods

6.2.1 Experimental Design

Richards (1991) tested unabsorbed ammonia levels around cat litter using a simple protocol and a Dragor Gas Detector Pump. He added differing amounts of ammonia cleaner to each litter, left them for a period of time and then measured the amount of ammonia that remained unabsorbed by each litter. This protocol was adapted for the current experiment, with some modifications to the design. Since Massey University does not have a Dragor Gas Detector Pump, the experiment utilised different apparatus and was carried out in preserving jars using filter papers to absorb the ammonia, which was then read through an auto analyser. This procedure was originally developed by Banerjee, et al., (2002) who used it to test ammonia levels in the soil.

An ammonia-based cleaner was used in place of cat urine since cat urine may have varying amounts of ammonia in it depending on the cat's diet and how much food the cat eats (Cottam, et al. 2002). A cleaner contains consistent amounts of ammonia and is readily available.

6.2.1.1 Calibration Process

Before the experiment could be performed, we needed to determine the amount of ammonia that needed to be added to each of the litters. The amount required had to be large enough to be absorbed by the litter, but also allow sufficient ammonia to remain within the container and be absorbed by the filter paper. The source of ammonia used for experiment was Handy Andy Ammonia Cleaner (Clorox, Mt Wellington, New Zealand). A one litre preserving jar was used for each replicate in this experiment with holes drilled in the lids (and plugged with bungs) to allow the addition of the cleaner. A 50 ml beaker was placed inside and to one side of the preserving jar. A 110 mm filter paper, fully soaked in 2 N sulphuric acid (H_2SO_4), was then shaped in a cone and placed into the 50 ml beaker in the preserving jar (Figure 6.1).

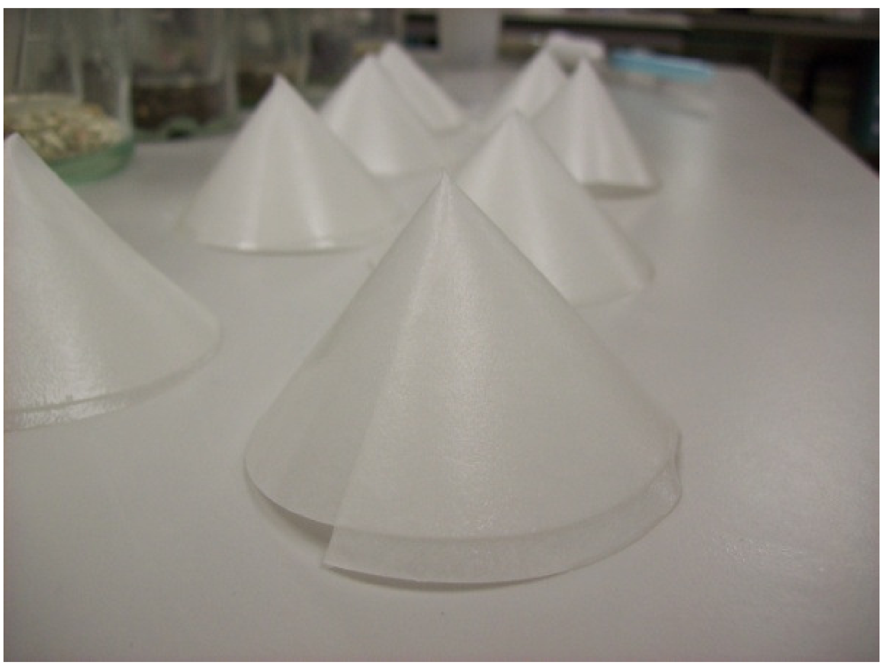


Figure 6.1: Filter papers prepared for the study.

The lid was then placed on the preserving jar (with the bung in place) to seal the container and prevent the ammonia from leaking out (Figure 6.2). A 6 ml syringe fitted with an 18 gauge 3½" needle was used to insert the ammonia cleaner into the jars. A 2 M potassium chloride (KCl) solution was used to extract the ammonia from the filter paper. A urine jar containing 20 ml 2 M KCl was used for this process.

Figure 6.2: Experimental apparatus used for the calibration process.

6.2.1.2 Experiment

The same apparatus used in the calibration process was used for the main experiment, but 50 g of cat litter was added to the base of the preserving jar before the beaker was placed in it. The concentration of cleaner used in the main experiment was determined during the calibration process and this amount was used throughout the remainder of the experiment.

6.2.2 Experimental Procedure

6.2.2.1 Calibration Process

The range of concentrations of the cleaner was tested in a series of preserving jars. The concentrations tested were 100% cleaner, 1:1 dilution of cleaner:water, and further dilutions of 1:2, 1:4, and 1:8. The cleaner: water concentrations were made up in a 50 ml beaker. 5 ml of each cleaner and/or water solution were drawn up into the syringe, and then dispensed to the bottom of the jar using a needle inserted through the bung. Care was taken to prevent any cleaner hitting the filter paper and adversely affecting the results. The jars remained sealed for 30 minutes following the addition of the cleaner, after which time the filter papers were removed and placed in the urine collection container with 20 ml 2N KCl. The containers were shaken for 30 seconds and then left for a further 9 minutes 30 seconds.

The filter papers were removed and the 2N KCl solution was placed in a refrigerator at 4°C until analysis occurred.

6.2.2.2 Main Experiment

Approximately 50 g of each cat litter was placed evenly in the bottom of each preserving jar. Four preserving jars were prepared for each of the eight litters, one for each time interval: 10 minutes, 20 minutes, 40 minutes, and 80 minutes. A ninth preserving jar which received no litter was used as a control. This jar underwent the same experimental procedure as the others with the filter paper removed after 10 minutes. Only one control jar was used for each data set to obtain a maximal ammonia value to compare the performance of the litters against. The lids were then replaced to seal the containers. 5 ml of 1:1 cleaner:water mixture (determined from the calibration process) was drawn up into a syringe. This mixture was prepared in advance so the same batch was used for each trial and each time interval. A needle was then used to dispense the 5ml solution through the bung, and onto the cat litter at the bottom of the preserving jar. Only the cat litter on the opposite side of the preserving jar to the beaker containing the filter paper was wetted with the cleaner:water mixture to avoid dispensing any cleaner on the filter paper (Figure 6.3). The jars remained sealed for the appropriate amounts of time (listed above). After each time period had elapsed, the filter papers were removed and placed in the urine collection jar and processed as previously described for the calibration trial (see section 6.2.2.1) Three replicates were carried out to obtain average ammonia absorption values for each litter.

Figure 6.3: Experimental apparatus to show how ammonia was dispensed on to each cat litter.

6.2.2.3 Analysis

The filter papers were analysed using a Technicon auto analysis system (Technicon Sampler series 1, Technicon Peristaltic Pump series III, Technicon Temperature Controller, and Technicon Colorimeter series II). The sample data were then interpreted by the computer software NAP v 4.4.

6.2.3 Statistical Analysis

A general linear model ANOVA from Minitab 15 (Minitab Inc., State College, Pennsylvania) was used to determine if there was a difference between the eight tested litters in their ability to absorb ammonia, with level of significance set at $p < 0.05$. Data are presented as mean (± SD) unless otherwise stated.

6.3 Results

6.3.1 Calibration

The results from the calibration process were used to determine the appropriate concentration of cleaner used in the main experiment. The cleaner was mixed with water to obtain a range of concentrations. The concentration of cleaner selected had to provide ammonia levels in the filter paper, that when released into the 2N KCl, were in the detectable range of the auto analyser (i.e. not too high or too low). Table 6.1 gives the ammonia concentrations that were released from the filter papers into the KCl solution. The main area of concern is the net height of the ammonia peak: it needed to be below 100 in order for the machine to be able to read the sample, but not too low that the machine could not read it. A complicating factor was the effect of the litter in the main study, which will absorb some of the ammonia, further reducing the ammonia levels for the machine to measure. Therefore, it was decided to use the 1:2, cleaner to water concentration for the main experiment so that we obtained readings comfortably within the measurement range of the auto analyser.

Table 6.1: Results from the calibration process: the height of the ammonia peak and concentration of ammonia generated by addition of varying dilutions of Handy Andy cleaner to an empty preserving jar.

Cleaner:Water	Net Peak Height	Concentration (µg/ml NH_3-N)
1:8	21.2412	2.77
1:4	46.6218	6.08
1:2	55.7577	7.27
1:1	68.4247	8.93
100% Cleaner	88.9413	11.6

6.3.2 Main Experiment

The results of the main study (Appendix 6.1) show how the O-Litters compared to the commercial brands. In the majority of the experiments, the O-Litter products absorbed a higher amount of ammonia than the commercial brands, with the exception of Excellence Ultra-Hygienic Litter. Breeder Celect Recycled Paper was found to have higher concentrations of ammonia than the control in some situations. Tables 6.2 through 6.5 and Figures 6.4 through 6.7 show the results of three replicates for each time interval.

Table 6.2: Results from the 10 minute odour absorption experiment. Values represent the amount of ammonia remaining unabsorbed by the litter and captured by filter paper soaked with KCl in 10 minutes after the cleaner was added.

Litter	Experiment 1 µg/ml NH_3-N	Experiment 2 µg/ml NH_3-N	Experiment 3 µg/ml NH_3-N	Mean µg/ml NH_3-N	SEM
Control (C)	10.89	-	-	10.89	
Breeder Celect BC)	6.69	6.73	5.22	6.21	0.50
Excellence (EU)	1.58	1.86	6.37	3.27	1.55
VitaPet (VC)	2.06	2.61	1.75	2.14	0.25
VitaPet Clumping Litter (VP)	2.74	3.51	2.22	2.82	0.38
New Natural (NN)	1.77	2.62	1.48	1.96	0.34
Old Natural (NO)	1.93	3.23	1.47	2.21	0.53
Pellet A (NA)	1.3	1.86	0.87	1.34	0.29
Pellet B (NB)	1.48	1.69	0.67	1.28	0.31

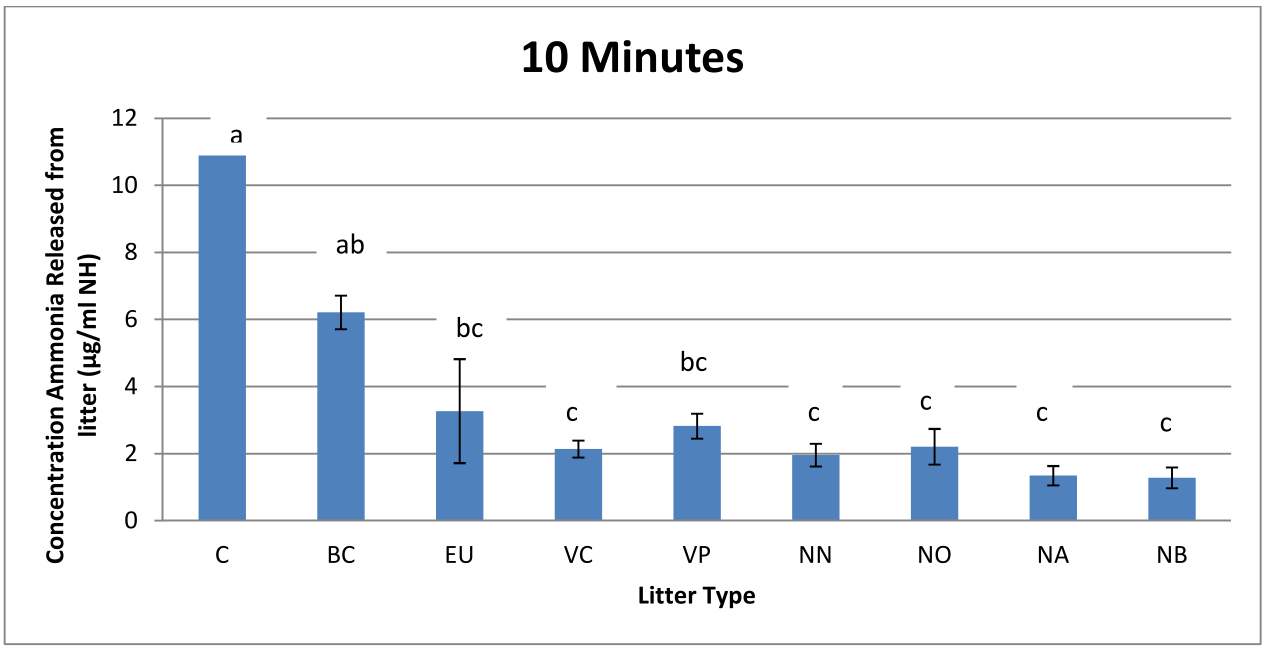


Figure 6.4: Amount of ammonia (Mean ± SEM) remaining unabsorbed by the litter and captured by filter paper soaked with KCl in 10 minutes after the cleaner was added. Matching letters above columns represent no significant difference ($p<0.05$).

