Biological Olfactory Repellents and their Potential to Deter Domestic Dogs (*Canis familiaris*) from Anticoagulant Rodenticides

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Abstract

The rapid decline in New Zealand's terrestrial biodiversity from the impacts of exotic mammalian predators has prompted the nationwide application of anticoagulant rodenticides. However, this application has increased the occurrence of accidental poisonings in non-target species, particularly the domestic dog (*Canis familiaris*). Odour excretions from predators can induce long-term feeding avoidance in prey species, however, predator odours and their potential as a dog repellent have not been investigated. The aim of this study was to investigate the potential for biologically-derived olfactory repellents to deter dogs from consuming toxic baits, in particular, rat poison. To complete this aim two experiments were conducted where dogs and rats were presented repellent-treated kibble and rat chow via a series of two-choice preference tests. The first experiment examined the repellent effects of African lion (*Panthera leo*), tiger (*Pantera tigris*), baboon (*Papio hamadryas*), domestic dog (*Canis familiaris*) faeces, and a commercial repellent, on dogs' feeding behaviour (N = 21). The second experiment examined if the repellents deterred rats (N = 10), the poison's target species. The results revealed that (1) the dogs ate significantly less when presented with lion or dog faeces; (2) baboon faeces, tiger faeces, and the commercial repellent had virtually no repellent effect on the dogs; and (3) overall food consumption by the rats did not differ between repellent types. In summary, this study demonstrates the potential use of animal faeces as a repellent, deterring dogs, but not rats, from poison.

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Chapter 1

Introduction

1.1 Research Significance

Rodents pose a significant risk to native biodiversity through spread of disease, predation and food competition (Tobin & Fall, 2004). In an attempt to control rodent populations, public and Government organizations worldwide have implemented the use of anticoagulant rodenticides (ARs) (Hadler & Buckle, 1992). The current peridomestic application of ARs has, unintentionally, increased the occurrence of accidental poisoning in non-target species, particularly the domestic dog (*Canis familiaris*) (Berny et. al., 2010a). Past studies have demonstrated that odour excretions from predators can induce long-term feeding avoidance in prey. However, investigation into predator odours and their potential as canid repellents remains an emerging (but far from complete) area of investigation. My research aims to fill this knowledge gap and to identify if predator odours could be used to deter non-target species, specifically dogs, from ARs. These results, if proven to be successful, could provide the initial framework for developing a bait deterrent for dogs. The following literature review describes: (1) rodents and their environmental and economic impact, (2) the advantages and disadvantages of rodent control methods, and (3) predator odours and their efficacy as a repellent.

1.2 Rodents

Over 40% of all mammalian species in the world are rodents (Kay & Hoekstra, 2008; Shiels & Witmer, 2017). Rodents are characterised by a pair of open-rooted, chisel-shaped incisors located in the upper and lower jaws (Kay & Hoekstra, 2008). These ever-growing teeth have a soft dentine inner layer and a hard enamel external layer and are used by rodents to gnaw food or objects (e.g., seeds, fruit, crop fields, wires, cables, etc.), excavate burrows and defend themselves (Kay & Hoekstra, 2008; Tobin & Fall, 2004).

Most living rodents are small, with a compact body and a long tail (Kay & Hoekstra, 2008). Their size range, however, is much larger than that of any other

mammalian order (Kay & Hoekstra, 2008). The smallest rodent, the pygmy mouse, can weigh as little as 7 g, while the world's largest rodent, the capybara, can weigh up to 50 kg (Kay & Hoekstra, 2008). In addition to their unique teeth and generally small to medium size, rodents are prolific breeders (Kay & Hoekstra, 2008). Because most rodents reach sexual maturity at a young age and ovulate immediately after birth (post-partum oestrus), they can reproduce at rates much quicker than most other mammalian species (Tobin & Fall, 2004; Kay & Hoekstra, 2008). This, in conjunction with their well-developed senses (e.g., taste, touch, hearing, and smell), wide-ranging diet, and adaptable nature has allowed rodents to successfully invade virtually every terrestrial ecosystem (Shiels & Witmer, 2017; Tobin & Fall, 2004).

1.2.1 Environmental and Economic Impacts

Rodents are important in seed dispersal, pollination and nutrient recycling (Shiels & Witmer, 2017; Tobin & Fall 2004). However, when rodents are introduced to an area outside of their natural home range, they can have severe ecological impacts (St Clair, 2011; Tobin & Fall, 2004). In New Zealand, for example, rodents have caused the decline and endangerment of several species of lizards (Towns, 1991), birds (O'Donnell, 1996) and plants (Allen *et al*., 1994).

Not only have rodents impacted New Zealand's biota, but they have also damaged ecosystems on an international scale (Angel *et al*., 2008; Caut *et al*., 2008; Hadfield *et al*., 1993). For example, predation by rats has caused the decline of seabird and snail populations' on Surprise Island (Canada) and Hawaii, respectively (Caut *et al*., 2008; Hadfield, Miller & Carwile, 1993). Grazing by mice has also resulted in a sedge species, *Uncinia compacta*, being extirpated from wetland areas on Marion Island, South Africa (Smith & Steenkamp, 1900 as cited by Angel *et al*., 2008). Furthermore, predation by mice has caused the local extinction of several invertebrate species on Antipodes Island, New Zealand (Angel *et al*., 2008). Although this is only a brief insight into the impacts rodents can have on native and endemic species, these studies demonstrate why rodent control methods are needed.

Alongside the environmental impacts, rodents have had a detrimental effect on the global economy by consuming food stocks and spreading diseases (Tobin & Fall, 2004; Leirs, 2003). Because rodents forage on the seeds, fruit, and foliage of a wide variety of crop types, they have severely impeded agricultural production in many countries worldwide (Leirs, 2003; Voznessenskaya *et al*., 2003). For example, in Tanzania, rodents consume 400,000 tonnes of maize each year, which corresponds to a financial loss of US$45 million (Leirs, 2003). Comparatively, in the United States, the apple industry loses US$90 million annually as a result of rodent damage (Voznessenskaya *et al*., 2003).

In addition to the consumption of food stocks, rodents also carry and spread a range of diseases such as leptospirosis, salmonellosis, and the bubonic plague. Globally these diseases (among others) have caused severe harm to economies due to related health care costs and the high rate of human and animal mortality (Jacob & Singleton, 2003). The severe economic and environmental impacts that rodents can have highlight the need for effective and targeted rodent control methods.

1.3 Rodent Control Methods

A wide variety of control methods have been developed to alleviate rodent damage and to reduce rodent populations world-wide (Tobin & Fall, 2004). In general, these methods can be categorised into two main types: non-poisonous measures and baited poisons (Meerburg *et al*., 2008; Tobin & Fall, 2004).

1.3.1 Non-poisonous Measures

Non-poisonous measures include traps, biological control and fertility control (Tobin & Fall, 2004). Traps are used in rodent control operations, as they are cost effective, target specific, and can be used in environmentally sensitive areas (Tobin & Fall, 2004). However, in recent years certain traps and trap setting methods have been deemed unsafe or inhumane by animal welfare organisations (Tobin & Fall, 2004). For example, glue board and gin traps have been banned/restricted from use in New Zealand and several states in the United States, as they cause severe pain, distress and suffering to the animal (Cowan & Brown, 2012). This, in conjunction with the inability to apply and maintain traps in certain areas, has prompted further research into other control methods such as biological

control and fertility control (Tobin & Fall, 2004).

Biological control involves the introduction of parasites, predators and disease organisms to reduce or mitigate the effects of pest populations (Tobin & Fall, 2004; Howarth, 1991). It is a cost-effective approach which can be applied over large or inaccessible areas (Howarth, 1991). However, if used incorrectly biological control can have severe ecological and economic effects (Tobin & Fall, 2004; Courchamp *et al*., 2003). Organisms introduced in biological control programs have the potential to feed on or invade all suitable hosts, including non-target species (Howarth, 1991). The outcomes of these encounters can be minor; however, they can also lead to the decline and extinction of numerous native organisms (Howarth, 1991). In the West Indies, for example, mongooses were introduced in the late eighteenth century to control rat populations (Tobin & Fall, 2003). Although these species were known predators of rats elsewhere, their introduction failed, and they have since caused the decline and extinction of numerous species of ground nesting birds, reptiles and amphibians (Tobin & Fall., 2004; Courchamp *et al*., 2003). A similar result was demonstrated in Europe, albeit with a bacteria species, *Salmonella enterditis*. *S. enterditis* is a highly virulent bacterium that was introduced in the 1800s to control rat populations. Unfortunately, as a result of its infectious nature it caused the illness and death of several human beings; thus its use as a rodenticide is now prohibited in most countries around the world (Hygnstrom *et al*., 1994).

Many methods have been developed for controlling the fertility of rodents over the past century (Chambers *et al*., 1999). These methods include castration or surgical sterilisation, the use of chemical sterilants, genetic manipulation, and agonists that block the function of hormones (Chambers *et al*., 1999; Hygnstrom *et al*., 1994). Although these methods are effective at reducing fertility, they require constant administration to maintain sterility at a population level. They can also have undesirable side effects and are difficult, expensive, and time consuming to administer (Chambers *et al*., 1999). Immunocontraception is a new technique which holds potential as a fertility control method for rodents. It is species-specific, self-disseminating, and works by promoting antibody production against various proteins necessary for reproduction (Chambers *et al*., 1999). Though it involves genetically modifying an organism and is in the initial stages of testing,

if performed correctly it could provide a long-term strategy for reducing rodent populations in various countries around the world (Hygnstrom *et al*., 1994). In addition to non-poisonous measures, rodents can also be controlled using poisons, in particular baited poisons (Meerburg *et al*., 2008; Shiels & Witmer, 2017).

1.3.2 Poisonous Measures

Baited poisons are highly toxic and can be applied over large areas (Meerburg *et al*., 2008; Tobin & Fall., 2004). If used correctly, or in conjunction with other control methods, these poisons can control and even eradicate entire pest populations. For example, the toxin sodium fluoroacetate (1080) has been used in New Zealand to control rat and possum populations (Murphy *et al*., 1998). Although some operations have proven effective at extirpating rodents (e.g., Murphy *et al*., 1998), 1080, like most other baited poisons, can have measurable, adverse effects on non-target wildlife (Berny *et al*., 2010b; Tobin & Fall., 2004).

Anticoagulant rodenticides are a form of baited poison, primarily used to control rodent populations in agricultural or urban settings (Meerburg *et al*., 2010; Gabriel *et al*., 2012). They work by inhibiting the action of vitamin K epoxide reductase; an enzyme responsible for the production and activation of blood clotting factors (Stone *et al*., 1999; Hadler & Buckle, 1992). Therefore, when consumed, these poisons can lead to the animal experiencing uncontrolled bleeding, weakness, lethargy and in most cases death (Stone *et al*., 1999; Hadler & Buckle, 1992).

There are two major classes of anticoagulant rodenticides: (1) first generation compounds, which require several doses to cause intoxication; and (2) second-generation compounds, which are more acutely toxic and only require a single dose to cause death (Gabriel *et al*., 2012). Although first generation compounds are effective at reducing rodent populations, species can develop bait shyness (i.e., learned avoidance) from ingesting sub-lethal doses of the poison. Consequently, a greater reliance has been placed on second generation anticoagulants to remove and eradicate rodent populations.

The increased application of second generation rodenticides, however, puts non-target animals, such as the domestic dog, at greater risk of anticoagulant toxicosis.

Anticoagulant toxicosis of non-target animals (or by-kill) occurs when the animal (1) directly consumes the bait (primary poisoning), or (2) consumes contaminated prey (secondary poisoning). Berny *et al*. (2010b) investigated the prevalence of anticoagulant poisonings in humans and animals in France from 2004 to 2007. The results from this study revealed that most (60%) reported cases of poisoning involved domestic dogs, while human poisonings were rare. A similar result was also demonstrated by Vandenbroucke *et al*. (2010), albeit in this study 79% (316) of the 400 enquiries made to the Belgium Poison Centre regarding anticoagulant poisonings involved domestic dogs. These studies, among others (e.g., Berny *et al*., 2010a; Caloni *et al*., 2003) highlight the need to establish an effective preventative measure that will deter non-target animals, particularly dogs, from consuming anticoagulant poisons.

1.4 Repellents

One method of avoiding non-target by-kill whilst dealing with nuisance or unwanted species is to use non-lethal repellents. A repellent can be defined as a substance or device that deters an animal from an area, place or object (Mason & Clark, 1992). There are four main types of repellents available: visual (sight), auditory (hearing), gustatory (taste) and olfactory (smell) (Mason, 1998). The effectiveness of each type of repellent, however, is dependent on the stimuli used and its mode of action (i.e., fear, pain, malaise or illness) (Wagner & Nolte, 2001). Visual, auditory and olfactory repellents generally startle or elicit fear in the target organism, while gustatory repellents usually cause pain, malaise or illness. The following sections will focus on olfactory repellents, providing a brief overview of how the mammalian olfactory system works, followed by examples of olfactory repellents and their use in the field.

1.4.1 Olfactory Repellents

When developing a repellent, it is integral to understand how the system targeted by the repellent works (Werner & Clark, 2003). The mammalian olfactory system has two anatomically and functionally separate sensory organs, the vomeronasal organ (VNO) and the main olfactory epithelium (MOE) (Lledo *et al*., 2005). The VNO is used by animals to detect pheromones (molecules released by one animal that affects the behaviour or physiology of other animals within the same species)

(Wyatt, 2003), while the MOE is used to detect general odorants (volatile organic compounds with a low molecular weight released from food or other animals) (Nielsen, 2017; Touhara & Vosshall, 2009; Brennan & Zufall, 2006). Both organs contain receptors (VNO: vomeronasal receptors; MOE: olfactory receptors) which, when activated, project axons to the main olfactory or accessory bulbs in the central nervous system, respectively (Touhara & Vosshall, 2009; Simpson, 1997). The glomeri on these structures then send the olfactory information to higher brain areas which elicit a behavioural or physiological response in the organism.

When an animal perceives an odour as a threat they often (1) inhibit their locomotor activity; (2) suppress grooming, foraging, and feeding behaviours; or (3) retreat to a strategic location. These behaviours are indicative of a stress induced fear response and are commonly seen in prey when exposed to a predator odour. For example, Swihart *et al* (1991) used urine from bobcats (*Lyra rufus*), coyotes (*Canis latrans*), and humans (*Homo sapiens*), to determine if these predator odours would successfully repel white-tailed deer (*Odocoileus virginianus*). The result from this study revealed that bobcat urine, followed by coyote urine substantially reduced browsing in this prey species. However, human urine (the main predator of white-tailed deer) had little to no deterrent effect. The authors attributed this result to the deer habituating to the presence of human stimuli. Similar results were found with a different prey species in a study conducted by Parsons and Blumstein (2010). In this study, the authors repeatedly exposed macropod marsupials (kangaroos, *Macropus rufus*) to olfactory scents (urine and faeces) from a sympatric predator, the dingo (*Canis lupus dingo*). Like the white-tailed deer, the macropods actively avoided the area treated with either urine or faeces (Parsons & Blumstien, 2010).

Arnould and Signoret (1993) investigated whether browsing damage to agricultural crops by sheep (*Ovis aries*) could be reduced using natural odours (i.e., dog faeces, pig faeces and foetal sheep fluid) and synthetic odours (i.e., odours derived from lion faeces and a commercial deer repellent). In this study, domestic sheep were individually presented with two troughs, each containing 30 g of maize. One trough was treated with a chemical product (i.e., faeces or commercial deer repellent), while the other remained as a control (i.e., containing

untreated maize). It was demonstrated that the odour of domestic dog faeces (a common predator of sheep) was highly repulsive and elicited feeding avoidance in the sheep. However, the odours from pig faeces, synthetic lion faeces, foetal fluid and the commercial deer repellent had little to no deterrent effect.

Swihart (1991) tested whether woodchuck (*Marmota monax*) damage to the stem of fruit trees could be reduced by applying predator odours (*bobcat urine*) to their stems, or by providing hardwood stakes as alternative scent marking sites. The results from this study revealed that topical application of bobcat urine reduced gnawing damage by 98.3%, relative to controls (untreated tree stems). The alternative scent marking sites, however, were insufficient at reducing damage to the stem of fruit trees.

Although the above studies demonstrate the effects that predator odours can have on their preys' behaviour, they, along with other studies (e.g., Cox *et al*., 2010; Rosell, 2001; Nolte *et al* 1993) have focused primarily on the behavioural responses of small or herbivorous prey species (i.e., white-tailed deer, macropods, and rodents). Little attention has been paid to apex predators (e.g., lions, tigers) and the effect that their odours can have on large carnivorous species lower down the food chain (e.g., canids).

1.4.2 Canid repellents

A limited number of studies have investigated olfactory repellents and their potential to deter domestic dogs or other members of the canid family. For example, Lehner *et al*. (1976) investigated the effects of 45 candidate repellents (natural and synthetic) and their potential to deter both coyotes and dogs from sheep. Although this study revealed several promising chemicals such as capsaicin, cinnamaldehyde, and other commercial products, none of them demonstrated long-term or widespread efficiency (i.e., they did not deter coyotes and dogs for an extended period of time). Wolski *et al*. (1984) demonstrated a similar result, albeit in this study several synthetic repellents were applied to garbage bags, in an attempt to reduce scavenging by dogs. Although these repellents were effective in a laboratory setting, they failed to prevent dogs from foraging on garbage bags in the field. To the author's knowledge, no further

studies on olfactory repellents for dogs have been conducted. Evidently, these studies have not investigated predator odours and their potential use as a canid repellent.

1.5 Summary

In conclusion, it is widely accepted that predator odours have driven the evolution of avoidance behaviour in a range of prey species (Banks *et al*., 2014). Several studies have demonstrated that in the presence of a predator odour, prey will reduce their foraging time and inhibit their activity (Cox *et al*., 2010; Rosell, 2001; Nolte *et al* 1993). However, investigations into potential olfactory repellents for canids has received little attention, with two known studies focusing on the use of synthetic/natural chemical compounds to deter canids. The increasingly frequent number of anticoagulant poisonings in non-target animals, in particular dogs, demonstrates that the development of an effective olfactory repellent that deters this species away from rodenticides would be of national and international value. Such a repellent and could be applied alongside conservation efforts to reduce the potentially fatal effects of anticoagulant rodenticides on non-target species.

1.6 Project Aim and Thesis Structure

The aim of this project is to investigate biologically-derived olfactory repellents in terms of their potential to deter dogs from consuming toxic baits, principally rat bait. To achieve this, two experiments were conducted, each with their own objectives and hypotheses:

Experiment One

- Objective 1: To determine if the selected olfactory repellents (lion, tiger, dog, and baboon faeces, and a commercial repellent) are effective at deterring dogs from eating dog food.
- Objective 2: To determine if there is differences in the volatile composition of the selected olfactory repellents by performing Gas-Chromatography-Mass-Spectrometry.
- Hypothesis 1: Domestic dog, tiger and lion faeces will deter dogs from consuming food. The commercial repellent and baboon faeces will not

deter dogs from dog food.

- Hypothesis 2: The volatile composition of the carnivorous species scat (lion, tiger, and dogs) will differ from the volatile composition of omnivorous species (baboon) scat, and the commercial repellent.

Experiment Two

- Objective: To determine whether the repellent(s) that deterred the dogs will also deter rats (*Rattus norvegicus*), the poison's target species, from consuming food.
- Hypothesis: The faeces from other animals will not deter rats from consuming food. The rats' food consumption will be similar across all repellent types.

1.6.1 Thesis Structure

Chapter one reviews and describes literature on rodents and the impacts they have on the environment and the economy, the advantages and disadvantages of different methods of rodent control, and anticoagulant rodenticides and their effects on non-target animals. Lastly, but most importantly, the chapter reviews the efficacy of olfactory repellents and their potential use as canid repellents. To summarise the chapter, the aims and objectives of the study are stated.

Chapter two describes Experiment One which examined the repellent effects of lion, tiger, baboon, dog faeces and a commercial repellent. In this chapter the results from a series of two choice preference tests with dogs, and an initial pilot study examining the volatiles of the repellents, are presented.