Table 6.3: Results from the 20 minute odour absorption experiment. Values represent the amount of ammonia remaining unabsorbed by the litter and captured by filter paper soaked with KCl in 20 minutes after the cleaner was added.

Litter	Experiment 1 µg/ml NH_3-N	Experiment 2 µg/ml NH_3-N	Experiment 3 µg/ml NH_3-N	Mean µg/ml NH_3-N	SEM
Control (C)	10.83	-	-	10.83	
Breeder Celect Recycled Paper (BC)	8.50	8.09	4.75	7.11	1.19
Excellence (EU)	1.24	4.48	1.01	2.24	1.12
VitaPet (VC)	2.03	4.05	1.98	2.69	0.68
VitaPet Purrfection Clumping Litter (VP)	3.02	6.07	2.33	3.81	1.15
New Natural (NN)	1.41	2.72	1.62	1.92	0.41
Old Natural (NO)	2.07	2.20	1.65	1.97	0.17
Pellet A (NA)	1.77	1.93	1.07	1.59	0.26
Pellet B (NB)	1.72	2.57	1.35	1.88	0.36

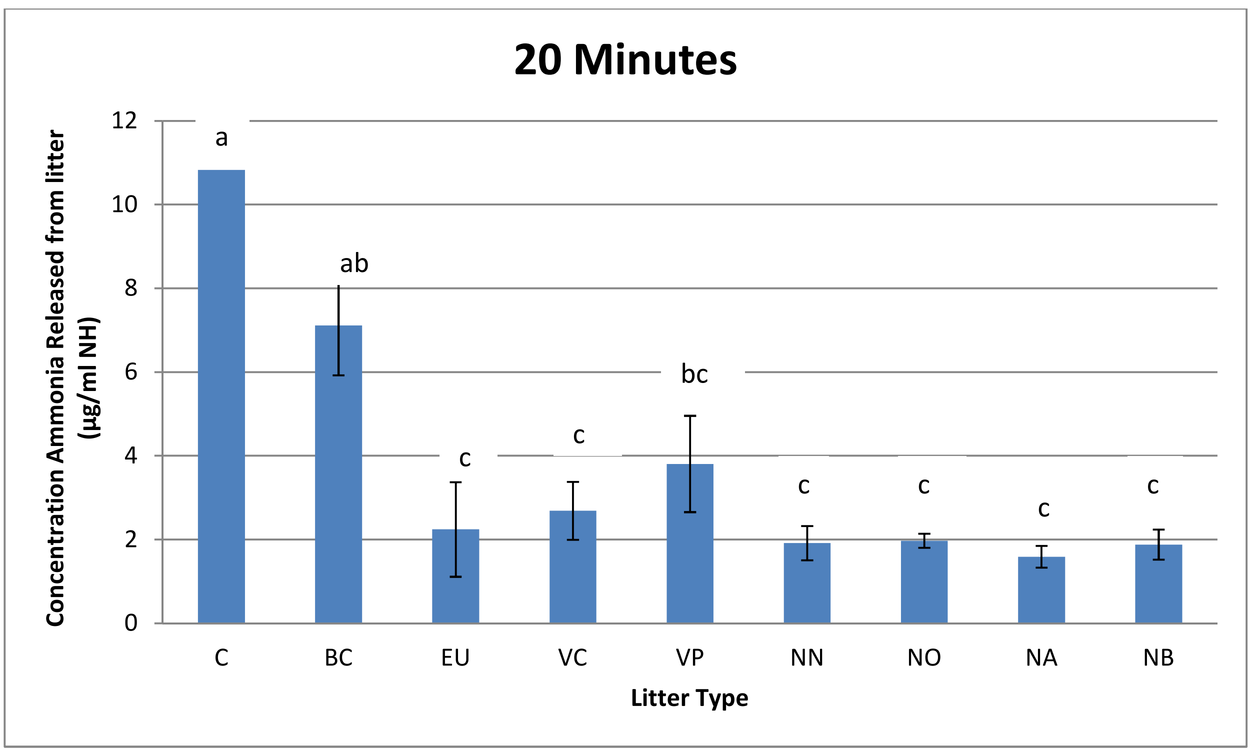


Figure 6.5: Amount of ammonia (Mean ± SEM) remaining unabsorbed by the litter and captured by filter paper soaked with KCl in 20 minutes after the cleaner was added. Matching letters above columns represent no significant difference ($p<0.05$).

Table 6.4: Results from the 40 minute odour absorption experiment. Values represent the amount of ammonia remaining unabsorbed by the litter and captured by filter paper soaked with KCl in 40 minutes after the cleaner was added.

Litter	Experiment 1 µg/ml NH_3-N	Experiment 2 µg/ml NH_3-N	Experiment 3 µg/ml NH_3-N	Mean µg/ml NH_3-N	SEM
Control (C)	10.76	-	-	10.76	
Breeder Celect Recycled Paper (BC)	10.81	11.51	10.63	10.98	0.27
Excellence (EU)	2.22	2.91	1.41	2.18	0.43
VitaPet (VC)	2.88	3.29	2.85	3.01	0.14
VitaPet Purrfection Clumping Litter (VP)	4.31	6.87	2.81	4.66	1.19
New Natural (NN)	3.10	1.83	2.28	2.40	0.37
Old Natural (NO)	3.47	2.23	2.14	2.61	0.43
Pellet A (NA)	2.23	1.70	1.45	1.79	0.23
Pellet B (NB)	2.45	1.82	1.61	1.96	0.25

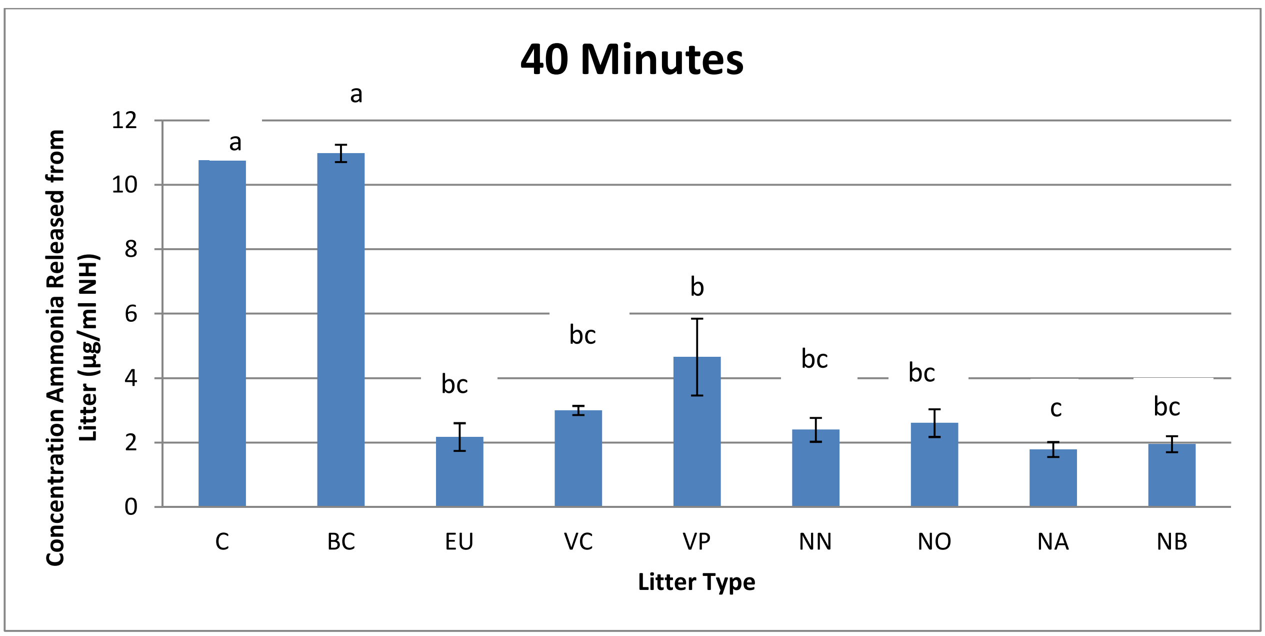


Figure 6.6: Amount of ammonia (Mean ± SEM) remaining unabsorbed by the litter and captured by filter paper soaked with KCl in 40 minutes after the cleaner was added. Matching letters above columns represent no significant difference ($p<0.05$).

Table 6.5: Results from the 80 minute odour absorption experiment. Values represent the amount of ammonia remaining unabsorbed by the litter and captured by filter paper soaked with KCl in 80 minutes after the cleaner was added.

Litter	Experiment 1 µg/ml NH_3-N	Experiment 2 µg/ml NH_3-N	Experiment 3 µg/ml NH_3-N	Mean µg/ml NH_3-N	SEM
Control (C)	10.71	-	-	10.71	
Breeder Celect Recycled Paper (BC)	11.49	11.02	11.47	11.33	0.15
Excellence (EU)	5.40	2.22	1.53	3.05	1.19
VitaPet (VC)	6.46	7.54	3.04	5.68	1.36
VitaPet Purrfection Clumping Litter (VP)	5.20	9.06	4.59	6.28	1.40
New Natural (NN)	2.88	1.63	2.48	2.33	0.37
Old Natural (NO)	5.80	2.32	3.21	3.78	1.04
Pellet A (NA)	5.03	2.44	2.14	3.20	0.92
Pellet B (NB)	3.27	1.70	1.59	2.19	0.54

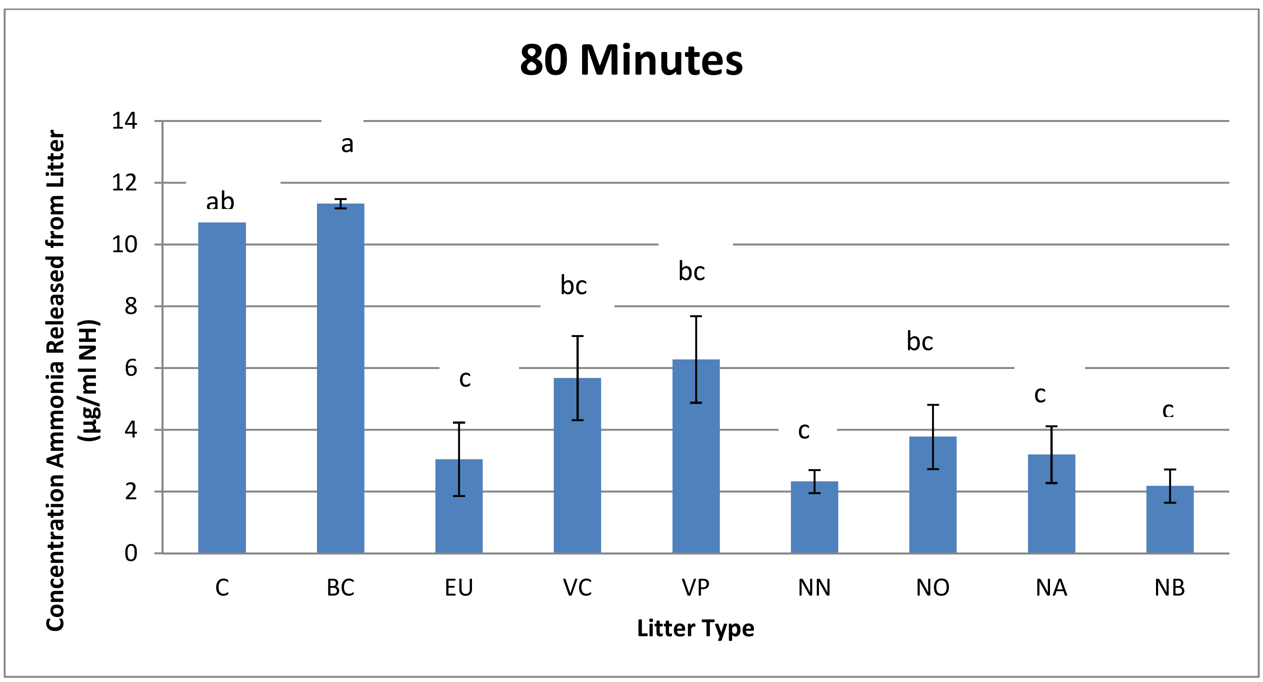


Figure 6.7: Amount of ammonia (Mean ± SEM) remaining unabsorbed by the litter and captured by filter paper soaked with KCl in 80 minutes after the cleaner was added. Matching letters above columns represent no significant difference ($p<0.05$).