Chapter three details Experiment Two which was performed to determine if the repellents that were 'most effective' on dogs, would also deter rats. In this experiment, a series of two choice preference tests were performed and the rats' feeding and exploratory behaviours were evaluated.

Chapter four, the final chapter, is a general discussion synthesising the findings from both experimental chapters. The conclusions from this study will be compared and discussed in light of current scientific literature.

Chapter 2

Biological Olfactory Repellents for Domestic Dogs

2.1 Introduction

Recent studies have demonstrated that domestic dogs are frequently poisoned by ARs (Vandenbrouke *et al*., 2010; Berny *et al*., 2010b; Albo & Nebbia, 2004). As described in Chapter 1, these poisons inhibit the production and activation of blood clotting factors, leading to uncontrolled bleeding, lethargy, and in most cases death (Stone *et al*., 1999; Hadler & Buckle, 1992). At present, two methods exist to help prevent accidental poisonings in non-target species, these being (1) careful placement of bait out of reach to non-target animals, and (2) the removal of dead/contaminated prey to avoid secondary poisoning. Despite the simplicity of these methods, the fact that non-target poisonings still occur suggests that these techniques are not always followed by rodenticide users, putting non-target species such as domestic dogs at risk of accidental poisoning.

Excretory products, such as urine and faeces, are often used by species to convey information on their social or reproductive status, age, sex, or group composition (Simpson, 1997). Though usually intended for conspecifics, the odours released from these products can also be detected by other species. It has been demonstrated in recent research that the excretory products from predators can deter small, herbivorous prey (Cox *et al*., 2010; Rosell, 2001; Nolte *et al* 1993). However, to the author's knowledge, predator odours and their deterrent effects on canid species are yet to be explored.

Dogs were domesticated at least 15,000 years ago (Miklósi, 2014) and have no natural predators. However, African wild dogs (*Lycaon pictus*), a distant relative of domestic dogs, are sometimes depredated by African lions (*Panthera leo*) (Darnell *et al*., 2014). Darnell *et al* (2014) investigated the spatial and temporal patterns of African wild dogs, and found that they actively avoided areas occupied by African lions. However, when in the presence of another competitor species, spotted hyenas (*Crocuta crocuta*), the behaviour of the African wild dogs was

unaffected. This study demonstrated that the behaviour of African wild dogs can be affected by the presence of predators in some cases. However, information on the sensory cues that may be influencing the avoidance behaviour is lacking. Given that smell is a dominant sense in both domestic dogs and African wild dogs, it is hypothesized that scent cues may have a strong influence on their behaviours. Furthermore, because domestic dogs and African wild dogs have a close genetic relationship, it is hypothesised that domestic dogs may also actively avoid African lion scent cues.

The aim of this experiment was to determine if the faeces from two predatory species (lion, *Panthera leo* and tigers, *Panthera tigris*), an omnivorous species (baboon, *Papio hamadryas*), and conspecifics (i.e., other dogs), would deter dogs from consuming their food. A commercial dog and cat repellent was also included in this investigation. A further objective was to determine if the different scat samples contained unique volatile constituents. It was hypothesised that domestic dog, tiger and lion faeces would deter dogs from their food, while the commercial repellent and baboon faeces would not.

2.2 Methods

In this experiment, dogs were presented with a series of paired choice preference tests to determine the repellent effects of five faecal types (lion, tiger, baboon, and dog) and a commercial dog and cat repellent. In addition to this, an initial pilot study was conducted using Gas Chromatography-Mass Spectrometry to determine if any faecal types had unique volatiles present.

2.2.1 Dog Experiment

2.2.1.1 Subjects

Thirty-nine domestic dogs between the age of 1 and 13 years of age, were recruited from the general pet dog population in Hamilton, New Zealand. The dogs were recruited using social media, word of mouth, and posters displayed at the University of Waikato, dog day-care facilities and veterinary clinics within the Hamilton area. Eight dogs were used in a pilot study to develop the methodology (see Appendix A for full details), and 21 dogs were used in the full study (Table 2.1). Ten dogs were withdrawn from this study due to showing signs of distress

(see Appendix A for full details on criteria for exclusion). The dogs' owners were given an information sheet that outlined the topic and requirements of the study before the trials started (See Appendix D for participation information). The owners were also given the opportunity to ask the researcher questions before signing a consent form. This study had approval from the University of Waikato Animal Ethics Committee (protocol number 1030).

Table 2.1: Details of the dogs who participated in this experiment (age, sex, breed).

Dog	Age (years)	Sex	Breed
Pac	10	Male[n]	Border collie x Shetland sheepdog
Rebel	3	Male[i]	Border collie
Tat	2	Male[n]	Golden retriever x border collie
Feature	2	Male[n]	Border collie
Arie	6	Male[n]	Heading dog cross
Clutch	5	Male[n]	Catahoula x greyhound
Xena	6	Female[s]	Border collie cross
Trigg	3	Male[n]	Labrador retriever
Raven	2	Female[s]	Labrador retriever
Maggie	1.5	Female[s]	Labrador retriever
Lulu	10	Female[s]	Shihtzu
Jett	2	Male[n]	Labrador retriever x huntaway
Kaspar	3	Male[n]	Golden retriever
Zoe	12	Female[s]	Labrador retriever
Zander	5	Male[n]	Labrador retriever
Ice	6	Male[n]	Sharpei x Labrador retriever
Bru	5.5	Female[s]	Border collie x Samoyed
Chief	5.5	Male[n]	Border collie x Samoyed
Lexie	2	Female[s]	Staffordshire terrier cross
Bella	8	Female[s]	Labrador retriever
Rex	1	Male[n]	Labrador retriever x golden retriever

Note: neutered[n], spayed[s], intact[i].

2.2.1.2 Study location and equipment

This experiment took place in an animal facility at the University of Waikato, Hamilton, New Zealand. The building had five rooms: a vivarium, two storage rooms, an observational room, and an experimental room (where the trials took place, 3.8 m (w) by 4 m (l); Figure 2.1). PVC insulation tape (18 mm (w)) was used to separate the experimental room into a four by four grid. Supplementary to the grid, two squares (1 m^2) containing two smaller quadrants (50 cm^2) were positioned in the centre of the experimental room. These quadrants were used by the researcher to determine the amount of time each dog spent in proximity to, and

investigating, the repellent/control feeding apparatuses, respectively (see 2.2.16 Video Analysis, below). Two security cameras (Xpose, Model: QC8612) and a GoPro HERO4 (silver) were fixed onto the walls of the arena (Figure 2.1).

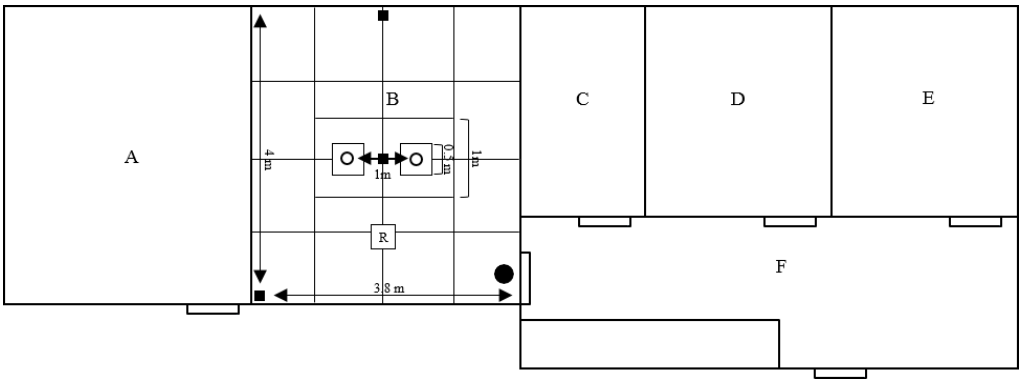

Figure 2.1: Layout of the building and the experimental test arena. A = storage room; B= experimental room; C = observation room; D = storage room; E = vivarium; F = preparation/sink area. In the experimental room: black squares = cameras; lines = taped grid lines; R = release point; hollow circles = feeding apparatuses; black circle = researcher's position during the trials.

A novel feeding apparatus was developed to determine if the selected repellents would deter dogs from consuming dog food based on smell alone (details of its development are in Appendix A). A shallow, rubber based stainless steel bowl (Yours Droolly brand, 13.5 cm diameter) was positioned in the centre of a green plastic saucer (20 cm diameter, 2.5 cm high; Figure 2.2). Insect mesh (Polar-Bear Insect Mesh, silver) was used to cover the voided space between the bowl and the saucer. A total of seven feeding apparatuses were made; one for each repellent type, and two to act as controls.

A child proof gate (170 cm (h) x 72 cm (w)) was placed in the door frame of the experimental test arena. This was used to prevent the dogs from exiting the room during the trial, and to reduce the chance of the dogs developing any stress or anxiety due to being in an enclosed area. A stopwatch was also used to time each trial.

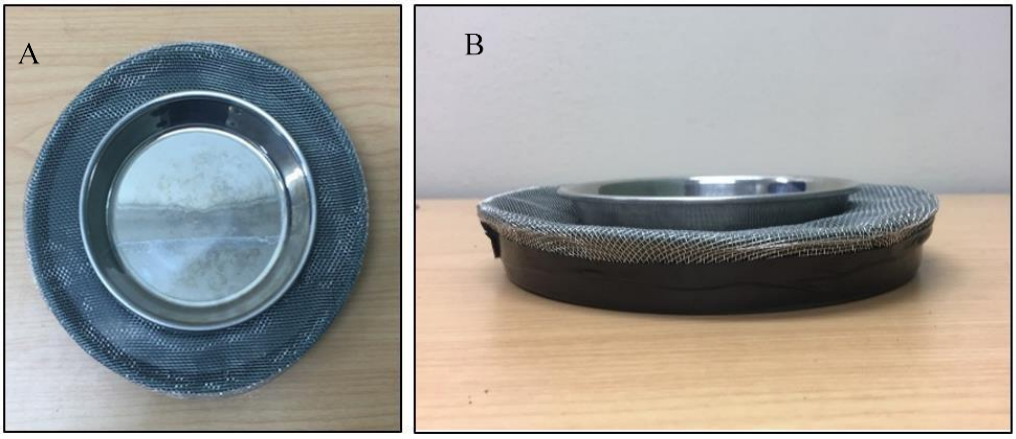

Figure 2.2: Bird's eye view (A) and side view (B) of the feeding apparatuses used in the trials. The repellent solution was placed in the saucer underneath the wire mesh (preventing the dogs from gaining direct access to the repellents) and the food was placed in the stainless-steel bowl.

2.2.1.3 Repellent Sample Collection

Faeces were collected in this experiment as they were easier for the zoo keepers to collect than urine. Lion and tiger faeces were used in this experiment to determine if the faeces from carnivorous species were more effective at deterring dogs from their food than the faeces from omnivores (baboon), conspecifics (dog) or a dog and cat commercial repellent.

The selected repellents were collected from three different locations. The first location was Auckland Zoo, New Zealand, where the exotic animal faeces were collected. Daily collections of tiger, lion, and baboon faeces were made over a four-week period by zoo keepers at Auckland Zoo. The faeces were stored in separate buckets (10 L) and frozen immediately after collection. The zoo keepers were asked to record the time and date each sample was collected and frozen, as well as the animals' diet, age, and sex (if possible) (refer to appendix F). In total five kilograms of faeces were collected per species. The faeces were transported from Auckland Zoo to the University of Waikato's Physical Containment Facility under movement authority number CL10367. During transportation the faeces were sealed in two buckets. To comply with the New Zealand's Biosecurity Act (1993) and to kill any unwanted pathogens or bacteria, all exotic animals' faeces were autoclaved using a verified (Medisys) destruction cycle (121^{o}C for 60 minutes) before leaving the Physical Containing Facility and becoming available for use in this experiment.

The domestic dog faeces were collected from Animal Lodge in Hamilton, New Zealand, a boarding facility for cats and dogs. Daily collections of dog faeces were made for a week, twice. All samples were placed into a one litre autoclavable container and stored in the refrigerator immediately after collection. To ensure all faeces samples were treated the same (standardised), the dog faeces were also autoclaved using a verified (Medisys) destruction cycle (121^{o}C for 60 minutes).

Skunk Shot Cat and Dog Repellent (Skunkshot Scatter, 250 ml bottle) was purchased from Mitre 10 Mega in Hamilton, New Zealand. This repellent is/was designed to prevent territorial marking and to keep cats and dogs away from certain areas or objects. An internet search was conducted to determine the efficacy of the repellent and its potential use in this study. The key words used in the internet search were: 'Skunkshot cat and dog repellent' and 'customer reviews'. This repellent was selected based on good customer reviews online and its non-toxic chemistry.

All faecal samples were stored in a freezer (-18^{o}C) until they were required for sample preparation and use in the experiment. The commercial repellent was held at room temperature, in an air tight container.

2.2.1.4 Repellent Sample Preparation

The faecal samples were defrosted 24-48 hours before making the repellent solutions. Breville scales (model BSK200B) were used to weigh 40 g of each species' faecal matter. Each faecal type was mixed with 100 ml of water and blended using a Home and Co Mini Blender (600 ml, 300 watt) for approximately two minutes. The resulting solution were poured through a coarse sieve into a 1 L plastic container (one container per repellent type). The containers were labelled with the date, sample type, and the researcher's name and stored in the chiller (4^{o}C) until they were required for use in the experiment. The repellent solutions were stored for a maximum of two weeks. All containers, the blender and the sieve were washed thoroughly with hot water and dishwashing liquid between treatments to avoid cross contamination of samples. The amount of commercial repellent presented to the dogs was based on the manufacturer's instructions: one teaspoon of repellent per square metre. Fifty teaspoons of commercial repellent were weighed using Denver Instrument Scales, and the average weight was

calculated as being 4 g. Using the equation: $A = \pi r^2$, the area of the feeding apparatus was determined. To establish the amount of commercial repellent to be used per trial, the area of the feeding apparatus was multiplied by the average weight of a teaspoon of commercial repellent (0.031 x 4 = 0.125 g per trial)

The possibility of using non-toxic versions of rat bait was explored, however, the cost involved in producing these baits was prohibitive; therefore dog food (Royal Canin Mini Exigent) was used as a bait substitute. To determine the quantity of Royal Canin Exigent food each dog received, the recommended daily feeding amounts were plotted into Microsoft Excel and a linear regression was performed. The dog weight (kg) was used as the independent variable (x-axis) and the amount of food (g) was used as the dependent variable. The linear equation: 10.933x + 18.622, produced from the regression was used to quantify the total amount of food each dog received. To determine the amount of food per bowl, the total amount of food (the calculated daily allowance) was divided by total number of presentation (24 presentations).

2.2.1.5 Experimental Procedure

Twelve two choice preference tests were performed by each dog over two sessions. Both sessions took place on the same day and each session comprised of six two-minute trials: an initial habituation period, and then the random presentation of five repellent-control combinations. All dogs were food deprived for 2-6 hours before the tests took place and all trials were video recorded. If the owners were present during the trials, they were asked to stand in a separate room, where they could not talk or interact with their dog.

To acclimatise the dogs to the test arena and feeding apparatuses, a habituation period was performed at the start of each session. In these habituation periods, two feeding apparatuses containing dog biscuits and 100 ml of water were positioned in the centre of the test arena, one meter apart. The dogs were led into the arena by the researcher and let go at the release point (Figure 2.1). The researcher remained in the arena during the trial and faced the wall of the front, right corner to avoid giving unintentional cues. After two minutes, the dog was removed from the arena and remained on a leash, outside, until it was required for the next trial. If it was raining, the dogs were kept inside in a separate room to where the samples

were prepared.

This procedure was repeated for the five remaining trials; however, in each of these trials the dogs were presented with one repellent-treated apparatus (dog biscuits + 100 ml repellent) and one control feeding apparatus (dog biscuits + 100 ml water). To minimise odour dissipation the researcher covered all repellent food bowls with glad wrap between trials and replaced all repellent solutions with a fresh solution every three hours on experimental days.

When the trials were completed, the test arena and all food/water bowls were cleaned thoroughly with Sterigene and dishwashing liquid, respectively.

2.2.1.6 Video Analysis

Preliminary video analysis was conducted on five pilot study videos using Windows Media® Player. The behaviours observed in these videos were recorded and an ethogram of the dogs' behaviours was made (Table 2.3). Solomon Coder (version: beta 17.03.22), a free behaviour coding program was used for formal video analysis. A configuration sheet was created to display all ethogram behaviours and arena locations as 'buttons' (Figure 2.3, Table 2.2, and Table 2.3). These buttons were allocated to one of three coding variables: instant (event behaviours, that occurred in an infinitesimal moment in time) (Table 2.3), continuous (state behaviours, that occurred over a measurable period of time) (Table 2.3), and position (the dog's location in the arena) (Table 2.2). When a behaviour occurred, or the dog was in a certain area of the arena, the corresponding button was pushed and the behaviour/area was recorded. When the video was completed the configuration sheet was saved and the output data were exported to Excel and used for statistical analysis. A total of 9.2 hours of video footage were watched; 8.4 for formal video analysis, and 50 minutes (10%) were re-watched for randomly selected intra-observer reliability.

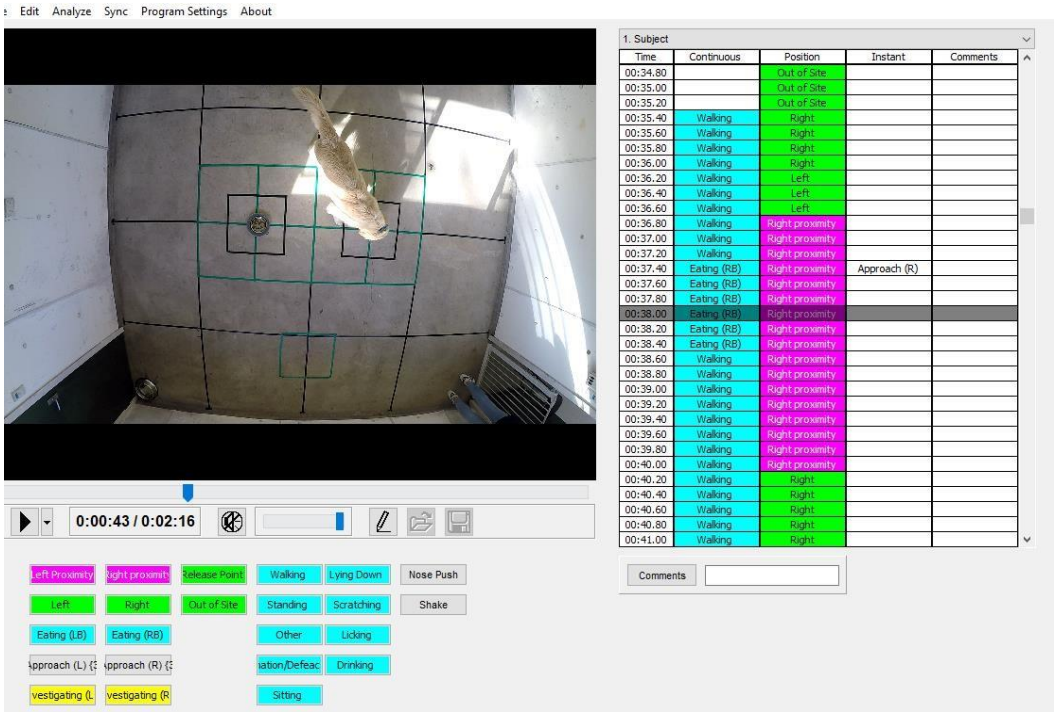

Figure 2.3: A screen shot of a video being analysed using Solomon Coder software. The video is displayed on the left side of the image and the table of recorded behaviours is on the right side. The 'buttons' for each behaviour and location of the arena are located on the lower half of this image. Grey button = event behaviours; blue and yellow buttons = state behaviours; green and pink buttons = arena position.

Table 2.2: Locations used to determine where the dogs were during the trial.

Arena Position	**Definition**
Left	The dog was on the left-hand side of the arena.
Right	The dog was on the right-hand side of the arena.
Proximity- Right Food Bowl	The dog was less than 50 cm away from the right feeding apparatus.
Proximity– Left Food Bowl	The dog was less than 50 cm away from the left feeding apparatus.
Release point	The dog was positioned at the release point.

Note: only the amount of time spent in proximity to left and right feeding apparatuses were used in statistical analyses.

Table 2.3: Ethogram of the dog behaviours recorded during the trials. Recording type is whether the behaviour was recorded as a continuous behaviour (C), or an instantaneous behaviour (I).

Behaviour	Definition	Recording type
Walking	Quadrupedal movement along a horizontal surface. Dog moves at a slow to moderate speed, alternately bearing weight on hind or fore feet. Movement occurs in a forward or backwards motion.	C
Standing[S]	Dog remains in a stationary position for more than one second. All four legs are in an extended position holding the dog's abdomen off the ground.	C
Sitting[S]	Supported by two extended forelimbs and two flexed back limbs. The dog's abdomen remains out of contact with the ground surface and the dog's rear end is placed on the ground.	C
Lying down[S]	Hind limbs are tucked under the body and forelimbs are extended in front of the body. Abdomen is in contact with the ground.	C
Drinking[O]	Animal uses its tongue to consume water from a dog bowl.	C
Eating	Food enters the dog's mouth and is ingested. The dog's jaw moves repeatedly in an upwards and downwards action.	C
Scratching[O]	The paw of the dog makes repeated contact with its face or body. Head is generally angled towards the moving limb.	I
Licking[O]	The dog's tongue comes in contact with an object, water or food.	I
Shaking[O]	Dog shakes its body from side to side whilst remaining in a stationary position.	I
Urination/ defaecation	Dog urinates or defecates in the experimental room. Can include territorial marking.	C
Investigating	Dog is sniffing, licking or exploring less than one metre away from the left (investigating left) or right (investigating right) feeding apparatus. Head is usually orientated towards the ground or the feeding apparatus.	C
Nose push	Dog uses its nose to move the left (left nose push) or right (right nose push) feeding apparatus. Each time the dog pushed and removed its nose from the object it was classified as an individual nose push.	I

Behaviour	Definition	Recording type
Approach	Dog sniffs, eat or licks the left (approach left) or right (approach right) feeding apparatus for more than 0.5 seconds. The head is orientated towards and is less than 50 cm away from the feeding apparatus.	C
Out of sight	Not visible. Behaviour or area cannot be defined.	C

Note: For statistical analysis, less conspicuous behaviours were grouped together, as either stationary[s], or other[o].

2.2.1.7 Statistical Analysis

For each session, the difference in the percentage of food consumed from the control food bowl and the repellent food bowl was calculated for each repellent and each dog. A mixed effects model was then applied to these differences, with the dog as a random effect (to account for the five observations per dog) and the repellent as the fixed effect. Although there was significant evidence against the assumption of normality, resampling routines produced similar results to those gained from this analysis. This suggests that the results from this parametric test are justified due to the central limit theorem.

When significance tests were conducted it was assumed that the data points represented 21 separate observations where each dog participated in five repellent trials (total 105 observations).

A one-way ANOVA was performed to test for significant differences in the mean response between the repellent treatments. However, to determine if there was a significant difference in the percentage of food consumed from the control food bowl and the percentage of food consumed from the repellent food bowl, for each repellent-control combination a single-step method for simultaneous tests of multiple general linear hypotheses was performed. On the results that were highlighted as significant, simultaneous 95% confidence intervals were applied.

The time the dogs spent investigating and the time they spent eating from the repellent and control food bowl for each repellent-control combination was converted into percentage form to account for small deviations in the total trial

time (120 s ± 12 s). Because the data were measured on an interval scale and did not meet the assumption of normality (Shapiro-Wilk p-value <0.05 for all repellent-control combinations), a Wilcoxon matched pairs test was performed on these data sets.

The latency to approach data was not converted into percentage form, albeit it still did not meet the assumption of normality when the Shapiro-Wilk test was performed. As a result, a Wilcoxon matched pair tested was performed to determine if there was a significant difference in the latency to approach the repellent food bowl and the latency to approach the control food bowl for each repellent-control combination. It is noted that the blanks in the data set (i.e., when the dogs did not approach both or one of the food bowls) were excluded for analysis. It was considered as to whether these blanks should be replaced with a 0 indicating the dogs approached the repellent immediately or the total trial time (120 s), however, these options were likely to skew the results and therefore were not used.

Descriptive statistics, tables and graphs were formulated to describe the frequency to approach data and the 'other' behaviours performed by the dogs.

To determine if the researcher's video analysis was reliable, and to determine the measure of agreement between different observers, intra-observer and inter-observer reliability were performed. For this, 50 minutes (10% of 8.4 h) of video footage was randomly selected from the sample population and re-watched by the researcher and another independent observer. This independent observer was trained by the researcher on Solomon Coder, and given the ethogram explaining all recorded behaviours. Pearson's correlation revealed that all recorded behaviours had a strong positive correlation (intra-observer: $p = 0.80$; inter-observer: $p = 0.85$), except for the release point (intra-observer: $p = 0.84$; inter-observer: $p = 0.13$).

All statistical analyses were performed using R and Statistica (Version 13). All figures were made using R, Graphpad, or Microsoft Excel. The specified level of alpha for all statistical tests was 0.05.

2.2.2 Gas Chromatography-Mass Spectrometry

2.2.2.1 Sample preparation

The faecal samples were defrosted 24 h prior to the experiment taking place. One gram (± 0.1 g) of the commercial repellent and one gram of each species' faecal matter was placed into an individual glass vial and capped immediately with a lid containing a rubber septum. The vials were incubated in an oven set at 80°C for a 30-120-minute period. A total of 12 samples were prepared; two per faecal type, two for the commercial repellent and two blanks. Once the samples were heated, a needle (24 gauge) with a 10 ml syringe was injected through the septum and purged three times to remove air from the syringe; 3 ml of headspace was then drawn up. The extracted headspace was then manually injected into the GC-MS machine.

2.2.2.2 GC-MS conditions

GC-MS analysis was carried out on a Hewlett Packard HP 6890 GC-MS. A Zebron ZB5 capillary column (250 µm inner diameter, 0.25 µm film thickness and 30.0 m length) was used for separation of volatile compounds. Helium was used as a carrier gas. The GC oven was initially set at 40°C; it was ramped to 80°C at a rate of 5°C/minute, then to 100°C over 30 minutes (with a total analysis time of 8.67 min). There was a solvent delay of 0.5 min. The MS was scanned from m/z 33.0 to 550.0. Blank vials were analysed to exclude any peaks found in the laboratory air. An internal standard was not used for this pilot study because this was an initial exploration of these repellent substances and it was unknown what compounds would be emitted.

2.2.2.3 Data processing

MSD Chem Station software and the National Institute of Standards and Technology (NIST) mass spectral library (version 2.0) were used to tentatively identify the volatile organic compounds (VOC) present in the samples.

2.3 Results

2.3.1 Amount of food consumed

The amount of food consumed from the repellent and control food bowl varied per repellent-control combination. In session one the dogs ate the least amount of food on average from the repellent food bowl when it is contained dog faeces (dog = 44.85 ± 20.11%, control = 78.57 ± 18.45%) (Mean ± 95% confidence intervals). This was followed by the repellent food bowl containing lion faeces (lion = 61.36 ± 20.27%, control = 88.51 ± 13.93%), tiger faeces (tiger = 65.22 ± 19.88, control = 78.25 ± 18.57), the commercial repellent (commercial = 81.73 ± 16.54%, control = 99.56 ± 0.92%) and baboon faeces (baboon = 86.12 ± 15.88%, control = 94.83 ± 9.91%). In session two, a similar pattern in consumption was observed. The dogs consumed the least amount of food from the repellent food bowl containing dog faeces (dog = 41.42 ± 21.30%, control = 86.24 ± 14.28%), followed by lion faeces (lion = 63.14 ± 20.38%, control = 85.25 ± 16.25%), tiger faeces (tiger = 65.60 ± 19.22%, control = 80.43 ± 18.21%), baboon faeces (baboon = 81.15 ± 17.17%, control = 88.3 ± 13.83%) and the commercial repellent (commercial = 82.65 ± 14.70%, control = 89.45 ± 13.70%) (Figure 2.4).

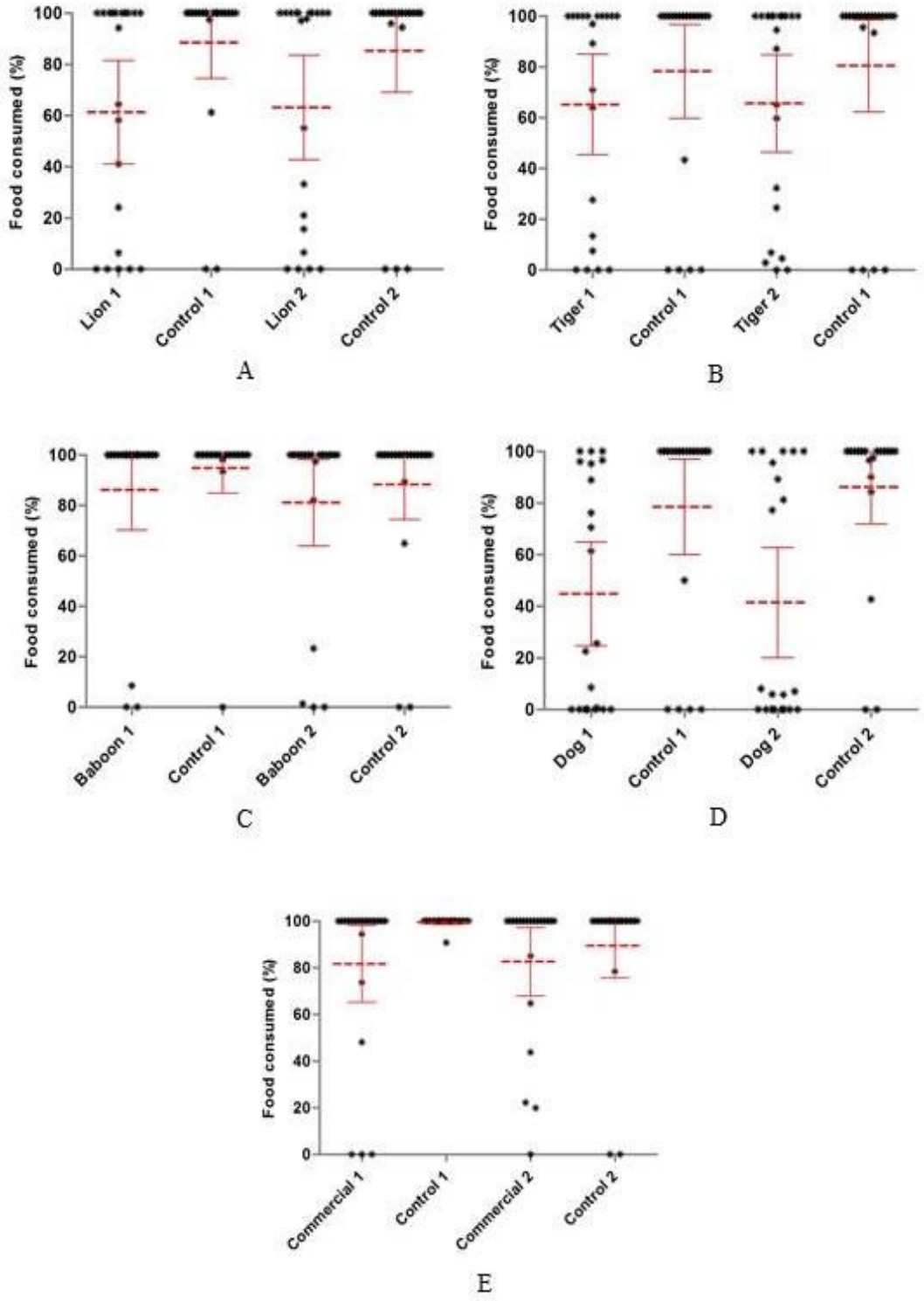

Figure 2.4: Mean percentage of food consumed by the dogs in session one (1) and session two (2) when presented the lion faeces (A), tiger faeces (B), baboon faeces (C), dog faeces (D) and the commercial repellent (E). Black dots= Individual data points. Error bars are 95% confidence intervals.

When examining if there were differences in the percentage of food consumed between the repellent and control apparatuses across all repellents (Figure 2.5), a one-way ANOVA showed that on average there was no significant difference between the repellent types ($F_{4,16} = 1.9217$, $p = 0.156$).

However, of more interest, was to determine if a single repellent produced a significant difference in the percentage of food consumed from the control food bowl and the percentage of food consumed from the repellent food bowl. After adjusting for multiple comparisons, tests of the hypothesis: a repellent has no significant effect on the amount of food consumed from the repellent and control food bowl, revealed that a significantly lower proportion of food was consumed from the repellent food bowl when it contained dog ($p = < 0.001$) and/or lion faeces ($p = 0.008$), than the control. When 95 % confidence intervals were applied, they revealed that between 11.7% and 55.8% less of the food was consumed from the food bowl containing dog faeces, and 5.1% and 49.2% less of the food was consumed from the food bowl containing lion faeces. No other significant differences were found.

In session two the results from an ANOVA revealed that there was at least one repellent that caused a significant difference in the percentage of food consumed form the repellent food bowl and the control food bowl ($F_{4,16} = 6.8882$, $p = 0.002$). A Tukey's post-hoc test revealed that dog faeces had a significantly more negative effect on the percentage of food consumed, than baboon faeces ($p = <0.001$), the commercial repellent ($p = <0.001$), and tiger faeces ($p = <0.004$).

Again when the null hypothesis; no repellent caused a significant difference in the amount of food consumed from the repellent and control food bowls, was examined, it was revealed that the dogs consumed significantly less food from the repellent food bowl when it contained dog faeces ($p = <0.001$) and lion faeces ($p = 0.017$), than the control food bowl. When 95 % confidence intervals were applied they revealed that between 25.6% and 64.0% less of the food was consumed from the food bowl containing dog faeces, and 2.9% and 41.3% less of the food was consumed from the food bowl containing lion faeces.

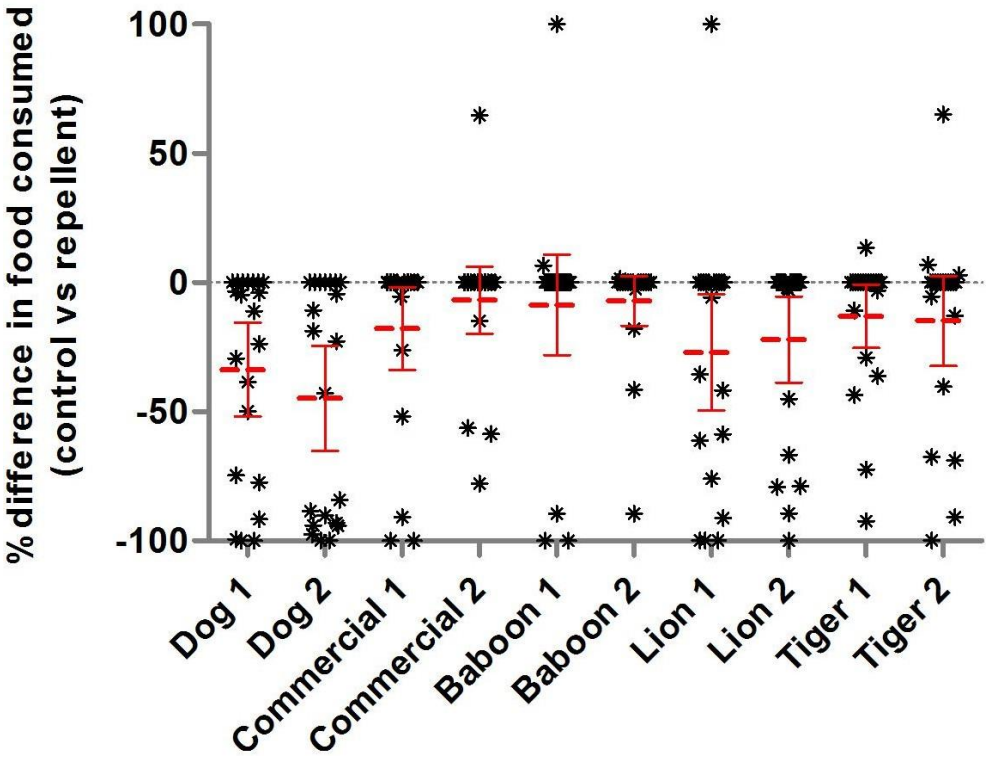

Figure 2.5: Mean percent difference in the amount of food the dogs consumed from the control food bowl and the amount of food they consumed from the repellent food bowl, for each repellent type (dog faeces, the commercial repellent, baboon faeces, lion faeces, and tiger faeces) in session one (1) and session two (2). Error bars are 95% confidence intervals.

2.3.2 Behaviour data

2.3.2.1 Time spent eating

On average, in session one, the dogs spent more time eating from the repellent food bowls, than the control food bowls (dog = 11.75 ± 6.21%, control = 6.47 ± 2.11%; tiger = 11.09 ± 3.51%, control = 6.30 ± 1.83%; baboon = 10.65 ± 2.95%, control 7.99 ± 1.55%; lion = 8.09 ± 3.08%, control = 7.64 ± 1.92%), except when presented the commercial repellent (commercial = 6.41 ± 1.72%, control = 9.02 ± 1.83%) (Mean ± 95% confidence intervals). In session two similar results was observed, albeit the dogs spent more time eating from all repellent food bowls, than control food bowls (dog = 10.96 ± 6.09%, control = 6.68 ± 1.81%; lion = 9.10 ± 4.54%, control = 7.09 ± 2.10%; tiger = 8.79 ± 3.14%, control = 5.89 ± 1.79%; baboon = 8.18 ± 2.59%, control = 6.92 ± 1.93%; commercial = 7.02 ± 1.49, control = 6.89 ± 1.64%) (Figure 2.6).

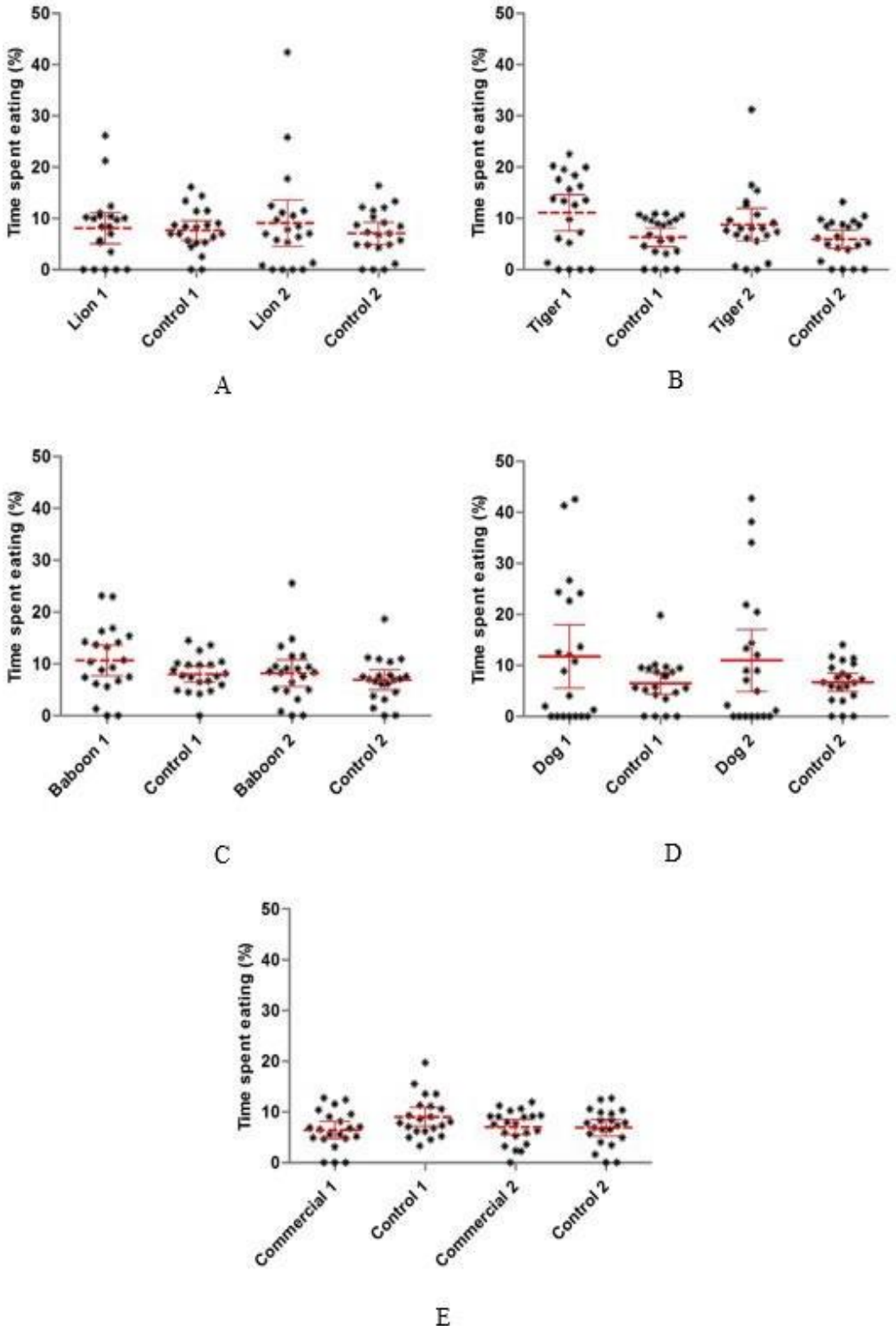

Figure 2.6: Mean amount of time the dogs spent eating in session one (1) and session two (2) when presented the lion faeces (A), tiger faeces (B), baboon faeces (C), dog faeces (D), and the commercial repellent (E). Black dots = Individual data points. Error bars are 95% confidence intervals.