6.3.3 Ranked Results

Table 6.6 provides the average ammonia concentration for each time interval and the rank that each litter had based on the absorption capacity of the ammonia. The two pelleted versions of O-Litter were given the same rank, rank 1, because it was dependent on the time interval for which place they belonged.

Table 6.6: Ammonia concentration (Mean ± SEM) for each litter at each time interval with an overall rank of litters based on ammonia absorption. Matching letters within columns represent no significant difference (p<0.05).

Litter	10 Minutes µg/ml NH_3-N	20 Minutes µg/ml NH_3-N	40 Minutes µg/ml NH_3-N	80 Minutes µg/ml NH_3-N	Rank
Control	10.89^{a}	10.83^{a}	10.76^{a}	10.71^{ab}	
Breeder Celect Recycled Newspaper	6.21 ± 0.50^{ab}	7.11 ± 1.19^{ab}	10.98 ± 0.27^{a}	11.33 ± 0.15^{a}	8
Excellence Ultra-Hygienic Litter	3.27 ± 1.55^{bc}	2.24 ± 1.12^{c}	2.18 ± 0.43^{bc}	3.05 ± 1.19^{c}	4
VitaPet Cat Litter	2.14 ± 0.25^{c}	2.69 ± 0.68^{c}	3.01 ± 0.14^{bc}	5.68 ± 1.36^{bc}	6
VitaPet Purrfection Clumping Litter	2.82 ± 0.38^{bc}	3.81 ± 1.15^{bc}	4.66 ± 1.19^{b}	6.28 ± 1.40^{bc}	7
New Natural O-Litter	1.96 ± 0.34^{c}	1.92 ± 0.41^{c}	2.40 ± 0.43^{bc}	2.33 ± 0.37^{c}	3
Old Natural O-Litter	2.21 ± 0.53^{c}	1.97 ± 0.17^{c}	2.61 ± 0.43^{bc}	3.78 ± 1.04^{bc}	4
O-Litter Pellet A	1.34 ± 0.29^{c}	1.59 ± 0.26^{c}	1.79 ± 0.23^{c}	3.20 ± 0.92^{c}	1
O-Litter Pellet B	1.28 ± 0.31^{c}	1.88 ± 0.36^{c}	1.96 ± 0.25^{bc}	2.19 ± 0.54^{c}	1

6.4 Discussion

6.4.1 Experiment

The results of this experiment support the hypothesis that O-Litter will make a marketable cat litter because it has odour control capabilities that are similar or superior to that of the commercial litter varieties tested. The O-Litter products, on average, had a consistently higher ammonia absorption capacity than the commercial litters that were tested, with the exception of Excellence Ultra-Hygienic Litter.

It was assumed that the reading obtained from the control jar was representative of the maximal levels of ammonia produced from the addition of the cleaner, we can assess the relative efficiencies of the litters to absorb ammonia at 10, 20, 40 and 80 minute intervals.

In the 10 minute trial, two litters consistently showed the highest ammonia absorption rates; the O-Litters Pellet A and Pellet B. Pellet B absorbed 88.3% of the ammonia administered and Pellet A 87.8%.

The worst performing litter was Breeder Celect Recycled Paper which only absorbed 43.0% of the ammonia.

In the first replicate, the majority of litters showed similar ammonia absorption capacities (74.8-88.1%) with the exception of Breeder Celect Recycled Paper which showed absorbed ammonia levels two times lower than the others (38.6%: Table 6.5 and Figure 6.7.). In the second and third replicates, Pellet B had the highest ammonia absorption capacity, 84.5% and 93.8% respectively. In the third replicate, the jar containing the Excellence Ultra-Hygienic litter recorded an unexpectedly high ammonia level (6.37ug/ml). This may have been caused by some of the ammonia cleaner accidentally being dispensed on the filter paper. The levels of ammonia recorded in the first two replicates of the same litter were over three times lower (1.58 and 1.86 ug/ml respectively).

In the 10 minute ammonia absorption experiment, Breeder Celect and the empty control jar, showed similar measured ammonia levels (p=0.09), indicating that the litter absorbed very little ammonia. However, the ammonia absorption capacity of the Breeder Celect was not significantly different from Excellence (p=0.21) and VitaPet Purrfection (p=0.08). In contrast, VitaPet Cat Litter (p=0.02), Old natural O-litter (p=0.02), New natural O-litter (p=0.01), Pellet A (p=0.00) and Pellet B (p=0.00) all absorbed significantly more ammonia.

In the 20 minute experiment, Breeder Celect Recycled Paper and VitaPet Purrfection Clumping Litter again were the highest for ammonia released from the litter, only absorbing 34.4% and 64.9% (Table 6.4 and Figure 6.6.). The remaining litters had very similar results though.

In the first and third replicate, the six remaining litters (Excellence Ultra-Hygienic, VitaPet Cat Litter, New and Old Natural and Pellet A and B), had ammonia release between 1.01 and 2.07 $\mu gX.ml^{-1}$ (90.8% and 81% absorption). The second trial had a greater range of ammonia release from 1.93 to 4.48 $\mu gX.ml^{-1}$(82.3% and 58.9% absorption). The reasoning for the difference between the replicate values is unclear. Some of the ammonia cleaner may have accidently been deposited on the filter paper itself, decreasing the cat litter absorption rate. The litters that absorbed the most ammonia were again, Excellence Ultra-Hygienic Litter and Pellet A.

The 20 minute ammonia absorption experiment was similar to the 10 minute experiment with Breeder Celect and the control showing no statistical significant difference (p=1.0), indicating that the litter absorbed very little ammonia. The remaining litters had no significant difference between them ($p \geq 0.05$).

In the 40 minute ammonia absorption experiment, Breeder Celect Recycled Paper had the highest ammonia released, with it having a higher ammonia level than the control (10.98 vs 10.76 µg/ml NH_3-N), followed by VitaPet Purrfection Clumping Litter, absorbing only 56.7% (Table 6.3 and Figure 6.5.). The two litters that absorbed the most ammonia were Pellet A (83.4%) and Pellet B (81.8%).

When looking at the statistical significant difference for the 40 minute experiment, Breeder Celect and the control had no significant difference between them (p=1.00). The remaining of the litters, with one exception, had no significant difference between them. The exception is with O-litter pellet A and VitaPet Purrfection Clumping litter which had a statistical significant difference of 0.02.

The 80 minute trial had similar results to the 40 minute trial, with the Breeder Celect Recycled Paper having an ammonia concentration that was higher than the control (Table 6.2 and Figure 6.4.). The reason for this is unsure unless the litter itself has ammonia in it. VitaPet Purrfection Clumping Litter had the second highest ammonia released, only absorbing 41.4%, followed by VitaPet Cat Litter, which absorbed 47%. Excellence Ultra-Hygienic Litter was the only litter that released less ammonia than some of the O-Litter products, absorbing 71.6% of the ammonia. Of the litters used for this experiment, the New Natural waste products absorbed the highest amount of ammonia (78.3%), therefore releasing less into the filter paper for analysis.

When comparing the litters based on statistical significant difference, Breeder Celect Recycled Paper, VitaPet, and VitaPet Purrfection Clumping Litter were not significantly different than the control (p=1.00; 0.73; and 1.00). This means that these litters did not do a good job absorbing the ammonia. The remaining litters had a P-value of less than, or equal to, 0.05, showing that they did have significant difference. Of the four O-Litter products, when looking at the results from the auto analyser, there is some difference, however based on the statistical test performed, there is no statistical significant difference between them (p=1.00).

In most cases, the Excellence Ultra-Hygienic Litter and Pellet A and B had the highest rate of ammonia absorption. In some cases, the concentration of ammonia was much higher than the other two sets in that time interval. This may have been caused by some of the ammonia cleaner accidentally being dispensed directly on the filter paper.

When comparing time intervals, it is noticed that there is a lower ammonia concentration in the 10 minute interval and it increases as the time increases. This gives more time for the ammonia to be released from the litter. It was expected in some cases for the ammonia concentration to decrease as time progressed because the litter was able to absorb it better. However, this was not the case.

6.4.2 Ranked Results

The two pelleted versions of O-Litter (Pellet A and Pellet B) were given a rank of one since they were both had the lowest concentration of ammonia in the filter paper, therefore more was absorbed by the litter. It was dependent on the time interval for which litter was the best. Pellet version A performed better than B in the 20 and 40 minute trial where as pellet B performed better than A in the 10 and 80 minute trial. The remaining litters had concentrations that made results easy to determine, showing that O-Litter products took the first four ranks for odour control. The recycled newspaper variety of cat litter, Breeder Celect, was the worst at absorbing the ammonia, and in some cases even had a higher ammonia concentration than the control.

When comparing the time course of each litter experiment, it was noticed that there was a lower ammonia concentration after 10 minutes of exposure which increased as the time of exposure increased. This gives more time for the ammonia to be released from the litter. It was expected in some cases for the ammonia concentrations to decrease as time progressed because the litter was able to absorb it better. However, this was not the case.

These results suggest that O-Litter products can meet the odour controlling requirements of owners and cats. Other cat litters that are already on the market do not perform as well as the different forms of O-Litter. The relative performance of the litters may also aid in the pricing of the litter since the company can say that O-Litter has high odour controlling properties which several other commercial brands cannot meet.

6.4.3 Future Recommendations

Since the concentration of ammonia can vary with cat's urine, an ammonia-based cleaner was used in this study. If cat urine was used, different results may have been obtained. For the purpose of this experiment however, it was felt that using an ammonia-based cleaner would be more beneficial because it was easier to obtain, had a consistent ammonia concentration, and was easier to use. A future experiment using cat urine may be the next step before sending this litter to market.

6.5 Conclusion

Having a cat litter that controls the odour of urine and faeces is a highly marketable characteristic. Although this experiment only tested the absorption or neutralisation of one odour (ammonia) by the litters, the results are extremely promising and similar results may well be obtained in further experiments conducted with cat urine or faeces. The results from this experiment support the hypothesis that O-litter will make a marketable cat litter since its ability to absorb ammonia is equal to, if not better than, many commercial brands.