The results from a Wilcoxon matched pair test demonstrated that in session one, the dogs spent significantly more time eating from the repellent food bowl than

the control food bowl when presented baboon (N = 18, p = 0.049) and tiger (N = 18, p = 0.010) faeces. The opposite, however, was observed for the commercial repellent; the dogs spent significantly less time eating from the repellent food bowl than the control food bowl (N = 20, p = 0.033). No significant difference in time spent eating from the repellent and control food bowls was found when the dogs were presented with lion (N = 18, p = 0.983) and dog (N = 17, p = 0.332) faeces.

In session two the dogs spent significantly more time eating from the control than the repellent food bowl when presented with the baboon (N = 19, p = 0.033) and tiger (N = 19, p = 0.014) faeces. However, there was no significant difference in the time the dogs spent eating from either the control and repellent food bowls when presented with lion faeces (N = 18, p = 0.349), dog faeces (N = 19, p = 0.334), and the commercial repellent (N = 20, p = 0.737).

2.3.2.2 Time spent investigating

In session one the dogs spent more time on average investigating the repellent food bowls, than the control food bowls (tiger = 4.88 ± 2.77%, control = 2.87 ± 2.21%; lion = 2.96 ± 1.32%, control = 2.45 ± 1.96%; commercial = 3.15 ± 1.53%, control = 2.40 ± 1.93%), especially when the repellent food bowl contained baboon (baboon = 9.4 ± 4.22%; control = 4.08 ± 3.35%), or dog faeces (dog = 5.89 ± 3.52 %; control = 3.98 ± 3.52%). In session two the results were more variable. The dogs spent more time investigating the repellent food bowl when it contained tiger (tiger = 1.79 ± 1.26%, control = 1.07 ± 0.74%) and dog faeces (dog = 3.07 ± 2.17%, control = 0.52 ± 0.39%), but less time when it contained lion faeces (lion = 0.74 ± 0.58%, control = 1.09 ± 0.80%), baboon faeces (baboon = 3.08 ± 1.85, control = 5.31 ± 5.86%), and the commercial repellent (commercial = 1.70 ± 1.64%, control = 2.30 ± 1.25) (Figure 2.7).

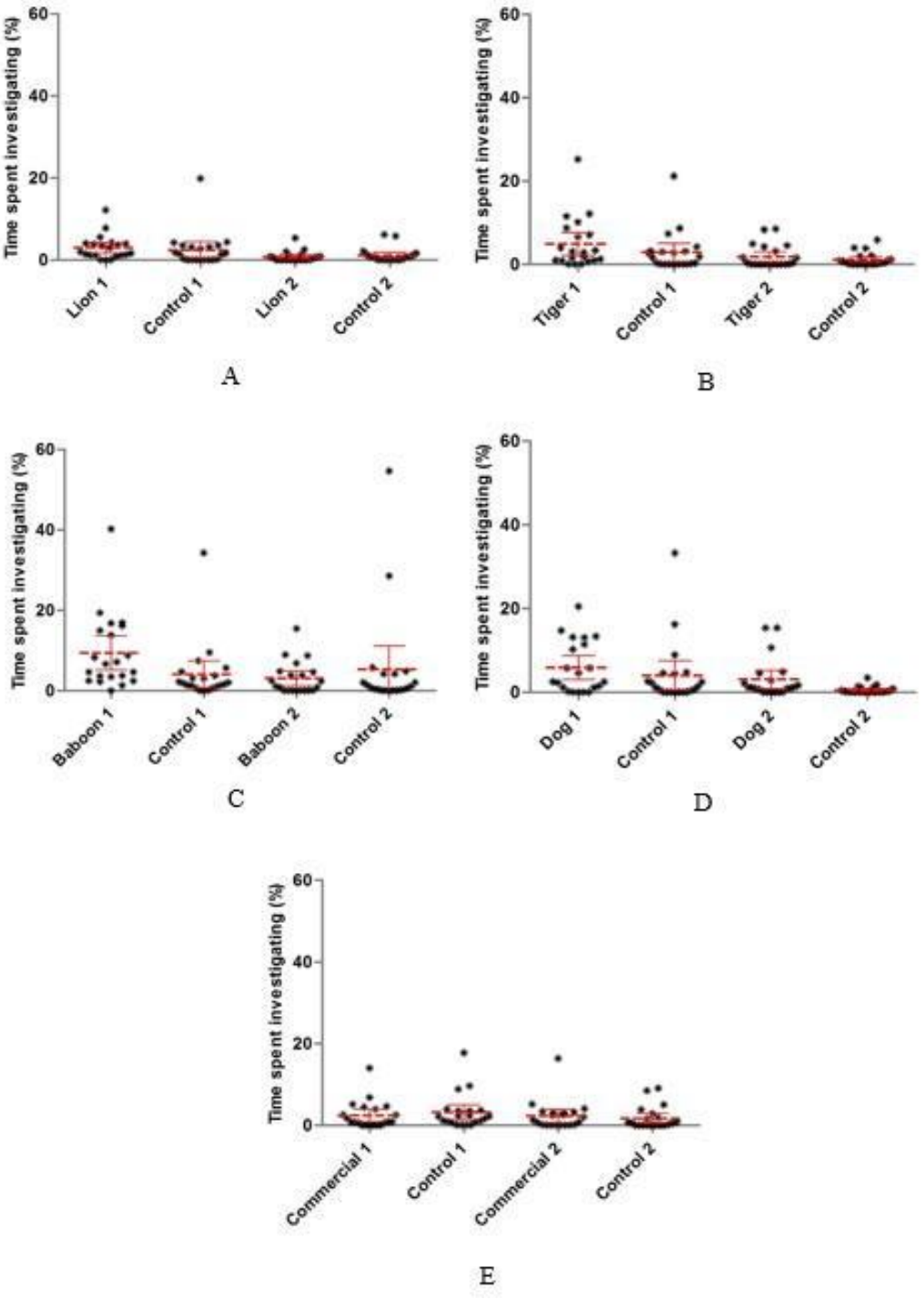

Figure 2.7: Mean percentage of time the dogs spent investigating in session one (1) and session two (2) when presented the lion faeces (A), tiger faeces (B), baboon faeces (C), dog faeces (D) and the commercial repellent (E). Black dots = Individual data points. Error bars are 95% Confidence Intervals.

A Wilcoxon matched pair test revealed that in session one, the dogs spent significantly more time investigating the repellent food bowl than the control food bowl when presented tiger (N = 19, p = 0.022), lion (N = 20, p = 0.030), dog (N = 17, p = 0.062), and baboon (N = 21, p = 0.002) faeces. No significant difference was found in the time spent investigating the control or repellent food

bowls when the dogs were presented the commercial repellent.

In session two the dogs spent significantly more time investigating the repellent than the control apparatus when presented with dog faeces (N = 18, p = 0.011). No significant differences between the time spent investigating the repellent or control apparatuses were found when presented with tiger faeces (N= 14, p= 0.18), lion faeces (N = 13, p = 0.173), baboon faeces (N = 16, p = 0.092), and the commercial repellent (N = 15, p = 0.363). Thus, for these latter faeces types the null hypothesis, that the median of the differences of time spent investigating equals or is similar to zero, was accepted.

2.3.2.3 Latency to approach

On average, in session one, the dogs took longer to approach the repellent food bowl when it contained tiger (tiger = 12.70 ± 6.72 s, control = 10.52 ± 10.72 s), lion (lion = 10.46 ± 4.88 s, control = 590 ± 2.61 s), and dog faeces (dog = 6.94 ± 2.62 s, control = 3.92 ± 2.83 s), than the control food bowl. However, when the repellent food bowl contained baboon faeces (baboon = 5.31 ± 3.53 s, control = 14.51 ± 6.30 s), and the commercial repellent (commercial = 7.36 ± 4.23 s, control = 9.90 ± 4.88 s) the dogs took less time to approach the repellent food bowl, than the control food bowl. In session two the results varied; the dogs took longer to approach the repellent food bowl when it contained lion faeces (lion = 11.71 ± 6.75 s, control = 7.47 ± 4.31 s), the commercial repellent (commercial = 10.66 ± 8.76 s, control = 8.92 ± 4.80 s), and dog faeces (dog = 6.38 ± 2.87 s, control = 6.21 ± 3.43 s), and less time when it contained baboon faeces (baboon = 5.61 ± 2.84 s, control = 15.41 ± 11.29 s), and tiger faeces (tiger = 7.68 ± 2.63 s, control = 8.47 ± 7.11 s) (Figure 2.8).

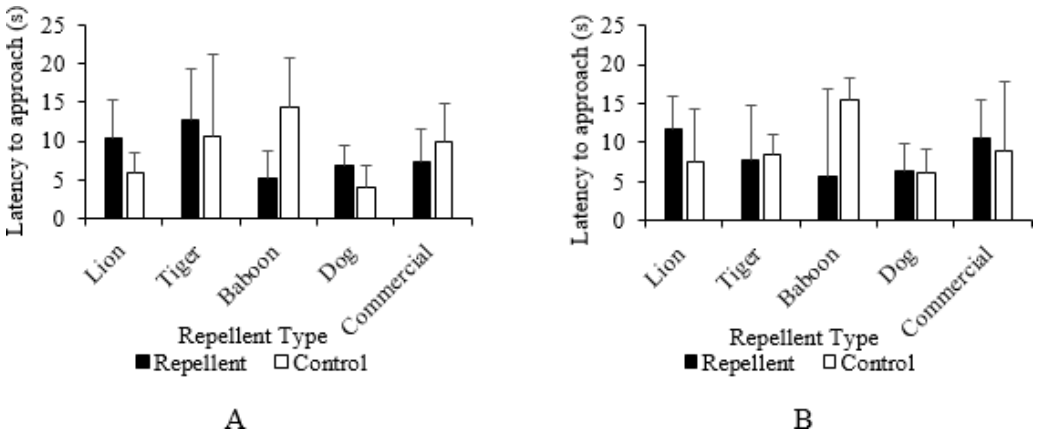

Figure 2.8: Mean latency for the dogs to first approach the feeding apparatuses in session one (A) and session two (B), when presented with lion faeces, tiger faeces, baboon faeces, dog faeces and the commercial repellent. Error bars are 95% confidence intervals.

In session one, a Wilcoxon matched pair test revealed that the dogs took significantly longer to approach the control food bowl compared to the repellent food bowl when they were presented baboon faeces (N = 20, p = 0.023). No other statistically significant differences in latency to approach were evident in session one (tiger: N = 17, p = 0.463; commercial: N = 19, p = 0.732; dog: N = 17, p = 0.124; lion: N = 19, p = 0.142) or session two (commercial: N = 19, p = 0.794; baboon: N = 18, p = 0.107; tiger: N = 16, p = 0.587; dog: N = 18, p = 0.616; lion: N = 18, p = 0.151). Thus, for these repellent-control combinations the median of the differences was equal to zero and the null hypothesis was accepted.

2.3.2.4 Frequency of approach

When comparing the total number of approaches the dogs made to the repellent bowl to the total number of approaches to the control bowl, it is evident that in session one the dogs approached the control more times than the repellent. In session two the opposite was observed, with the dogs approaching the repellents more times than the controls (Table 2.4).

Table 2.4: Total number of approaches to the repellent food bowl and the control food bowl in Session 1 and Session 2.

Type	Repellent	Control	Total
Session 1	276	291	561
Session 2	202	174	376

When comparing the mean number of approaches to each repellent type, the results varied marginally (Figure 2.9). On average, in session one, the dogs approached baboon faeces the most (μ = 2.76 ± 0.70) (mean ± confidence intervals), followed by dog faeces (μ = 2.67 ± 0.98) and the commercial repellent (μ = 2.67 ± 0.74), tiger (μ = 2.62 ± 2.67), and lion faeces (μ = 2.43 ± 0.81). In session two the total number of approaches decreased, however, the data followed a similar trend. The dogs approached the baboon faeces (μ = 2.14 ± 2.14) and the dog faeces (μ = 2.14 ± 0.92) the most, followed by the commercial repellent (μ = 1.90 ± 0.38), lion faeces (μ = 1.76 ± 0.57), and tiger faeces (μ = 1.66 ± 0.44).

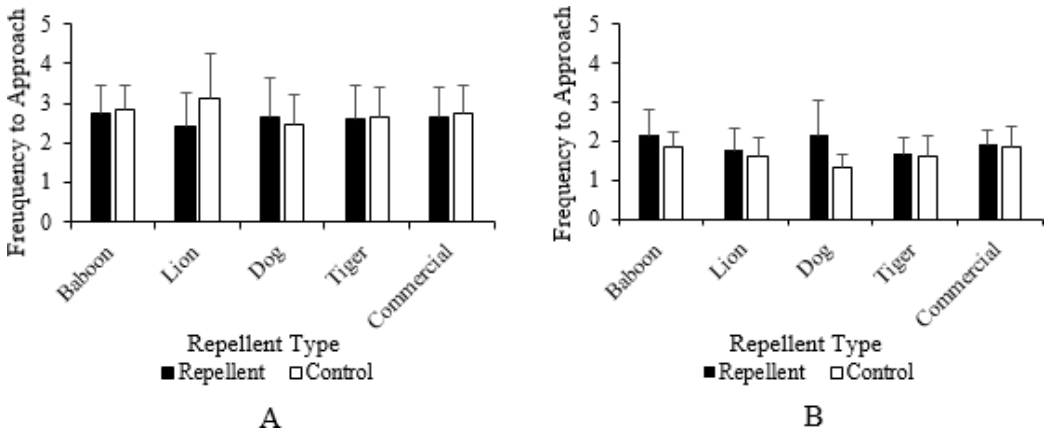

Figure 2.9: The mean number of times that the dogs (N = 21) approached the repellent and control feeding apparatus in session one (A) and session two (B) when presented baboon, lion, dog, and tiger faeces and the commercial repellent. Mean ± 95% confidence intervals.

2.3.2.5 Other behaviours

On average the dogs spent the majority of their time, regardless of session or repellent type, being stationary (Session 1: baboon = 36.28%, lion = 44.79%, dog = 40.39%, tiger = 36.10%, commercial = 44.30%; Session 2: baboon = 49.68%, lion = 55.03%, dog = 49.97%, tiger = 57.34%, commercial = 57.69%) (Refer to Table 2.2 for behaviours classed as being stationary) or walking (Session 1: baboon = 30.22%, lion = 29.66%, dog = 29.26%, tiger = 32.68%, commercial = 32.71%; Session 2: baboon = 21.58%, lion = 22.39%, dog = 21.01%, tiger = 22.08%, commercial = 22.44%). They spent a smaller proportion of their time out of sight of the cameras, performing other behaviours (refer to Table 2.3 for behaviours classed as 'other'), and urinating or defecating (Figure 2.10).

When comparing the dogs' behaviour across sessions, they spent less time being stationary and more time walking in session one than in session two, regardless of the repellent type (Figure 2.10 A & B). The results for the time spent urinating/defecating and performing other behaviours, however, were more variable. The dogs urinated/defecated for a longer duration of time in session one than session two, particularly when presented with dog and tiger faeces (Figure 2.10 C & D). Furthermore, the dogs spent more time performing other behaviours in session two than in session one, when presented with baboon, lion and dogs faeces and the commercial repellent.

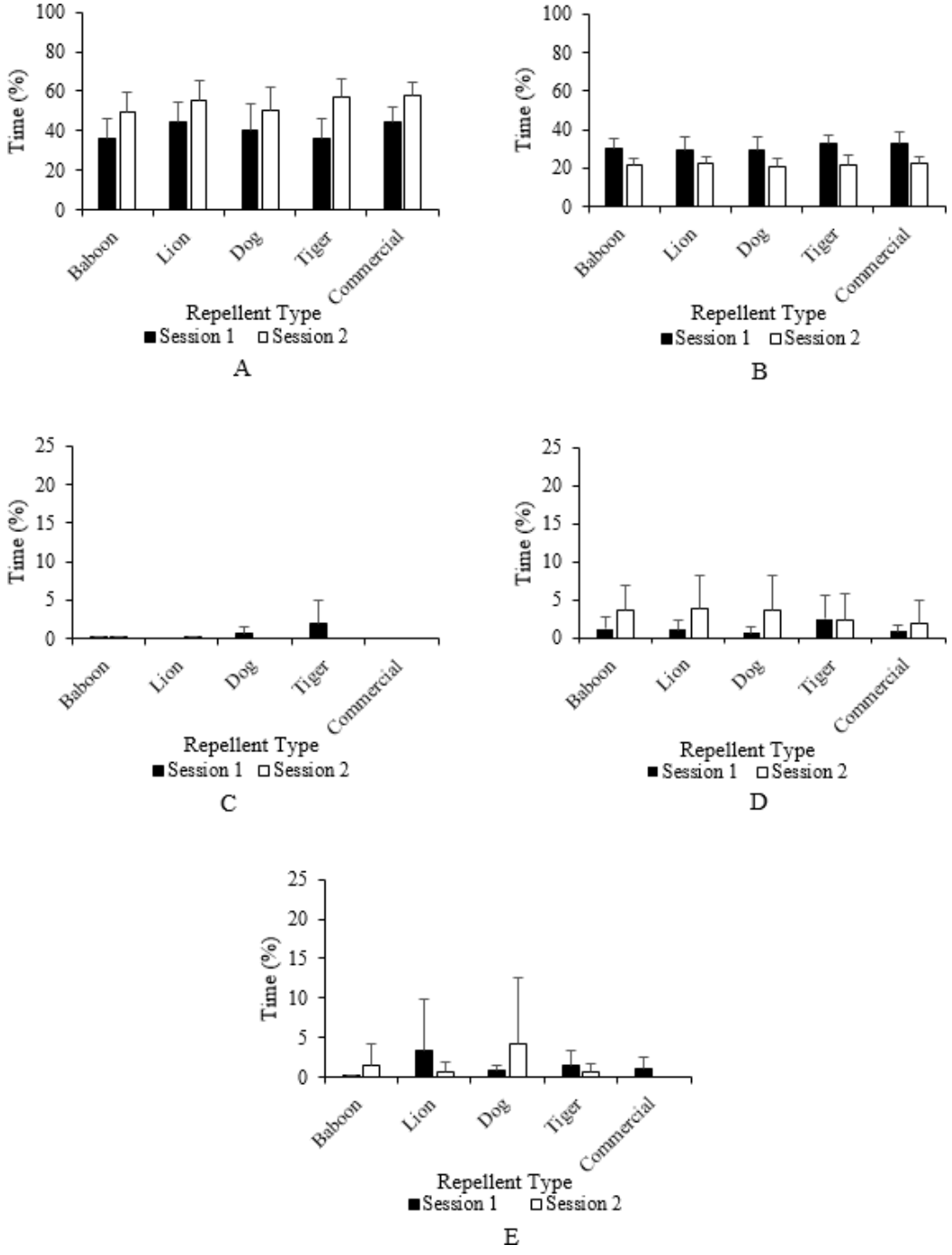

Figure 2.10: The mean percentage of time (%) the dogs (N = 21) spent being stationary (A), walking (B), urinating/defecating (C), performing 'other' behaviours (D), and being out of sight (E), in session one and session two when presented baboon faeces, lion faeces, dog faeces, tiger faeces and the commercial repellent. Error bars are 95% confidence intervals.

2.3.3 Gas Chromatography-Mass Spectrometry

This integration method revealed that there were a different number of peaks observed in each sample. For instance, 17 peaks were identified in the baboon; 14 in the tiger; 12 in the lion; 8 in the dog and only 6 in the commercial (Figure 2.11). However, because this was an initial pilot study and unstandardized methods were used (e.g., varied incubation period and injection technique, no standards run), we can-not determine with 100% certainty what Volatile Organic Compounds (VOCs) were present in the samples, or their relative abundances. The following descriptions are therefore the 'most likely' volatiles present in the faecal samples and the commercial repellent. This was based on comparisons made between the mass spectral patterns of the volatile of interest and the mass spectral pattern of volatiles recorded in the NIST library.

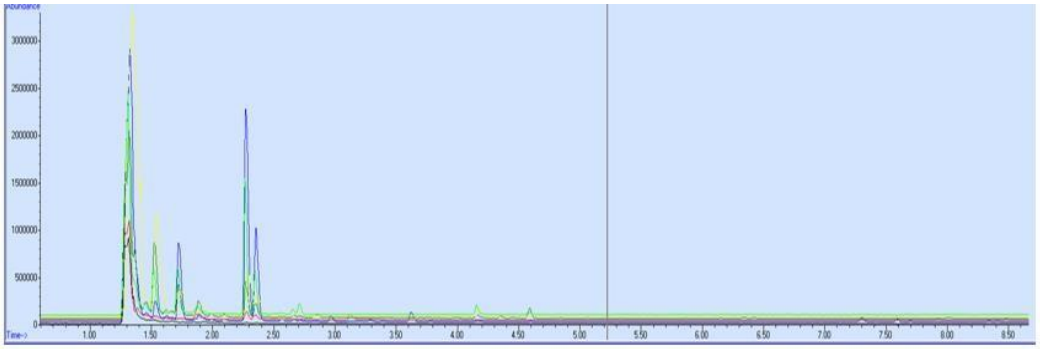

Figure 2.11: An overlay of chromatograms generated from the GCMS analysis of the animal faeces and the commercial repellent. Black line = blank, red line = commercial, dark green line = dog, light green line = tiger, yellow line = lion, blue line = baboon. Y axis = relative abundance (Intervals of 50000); X axis = retention time (minutes).

When evaluating the chromatogram, it was evident that certain samples had peaks at a retention time where others did not (Figure 2.12). For example, the commercial sample had a peak at a retention time of 3.10 minutes; the baboon sample at retention times 2.97, 3.28, 3.60 minutes; and the dog sample at a retention time of 4.35 minutes. Based on similarities in the mass spectral pattern these volatiles were tentatively identified as 1-Pentanol, Butanoic acid, Dimethl disulphide, Toluene, and Propionic acid, respectively. However, to be certain these compounds are the correct assignment we would need to run internal standards.

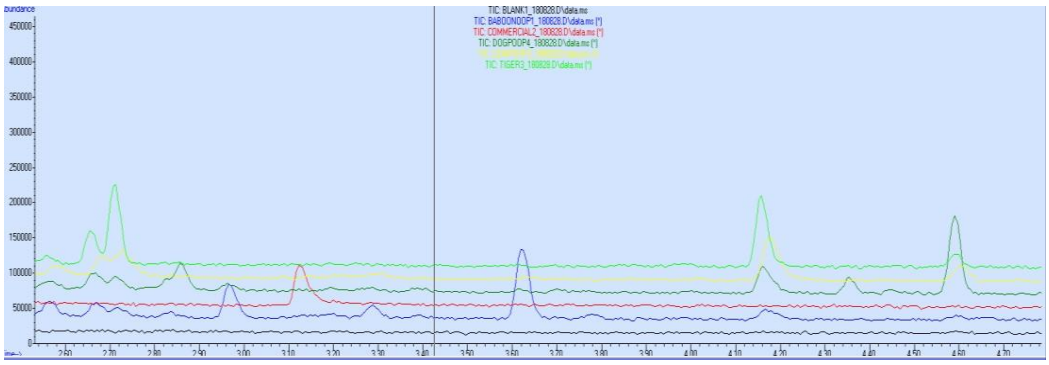

Figure 2.12: An overlay of chromatograms generated from the GCMS analysis of the animal faeces and the commercial repellent between retention times 2.60 to 4.40. Black line = blank, red line = commercial, dark green line = dog, light green line = tiger, yellow line = lion, blue line = baboon. Y-axis = relative abundance (Intervals of 50000); x-axis = retention time (minutes).

2.4 Discussion

The results from this experiment revealed that dog faeces was the most effective olfactory repellent. When this repellent solution was presented to the dogs, the dogs ate significantly less food from the food bowl containing the faeces than the food bowl containing water (the control). A similar result was also demonstrated when the dogs were presented with lion faeces. These findings suggest that both dog faeces and lion faeces have the potential to act as dog repellents, and could be applied to AR baits to prevent accidental dog poisonings. When the dogs were presented with baboon faeces, tiger faeces and the commercial repellent, however, there was no significant difference in the amount of food consumed from the repellent and control food bowls. This suggests that the dogs were not averse, or showed little aversion to, the odours released from these excretory products and the commercial repellent. The fact that the commercial repellent lacked any significant repellent effect in this study raises concerns about its use to deter dogs from consuming ARs. Results from this study suggest that using this commercial repellent alone is unlikely to affect AR ingestion by dogs, making its application to the bait ineffective and redundant in terms of improving dog safety.

The time that the dogs spent eating from the control and repellent food bowls was recorded to determine (1) if the selected repellents affected the dogs' feeding rate, and (2) if the dogs' feeding rate matched the amount of food they consumed. The results demonstrated that the dogs spent significantly more time consuming the food from the repellent food bowl when it contained baboon or tiger faeces,

although the dogs ate similar quantities of food from the repellent and control food bowls. It is possible that the dogs spent more time eating from the repellent food bowl when presented with baboon or tiger faeces as they did not associate any risk with these scents (i.e., the predators themselves).

Interestingly, when the dogs were presented with the commercial repellent in session one, they spent significantly more time feeding from the control food bowl, than the repellent food bowl. However, the amount of food consumed from both food bowls was similar. This result was unexpected and could suggest that the dogs' feeding rate increased significantly when the commercial repellent was present, or that the control was contaminated by another odour. The latter explanation, however, is unlikely as the experimental equipment was washed thoroughly after the completion of each dog's trials. Further, if the control was contaminated then similar behavioural responses would have been observed across all trials.

The time that the dogs spent investigating the repellents was an important measure in this study as it allowed the researcher to determine if the dogs were interested in, or if their exploratory behaviour was affected by, the odours released from the repellents. The dogs spent significantly more time investigating the repellent food bowl in session one when it contained lion, tiger, baboon, and dog faeces. When presented with the commercial repellent, however, no significant difference in the time spent investigating the repellent and control food bowl was found. This suggests that during this first presentation, the dogs attended to the volatiles released from natural products (faeces) more than any volatiles released from commercially synthesised repellent (the commercial repellent). This may be explained by the finding that the commercial repellent had very few peaks when GC-MS was performed; it could be that the volatiles, or lack of volatiles, in this sample were not detected by the dogs, while the abundance of volatiles in the other samples were, and hence the increased time spent investigating the natural repellents.

In session two, the results were somewhat different; the dogs only spent significantly more time investigating the repellent food bowl when it contained dog faeces. Because this response to dog faeces was consistent across both

sessions, it can be hypothesised that the dogs' responses might indicate that they recognise the scents as relating to conspecifics. This kind of response (attending to excretory products of conspecifics) has been documented in other studies. For example, Bekoff (2001) conducted a study on a single male dog named Jethro to determine if the urine from other animals influenced scent marking in dogs. In this study, urine saturated snow was moved from place-to-place over a five-year period and Jethro's responses to the urine were evaluated. The results revealed that Jethro spent more time sniffing and countermarking the urine of other male dogs, or the urine of female conspecifics, than he did his own. Lisberg and Snowdon (2011) also conducted a study on the excretory products of dogs, examining if intact males countermark female urine to guard potential mates, or if dogs countermarked competitively. Their results showed that dogs with a high social status, regardless of the sex, urinated more frequently than dogs with a lower social status. Thus, these studies demonstrate that dogs' excretory products do function as communicatory signals, conveying information on an individual's social status, sex and territory. This may explain the additional time that the dogs in this study spent investigating the repellent bowl containing dog faeces.

To determine if the dogs took longer to approach the repellent feeding apparatus or the control feeding apparatus, their latency to first approach was recorded. It was found that the dogs took similar amounts of time to approach the repellent and control feeding apparatus in all but one instance (session one: baboon vs. control). This suggests that the dogs were not neophobic to the repellents used in this study. A similar conclusion on neophobia and exploration in dogs was drawn by Moretti *et al* (2015). Those authors found that dogs, especially when in groups, were quicker to approach but showed less interest in novel objects, than wolves. The authors suggested that this was a consequence of domestication; while wolves have encountered various degrees of harassment and exploitation from humans during the last centuries, potentially selecting for greater neophobia, dogs have evolved with humans and thus should be inherently less neophobic than wolves.

The frequency of approach and the duration of time spent performing 'other' behaviours were the final measures taken in this study. It was evident from the results that the number of approaches corresponded to the dogs' activity levels. For example, the dogs approached the food bowls more in session one as they

spent less time being stationary and more time walking, than in session two.

2.4.1 Gas Chromatography-Mass Spectrometry

The GC-MS analysis conducted in this study was a starting point for working towards a standardised method that will allow volatiles to be identified in, and comparisons to be made between, lion, tiger, baboon and dog faeces, and the commercial repellent. Whilst this was only a pilot study, the results revealed that there were clear differences in the VOCs found in each repellent type. These differences could account for the varied behavioural responses observed by the dogs, although more investigation is required. Future work should consider (1) using fibres to extract volatiles, (2) using a standardised method, and lastly (3) running internal standards to confirm the identity of the volatiles present in the samples.

2.4.2 Limitations

There were some practical limitations to this experiment. Firstly, the dogs had different feeding histories. One food type was used in this experiment, Royal Canin Mini Exigent, and it is possible that certain dogs did not find this food palatable and therefore may not have eaten or ate significantly less food during the trials. A pilot study using several different brands of dog food to determine what food the dogs find most palatable could be conducted in future studies. This is recommended as it has been shown that dogs' preferences for food can vary depending on their age, sex, and nutritional requirements (Tobie *et al*., 2015). However, two steps were taken to minimise this problem in this experiment: (1) only dogs known to eat kibble/dog biscuits were used in this study (c.f., dogs on raw food diets), and (2) the owners were asked to subject their dog to a two-six hour food deprivation period before the trials began. In most, but not all cases, the owners complied with the latter requirement.

Despite the researcher's best efforts to recruit dogs, the sample size in this experiment was small (N = 21). Tobie *et al* (2015) state that more than 30 individuals should be used in preference testing experiments to get robust statistical results. Although a larger sample size would have been preferred, a power study performed on the pilot study data did reveal that only 16 individuals

were required to detect an average time difference of 5 seconds between being proximate to the repellent side and being proximate to the non-repellent side (Appendix A). This number of individuals was therefore used as a guide to the minimum number of dogs required in this study (refer to Appendix C for details).

The position of the researcher and the transparency of the baby gate in the experimental room (front right-hand corner) may have influenced the dogs' location during the trial. Anecdotal observations of the video footage suggest that the dogs spent most of their time on the right-hand side of the arena either sitting, lying down or standing at the gate. This is likely a result of the dogs associating the baby gate as an exit point. Thus, after they had completed exploring the arena or eating from the food bowl, the dogs positioned themselves there.

Two limitations were identified in the method used for GC-MS. Firstly, the samples were left in the oven for different periods of time before analysis. This could have resulted in some samples (those that remained in the oven for longer) reaching an equilibrium of compounds in the headspace and solid sample, which would result in a higher concentration of volatile compounds being injected into the GC-MS, than others (i.e., those that remained in the oven for a shorter period of time). Secondly, the injection technique was also considered a limitation, as the researcher's inexperience with this technique could have resulted in only partial release of the extracted headspace (i.e., not all 3 mL extracted from the sample were injected into the injection port), or inconsistent injection speed. This may have caused the sample to enter the column in a wide band which can cause peak broadening. This was seen in some chromatograms. To improve the experimental method used for GC-MS for future studies, the incubation period (oven time) should be consistent across all samples, and a consistent injection volume should be used for all samples. In addition, an internal standard should be used to allow standardisation of the results, as this would give us more insight into exactly what VOCs each repellent sample contains.

2.4.3 Conclusions

In summary, this experiment has demonstrated that dog and lion faeces are the most effective biological olfactory repellents, as in the presence of these faecal types the dogs ate significantly less food. This finding suggest that the volatiles derived from these products have the potential to be used to prevent non-target species, such as dogs, from consuming anticoagulant poisons. However, to further test the theory that natural olfactory repellents might have practical value in terms of reducing AR by-kill, it is important to determine if these products deter the poison's target species (i.e., rats).

Chapter 3

Repellent Effects of Predator Odours on Rats

3.1 Introduction

Rodents are found in virtually every terrestrial ecosystem (Tobin & Fall, 2004). Although native rodents can play an important role in ecosystem functioning (Shiels & Witmer, 2017; Tobin & Fall, 2004), introduced rodents have been documented to cause severe ecological and economic impacts (St Clair, 2011; Tobin & Fall, 2004). Anticoagulant rodenticides are commonly employed to control invasive rodent populations (Murphy *et al*., 1998); however, the acute toxicity of these poisons can result in by-kill of non-target species, such as the domestic dog (Berny *et al*., 2010b; Vandenbrouke *et al*., 2010; Albo & Nebbia, 2004).

A potential method to prevent by-kill is through the use of repellents. These repellents, however, must not deter the poisons' target species. Past research has investigated predator odours and their impact on the feeding and exploratory behaviour of rats. For example, Burwash *et al* (1998) demonstrated that Hawaiian roof rats (*Rattus rattus*) reduced their food consumption and activity levels in presence of synthetic predator odours, namely DMDIT, TMT, and MMP. In contrast, Bramley and Waas (2001) found that in the presence of both synthetic and natural predator odours, wild rats (*R. rattus* and *R. exulans*) did not alter their feeding or exploratory behaviours. Evidently these studies demonstrate that rats' responses to certain predator odours can be variable and that there is a need for further research in this field.

The aim of this chapter is to determine if the dog, lion, and tiger faeces, the repellents that were most effective on dogs, deter rats from consuming food. It was hypothesised that rats, as a generalist species, would not inhibit their activity or consume less food in the presence of these faecal types.

3.2 Method

3.2.1 Subjects

Ten female rats (Sprague-Dewley strain) were sourced from Ruakura AgResearch, Hamilton, New Zealand. At the time of testing the rats were 7-weeks of age and had an average weight of 256.8 g ± 0.5 g (Mettler Toledo BD1201). This study had approval from the University of Waikato's Animal Ethics Committee (protocol number: 1030).

3.2.2 Study location, animal husbandry, and equipment

This experiment took place in an animal facility at the University of Waikato, Hamilton, New Zealand. The building had five rooms: a vivarium, two storage rooms, an observational room, and an experimental room. The vivarium was where the rats were housed during non-experimental and experimental rest periods (periods when experiments were running, but the rats were placed in their home cages between trials). This room was temperature controlled (22 ± 2 $^{\circ}$C), with a set reverse 12-h light:12-h dark cycle (lights on at 7 pm, lights off at 7 am).

During the non-experimental periods, the rats were housed in four cages, 39 cm (l) x 25 cm (w) x 16 cm (h), with each cage containing two to three rats (Figure 3.1). The rats had *ad-libitum* access to food (standard laboratory rat chow, brand: Specialty Feeds) and water, and were checked daily to ensure that they were free of illness or injury. The rat cages were cleaned twice a week, or when required. During cleaning, all rats were placed in spare cages, the bedding material (wood shavings) was removed from the soiled cages and the cages were cleaned with hot water and dishwashing detergent. Once clean, the cages were returned to the vivarium and left to dry.

Throughout the experimental rest period the rats were housed in individual wire cages (same dimensions as above). All rats had *ad-libitum* access to water, however, their food intake was restricted (their feeding regime during the experimental period is detailed in section 3.2.5 Experimental procedure, below). To minimise disturbance during the experiment, the rats' cages were only cleaned if required (i.e., soiled bedding), following the same cleaning procedure as

described above. All rat cages (non-experimental and experimental) contained a retreat, wood shavings and a water bottle that was replenished with fresh water each day (Figure 3.1).

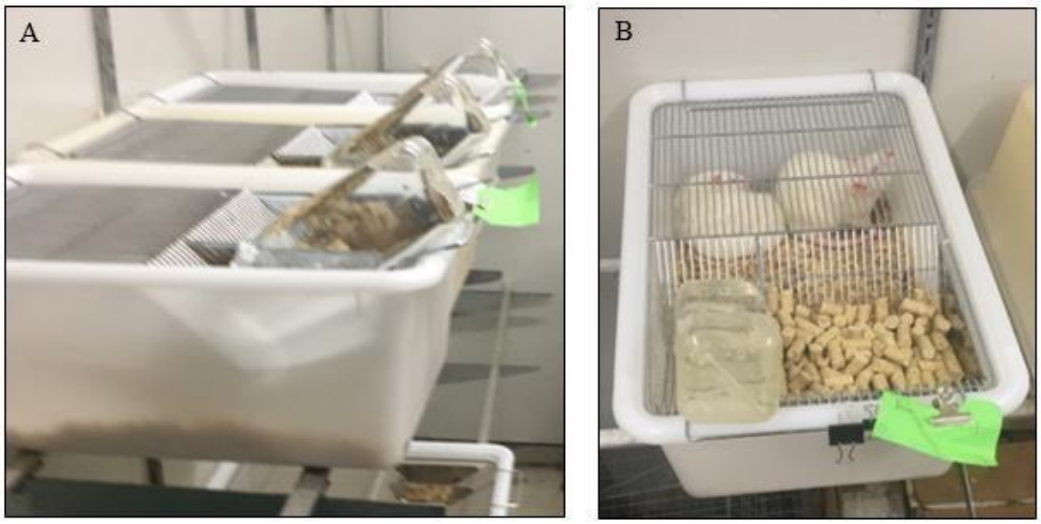

Figure 3.1: Example of how the rats' home cages were laid out in the vivarium (A), and an example of how their home cages were set up (B).

Five polycarbonate plastic containers, 55 cm (w) x 86 cm (l) x 51 cm (h), were placed in the experimental room (4 m x 3.8 m) and used as test arenas for this study (Figure 3.2 C). Each container was lined with brown paper and contained a retreat (a PVC tube with shredded paper), a water bowl and two feeding apparatuses. The feeding apparatuses were 400 mm (l) x 110 mm (w) x 110 mm (h) and were made from clear polycarbonate plastic (Figure 3.2). A plastic partition with 64 holes (8 mm diameter) was used to separate each feeding apparatus into two small chambers: one chamber with a small tray for holding a repellent (odour chamber) and the other with a small tray for holding food (food chamber). The trays used in the odour chamber and the food chamber were allocated to a specific repellent type or arena, respectively. This was performed to minimise the potential of cross contamination of odours. To prevent the rats from escaping during their trials two glass panes were placed over the top of the arenas. A security camera was positioned above each arena (two Techview cameras, and three Xpose cameras) and a recorder (Techview H.264 Channel HD DVR) were used to record the trials.