Chapter 7: General Discussion

The purpose of this thesis was to outline the development and testing of a novel cat litter from a waste product of the olive oil industry. Indoor cat ownership in New Zealand is rising, therefore the usage of cat litter is increasing accordingly. This increased use of cat litter has led to an increase in the amount of non-biodegradable waste in landfills, and concern regarding the effect this is having on the environment. Therefore, creating a cat litter that is biodegradable would benefit the environment and provide a marketing strategy for the producer. In addition, since the litter has been developed from the waste products from the olive oil industry, developing the litter will be reducing that waste in landfills.

There are a few existing cat litters based on recycled products (e.g. newspaper and corn husk), but these products are limited and few are available in New Zealand. In addition, besides information obtained from patents, little research has been published relating to cat litter production/creation and cat preferences for one litter over another.

Four experiments were performed on the O-Litter products to determine whether they would make a marketable cat litter: a pilot study to establish a preference testing protocol, a larger study identifying cat preference for a range of litters, and two laboratory tests of litter absorbency and odour control. Each of these experiments produced useful information for both the research side of cat litter and cat preferences and also information that would be of importance for the producer, The Village Press. The following summarises each experiment and the results obtained.

The purpose of the first experiment, the pilot study (chapter 3), was to determine the length of time each litter combination needed to remain in the cage to accurately determine a preference and make sure the cats do not demonstrate side preferences. According to the criteria used in this study, most cats expressed a preference within the first 24 hours. Two cats here, Raven and Jade, did not meet the criteria for a preference, using both litters provided almost equally. Also, Lea and Rach switched litters between day six and eight. This switch most likely reflected poor urine absorbency or odour control of the originally preferred litter (this was shown to be the most likely case from the experiments performed in chapters 5 and 6). The results also demonstrated that the cats did not have a side preference, using their preferred litter regardless of which side of the cage the litter was on.

Although the data suggests that only a 24 hour period was necessary for each litter combination to remain in the cage, it was decided that 48 hours would be better as it would allow the cats to get use to each new litter combination. In addition, it would allow the boxes to be rotated from one side to other to verify that the cats did not have a side preference instead of a litter preference.

Although determining litter preferences was not the purpose of this pilot study, a record was made of each cat's preference between the two litters offered. Preferences were determined by two methods: the change of weight and the number of excrements. The commercial brand and the natural form of O-litter were preferred over the two pelleted forms. Regardless of litter type, all cats offered an empty box chose the litter exclusively, suggesting that cats prefer to bury their excrements in some sort of material and also that they do not have an aversion to the O-Litter products. This provided a starting point for the next experiment which was to determine the cats litter preferences between all litter choices.

The purpose of the first main experiment (chapter 4) was to compare three different O-Litter products against a commercial brand in terms of cat preferences. Three versions of O-Litter were offered: natural, pellet A, and pellet B; along with a bentonite commercial brand: VitaPet Cat Litter and a negative control (empty litter box). Each of the 10 cats received each of the ten combinations for a 48 hour period. The change of weight and number of excrements were recorded to determine which litter the cats preferred.

Preferences were evaluated at the individual cat level and at a population level. In all cases, any litter was chosen over the empty box, showing that the cats preferred to leave their excrements in some sort of litter. At the individual level, cats preferred the commercial litter and natural waste product equally on the basis of weight change. Based on the number of excrements, the commercial litter was the most preferred. At the population level, the commercial litter was the most preferred followed by the natural waste product.

Importantly, this experiment showed that the cats did not have an aversion to the O-Litter products, even though the commercial brand was the most preferred litter overall. Of the three O-Litter products tested, the natural waste material was the most preferred. This suggests that the waste product does not need to go through any further processing based on the cats' preferences.

The natural form of the O-Litter products may have been the most preferred because it has a similar texture to the saw dust that is normally used for their cat litter. The pelleted forms of the litter were a new thing for the cats, and with more time, the cats may have eventually preferred these litters over the natural.

The purpose of the experiment for chapter 5 was to compare the absorbency of O-Litter products with that of commercial brands. In this experiment, four O-Litter products: New Natural, Old Natural, Pellet A, and Pellet B were compared with four commercial brands: Breeder Celect, Excellence Ultra-Hygienic, VitaPet Cat Litter, and VitaPet Purrfection. An equal volume (500 ml) of litter was placed in litter trays and weighed, before 1000ml of water was added. After 30 minutes, the litters were strained to determine the amount of water absorbed by each. The results showed that VitaPet Purrfection had the highest absorbency, absorbing almost all of the water. This was expected since this is a clumping variety and such litters are known for their high liquid absorbency. The two pelleted versions of O-Litter and the Breeder Celect commercial litter had the next highest absorbency. Excellence Ultra-Hygienic and the New Natural waste product had the lowest absorbency. The New Natural O-Litter had the lowest absorbency rate. The reason for this is unclear since both the new and old natural forms of O-Litter went through the same processing.

This experiment showed that the pelleted versions of the O-Litter performed better in terms of absorbency than the two natural waste products. The pelleted varieties also performed either as well as or better than, the commercial brands with the exception of the clumping litter. The O-Litter varieties may have had a high absorption rate because the material started with a high volume of liquid, which was removed, then the waste material was dried even further. This allowed for the material to absorb a large amount of liquid again. These results showing how the O-Litter varieties absorbed liquid are valuable for The Village Press and will allow them to decide how best to process the waste products into a cat litter. Pelleting the olive oil waste products appears to significantly increase its absorption capacity.

Odour control is another important characteristic in the development of a cat litter (chapter 6). In this experiment the same litter varieties were used as in chapter 5, but in this experiment I was comparing the odour controlling capabilities of the various litters. Each litter was placed in its own preserving jar where an ammonia source was added and then left for the allotted amount of time. This study found that the two pelleted versions of O-Litter consistently performed better than all of the other the other litters. This may be due to the pelleted varieties being able to absorb more odour into the pellets than the non-pelleted varieties.

Overall, these experiments show that some of the O-Litter products have great potential for being developed into a novel cat litter. Olive oil waste products were comparable to some of the commercial brands and in some cases out-performed the commercial brands. In the preference experiment the commercial litter was the most preferred; in the absorbency experiment the clumping litter absorbed the most liquid; and in the odour control experiment the pellet varieties absorbed the most ammonia.

Determining the best type of O-Litter product depends on which characteristic is considered the most important. Based on cat preference testing, the natural form was the most preferred of the O-Litter varieties. However, the study also showed that cats would use either pelleted form when the alternative was an empty box. The pelleted varieties had better absorbency than the natural forms. In terms of odour control, there was no significant difference between the four O-litter products. On the whole, the pelleted versions would make a better litter considering that cats will use them if given no other litter, and they performed better for absorbency and odour control. Very little difference was noticed between the two pelleted varieties, therefore the additional processing of pellet A is not necessary.

There were some limitations in each experiment that may have affected the results. In the preference experiment, the previous experience of the cats with sawdust litter and the order in which the litter combinations were provided may have affected the cat's preferences. All cats were given the litter combinations in the same order. This may have resulted in an order effect, with cats choosing the most familiar litter. However, there was no indication of this in the results. For both the absorption and odour control experiments, cat's urine was not used because other sources were easier to obtain and could be standardised. However, if cat's urine was used, different results may have been obtained.

There are several areas where additional research would be useful. For the preference experiment, monitoring of behaviour could provide more information on the cats' interaction with the various litters. For example, it would be interesting to know how long they spend burying their excrements in each litter, which would give an indication of the litter's ability to mask the scent of the urine and faeces. For the absorbance and odour control experiment, using cat's urine may provide a better indication of how the litters will perform once the cats are using them.

Additional experiments on the clumping ability, dust formation, and tracking should be performed. These experiments would aid in marketing strategies and show that this biodegradable cat litter is of a high quality and competes with other litters already on the market. In addition, in-home trials should be performed to evaluate owner preferences. Obtaining owner feedback about the litter can provide information on what needs to be changed and identify particular strong points about the litter which can then be used for marketing. This thesis provided information on some of the characteristics of cat litter that the cats preferred, but in the end, it is the cat owner that will be making the purchase, therefore finding what is preferred of the owner is valuable. These characteristics were not evaluated in this thesis due to lack of time but the information gained would be useful for the marketing of the cat litter.

Conclusions

Although O-Litters may have only out-performed the commercial brands in the odour control tests carried out in this thesis, they were comparable in most cases to the commercial litters. Cats did not have an aversion to the O-litters and used them when that was the only litter provided. In laboratory tests, O-Litters performed adequately in terms of absorbing liquid and excellently in terms of odour control. The information gained from this research suggests that the waste products of the olive oil industry can be used as a marketable cat litter and that pelleting the raw material improves its value as a cat litter.

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Appendix 3.1

Cat use of the litter with records of weight before (empty) and after (full) use, the change in weight, and the faecal and urine output. The side of the cage the litter box was placed is also recorded.

Cat	Litter	Side	Day	Full wt (g)	Empty wt (g)	Wt Δ (g)	# F	# U
Ra	a	L	0		1523		0	0
Ra	a	R	1	1552	1552	29	0	0
Ra	a	L	2	1567	1567	15	0	0
Ra	a	R	3	1573	1573	6	0	0
Ra	a	L	4	1573	1573	0	0	0
Ra	a	R	5	1566	1566	-7	0	0
Ra	a	L	6	1566	1566	0	0	0
Ra	a	R	7	1566	1566	0	0	0
Ra	a	L	8	1567	1567	1	0	0
Ra	a	R	9	1571	1571	4	0	0
Ra	a	-	10	1574		3	0	0
Ra	c	R	0		456		0	0
Ra	c	L	1	583	2054	127	1	1
Ra	c	R	2	2149	2149	95	0	0
Ra	c	L	3	2272	2256	123	1	1
Ra	c	R	4	2392	2297	136	1	1
Ra	c	L	5	2402	1928	105	1	1
Ra	c	R	6	2038	1852	110	1	1
Ra	c	L	7	1934	1763	82	1	1
Ra	c	R	8	1877	1669	114	1	1
Ra	c	L	9	1735	1311	66	1	1
Ra	c	-	10	1482		171	1	1
Cat	**Litter**	**Side**	**Day**	**Full wt (g)**	**Empty wt (g)**	**Wt Δ (g)**	**# F**	**# U**
Tui	a	L	0		1574		0	0
Tui	a	R	1	1601	1601	27	0	0
Tui	a	L	2	1615	1615	14	0	0
Tui	a	R	3	1624	1624	9	0	0
Tui	a	L	4	1623	1623	-1	0	0
Tui	a	R	5	1678	1558	55	0	1
Tui	a	L	6	1550	1550	-8	0	0
Tui	a	R	7	1545	1545	-5	0	0
Tui	a	L	8	1571	1497	26	0	1
Tui	a	R	9	1495	1495	-2	0	0
Tui	a	-	10	1497		2	0	0

Tui	n	R	0		997		0	0
Tui	n	L	1	1143	1110	146	1	1
Tui	n	R	2	1238	1052	128	1	1
Tui	n	L	3	1207	1352	155	1	1
Tui	n	R	4	1508	1196	156	2	1
Tui	n	L	5	1222	1127	26	1	1
Tui	n	R	6	1336	943	209	2	1
Tui	n	L	7	1017	1668	74	1	1
Tui	n	R	8	1764	1551	96	1	1
Tui	n	L	9	1733	1474	182	2	1
Tui	n	-	10	1593		119	1	1
Cat	**Litter**	**Side**	**Day**	**Full wt (g)**	**Empty wt (g)**	**Wt Δ (g)**	**# F**	**# U**
Raven	a	L	0		1606		0	0
Raven	a	R	1	1572	1572	-34	0	0
Raven	a	L	2	1583	1583	11	0	0
Raven	a	R	3	1690	1621	107	1	1
Raven	a	L	4	1610	1610	-11	0	0
Raven	a	R	5	1619	1619	9	0	0
Raven	a	L	6	1695	1502	76	0	1
Raven	a	R	7	1596	1393	94	1	1
Raven	a	L	8	1353	1353	-40	0	0
Raven	a	R	9	1477	1265	124	1	1
Raven	a	-	10	1325		60	0	1
Raven	b	R	0		1622		0	0
Raven	b	L	1	1779	1737	157	1	1
Raven	b	R	2	1800	1588	63	0	2
Raven	b	L	3	1608	1480	20	0	1
Raven	b	R	4	1595	1352	115	1	1
Raven	b	L	5	1420	1248	68	1	1
Raven	b	R	6	1348	1171	100	1	1
Raven	b	L	7	1201	1098	30	0	1
Raven	b	R	8	1167	1015	69	1	1
Raven	b	L	9	1041	987	26	0	1
Raven	b	-	10	1067		80	0	1
Cat	**Litter**	**Side**	**Day**	**Full wt (g)**	**Empty wt (g)**	**Wt Δ (g)**	**# F**	**# U**
Aura	a	L	0		1348		0	0
Aura	a	R	1	1441	1441	93	0	1
Aura	a	L	2	1551	1366	110	1	1
Aura	a	R	3	1443	1306	77	2	1