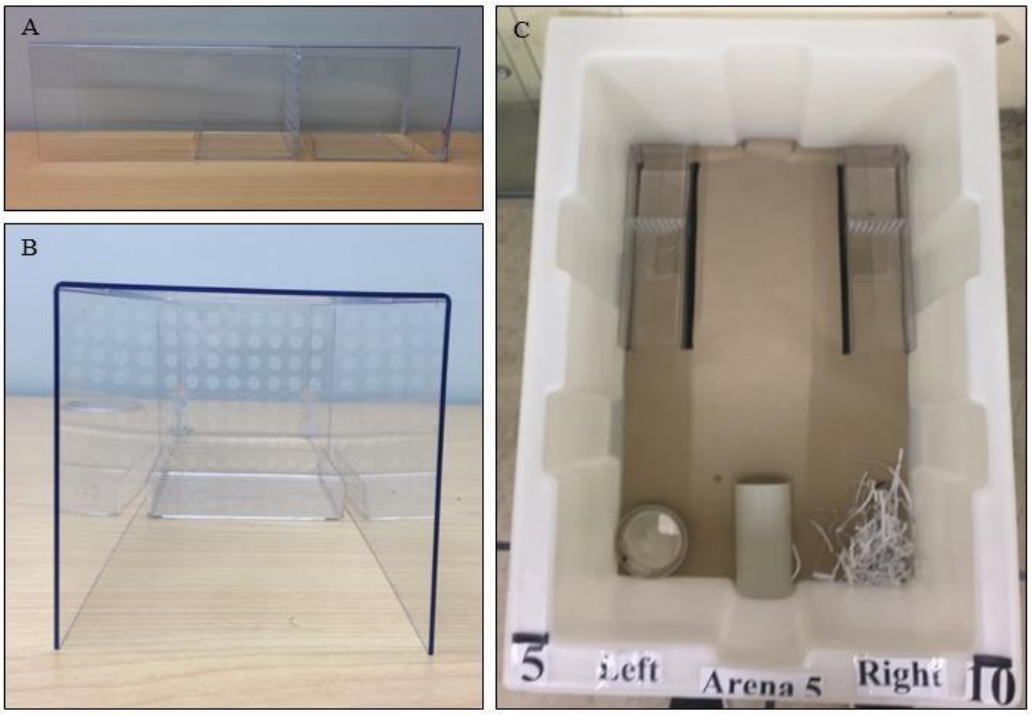

Figure 3.2: Examples of the feeding apparatus (A = side view; B = front view) and arena layout (C). Food was presented in the tray in front of the partition, and the repellent/control solutions were presented in the tray behind the partition.

The test arenas were cleaned daily at the end of each experimental session, once the rats were removed and taken back to their home cages. A full clean (i.e., all equipment was removed and washed using hot water and dishwashing detergent and then dried, and flooring (brown paper) and nesting material were replaced) was performed every second day, after each repellent treatment (see section 3.2.5 Experimental procedure, below). A partial clean (i.e., removal of animal waste products) was performed between days. Gloves were worn during all cleaning procedures, and a dust pan and shovel was used to remove unwanted material from the floor.

3.2.3 Repellent sample preparation

The faecal types selected for inclusion in this experiment were the ones that were most effective at repelling dogs from their food (dog, lion, and tiger faeces) (refer to section 2.3 in Chapter 2). These faecal types were sourced and prepared as described in Chapter 2. However, in this chapter the repellent solutions were kept in a freezer (-18^{o} C) and stored for a maximum period of 16 days.

3.2.4 Food Sample Preparation

A total of 120 food bags were prepared for this experiment. Ten grams of standard laboratory rat chow (same food as the rats received in their home cages) were weighed (Sartorius scales, Model: ISO9001) and placed into a zip-lock bag. The bag was then labelled with the rat, trial and arena number and the weight of each sample (4 d.p.).

3.2.5 Experimental Procedure

Each rat participated in six two-choice preference tests. All preference tests were five hours long (7 am – 12 pm) and took place over six consecutive days (one per day). On days one, three and five the rats were placed into the test arena and presented with two feeding apparatuses containing water (100 ml) which acted as the control treatment. On alternate days (days two, four and six), the rats were put in the arena and presented with a repellent treatment (dog, lion, or tiger faeces). For the repellent trials, one feeding apparatus contained 100 ml of water (control) and the other contained 100 ml of the repellent solution. All solutions (water or repellent) and food (10 g standard laboratory rat chow) were placed in the odour chambers and food chambers, respectively, approximately five minutes before the start of the trials.

The rats were tested in two groups of five. The first group performed all trials to completion before the second group began. The order of repellent presentation was randomised for each group, while the side of presentation (left or right) was pseudo-randomised to prevent all repellent treatments occurring on the same side (left or right). Because all of the test arenas were housed within the one room, all rats were presented the same repellent treatment on the same day to avoid cross-contamination of odours.

For the experiment, each rat was allocated a specific arena in which they performed all trials. All rats were subjected to a 12-h acclimation period in their own arena the night before the first trial (7 pm - 7 am), to allow the rats to habituate to the test arena. The rats were placed in the test arena at 7 am each morning and remained there until the trial was complete at 12 pm on the same day. Once complete, the rats were placed in their home cages, transported back to the

vivarium and given *ad-libitum* access to food and water. The food remaining in each rat's experimental test arena (cached or food in the repellent and control feeding apparatus) was collected and taken to the laboratory and weighed. At 7 pm each night (12-h before each trial) the rats' food was removed from their home cages. This food deprivation period was performed to ensure that the rats were not satiated at the time of testing on the following days.

The methodology used in this experiment was developed from a pilot study performed with the same animals. This pilot study took place 12 days before the formal experiment began (refer to Appendix E for more details on the pilot study).

3.2.6 Video Analysis

All videos were converted from .264 files to AVI files using AVI Generator. For formal video analysis the researcher watched the first hour of each session using Solomon Coder (version: beta 17.03.22); a free behavioural coding program (total of 60 h of video footage). A configuration sheet was created on Solomon Coder to display all operational definitions as 'buttons'. These buttons were allocated to one of three coding variables: frequency (the number of times the rats made a full entry or a partial entry into the repellent and control feeding apparatus), duration (the amount of time each rat spent in the left or right feeding apparatus), and total time (the total amount of time each video was analysed for). When a rat entered the feeding apparatuses the button corresponding to the rat's behaviour was pushed. To record all instances of the behaviour behavioural sampling with continuous recording was used.

3.2.7 Statistical analysis

The amount of food taken from the feeding apparatuses was converted into percentage form to account for small deviations in the amount of food given to each rat during each trial (10 g ± 0.8 g). Because the data were measured on an interval scale and thus did not meet the assumptions of normality (Shapiro-Wilk p-value <0.05 for all repellent-control combinations), a Wilcoxon matched-pairs test, a non-parametric alternative to the t-test for dependent samples, was performed (it is acknowledged that the test may be limited due to the small sample size).

The amount of food consumed was calculated by deducting the amount of food cached by the rats from the total amount of food presented to the rats (e.g., 20 ± 0.16 g). A Kruskal-Wallis ANOVA was used to compare the total amounts of food consumed by the rats when they were presented with the three different repellent types (dog, lion, tiger faeces). This test was selected for use (c.f. a one-way ANOVA) as the data set did not meet the assumptions of normality or homogeneity of variances (Shapiro-Wilk p-value < 0.05; Brown-Forsythe p-value > 0.05) and had one independent (amount consumed) and three grouping variables (lion, tiger, dog). The amount of food taken and the amount of food consumed were classed as two different variables, as from video footage alone the researcher could not determine when the rat was eating.

A Wilcoxon matched pairs test was performed on the rats' behaviour data: both the frequency of full and partial entries into the feeding apparatus, and the duration spent in the feeding apparatuses for each repellent-control combination. This test was performed as the frequency and the duration data for each repellent-control combination (even when log transformed by the logarithm of base 10) did not meet the assumption of normality (Shapiro-Wilk p-value <0.05).

To determine if the researcher's video analysis was reliable, six hours of video footage (10% of 60 hours) was randomly selected from the entire sample population for re-analysis (intra-observer reliability). Pearson's correlation co-efficient revealed that there was a strong positive correlation for all operational behaviours (duration: $p = 0.999$, partial entries: $p = 1$, full entries: $p = 1$).

All statistical analyses were performed using Statistica (Version 13), and all figures were made with Graphpad or Microsoft Excel. The specified level of alpha for all statistical tests was 0.05.

3.3 Results

3.3.1 Amount of food taken

Figure 3.3 demonstrates the amount of food taken by the rats. On average the rats took more food from the repellent feeding apparatus when it contained lion faeces, than the control feeding apparatus (lion = 75.86 ± 26.79%, control = 68.99 ± 19.53%) (Mean ± 95% confidence intervals) (Figure 3.3 C). They took similar quantities of food from the repellent and control feeding apparatus, when the repellent feeding apparatus contained dog faeces (dog = 72.88 ± 27.85%, control = 72.42 ± 23.20%) (Figure 3.3 B), and they took less food from the repellent feeding apparatus when it contained tiger faeces (tiger = 73.54 ± 22.59%, control = 87.74 ± 18.00%) (Figure 3.3 A).

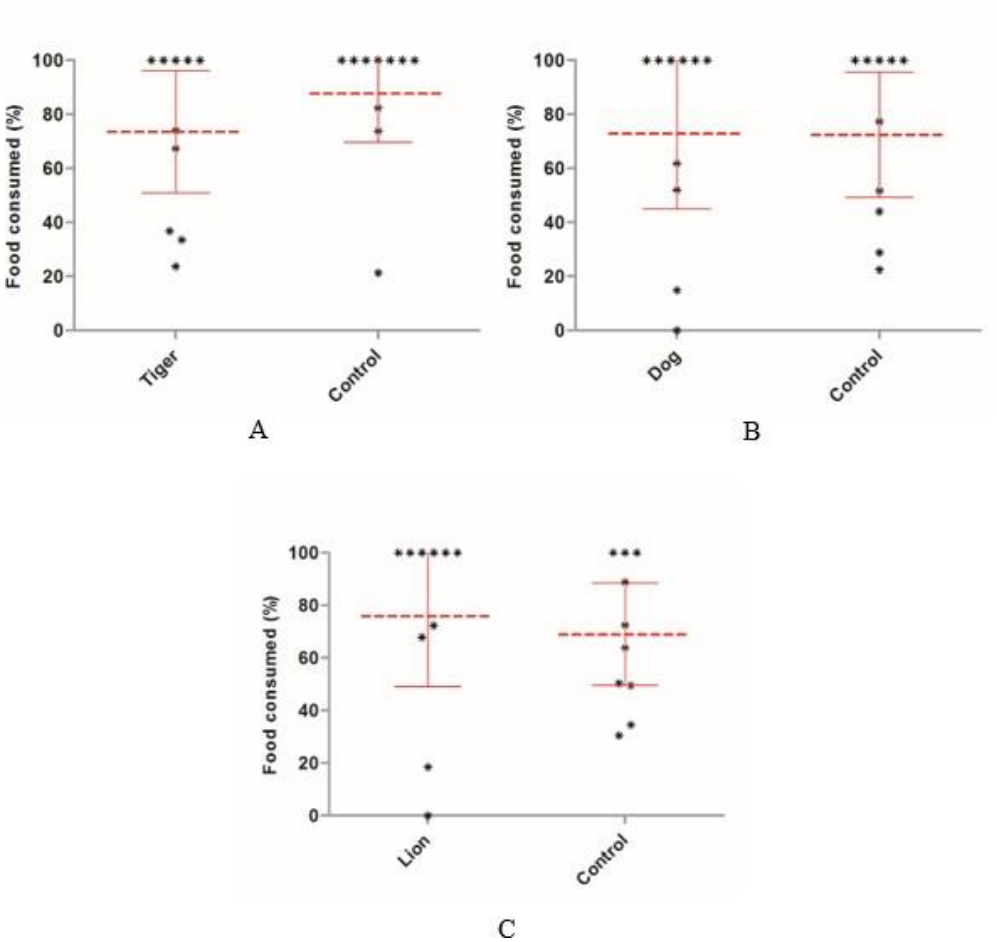

Figure 3.3: Average percentage of food taken from the repellent and control feeding apparatuses when the rats (N = 10) were presented with tiger (A), dog (B), or lion (C) faeces. Error bars are 95% confidence intervals.

The results from a Wilcoxon matched pair test, however, revealed that there was no significant difference amount of food taken from the repellent and

control feeding apparatus for each repellent-control combination (dog: N = 7, *p* = 0.866; lion: N = 7, *p* = 0.40; tiger: N = 5, *p* = 0.138) (Figure 3.4).

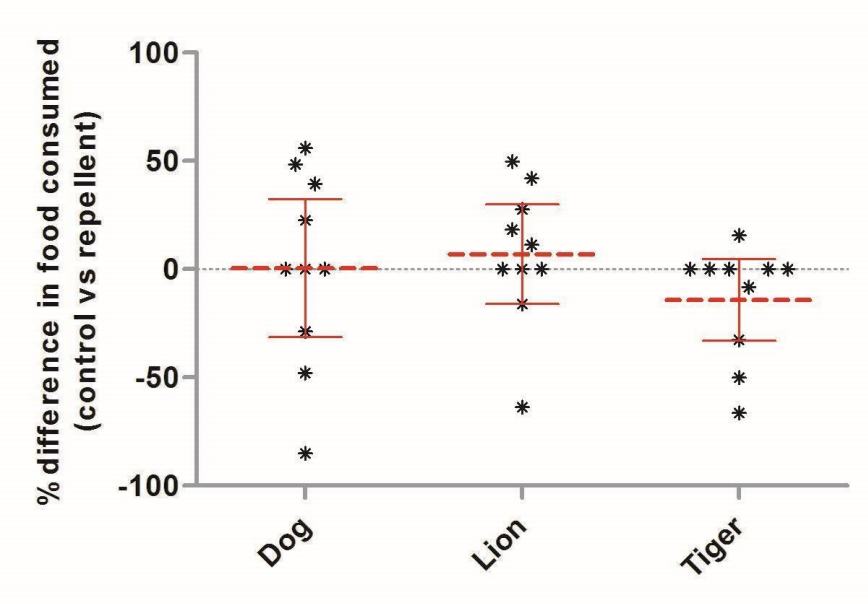

Figure 3.4: Median difference (± interquartile range) in amount of food consumed from the repellent food bowl and the control food bowl when presented tiger, lion and faeces.

3.3.2 Amount of food consumed

On average the rats consumed more food in the presence of lion faeces (63.32 ± 15.68%), than in the presence of tiger faeces (57.16 ± 17.19%), and dog faeces (54.85 ± 18.08%) (Mean ± 95% confidence intervals) (Figure 3.5). However, a Kruskal-Wallis ANOVA revealed that there was no significant difference in the total amount of food consumed by the rats when the three different repellent types (dog, lion, and tiger faeces) were present in the arena (Kruskal-Wallis: N = 30, *p* = 0.436).

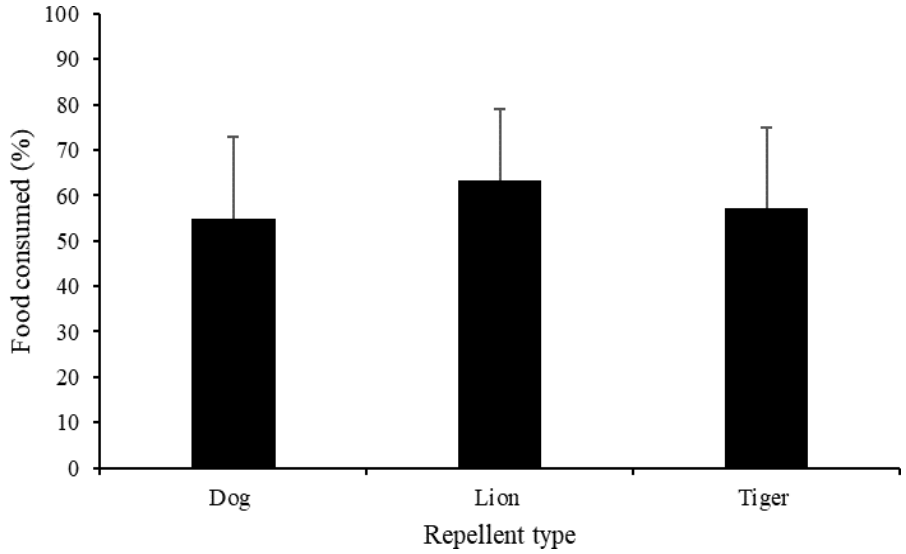

Figure 3.5: Average percentage of food consumed (total amount of food presented to the rats minus cached food) when dog faeces, lion faeces, and tiger faeces was present in the test arena. Error bars are 95% confidence intervals.

3.3.3 Frequency of Approach

The average number of full and partial entries into the feeding apparatuses for each repellent-control combination varied. When presented with tiger and dog faeces, the rats made fewer full entries into the repellent feeding apparatus than the control feeding apparatus (dog = 2.8 ± 1.38, control = 4 ± 1.31; tiger = 2.4 ± 1.13, control = 3.3 ± 1.31) (Mean ± 95% confidence interval) (Figure 3.6). However, when the rats were presented with lion faeces, a similar number of full entries were made into the repellent and the control feeding apparatuses (lion = 3.8 ± 1.61, control = 3.7 ± 1.39) (Figure 3.6 A).

The average number of partial entries was much lower than the average number of full entries for each repellent-control combination. In general, the rats performed more partial entries into the repellent feeding apparatus, than the control feeding apparatus, when presented tiger (tiger = 0.4 ± 0.50, control = 0.1 ± 0.23) and lion faeces (lion = 0.4 ± 0.88, control = 0.2 ± 0.37) (Figure 3.6). When presented with dog faeces, however, the rats made fewer partial entries into the feeding apparatus containing the repellent (dog = 0.2 ± 0.30, control = 0.5 ± 0.70) (Figure 3.6 B).

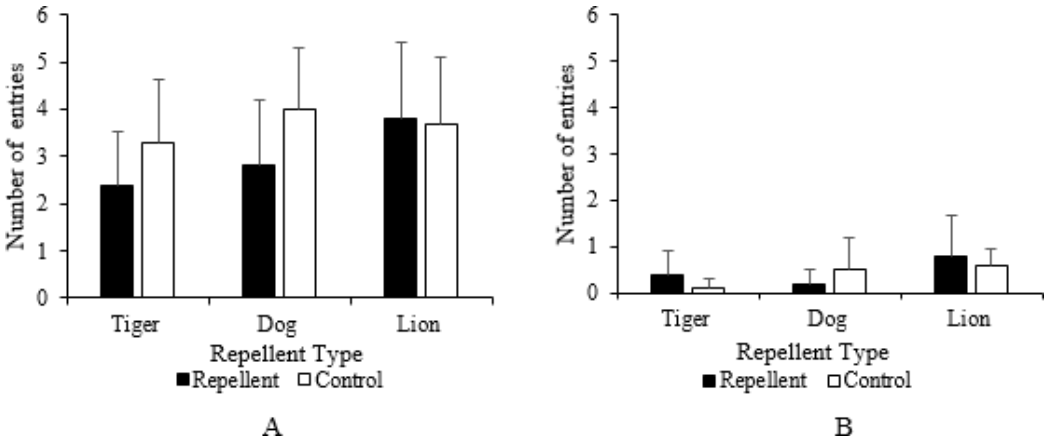

Figure 3.6: Mean frequency of the rats' full entries (A) and partial entries (B) into the repellent and control feeding apparatuses when presented with tiger, dog and lion faeces. Error bars are 95% confidence intervals.

3.3.4 Time Spent in the Feeding Apparatuses

The rats spent a small proportion of the total session time in the feeding apparatuses. When comparing the amount of time spent in the repellent and control feeding apparatus for each repellent–control combination it was evident that when presented dog faeces and lion faeces the rats spent a marginally more time in the repellent feeding apparatus, than the control feeding apparatus (dog = 88.84s, control = 76.90s; lion = 55.01s, control = 44.69s) (geometric mean) (Figure 3.7). However, when presented tiger faeces the opposite was observed; the rats spent a larger proportion of their time in the control feeding apparatus than the repellent feeding apparatus (tiger = 40.42 s, control = 158.90 s) (geometric mean).

On certain occasions the rats were observed to construct nests in the repellent and control feeding apparatuses. Thus, the outliers shown on Figure 3.7 are most likely due to these nesting occurrences.

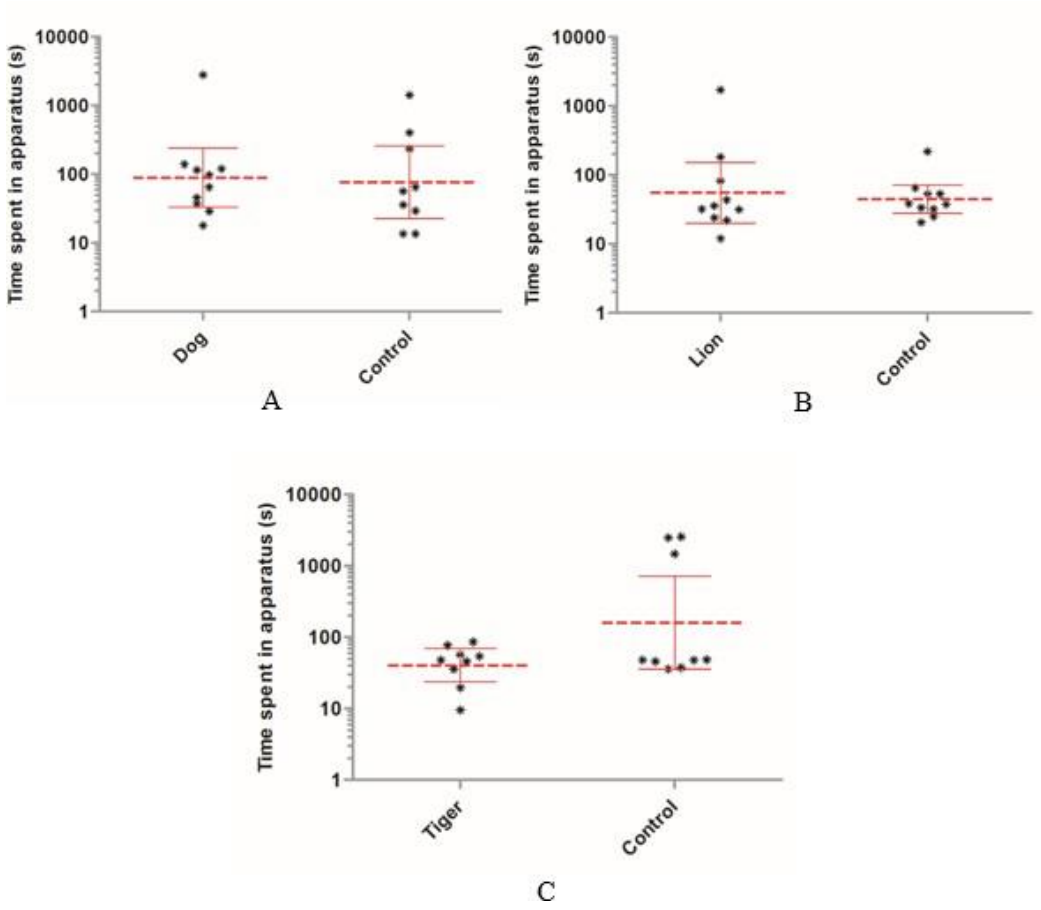

Figure 3.7: Average amount of time the rats (N =10) spent in the repellent and the control feeding apparatus when the repellent food bowl contained dog faeces (A), lion faeces (B), and tiger faeces (C). Black dots represent individual data points. Y-axis = log scale of time spent in apparatus. Geometric mean ± 95% confidence intervals.

A Wilcoxon matched pairs test, however, revealed that there was no significant difference in the amount of time spent in the control and repellent feeding apparatuses for all repellent control combinations (dog: N = 10, p = 0.33; tiger: N = 9, p = 0.26; lion: N = 10, p = 0.39).

3.4 Discussion

The current study revealed that the rats did not alter their feeding or exploratory behaviours in presence of three predator odours; lion, tiger, and dog faeces. Regardless of the repellent type, the rats spent a similar amount of time in and retrieved a similar quantity of food from, the repellent and control feeding apparatuses. These results are consistent with those found by Bramley and Waas (2001), however, in that study wild rat strains were used (*Rattus rattus* & *Rattus exulans*) and two experiments were conducted. In the first experiment, the rats were exposed to nine odours (three synthesized predator smells, three natural

herbivore smells: guinea pig faeces, rabbit urine, red deer urine, and three natural predator smells: cat urine and faeces, mongoose faeces) using a Y-maze, and their behavioural responses to those odours were evaluated. In the second experiment, two synthesised predator odours (containing the volatile ingredients of urine and faeces) were applied to purpose-built feeders in the field. The results from the Y-maze experiment revealed that in the presence of the natural odours no avoidance behaviour was demonstrated, in fact the rats spent more time in the arms containing the natural odours (herbivorous and carnivorous), than the arms containing the synthesised semiochemicals. The results from the field study were similar, no avoidance behaviour was demonstrated in the rats and further their feeding behaviour were unaffected by the presence of two synthetic predator odours.

In addition to the time spent in, and the amount of food taken from, the repellent and control feeding apparatus, the number of partial and full entries were also recorded. The results revealed that the rats made a similar number of entries (partial and full) into the repellent and control feeding apparatus, regardless of the repellent type. These findings are similar to those made by Banks (1998), who investigated the response of a native Australian bush rat (*Rattus fuscipes*) to the odour of an introduced predator, the red fox (*Vulpes vulpes*). In this study, a first experiment measured the trapping success using both clean and scented traps (fox faeces tainted with urine); and a second experiment offered a choice of both clean and scented traps at bait stations. The results revealed that the rats displayed no avoidance of the predator odour, making a similar number of entries into both clean and scented traps. Although different methodologies were used across this current study, and the studies conducted by Bramley and Waas (2001) and Banks (1998) similar conclusion were drawn; predator odours had no repellent effects on rats.

3.4.1 Limitations and Future Recommendations

Before the full study began, the rats participated in a pilot study to finalise the design and methodology of the experiment (see Appendix E for details). The same equipment was used in both the pilot study and the full experiment (excluding the glass panes used as a lid in the full experiment), and the rats were presented with the same predator odours. This prior exposure to the odours, however, may have influenced the results in this full study, as (1) the rats could have habituated to the odours, and (2) they could have learnt that the repellents were unaccompanied by a real threat (e.g., the predators themselves). If this experiment were to be repeated it is recommended that two different groups of rats are used, one for developing methods in the pilot study, and the other for use in the full study. Practical and ethical limitations precluded doing this, in the current study.

Specific strains of rats may respond differently to the same predator odour. Day *et al* (2004) discovered that Sprague-Dawley strains are relatively insensitive to cat odours, while Dielenberg and McGregor (2001) found that cat odours elicit strong defensive behaviours in Wistar, Lewis and Hooded rats. Thus, it cannot be assumed (without further testing) that all rat strains behave in the same way when presented a predator odour. Therefore, the results from this study should be interpreted with that understanding. Future research should explore the repellent effects of tiger, lion and dog faeces on wild rats (*R. rattus* and *R. nrovegicus*), the poison's target species. This would determine if the volatiles present in these faeces have the potential to deter dogs but not wild rats from consuming rat bait.

Regular handling and transportation of the rats could have increased their stress levels and further affected their behaviour. Barret and Stockham (1963) and Gärtner *et al*. (1980) demonstrated that when male rats were handled or moved, respectively, their corticosterone levels (a stress hormone released from the adrenal cortex) increased. To reduce stress, the rats should have been subjected to a minimum of two weeks of pre-test handling (following standard procedures), to densensitise them to these procedures.

Two experimental design limitations were identified. Firstly, rats may display a side bias (consistently moving to the left or right initially or more frequently)

rather than an aversion to the odours. Given that the rats were only presented each repellent once, any side bias could have impacted the results. Logistical constraints, however, prevented the experiment from being repeated; and the long period of exposure (five hours) that the rats experienced when presented with each repellent, hopefully reduced any such impact. Secondly, there was a chance of an order effect (the potential for the rats to respond differently based on the order of odour presentation), but pseudo-randomisation was used to reduce this effect, although complete randomisation would be best to use in future projects.

3.4.2 Summary

In summary of these findings it can be concluded that the odours that were most effective at repelling dogs from their food, had no repellent effect on rats. This means that there could be future implications for using these faecal products as a repellent to deter dogs, but not the poisons target species (rats) from consuming anticoagulant rodenticides.

Chapter 4

General Discussion

4.1 Key Findings

The overall aim of this thesis was to determine if biologically derived olfactory repellents have the potential to prevent dogs from consuming anticoagulant rodenticides, in particular rat poison. The results from the experiments conducted in this study revealed that dog and lion faeces were the most effective dog repellents. In the presence of these faecal types the dogs ate significantly less food, indicating they did have a repellent effect. In contrast, dog, lion or tiger faeces did not affect the feeding or exploratory behaviours of rats. The rats took a similar quantity of food and spent a similar amount of time in the repellent and control feeding apparatuses, showing that these excretory products did not have a repellent effect on this species. Together, these results provide compelling evidence that the volatiles in lion and dog faeces have the potential to act as a dog repellent whilst not repelling the poison's target species, and with further research these repellents could potentially be applied to rat bait to reduce the number of accidental dog poisonings.

Although dog faeces was considered the most effective repellent in the dog experiment (Chapter 2), it did not deter all dogs from consuming their food. A study conducted by Hart et al. (2018) revealed that a small proportion (16%) of dogs consume their own faeces or the faeces of conspecifics. This coprophagic behaviour may therefore explain why dog faeces did not suppress the feeding of certain dogs.

While the GC-MS work in this study was preliminary, the results were informative in terms of revealing that there were different VOCs present in the different repellents. However, it would be worthwhile repeating the GC-MS analysis in a full investigative study using standardised methods. This would allow us to identify what compounds in both the dog and lion faeces might be responsible for the observed repellent effect in dogs (but not rats).

4.2 Conspecific and hetero-specific communication

4.2.1 Behavioural interactions

An animal's response to odour stimuli is dependent on the strength and familiarity of those stimuli. In experiment one of this study, the dogs were presented with four unfamiliar odours (lion, tiger, and baboon faeces and the commercial repellent) and one familiar odour (dog faeces) over two sessions. In session one, the dogs spent significantly more time investigating the repellent food bowl than the control when it contained baboon, lion, tiger, and dog faeces; while in session two, they only spent significantly more time investigating the repellent food bowl when it contained dog faeces. Harris and Knowlton (2001) suggested that weak or unfamiliar stimuli may result in the animal either ignoring or approaching the stimuli to gather more information. Perhaps in session one the dogs investigated all faecal types to the same extent because they were novel and unfamiliar, but they were also natural products, which may explain why the synthetic commercial repellent did not elicit the same investigative response (The GS-MS data also indicated that there were fewer VOCs present in the commercial repellent). In session two, conspecific communication may explain why the dogs spent longer investigating dog faeces; suggesting that they were able to interpret the volatiles being released, as discussed in Chapter 2 (Lisberg & Snowdon, 2011; Bekoff, 2001). The reduction in investigative behaviours towards other faecal types over the two sessions may be because dogs did not evolve in the presence lions, tigers, or baboons so they were unable understand signals from these hetero-specific species.

4.2.2 Food consumption

The 'common constituents' hypothesis proposes that faecal material from predators contain sulphurous compounds from the digestion of meat which can be recognised by prey even if the predator is unfamiliar (Banks *et al*., 2014). Assuming that both dogs and lions were fed meat (Appendix F), this hypothesis may explain why the dogs consumed significantly less food in the presence of these faecal types. However, it does not explain why tiger faeces, which also contained digested meat products, did not elicit the same level of feeding suppression in the dogs. This hypothesis also does not account for the lack of

behaviour change observed in the rats in response to predator odours.

Variances in the animals' (from whom the faeces were collected) diets may also provide a simple explanation for why the dogs reacted differently to different repellent types (Apfelbach *et al*., 2005; Apfelbach *et al*., 2015). A study investigating prey (goats, *Capra hicus* and eastern grey kangaroos, *Macropus giganteus*) responses to predator odours (tigers and Tasmanian devils, *Sacrophilus harrisii*) revealed that when prey species were presented with predatory faecal matter containing conspecifics, they decreased their feeding events. Thus, any variation in diets of the species used in this study may explain why the dogs consumed more or less in the presence of different biological samples (refer to Appendix F for more information on the animals' diet). Comparatively, rats have a more generalist diet, which may account for why the rats did not alter their feeding behaviour in the presence of lion, tiger, and dog faeces.

4.3 Persistence and decay of odours

Odours decay over time, the rate at which they decay, however, is reliant on the structure of the chemical cue and its resistance to different environmental/abiotic parameters (i.e., rain, UV radiation, bacterial decomposition, humidity) (Parsons *et al*., 2018). In general, when an odour is deposited or released into the environment the volatile compounds evaporate first. When these compounds evaporate, however, it can weaken the odour and potentially modify its meaning. For example, an aged signal may become an attractant, as demonstrated by Parsons *et al* (2012), or it may indicate to prey that a predator is no longer present (Parsons et al., 2018). In this study the dogs tended to consume more food in the second session when presented baboon, lion, tiger faeces and the commercial repellent, than in the first session. Although this result may suggest that the dogs were habituating to the stimuli (i.e., decreased their response overtime, as they knew there was no accompanied threat), it could also suggest that chemical decomposition occurred. Thus, the odours were less repugnant, or their meanings were modified, decreasing their repellent effect and resulting in the dogs consuming more food.

4.4 Limitations

The biological samples in this study were autoclaved at 80ºC and then frozen. The former was performed to comply with New Zealand's Biosecurity Act (1993), and to kill any pathogens or bacteria in the samples that may have posed health risks to the participating animals; while the latter was performed to retard degradation of the faeces between successive trials. Although no other methods could have been used in this study, it is worth noting that heating and freezing biological samples can enhance chemical breakdown (Parsons *et al*., 2018). Thus, it is possible that the odours may have been modified or their strength weakened because of these prevention (heating) and preservation (freezing) methods.

Cross contamination of odours may have occurred in this experiment, as the odours released from the excretory products were not confined. Thus, the odours could have dissipated throughout the test arena or arenas used in the dog and rat experiments, respectively, and this could have influenced the dogs' or rats' feeding or exploratory behaviours. However, steps were taken to minimise the risk of cross contamination (e.g., washing the experimental equipment thoroughly after each dog (dog experiment) or each trial (rat experiment)) (refer to the methods sections of Chapter 2 and Chapter 3 for more information). Additionally, it is thought that the concentration of the solutions when in close proximity to the food, would have negated any minor cross contamination.

The time of day at which experiments were run may also have been a limitation of this study. Although minor, this factor could have influenced how much food the animals consumed, particularly the dogs. Because most dog owners feed their dogs at certain times of day, certain dogs may not have been hungry when the trials took place. To minimise this potential limitation, the owners were asked to with-hold food from their dogs 2-6 h before the trials began; however, it is known that in some instances the owners did not comply with this requirement. Because rats are nocturnal feeders (Tobin & Fall, 2004), they were held on a reverse day/night cycle, thus the experiment was performed at a biologically appropriate time for them.

The cost of manufacturing non-toxic bait prohibited its use in this research project.

Consequently, commercial dog food and rat chow were used as the experimental foods. It is possible, however, that these products are more palatable than rat bait (at least for the dogs), and thus testing these repellents on such food types may actually be a stronger test of the repellents' efficacy.

4.5 Future recommendations

To improve this research and to further explore the efficacy of predator/ conspecific odours as a rat bait repellent for dogs, several recommendations can be made. The first recommendation would be to repeat the experiments in this study, albeit with increased samples sizes. Although statistically significant results were gained in this study, increased sample sizes would increase statistical validity and possibly give more accurate results.

The second recommendation would be to test the selected repellents on wild rats. This is an important recommendation as it has been demonstrated that laboratory strains can respond differently to the same odour (Day *et al*., 2004; Dielenberg and McGregor, 2001). Therefore, the results produced in this study (that the rats' feeding and exploratory behaviours were not affected by lion, dog or tiger faeces) may not be replicated when wild rats are used.

The third recommendation would be to try to identify the volatile compounds responsible for the avoidance behaviour in dogs, using a standardised GC-MS method. Identifying these compounds could allow for the formation of synthetic 'super scents', which are more resistant to environmental parameters (e.g., wind, rain, UV, etc.), and are more readily available than faeces.

The final recommendation would be to test the efficacy of these repellents or synthetically derived counterparts in the field. This is integral to the development of an effective repellent.

4.6 Conclusion

Developing a repellent that can prevent dogs, but not rats, from eating rat poison will be of national and international significance, as it could potentially reduce the number of accidental dog poisonings that result from the application of

anticoagulant rodenticides. The results from this study provide evidence that the volatiles contained in dog and lion faeces have the potential to deter dogs from consuming rat poison. In the presence of these faecal types the dogs consumed significantly less food, while the rats' feeding and exploratory behaviours were unaffected. Thus, the findings from this study provide the initial framework for investigating natural olfactory repellents for dogs, and their potential application to anticoagulant rodenticides (rat bait).

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Appendices

Appendix A: Dog Pilot Study

Introduction

The purpose of this pilot study was to determine the methods, equipment and number of subjects required for the main experiment in this thesis, Chapter 2: Natural Olfactory Repellents for Dogs. The aim was to find an effective olfactory repellent that would deter dogs from consuming food. The advantages and disadvantages of each method were assessed, and the most effective method was used in the main experiment (Chapter 2).

General Information

Subjects

Dogs were recruited for this pilot study via word of mouth, posters, and social media posts. A total of 15 dogs were trialed; six females and nine males, ranging from one to 13 years of age (Table 1). Exclusion criteria (see below) were used to withdraw dogs that did not perform or were not suited to the experimental procedures, and as a result only eight dogs participated in more than two sessions in this pilot study.

Dog were withdrawn from the study when they: (1) appeared uncomfortable or stressed (e.g., displayed heavy and rapid breathing, barked repeatedly or paced backwards and forward), (2) whined or repeatedly jumped on the gate, (3) did not consume or display an interest in the food for more than six consecutive trials, and lastly (5) if the owner terminated the trial.

Table 1: Details of dogs who participated in the Pilot Study

Subject	Age (years)	Sex	Breed	Method
Flint*	8	Male	Heading dog	3
Mica	7	Female	Australian cattle dog x heading dog	1,2,3
Kimchi*	12	Female[s]	Papillion x	2
Josh	11	Male[n]	Dalmatian	3
Toshka*	13	Female[s]	Siberian husky	3
Cocoa	11	Female[s]	Labrador retriever	3
Laddie	11	Male[n]	Standard collie	3
Tui	2	Male[n]	Huntaway x kelpie x border collie	3
Merlin*	10	Male[n]	Standard collie x Border collie	3
Ranbo*	6	Male[n]	Chihuahua	3
Hunny	10	Female[s]	Bulldog	3
Pringle*	1	Female[s]	Corgi	3
Bovril	10	Male[n]	Labrador retriever x spaniel	3
Dillion*	5	Male[n]	Fox terrier	3
Stu*	8	Male[n]	Miniature pinscher	3

[S]Spayed, [n] Neutered, *Trial terminated

Study location

This pilot study took place in an animal facility located at the University of Waikato. The building consisted of five rooms; a vivarium (rodent room), two storage rooms, an observational room and an experimental room. The experimental room was 4 x 3.8 m. Two one metre squares were positioned in the centre of the experimental room using tape. These squares were used as a measure of proximity to the repellent and the control (Figure 1).