Aura	a	L	4	1385	1256	79	1	1
Aura	a	R	5	1293	1078	37	1	1
Aura	a	L	6	1209	1845	131	1	1
Aura	a	R	7	1936	1834	91	1	1
Aura	a	L	8	1891	1799	57	1	1
Aura	a	R	9	1858	1746	59	1	1
Aura	a	-	10	1795		49	1	1
Aura	e	R	0		0		0	0
Aura	e	L	1	0	0	0	0	0
Aura	e	R	2	0	0	0	0	0
Aura	e	L	3	0	0	0	0	0
Aura	e	R	4	0	0	0	0	0
Aura	e	L	5	0	0	0	0	0
Aura	e	R	6	0	0	0	0	0
Aura	e	L	7	0	0	0	0	0
Aura	e	R	8	0	0	0	0	0
Aura	e	L	9	0	0	0	0	0
Aura	e	-	10	0		0	0	0
Cat	**Litter**	**Side**	**Day**	**Full wt (g)**	**Empty wt (g)**	**Wt Δ (g)**	**# F**	**# U**
Jade	n	L	0		1036		0	0
Jade	n	R	1	1166	1144	130	1	1
Jade	n	L	2	1187	978	43	2	1
Jade	n	R	3	1047	1504	69	2	1
Jade	n	L	4	1550	1488	46	1	1
Jade	n	R	5	1499	1450	11	2	0
Jade	n	L	6	1463	1437	13	1	0
Jade	n	R	7	1476	1423	39	2	0
Jade	n	L	8	1456	1380	33	0	1
Jade	n	R	9	1417	1318	37	0	1
Jade	n	-	10	1337		19	0	1
Jade	c	R	0		574		0	0
Jade	c	L	1	595	1747	21	0	0
Jade	c	R	2	1895	1895	148	0	1
Jade	c	L	3	2036	1870	141	0	1
Jade	c	R	4	1921	1921	51	0	1
Jade	c	L	5	1972	1721	51	0	1
Jade	c	R	6	1848	1565	127	1	1
Jade	c	L	7	1657	1318	92	0	1
Jade	c	R	8	1368	2036	50	1	1
Jade	c	L	9	2154	1931	118	1	1

Jade	c	-	10	2004		73	1	1
Cat	**Litter**	**Side**	**Day**	**Full wt (g)**	**Empty wt (g)**	**Wt Δ (g)**	**# F**	**# U**
Lea	b	L	0		1480		0	0
Lea	b	R	1	1514	1514	34	0	0
Lea	b	L	2	1528	1528	14	0	0
Lea	b	R	3	1538	1538	10	0	0
Lea	b	L	4	1538	1538	0	0	0
Lea	b	R	5	1538	1538	0	0	0
Lea	b	L	6	1619	1429	81	0	1
Lea	b	R	7	1616	2688	187	0	1
Lea	b	L	8	2850	2649	162	1	1
Lea	b	R	9	2714	2498	65	0	1
Lea	b	-	10	2626		128	2	1
Lea	c	R	0		400		0	0
Lea	c	L	1	594	2226	194	1	2
Lea	c	R	2	2494	2366	268	1	1
Lea	c	L	3	2733	2434	367	3	1
Lea	c	R	4	2653	2610	219	1	1
Lea	c	L	5	2752	2446	142	1	1
Lea	c	R	6	2615	2091	169	2	1
Lea	c	L	7	2139	1994	48	2	1
Lea	c	R	8	1932	1867	-62	0	1
Lea	c	L	9	1888	1783	21	1	0
Lea	c	-	10	1736		-47	0	0
Cat	**Litter**	**Side**	**Day**	**Full wt (g)**	**Empty wt (g)**	**Wt Δ (g)**	**# F**	**# U**
Billi	c	R	0		544		0	0
Billi	c	L	1	682	2219	138	0	1
Billi	c	R	2	2335	2284	116	1	1
Billi	c	L	3	2458	2347	174	1	1
Billi	c	R	4	2557	2496	210	1	1
Billi	c	L	5	2571	2571	75	0	1
Billi	c	R	6	2821	2073	250	2	1
Billi	c	L	7	2111	1924	38	1	1
Billi	c	R	8	2149	1918	225	1	1
Billi	c	L	9	2190	1798	272	1	1
Billi	c	-	10	2096		298	1	1
Billi	e	L	0		0		0	0
Billi	e	R	1	0	0	0	0	0
Billi	e	L	2	0	0	0	0	0

Billi	e	R	3	0	0	0	0	0
Billi	e	L	4	0	0	0	0	0
Billi	e	R	5	0	0	0	0	0
Billi	e	L	6	0	0	0	0	0
Billi	e	R	7	0	0	0	0	0
Billi	e	L	8	0	0	0	0	0
Billi	e	R	9	0	0	0	0	0
Billi	e	-	10	0		0	0	0

Cat	Litter	Side	Day	Full wt (g)	Empty wt (g)	Wt Δ (g)	# F	# U
Beeva	b	R	0		1440		0	0
Beeva	b	L	1	1577	1470	137	1	1
Beeva	b	R	2	1597	1289	127	0	1
Beeva	b	L	3	1480	1015	191	2	1
Beeva	b	R	4	1157	2738	142	1	1
Beeva	b	L	5	2872	2529	134	1	1
Beeva	b	R	6	2716	2288	187	1	1
Beeva	b	L	7	2451	2015	163	2	1
Beeva	b	R	8	2167	1753	152	1	2
Beeva	b	L	9	1933	1518	180	1	1
Beeva	b	-	10	1686		168	2	1
Beeva	e	L	0		0		0	0
Beeva	e	R	1	0	0	0	0	0
Beeva	e	L	2	0	0	0	0	0
Beeva	e	R	3	0	0	0	0	0
Beeva	e	L	4	0	0	0	0	0
Beeva	e	R	5	0	0	0	0	0
Beeva	e	L	6	0	0	0	0	0
Beeva	e	R	7	0	0	0	0	0
Beeva	e	L	8	0	0	0	0	0
Beeva	e	R	9	0	0	0	0	0
Beeva	e	-	10	0		0	0	0

Cat	Litter	Side	Day	Full wt (g)	Empty wt (g)	Wt Δ (g)	# F	# U
Esta	n	R	0		1097		0	0
Esta	n	L	1	1134	1134	37	0	1
Esta	n	R	2	1214	1101	80	1	1
Esta	n	L	3	1237	999	136	1	1
Esta	n	R	4	1081	846	82	0	1
Esta	n	L	5	875	1797	29	1	1
Esta	n	R	6	1917	1689	120	1	1

Esta	n	L	7	1853	1577	164	2	1
Esta	n	R	8	1609	1498	32	1	1
Esta	n	L	9	1581	1407	83	1	1
Esta	n	-	10	1432		25	0	1
Esta	e	L	0		0		0	0
Esta	e	R	1	0	0	0	0	0
Esta	e	L	2	0	0	0	0	0
Esta	e	R	3	0	0	0	0	0
Esta	e	L	4	0	0	0	0	0
Esta	e	R	5	0	0	0	0	0
Esta	e	L	6	0	0	0	0	0
Esta	e	R	7	0	0	0	0	0
Esta	e	L	8	0	0	0	0	0
Esta	e	R	9	0	0	0	0	0
Esta	e	-	10	0		0	0	0

Cat	**Litter**	**Side**	**Day**	**Full wt (g)**	**Empty wt (g)**	**Wt Δ (g)**	**# F**	**# U**
Rach	b	L	0		1446		0	0
Rach	b	R	1	1478	1478	32	0	0
Rach	b	L	2	1495	1495	17	0	0
Rach	b	R	3	1505	1505	10	0	0
Rach	b	L	4	1543	1543	38	0	0
Rach	b	R	5	1538	1538	-5	0	0
Rach	b	L	6	1535	1535	-3	0	0
Rach	b	R	7	1524	1524	-11	0	0
Rach	b	L	8	1490	1390	-34	1	1
Rach	b	R	9	1511	1249	121	0	2
Rach	b	-	10	1281		32	0	1
Rach	n	R	0		1212		0	0
Rach	n	L	1	1261	1261	49	0	1
Rach	n	R	2	1395	1125	134	1	1
Rach	n	L	3	1300	1034	175	1	1
Rach	n	R	4	1127	2155	93	1	1
Rach	n	L	5	2297	2052	142	1	1
Rach	n	R	6	2245	1798	193	1	1
Rach	n	L	7	1932	1708	134	1	1
Rach	n	R	8	1895	1741	187	0	1
Rach	n	L	9	1811	1537	70	1	1
Rach	n	-	10	1609		72	1	1

Appendix 4.1

Daily Litter Box Measurements

Cat	Combination	Litter	Day	Full wt (g)	Empty wt (g)	Wt Δ (g)	# F	# U
Ra	a+c	a	1		926		0	0
Ra	a+c	a	2	935	935	9	0	0
Ra	a+c	a	3	941		6	0	0
Ra	a+c	c	1		860		0	0
Ra	a+c	c	2	1051	878	191	1	1
Ra	a+c	c	3	993		115	0	1
Ra	b+n	b	3		1048		0	0
Ra	b+n	b	4	1054	1054	6	0	0
Ra	b+n	b	5	1066		12	0	0
Ra	b+n	n	3		1122		0	0
Ra	b+n	n	4	1384	1311	262	1	1
Ra	b+n	n	5	1445		134	1	1
Ra	n+e	n	5		654		0	0
Ra	n+e	n	6	911	435	257	2	2
Ra	n+e	n	7	630		195	1	1
Ra	n+e	e	5		87		0	0
Ra	n+e	e	6	87	87	0	0	0
Ra	n+e	e	7	87		0	0	0
Ra	b+e	b	7		744		0	0
Ra	b+e	b	8	972	576	228	1	2
Ra	b+e	b	9	773		197	1	2
Ra	b+e	e	7		0		0	0
Ra	b+e	e	8	0	0	0	0	0
Ra	b+e	e	9	0		0	0	0
Ra	c+e	c	9		988		0	0
Ra	c+e	c	10	1178	1059	190	1	1
Ra	c+e	c	11	1229		170	1	1
Ra	c+e	e	9		0		0	0
Ra	c+e	e	10	0	0	0	0	0
Ra	c+e	e	11	0		0	0	0
Ra	b+c	b	11		933		0	0
Ra	b+c	b	12	933	933	0	0	0
Ra	b+c	b	13	937		4	0	0
Ra	b+c	c	11		1118		0	0
Ra	b+c	c	12	1382	1084	264	1	1
Ra	b+c	c	13	1232		148	1	1
Ra	n+c	n	13		913		0	0

Ra	n+c	n	14	1021	931	108	0	1
Ra	n+c	n	15	984		53	0	1
Ra	n+c	c	13		1047		0	0
Ra	n+c	c	14	1195	1075	148	1	1
Ra	n+c	c	15	1216		141	1	1
Ra	a+e	a	15		772		0	0
Ra	a+e	a	16	1049	575	277	1	2
Ra	a+e	a	17	851		276	1	2
Ra	a+e	e	15		0		0	0
Ra	a+e	e	16	0	0	0	0	0
Ra	a+e	e	17	0		0	0	0
Ra	a+b	a	17		579		0	0
Ra	a+b	a	18	585	585	6	0	0
Ra	a+b	a	19	605		20	0	1
Ra	a+b	b	17		1156		0	0
Ra	a+b	b	18	1312	1032	156	1	2
Ra	a+b	b	19	1108		76	0	1
Ra	a+n	a	19		992		0	0
Ra	a+n	a	20	1000	1000	8	0	0
Ra	a+n	a	21	1038		38	0	1
Ra	a+n	n	19		750		0	0
Ra	a+n	n	20	990	533	240	1	1
Ra	a+n	n	21	670		137	1	1
Tui	a+n	a	1		1043		0	0
Tui	a+n	a	2	1055	1055	12	0	0
Tui	a+n	a	3	1095		40	0	1
Tui	a+n	n	1		465		0	0
Tui	a+n	n	2	590	385	125	1	2
Tui	a+n	n	3	543		158	2	1
Tui	a+c	a	3		1376		0	0
Tui	a+c	a	4	1377	1377	1	0	0
Tui	a+c	a	5	1376		-1	0	0
Tui	a+c	c	3		1057		0	0
Tui	a+c	c	4	1251	998	194	2	1
Tui	a+c	c	5	1135		137	1	1
Tui	b+n	b	5		725		0	0
Tui	b+n	b	6	746	746	21	0	0
Tui	b+n	b	7	852		106	0	1
Tui	b+n	n	5		588		0	0
Tui	b+n	n	6	857	503	269	1	2
Tui	b+n	n	7	588		85	2	1

Tui	n+e	n	7		571		0	0
Tui	n+e	n	8	775	565	204	2	1
Tui	n+e	n	9	745		180	1	1
Tui	n+e	e	7		0		0	0
Tui	n+e	e	8	0	0	0	0	0
Tui	n+e	e	9	0		0	0	0
Tui	b+e	b	9		951		0	0
Tui	b+e	b	10	1092	779	141	2	2
Tui	b+e	b	11	1019		240	1	2
Tui	b+e	e	9		0		0	0
Tui	b+e	e	10	0	0	0	0	0
Tui	b+e	e	11	0		0	0	0
Tui	c+e	c	11		1167		0	0
Tui	c+e	c	12	1467	1126	300	3	2
Tui	c+e	c	13	1310		184	1	1
Tui	c+e	e	11		0		0	0
Tui	c+e	e	12	0	0	0	0	0
Tui	c+e	e	13	0		0	0	0
Tui	b+c	b	13		1041		0	0
Tui	b+c	b	14	1116	986	75	0	1
Tui	b+c	b	15	1076		90	0	1
Tui	b+c	c	13		1073		0	0
Tui	b+c	c	14	1257	1180	184	1	2
Tui	b+c	c	15	1278		98	1	1
Tui	n+c	n	15		571		0	0
Tui	n+c	n	16	702	570	131	2	1
Tui	n+c	n	17	588		18	1	0
Tui	n+c	c	15		1202		0	0
Tui	n+c	c	16	1251	1166	49	0	1
Tui	n+c	c	17	1302		136	0	2
Tui	a+e	a	17		807		0	0
Tui	a+e	a	18	1017	633	210	1	2
Tui	a+e	a	19	848		215	2	2
Tui	a+e	e	17		0		0	0
Tui	a+e	e	18	0	0	0	0	0
Tui	a+e	e	19	0		0	0	0
Tui	a+b	a	19		797		0	0
Tui	a+b	a	20	955	740	158	1	1
Tui	a+b	a	21	745		5	0	1
Tui	a+b	b	19		891		0	0
Tui	a+b	b	20	984	829	93	1	1

Tui	a+b	b	21	1013		184	2	1
Raven	a+b	a	1		1293		0	0
Raven	a+b	a	2	1398	1228	105	1	1
Raven	a+b	a	3	1376		148	2	1
Raven	a+b	b	1		945		0	0
Raven	a+b	b	2	939	939	-6	0	0
Raven	a+b	b	3	976		37	0	1
Raven	a+n	a	3		739		0	0
Raven	a+n	a	4	817	731	78	0	1
Raven	a+n	a	5	723		-8	0	0
Raven	a+n	n	3		940		0	0
Raven	a+n	n	4	1071	939	131	1	1
Raven	a+n	n	5	1100		161	1	1
Raven	a+c	a	5		1042		0	0
Raven	a+c	a	6	1292	1064	250	0	2
Raven	a+c	a	7	1178		114	0	2
Raven	a+c	c	5		1060		0	0
Raven	a+c	c	6	1121	1082	61	1	0
Raven	a+c	c	7	1150		68	2	0
Raven	b+n	b	7		1137		0	0
Raven	b+n	b	8	1262	1038	125	0	2
Raven	b+n	b	9	844		-194	1	1
Raven	b+n	n	7		260		0	0
Raven	b+n	n	8	392	181	132	1	1
Raven	b+n	n	9	582		401	0	2
Raven	n+e	n	9		786		0	0
Raven	n+e	n	10	1045	789	259	1	1
Raven	n+e	n	11	1053		264	1	2
Raven	n+e	e	9		0		0	0
Raven	n+e	e	10	10	0	10	1	0
Raven	n+e	e	11	0		0	0	0
Raven	b+e	b	11		1050		0	0
Raven	b+e	b	12	1256	708	206	1	2
Raven	b+e	b	13	867		159	1	2
Raven	b+e	e	11		0		0	0
Raven	b+e	e	12	0	0	0	0	0
Raven	b+e	e	13	0		0	0	0
Raven	c+e	c	13		935		0	0
Raven	c+e	c	14	1119	1059	184	2	0
Raven	c+e	c	15	1265		206	1	1
Raven	c+e	e	13		0		0	0

Raven	c+e	e	14	0	0	0	0	0
Raven	c+e	e	15	0		0	0	0
Raven	b+c	b	15		916		0	0
Raven	b+c	b	16	910	910	-6	0	0
Raven	b+c	b	17	935		25	1	0
Raven	b+c	c	15		1082		0	0
Raven	b+c	c	16	1262	1130	180	2	1
Raven	b+c	c	17	1222		92	0	2
Raven	n+c	n	17		829		0	0
Raven	n+c	n	18	897	804	68	1	1
Raven	n+c	n	19	846		42	1	1
Raven	n+c	c	17		826		0	0
Raven	n+c	c	18	901	837	75	0	1
Raven	n+c	c	19	927		90	0	1
Raven	a+e	a	19		950		0	0
Raven	a+e	a	20	1086	701	136	1	2
Raven	a+e	a	21	819		118	1	1
Raven	a+e	e	19		0		0	0
Raven	a+e	e	20	0	0	0	0	0
Raven	a+e	e	21	0		0	0	0
Aura	a+e	a	1		928		0	0
Aura	a+e	a	2	985	935	57	0	1
Aura	a+e	a	3	1072		137	1	1
Aura	a+e	e	1		0		0	0
Aura	a+e	e	2	0	0	0	0	0
Aura	a+e	e	3	0		0	0	0
Aura	a+b	a	3		813		0	0
Aura	a+b	a	4	917	780	104	1	1
Aura	a+b	a	5	787		7	1	0
Aura	a+b	b	3		1404		0	0
Aura	a+b	b	4	1494	1482	90	1	0
Aura	a+b	b	5	1626		144	1	1
Aura	a+n	a	5		842		0	0
Aura	a+n	a	6	974	715	132	2	1
Aura	a+n	a	7	815		100	0	1
Aura	a+n	n	5		594		0	0
Aura	a+n	n	6	687	646	93	0	1
Aura	a+n	n	7	932		286	1	1
Aura	a+c	a	7		818		0	0
Aura	a+c	a	8	979	900	161	2	1
Aura	a+c	a	9	971		71	0	2

Aura	a+c	c	7		1280		0	0
Aura	a+c	c	8	1414	1334	134	0	1
Aura	a+c	c	9	1386		52	1	0
Aura	b+n	b	9		762		0	0
Aura	b+n	b	10	808	770	46	1	1
Aura	b+n	b	11	824		54	0	1
Aura	b+n	n	9		829		0	0
Aura	b+n	n	10	950	858	121	1	1
Aura	b+n	n	11	1053		195	1	1
Aura	n+e	n	11		940		0	0
Aura	n+e	n	12	1165	1034	225	1	1
Aura	n+e	n	13	1149		115	1	2
Aura	n+e	e	11		0		0	0
Aura	n+e	e	12	0	0	0	0	0
Aura	n+e	e	13	0		0	0	0
Aura	b+e	b	13		1060		0	0
Aura	b+e	b	14	1252	906	192	1	2
Aura	b+e	b	15	1046		140	1	2
Aura	b+e	e	13		0		0	0
Aura	b+e	e	14	0	0	0	0	0
Aura	b+e	e	15	0		0	0	0
Aura	c+e	c	15		693		0	0
Aura	c+e	c	16	905	629	212	2	1
Aura	c+e	c	17	781		152	1	1
Aura	c+e	e	15		0		0	0
Aura	c+e	e	16	0	0	0	0	0
Aura	c+e	e	17	0		0	0	0
Aura	b+c	b	17		1015		0	0
Aura	b+c	b	18	1088	948	73	0	1
Aura	b+c	b	19	1004		56	0	2
Aura	b+c	c	17		793		0	0
Aura	b+c	c	18	941	896	148	1	1
Aura	b+c	c	19	937		41	2	1
Aura	n+c	n	19		780		0	0
Aura	n+c	n	20	801	780	21	1	0
Aura	n+c	n	21	815		35	1	1
Aura	n+c	c	19		329		0	0
Aura	n+c	c	20	429	276	100	1	1
Aura	n+c	c	21	368		92	0	1
Jade	n+c	n	1		491		0	0
Jade	n+c	n	2	522	461	31	0	1

Jade	n+c	n	3	540		79	0	1
Jade	n+c	c	1		986		0	0
Jade	n+c	c	2	1074	1031	88	1	0
Jade	n+c	c	3	1111		80	2	0
Jade	a+e	a	3		1270		0	0
Jade	a+e	a	4	1430	1220	160	1	2
Jade	a+e	a	5	1379		159	2	1
Jade	a+e	e	3		0		0	0
Jade	a+e	e	4	0	0	0	0	0
Jade	a+e	e	5	0		0	0	0
Jade	a+b	a	5		801		0	0
Jade	a+b	a	6	962	543	161	1	1
Jade	a+b	a	7	646		103	2	1
Jade	a+b	b	5		905		0	0
Jade	a+b	b	6	981	837	76	1	1
Jade	a+b	b	7	936		99	0	1
Jade	a+n	a	7		909		0	0
Jade	a+n	a	8	1041	870	132	2	2
Jade	a+n	a	9	944		74	0	2
Jade	a+n	n	7		559		0	0
Jade	a+n	n	8	682	634	123	0	1
Jade	a+n	n	9	690		56	1	1
Jade	a+c	a	9		1043		0	0
Jade	a+c	a	10	1085	1049	42	1	0
Jade	a+c	a	11	1134		85	2	1
Jade	a+c	c	9		832		0	0
Jade	a+c	c	10	939	871	107	0	1
Jade	a+c	c	11	968		97	1	1
Jade	b+n	b	11		867		0	0
Jade	b+n	b	12	896	794	29	0	1
Jade	b+n	b	13	822		28	0	1
Jade	b+n	n	11		777		0	0
Jade	b+n	n	12	898	846	121	1	1
Jade	b+n	n	13	918		72	1	1
Jade	n+e	n	13		922		0	0
Jade	n+e	n	14	1057	1016	135	0	2
Jade	n+e	n	15	1190		174	2	1
Jade	n+e	e	13		0		0	0
Jade	n+e	e	14	38	0	38	1	0
Jade	n+e	e	15	0		0	0	0
Jade	b+e	b	15		915		0	0

Jade	b+e	b	16	1066	833	151	2	1
Jade	b+e	b	17	963		130	1	1
Jade	b+e	e	15		0		0	0
Jade	b+e	e	16	0	0	0	0	0
Jade	b+e	e	17	0		0	0	0
Jade	c+e	c	17		876		0	0
Jade	c+e	c	18	1025	960	149	2	1
Jade	c+e	c	19	1058		98	1	2
Jade	c+e	e	17		0		0	0
Jade	c+e	e	18	0	0	0	0	0
Jade	c+e	e	19	0		0	0	0
Jade	b+c	b	19		1055		0	0
Jade	b+c	b	20	1116	1052	61	1	1
Jade	b+c	b	21	1082		30	1	0
Jade	b+c	c	19		524		0	0
Jade	b+c	c	20	671	563	147	1	1
Jade	b+c	c	21	742		179	2	2
Lea	b+c	b	1		871		0	0
Lea	b+c	b	2	912	754	41	0	1
Lea	b+c	b	3	842		88	0	1
Lea	b+c	c	1		1144		0	0
Lea	b+c	c	2	1348	1265	204	2	0
Lea	b+c	c	3	1303		38	1	0
Lea	n+c	n	3		968		0	0
Lea	n+c	n	4	1059	986	91	0	1
Lea	n+c	n	5	1037		51	1	1
Lea	n+c	c	3		1165		0	0
Lea	n+c	c	4	1259	1238	94	0	1
Lea	n+c	c	5	1359		121	1	1
Lea	a+e	a	5		859		0	0
Lea	a+e	a	6	1090	755	231	1	2
Lea	a+e	a	7	963		208	1	2
Lea	a+e	e	5		0		0	0
Lea	a+e	e	6	0	0	0	0	0
Lea	a+e	e	7	0		0	0	0
Lea	a+b	a	7		822		0	0
Lea	a+b	a	8	934	816	112	1	1
Lea	a+b	a	9	934		118	1	1
Lea	a+b	b	7		691		0	0
Lea	a+b	b	8	815	618	124	0	1
Lea	a+b	b	9	712		94	0	1

Lea	a+n	a	9		946		0	0
Lea	a+n	a	10	957	957	11	0	0
Lea	a+n	a	11	1036		79	0	1
Lea	a+n	n	9		834		0	0
Lea	a+n	n	10	1023	704	189	1	1
Lea	a+n	n	11	812		108	1	1
Lea	a+c	a	11		901		0	0
Lea	a+c	a	12	912	912	11	0	0
Lea	a+c	a	13	1041		129	0	1
Lea	a+c	c	11		1092		0	0
Lea	a+c	c	12	1276	1207	184	1	1
Lea	a+c	c	13	1340		133	1	0
Lea	b+n	b	13		927		0	0
Lea	b+n	b	14	1011	853	84	1	1
Lea	b+n	b	15	848		-5	0	0
Lea	b+n	n	13		836		0	0
Lea	b+n	n	14	963	826	127	0	1
Lea	b+n	n	15	980		154	0	0
Lea	n+e	n	15		624		0	0
Lea	n+e	n	16	796	584	172	1	1
Lea	n+e	n	17	721		137	1	1
Lea	n+e	e	15		0		0	0
Lea	n+e	e	16	0	0	0	0	0
Lea	n+e	e	17	0		0	0	0
Lea	b+e	b	17		1062		0	0
Lea	b+e	b	18	1233	867	171	1	2
Lea	b+e	b	19	983		116	1	1
Lea	b+e	e	17		0		0	0
Lea	b+e	e	18	0	0	0	0	0
Lea	b+e	e	19	0		0	0	0
Lea	c+e	c	19		566		0	0
Lea	c+e	c	20	661	644	95	1	1
Lea	c+e	c	21	727		83	1	1
Lea	c+e	e	19		0		0	0
Lea	c+e	e	20	0	0	0	0	0
Lea	c+e	e	21	0		0	0	0
Billi	c+e	c	1		739		0	0
Billi	c+e	c	2	986	581	247	1	1
Billi	c+e	c	3	826		245	1	1
Billi	c+e	e	1		0		0	0
Billi	c+e	e	2	0	0	0	0	0

Billi	c+e	e	3	0		0	0	0
Billi	b+c	b	3		808		0	0
Billi	b+c	b	4	870	820	62	1	1
Billi	b+c	b	5	1035		215	1	2
Billi	b+c	c	3		818		0	0
Billi	b+c	c	4	1099	734	281	1	1
Billi	b+c	c	5	764		30	0	1
Billi	n+c	n	5		655		0	0
Billi	n+c	n	6	674	674	19	0	0
Billi	n+c	n	7	683		9	0	0
Billi	n+c	c	5		1199		0	0
Billi	n+c	c	6	1401	1158	202	0	1
Billi	n+c	c	7	1382		224	2	1
Billi	a+e	a	7		841		0	0
Billi	a+e	a	8	1166	778	325	1	2
Billi	a+e	a	9	998		220	1	1
Billi	a+e	e	7		0		0	0
Billi	a+e	e	8	0	0	0	0	0
Billi	a+e	e	9	0		0	0	0
Billi	a+b	a	9		1008		0	0
Billi	a+b	a	10	1335	862	327	1	2
Billi	a+b	a	11	914		52	1	2
Billi	a+b	b	9		945		0	0
Billi	a+b	b	10	1038	850	93	1	1
Billi	a+b	b	11	1048		198	0	2
Billi	a+n	a	11		902		0	0
Billi	a+n	a	12	1070	849	168	1	2
Billi	a+n	a	13	883		34	0	0
Billi	a+n	n	11		801		0	0
Billi	a+n	n	12	1047	818	246	1	2
Billi	a+n	n	13	1042		224	0	2
Billi	a+c	a	13		719		0	0
Billi	a+c	a	14	866	660	147	1	1
Billi	a+c	a	15	808		148	1	2
Billi	a+c	c	13		1018		0	0
Billi	a+c	c	14	1382	846	364	1	1
Billi	a+c	c	15	965		119	1	1
Billi	b+n	b	15		1037		0	0
Billi	b+n	b	16	1079	979	42	0	1
Billi	b+n	b	17	1064		85	0	1
Billi	b+n	n	15		755		0	0

Billi	b+n	n	16	823	671	68	0	1
Billi	b+n	n	17	737		66	1	1
Billi	n+e	n	17		756		0	0
Billi	n+e	n	18	998	570	242	2	2
Billi	n+e	n	19	689		119	0	2
Billi	n+e	e	17		0		0	0
Billi	n+e	e	18	0	0	0	0	0
Billi	n+e	e	19	0		0	0	0
Billi	b+e	b	19		1031		0	0
Billi	b+e	b	20	1290	815	259	2	2
Billi	b+e	b	21	1101		286	2	2
Billi	b+e	e	19		0		0	0
Billi	b+e	e	20	0	0	0	0	0
Billi	b+e	e	21	0		0	0	0
Beeva	b+e	b	1		1203		0	0
Beeva	b+e	b	2	1316	1072	113	1	2
Beeva	b+e	b	3	1351		279	2	2
Beeva	b+e	e	1		0		0	0
Beeva	b+e	e	2	0	0	0	0	0
Beeva	b+e	e	3	0		0	0	0
Beeva	c+e	c	3		826		0	0
Beeva	c+e	c	4	1058	967	232	2	1
Beeva	c+e	c	5	1166		199	2	2
Beeva	c+e	e	3		0		0	0
Beeva	c+e	e	4	0	0	0	0	0
Beeva	c+e	e	5	27		27	0	1
Beeva	b+c	b	5		969		0	0
Beeva	b+c	b	6	1135	886	166	2	1
Beeva	b+c	b	7	1296		410	2	1
Beeva	b+c	c	5		1066		0	0
Beeva	b+c	c	6	1164	760	98	0	1
Beeva	b+c	c	7	832		72	0	1
Beeva	n+c	n	7		499		0	0
Beeva	n+c	n	8	639	564	140	1	0
Beeva	n+c	n	9	592		28	1	1
Beeva	n+c	c	7		1251		0	0
Beeva	n+c	c	8	1463	1182	212	1	1
Beeva	n+c	c	9	1402		220	1	1
Beeva	a+e	a	9		1003		0	0
Beeva	a+e	a	10	1222	860	219	1	2
Beeva	a+e	a	11	1027		167	1	2

Beeva	a+e	e	9		0		0	0
Beeva	a+e	e	10	0	0	0	0	0
Beeva	a+e	e	11	0		0	0	0
Beeva	a+b	a	11		974		0	0
Beeva	a+b	a	12	1049	1024	75	1	0
Beeva	a+b	a	13	1129		105	0	1
Beeva	a+b	b	11		981		0	0
Beeva	a+b	b	12	1081	639	100	0	1
Beeva	a+b	b	13	786		147	1	1
Beeva	a+n	a	13		699		0	0
Beeva	a+n	a	14	826	586	127	1	1
Beeva	a+n	a	15	649		63	1	1
Beeva	a+n	n	13		854		0	0
Beeva	a+n	n	14	1016	815	162	0	1
Beeva	a+n	n	15	919		104	1	2
Beeva	a+c	a	15		752		0	0
Beeva	a+c	a	16	929	689	177	2	1
Beeva	a+c	a	17	749		60	1	1
Beeva	a+c	c	15		937		0	0
Beeva	a+c	c	16	993	892	56	0	1
Beeva	a+c	c	17	974		82	0	1
Beeva	b+n	b	17		879		0	0
Beeva	b+n	b	18	1122	677	243	2	1
Beeva	b+n	b	19	734		57	1	2
Beeva	b+n	n	17		645		0	0
Beeva	b+n	n	18	722	574	77	1	1
Beeva	b+n	n	19	659		85	1	2
Beeva	n+e	n	19		766		0	0
Beeva	n+e	n	20	1038	528	272	1	2
Beeva	n+e	n	21	810		282	2	2
Beeva	n+e	e	19		0		0	0
Beeva	n+e	e	20	0	0	0	0	0
Beeva	n+e	e	21	0		0	0	0
Esta	n+e	n	1		414		0	0
Esta	n+e	n	2	434	423	20	0	1
Esta	n+e	n	3	506		83	0	1
Esta	n+e	e	1		0		0	0
Esta	n+e	e	2	0	0	0	0	0
Esta	n+e	e	3	0		0	0	0
Esta	b+e	b	3		773		0	0
Esta	b+e	b	4	873	732	100	1	1

Esta	b+e	b	5	822		90	1	1
Esta	b+e	e	3		0		0	0
Esta	b+e	e	4	0	0	0	0	0
Esta	b+e	e	5	0		0	0	0
Esta	c+e	c	5		699		0	0
Esta	c+e	c	6	832	718	133	1	1
Esta	c+e	c	7	876		158	1	2
Esta	c+e	e	5		0		0	0
Esta	c+e	e	6	0	0	0	0	0
Esta	c+e	e	7	0		0	0	0
Esta	b+c	b	7		786		0	0
Esta	b+c	b	8	831	758	45	0	1
Esta	b+c	b	9	768		10	1	0
Esta	b+c	c	7		1352		0	0
Esta	b+c	c	8	1497	1375	145	1	1
Esta	b+c	c	9	1474		99	0	1
Esta	n+c	n	9		636		0	0
Esta	n+c	n	10	650	637	14	1	0
Esta	n+c	n	11	730		93	0	2
Esta	n+c	c	9		1082		0	0
Esta	n+c	c	10	1235	1028	153	0	2
Esta	n+c	c	11	1113		85	2	1
Esta	a+e	a	11		889		0	0
Esta	a+e	a	12	995	849	106	1	1
Esta	a+e	a	13	987		138	1	2
Esta	a+e	e	11		0		0	0
Esta	a+e	e	12	0	0	0	0	0
Esta	a+e	e	13	0		0	0	0
Esta	a+b	a	13		735		0	0
Esta	a+b	a	14	861	643	126	0	1
Esta	a+b	a	15	749		106	0	1
Esta	a+b	b	13		879		0	0
Esta	a+b	b	14	910	884	31	1	0
Esta	a+b	b	15	898		14	1	0
Esta	a+n	a	15		851		0	0
Esta	a+n	a	16	902	800	51	1	1
Esta	a+n	a	17	827		27	0	1
Esta	a+n	n	15		779		0	0
Esta	a+n	n	16	874	773	95	0	1
Esta	a+n	n	17	778		5	0	1
Esta	a+c	a	17		758		0	0

Esta	a+c	a	18	771	761	13	1	0
Esta	a+c	a	19	762		1	0	0
Esta	a+c	c	17		1009		0	0
Esta	a+c	c	18	1110	978	101	0	2
Esta	a+c	c	19	1082		104	1	2
Esta	b+n	b	19		1219		0	0
Esta	b+n	b	20	1308	1163	89	1	1
Esta	b+n	b	21	1182		19	1	1
Esta	b+n	n	19		1015		0	0
Esta	b+n	n	20	1029	1004	14	0	1
Esta	b+n	n	21	1093		89	0	1
Rach	b+n	b	1		1119		0	0
Rach	b+n	b	2	1189	993	70	0	1
Rach	b+n	b	3	1052		59	0	2
Rach	b+n	n	1		566		0	0
Rach	b+n	n	2	664	575	98	0	1
Rach	b+n	n	3	686		111	1	1
Rach	n+e	n	3		780		0	0
Rach	n+e	n	4	980	846	200	1	1
Rach	n+e	n	5	1061		215	1	1
Rach	n+e	e	3		0		0	0
Rach	n+e	e	4	0	0	0	0	0
Rach	n+e	e	5	0		0	0	0
Rach	b+e	b	5		817		0	0
Rach	b+e	b	6	1068	668	251	2	2
Rach	b+e	b	7	960		292	2	2
Rach	b+e	e	5		0		0	0
Rach	b+e	e	6	0	0	0	0	0
Rach	b+e	e	7	0		0	0	0
Rach	c+e	c	7		889		0	0
Rach	c+e	c	8	1218	1045	329	1	1
Rach	c+e	c	9	1171		126	1	2
Rach	c+e	e	7		0		0	0
Rach	c+e	e	8	0	0	0	0	0
Rach	c+e	e	9	0		0	0	0
Rach	b+c	b	9		975		0	0
Rach	b+c	b	10	1021	830	46	0	1
Rach	b+c	b	11	861		31	0	1
Rach	b+c	c	9		1000		0	0
Rach	b+c	c	10	1248	1097	248	1	1
Rach	b+c	c	11	1313		216	2	1

Rach	n+c	n	11		862		0	0
Rach	n+c	n	12	925	825	63	0	1
Rach	n+c	n	13	827		2	0	0
Rach	n+c	c	11		1175		0	0
Rach	n+c	c	12	1343	1285	168	1	0
Rach	n+c	c	13	1491		206	0	1
Rach	a+e	a	13		771		0	0
Rach	a+e	a	14	1025	547	254	1	2
Rach	a+e	a	15	781		234	2	2
Rach	a+e	e	13		0		0	0
Rach	a+e	e	14	0	0	0	0	0
Rach	a+e	e	15	0		0	0	0
Rach	a+b	a	15		738		0	0
Rach	a+b	a	16	808	713	70	1	1
Rach	a+b	a	17	850		137	0	1
Rach	a+b	b	15		895		0	0
Rach	a+b	b	16	1032	746	137	0	1
Rach	a+b	b	17	802		56	1	1
Rach	a+n	a	17		769		0	0
Rach	a+n	a	18	836	742	67	1	1
Rach	a+n	a	19	795		53	1	1
Rach	a+n	n	17		569		0	0
Rach	a+n	n	18	745	456	176	0	1
Rach	a+n	n	19	532		76	0	1
Rach	a+c	a	19		933		0	0
Rach	a+c	a	20	968	873	35	0	1
Rach	a+c	a	21	992		119	0	1
Rach	a+c	c	19		649		0	0
Rach	a+c	c	20	796	724	147	1	1
Rach	a+c	c	21	789		65	1	1

Appendix 6.1

Ammonia absorption experiment results.

Sample #	Litter	Starting Weight (g)	Time Interval (min)	Height	Concentration (μgX.ml^{-1})
1	Breeder Celect Recycled Paper	50.44	80	89.4419	11.49
2	VitaPet Purrfection Clumping Litter	50.74	80	40.5082	5.2
3	Excellence Ultra-Hygienic Litter	50.38	80	42.0739	5.4
4	VitaPet Cat Litter	50.97	80	50.2729	6.46
5	Old Natural O-Litter	50.62	80	45.1562	5.8
6	Pellet B	50.63	80	25.4733	3.27
7	New Natural O-Litter	50.64	80	22.4118	2.88
8	Pellet A	50.62	80	39.1348	5.03
9	Breeder Celect Recycled Paper	50.48	40	84.1825	10.81
10	VitaPet Purrfection Clumping Litter	50.66	40	33.5579	4.31
11	Excellence Ultra-Hygienic Litter	50.38	40	17.2674	2.22
12	VitaPet Cat Litter	50.4	40	22.4587	2.88
13	Old Natural O-Litter	50.22	40	27.0201	3.47
14	Pellet B	50.33	40	19.0642	2.45
15	New Natural O-Litter	50.67	40	24.1076	3.1
16	Pellet A	50.42	40	17.3609	2.23
17	Breeder Celect Recycled Paper	50.23	20	66.197	8.5
18	VitaPet Purrfection Clumping Litter	50.15	20	23.5196	3.02
19	Excellence Ultra-Hygienic Litter	50.23	20	9.63627	1.24
20	VitaPet Cat Litter	50.35	20	15.801	2.03
21	Old Natural O-Litter	50.61	20	16.0827	2.07
22	Pellet B	50.33	20	13.4292	1.72
23	New Natural O-Litter	50.58	20	10.9825	1.41
24	Pellet A	50.52	20	13.7573	1.77
25	Breeder Celect Recycled Paper	50.46	10	52.1183	6.69
26	VitaPet Purrfection Clumping Litter	50.4	10	21.3399	2.74
27	Excellence Ultra-Hygienic Litter	50.64	10	12.2832	1.58
28	VitaPet Cat Litter	50.36	10	16.0373	2.06
29	Old Natural O-Litter	50.82	10	14.994	1.93
30	Pellet B	50.54	10	11.5047	1.48
31	New Natural O-Litter	50.43	10	13.7747	1.77
32	Pellet A	50.96	10	10.158	1.3
33	Breeder Celect Recycled Paper	50.84	80	85.7887	11.02
34	VitaPet Purrfection Clumping Litter	50.47	80	70.5456	9.06
35	Excellence Ultra-Hygienic Litter	50.68	80	17.3052	2.22
36	VitaPet Cat Litter	50.36	80	58.7428	7.54
37	Old Natural O-Litter	50.14	80	18.0734	2.32
38	Pellet B	50.99	80	13.2236	1.7
39	New Natural O-Litter	50.43	80	12.7283	1.63
40	Pellet A	50.6	80	18.9651	2.44

41	Breeder Celect Recycled Paper	50.82	40	89.6054	11.51
42	VitaPet Purrfection Clumping Litter	50.15	40	53.5193	6.87
43	Excellence Ultra-Hygienic Litter	50.34	40	22.6508	2.91
44	VitaPet Cat Litter	50.87	40	25.6521	3.29
45	Old Natural O-Litter	50.36	40	17.3379	2.23
46	Pellet B	50.44	40	14.1919	1.82
47	New Natural O-Litter	50.72	40	14.2331	1.83
48	Pellet A	50.18	40	13.2393	1.7
49	Breeder Celect Recycled Paper	50.21	20	62.963	8.09
50	VitaPet Purrfection Clumping Litter	50.96	20	47.2483	6.07
51	Excellence Ultra-Hygienic Litter	50.83	20	34.9111	4.48
52	VitaPet Cat Litter	50.81	20	31.5408	4.05
53	Old Natural O-Litter	50.29	20	17.1291	2.2
54	Pellet B	50.45	20	20.0136	2.57
55	New Natural O-Litter	50.28	20	21.1759	2.72
56	Pellet A	50.15	20	15.054	1.93
57	Breeder Celect Recycled Paper	50.7	10	52.4174	6.73
58	VitaPet Purrfection Clumping Litter	50.75	10	27.3418	3.51
59	Excellence Ultra-Hygienic Litter	50.85	10	14.4926	1.86
60	VitaPet Cat Litter	50.17	10	20.3302	2.61
61	Old Natural O-Litter	50.13	10	25.1832	3.23
62	Pellet B	50.74	10	13.1593	1.69
63	New Natural O-Litter	50.65	10	20.3875	2.62
64	Pellet A	50.86	10	14.4469	1.86
65	Breeder Celect Recycled Paper	50.64	80	89.3012	11.47
66	VitaPet Purrfection Clumping Litter	50.13	80	35.7635	4.59
67	Excellence Ultra-Hygienic Litter	50.59	80	11.8806	1.53
68	VitaPet Cat Litter	50.74	80	23.6347	3.04
69	Old Natural O-Litter	50.58	80	25.0011	3.21
70	Pellet B	50.63	80	12.4164	1.59
71	New Natural O-Litter	50.76	80	19.3079	2.48
72	Pellet A	50.88	80	16.6469	2.14
73	Breeder Celect Recycled Paper	50.46	40	82.749	10.63
74	VitaPet Purrfection Clumping Litter	50.15	40	21.9082	2.81
75	Excellence Ultra-Hygienic Litter	50.41	40	10.9888	1.41
76	VitaPet Cat Litter	50.27	40	22.1924	2.85
77	Old Natural O-Litter	50.49	40	16.6441	2.14
78	Pellet B	50.08	40	12.5089	1.61
79	New Natural O-Litter	50.8	40	17.7715	2.28
80	Pellet A	50.35	40	11.2855	1.45
81	Breeder Celect Recycled Paper	50.74	20	36.9808	4.75
82	VitaPet Purrfection Clumping Litter	50.4	20	18.1569	2.33
83	Excellence Ultra-Hygienic Litter	50.45	20	7.89967	1.01
84	VitaPet Cat Litter	50.69	20	15.3828	1.98
85	Old Natural O-Litter	50.47	20	12.8164	1.65

86	Pellet B	50.31	20	10.4822	1.35
87	New Natural O-Litter	50.59	20	12.6364	1.62
88	Pellet A	50.68	20	8.30286	1.07
89	Breeder Celect Recycled Paper	50.52	10	40.609	5.22
90	VitaPet Purrfection Clumping Litter	50.39	10	17.2612	2.22
91	Excellence Ultra-Hygienic Litter	50.21	10	49.5917	6.37
92	VitaPet Cat Litter	50.53	10	13.6556	1.75
93	Old Natural O-Litter	50.13	10	11.4691	1.47
94	Pellet B	50.22	10	5.25601	0.67
95	New Natural O-Litter	50.92	10	11.5293	1.48
96	Pellet A	50.4	10	6.74198	0.87
97	Control		10	84.7934	10.89
98	Control		20	84.2922	10.83
99	Control		40	83.791	10.76
100	Control		80	83.293	10.71