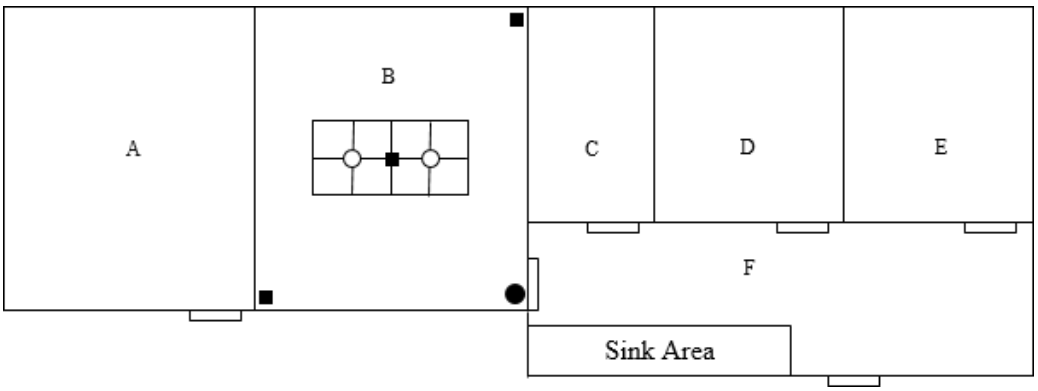

Figure 1: Layout of the animal facility located and the University of Waikato. Storage rooms (A & D); experimental room (B); observation room (C); vivarium (E); cleaning area (F). Black squares = cameras, black circle = researcher's position.

Experiment One

Aim

The aim of this method was to develop a practical method of applying the natural repellents to commercially available dog biscuits. Because the exotic animal faeces (lion, tiger, baboon) were not collected at this stage, this method was only tested using dog faeces.

Method

Subjects

A single dog, Mica, was used in this experiment (Table 1). Animal ethics approval was given for this research and applies to all studies in this thesis, including the appendices (further details in Chapter 2, protocol number 1030).

Equipment

Two dog bowls were used to hold the experimental and control food. Two cameras were used to film the trials. Denver Instrument Scales were used to weigh the faeces and blender was used to make the faeces solution. A tray was used to dry the treated food.

Sample preparation

Denver Instrument Scales (SI-234) were used to weigh 20 g of dog faeces. The faeces were placed into a blender (Home and Co mini blender) with 40 ml of water and blended for approximately two minutes. Fifteen grams of dog kibble (Brand: Black Hawk) was placed into the solution for one minute and then removed and left on a paper towel to dry overnight (approximately 12 hours). Once dry, the kibble was placed into a sealed container and refrigerated until it was required for use.

Feeding trial

Mica participated in one session which comprised of two trials. The first trial was a habituation period, which was performed to allow Mica to get use to the test arena. In this trial, two empty stainless-steel bowls were placed in the centre of the test arena 0.75 m apart. Mica was led into the arena by the researcher and released. After five minutes Mica was removed from the test arena and remained outside the arena on a leash until she was required for her next trial. In the second trial, Mica was presented with two bowls as in the first session, however in this trial, one bowl contained repellent-treated dog food and the other bowl contained untreated dog food.

Though this method of using the repellent was effective at deterring Mica from the food (i.e., no food was consumed), several limitations with this method were identified. These limitations were: (1) drying the repellent-treated kibble overnight could have resulted in the volatiles or odours dissipating, (2) if consumed by the test subject, any observed repellent effect may have been a result of taste aversion not odour aversion, and lastly (3) application of the repellent to the kibble was time consuming and the repellent-treated kibble could only be stored for a short period of time (two days). Consequently, this method was not used again and new methods were developed to reduce preparation time and ensure that the dog were being deterred by the smell of the repellent and not its taste.

Experiment 2

Aim

The aim of this part of the pilot study was to develop a method of presenting the odours to the dogs without affecting the taste of the food.

Method

Subjects

Two dogs participated in this experiment. These dogs were Mica and Kimchi (Table 1).

Equipment

Two dog food bowls were used to hold the food. A small glass jar was placed in the centre of each bowls (Figure 2). In one bowl, the glass jar contained water (45 ml) and in the other food bowl the glass jar contained the repellent solution (45 ml). The jars were held in position using adhesive Velcro dots. To ensure that the dogs could access the food from all directions, the food was evenly distributed around the outside of the jar.

Two cameras were used to film the trials. Denver Instrument Scales were used to weight the faeces and blender was used to make the faeces solution.

Figure 2: Food bowl containing a small glass jar. Solutions were placed in the glass jar and the food was placed around the outside of the jar, in the bowl.

Sample preparation

Breville scales were used to weight 25 g of lion, tiger, baboon, and dog faeces, and 25 g of the commercial repellent. The faeces were placed into a blender (Home and Co mini blender) with 45 ml of water and blended for two minutes. To remove large, unwanted material (e.g., bones, gravel, fur) the solution was poured through a coarse sieve into a sealable container. This procedure was repeated for all four faecal types.

Food preparation

Hill's Science Diet dog food was used in this experiment. To determine the quantity of food each dog received, the recommended feeding amounts on the packaging were divided by the total number of presentations (see equation below).

$$n = \frac{total\ food\ amount}{number\ \ of\ trials\ \ x\ number\ of\ bowls}$$

Feeding trial

Both dogs participated in a total of six, two choice preference tests. For these tests the dogs were presented with two stainless steel bowls containing food and repellent/water. All dogs were deprived of food for 6-12 hours before the trials began to ensure that they were sufficiently hungry at the time of testing.

The first round of each session was a habituation period. For this, two bowls containing food and 45 millilitres of water were placed in the centre of the test arena, one metre apart. The dogs were led into the test arena by the researcher, with the researcher stating 'what is it?' before releasing the dogs and walking to the corner of the test facility and facing the wall (to avoid giving unintentional cues). The researcher remained in the arena during all trials to reduce the stress levels of the dog and allow for easy capture once the trials were complete. When the two minute trial was complete, the researcher removed the dog from the arena and it remained tied up outside the room or held by the owner or an assistant until it was required for the next trial. This procedure was repeated for the five remaining experimental trials; however, in the subsequent experimental trials, the

dogs were presented with one repellent-treated (dog biscuits + 45 ml repellent) and one control feeding apparatus (dog biscuits + 45 ml water). The side of presentation was pseudo-randomised to ensure that the repellent was not presented more than three consecutive times on the same side, while the order of repellent presentation was randomised.

Due to this being a pilot study, no formal measures were taken. All results were observational.

Results

Kimchi approached the food bowls, but did not eat the food presented. Mica approached and ate the food presented; she also consumed the repellent mixture made from baboon faeces. This method was, therefore, deemed unsuitable by the researcher for the following reasons: (1) the dogs could consume the liquid solution during the trial (i.e., no mesh or lid was used to cover the solution), and (2) the position and height of the glass jar resulted in the odours being released from a small, localised area (the top of the glass jar) only. Thus, the dog may not have encountered the odour before consuming the food. Further modifications to the equipment were required to ensure that the repellent odour was smelt by the dog before food consumption took place.

Experiment 3

Aim

There were two aims associated with this method: (1) to develop a feeding apparatus that ensured that the dogs encountered the odour before investigating/consuming the food, and (2) to determine if an increased concentration of repellent solution would be more effective at deterring dogs from their food, as compared to a low concentration of repellent solution.

Method

Subjects

Eight dogs were used in this experiment (Table 1).

Equipment

To complete the first aim, a specialised feeding apparatus was made using a small dog bowl, a plastic saucer and wire mesh (Figure 3). The dog bowl was placed in the centre of the saucer. The repellent solution was poured into the saucer, and the wire mesh was used to prevent the dogs from consuming the repellent.

Figure 3: Feeding apparatus used in the experiment. Food was placed in the aluminum food bowl, the repellent solution was placed in the saucer underneath, and the mesh was used to prevent dogs from consuming the repellent.

To complete the second aim, eight dogs were presented with the repellents at a low concentration (40 g faeces: 100 ml water; 20 g commercial repellent) over four sessions (two sessions a day, over two different days). Four of the eight dogs

were then asked to come in and participate in an additional two sessions. However, in these sessions the dogs were presented with a higher concentration of repellent (80 g faeces: 100 ml water; 40 g commercial repellent). This was because it was hypothesised that the increased concentration of repellent would be more effective at deterring the dogs from the food.

Sample preparation

Breville scales were used to weigh the faeces (40 or 80 g). The faeces were placed into a blender (Home and Co mini blender) with 100 ml of water and blended for approximately two minutes. Large material (e.g., bone fragments) was removed by pouring the solution through a coarse sieve. The solution was then poured into a Nalgene jar (one litre) and stored in the chiller (4ºC) until it was required for use. Solutions were stored for a maximum of three weeks. The commercial repellent was weighed using Breville scales (20 or 40 g). Once weighed it was stored in an air-tight container at room temperature.

Feeding trial

The same method as described Chapter 2 was used in this experiment.

Results and Discussion

Table 2 displays the average amount of food consumed by eight dogs when presented with five different repellent types at a low (40 g/100 ml) concentration. Evidently dog faeces was the most effective repellent, with less than 50% of the dog food being consumed in these trials. This was followed by tiger (81% consumed) and lion faeces (89.5% consumed). The least effective repellents were baboon faeces and the commercial repellent, with less than 6.5% and 4% of the food remaining, respectively.

Table 2: Average amount of food consumed (%) when the dogs (N = 8) were presented with a repellent concentration of 40 g faeces and 100 ml of water (40:100) and 20 grams of commercial repellent, over four sessions.

Repellent type	Number of sessions	Average amount of repellent-treated food consumed (%)	Average amount of control food consumed (%)
Dog	4	47.5	90
Baboon	4	96	93.5
Lion	4	89.5	89.5
Tiger	4	81	90.5
Commercial	4	96.5	96

When comparing the average percentage of repellent-treated food consumed to the average percentage of control food consumed, it was evident that when the dogs were presented with dog, lion or tiger faeces, they preferred the control food. However, when presented with the commercial repellent or the baboon faeces, the repellent-treated food was preferred by the dogs. Anecdotal viewing of the video footage suggested that dogs were 'attracted' to the baboon faeces, with some dogs investigating the bowl after all of the food had been consumed.

It is evident from the results that more food was consumed at an increased repellent concentration (Figure 4). This is likely to be a result of the dogs habituating to the odours presented. Dog faeces was the most effective repellent at both a low and high concentration, with 58% and 19.5% of the food remaining, respectively. At a low concentration, tiger faeces repelled dogs from the food more effectively than lion faeces. However, at an increased concentration lion faeces repelled dogs more effectively than tiger faeces. The commercial repellent and baboon faeces were the least effective repellents, deterring no dogs from the food at an increased concentration.

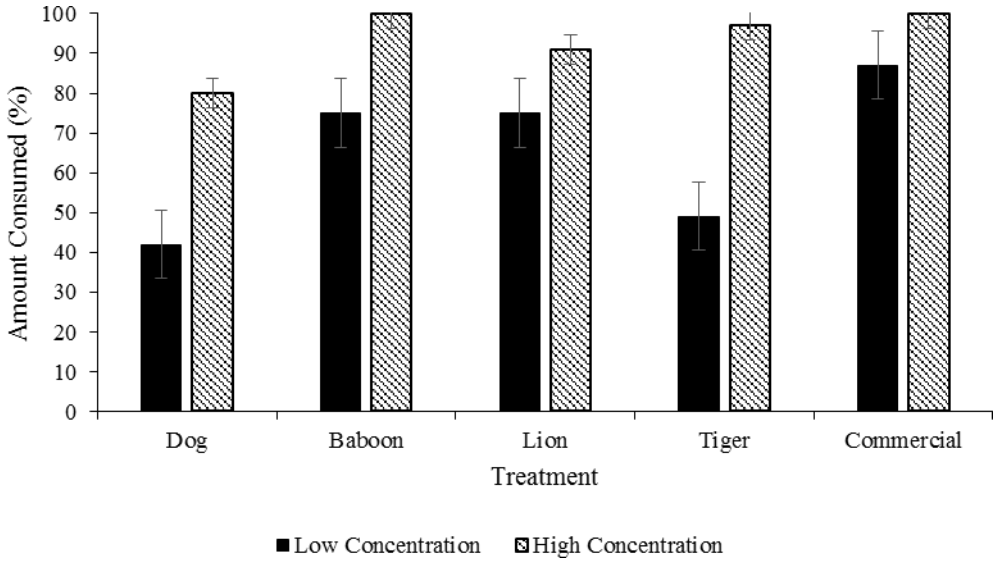

Figure 4: Average (± S.E.M) amount of food consumed by four dogs (Mica, Tui, Hunny and Josh) when presented in two sessions with a low repellent concentration (40 g faeces mixed with 100 ml water) followed by two sessions with a high repellent concentration (80 g faeces mixed with 100 ml water).

In summary, these findings did not support the hypothesis that an increased concentration of repellent would be more effective at deterring dogs from their food. Thus, the lower concentration of repellent was selected for use in the full experiment, described in Chapter 2.

To determine the number of dogs required for the main experiment, a power analysis was performed. For this analysis, the time spent in proximity to the left feeding apparatus (less than 50 cm away from left bowl) and the time spent in proximity to the right feeding apparatus (less than 50 cm away from right bowl) were used. A response variable was created by subtracting the time spent proximate to the repellent from the time spent proximate to the control (non-repellent). Thus, a positive difference implied that the repellent attracted the dogs' attention, while a negative difference implied that the repellent deterred the dogs' attention. A simple linear model using only the repellents gave a residual standard deviation of 14.18. Using this value, it was determined how big a difference (positive and negative) was required to give a significant result at five percent level (p = <0.05).

Figure 5 illustrates how many dogs are required to detect a significant difference at a five percent level. It was discovered that to detect an average difference in time of 10 s or 5 s between being proximate to the repellent side and being proximate to the non-repellent side (control) at a 5 percent level of significance, four or 16 dogs over two sessions were required, respectively. This number of dogs was therefore used as a minimum target in Chapter 2.

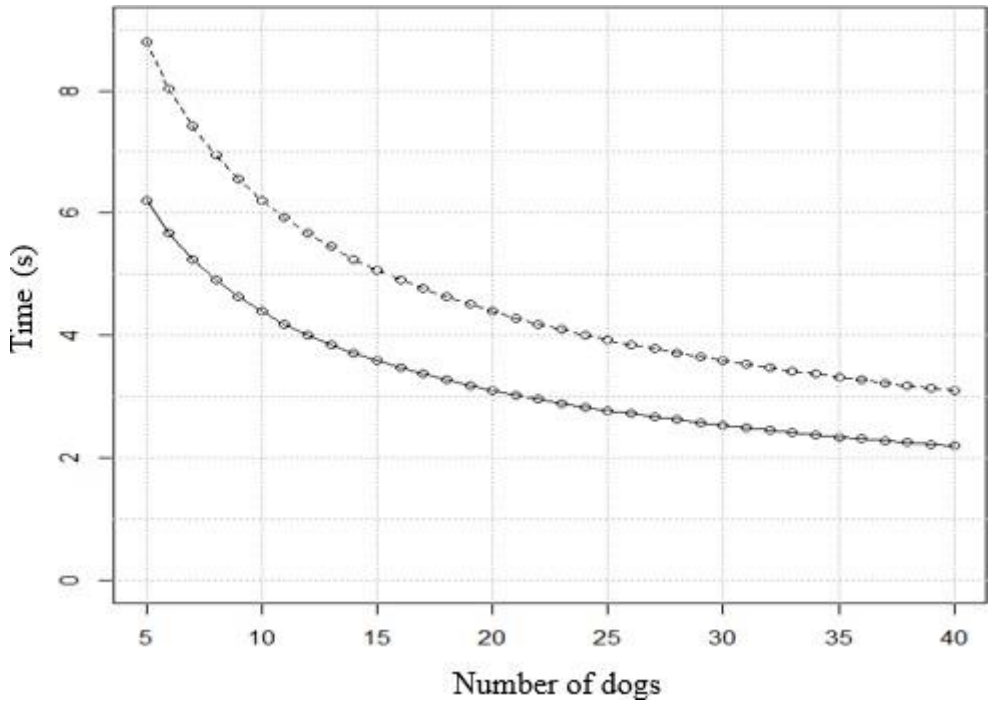

Figure 5: The number of dogs required to gain a statistically valid result at a five percent significance level ($p < 0.05$). The dashed line indicates the number of dogs required if two session were performed. The solid line indicates the number of dogs required if four sessions were to be performed.

Appendix B: Intra- and Inter-Observer Reliability

Table 1: Results from Pearson's Correlation on Intra-observer data. N = 25 videos.

Variable	Behaviour/Position	Correlation
Latency to Approach	Left Food Bowl	0.994060
	Right Food Bowl	0.997800
Behaviour	Walking	0.993618
	Standing	0.998791
	Sitting	0.996653
	Lying down	0.999410
	Eating left bowl	0.994813
	Eating Right Bowl	0.997236
	Investigating Left bowl	0.949830
	Investigating Right Bowl	0.997742
	Drinking	0.999624
	Scratching	1.000000
	Licking	1.000000
	Out of Sight	0.999883
	Other	0.990751
Frequency to Approach	Left Food Bowl	0.993900
	Right Food Bowl	0.990820
Arena Position	Out of Sight	0.998927
	Left	0.765220
	Right	0.968176
	Left Proximity	0.989733
	Right Proximity	0.983155
	Release Point	0.845421

Table 2: Results from Pearson's Correlation on Inter-observer data. N = 25 videos.

Variable	Behaviour/Position	Correlation
Latency to Approach	Left Food Bowl	0.909930
	Right Food Bowl	0.931438
Behaviour	Walking	0.993580
	Standing	0.995405
	Sitting	0.998541
	Lying down	0.999926
	Eating left bowl	0.972737
	Eating Right Bowl	0.973938
	Investigating Left bowl	0.993588
	Investigating Right Bowl	0.934101
	Drinking	0.999545
	Scratching	1.000000
	Licking	1.000000
	Out of Sight	0.863023
	Other	0.992239
Frequency	Left Food Bowl	0.980066
	Right Food Bowl	0.983429
Arena Position	Out of Sight	0.863023
	Left	0.970383
	Right	0.989914
	Left Proximity	0.998242
	Right Proximity	0.992593
	Release Point	0.129444
	Total Time	0.998626

Appendix C: Dog Participants

Table 1: Details of all dogs that participated in the full study.

Subject	Age (years)	Sex	Breed
Flint*	8	Male	Heading dog
Mica	7	Female	Australian cattle dog x heading dog
Kimchi*	12	Female[s]	Papillion x
Josh	11	Male[n]	Dalmatian
Toshka*	13	Female[s]	Siberian husky
Cocoa	11	Female[s]	Labrador retriever
Laddie	11	Male[n]	Standard collie
Tui	2	Male[n]	Huntaway x kelpie x border collie
Merlin*	10	Male[n]	Standard collie c Border collie
Ranbo*	6	Male[n]	Chihuahua
Hunny	10	Female[s]	Bulldog
Pringle*	1	Female[s]	Corgi
Bovril	10	Male[n]	Labrador retriever x spaniel
Dillion*	5	Male[n]	Fox terrier
Stu*	8	Male[n]	Miniature pinscher
Pac	10	Male[n]	Border collie x Shetland Sheepdog
Rebel	3	Male[i]	Border Collie
Tat	2	Male[n]	Golden Retriever x Border Collie
Feature	2	Male[n]	Border Collie
Arie	6	Male[n]	Heading dog cross
Clutch	5	Male[n]	Catahoula x Grey Hound
Xena	6	Female[s]	Border Collie x
Trigg	3	Male[n]	Labrador retriever
Raven	2	Female[s]	Labrador retriever
Maggie	1.5	Female[s]	Labrador retriever
Lulu	10	Female[s]	Shihtzu
Jet	2	Male[n]	Labrador retriever x Huntaway
Kaspar	3	Male[n]	Golden Retriever
Zoe	12	Female[s]	Labrador retriever
Zander	5	Male[n]	Labrador retriever
Ice	6	Male[n]	Sharpei x Labrador retriever
Bru	5.5	Female[s]	Border Collie x Samoyed
Chief	5.5	Male[n]	Border Collie x Samoyed
Lexie	2	Female[s]	Staffordshire Cross
Bella	8	Female[s]	Labrador retriever
Rex	1	Male[n]	Labrador x Golden Retriever
Vera*		Female[s]	Siberian Husky
Hank	5	Male[n]	Labrador retriever
Bently*	3	Male[n]	Labrador retriever x Staffordshire

[S]Spayed, [n] Neutered, *Trial terminated

Appendix D: Dog Trial Forms

University of Waikato
School of Science
PROJECT INFORMATION SHEET

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Dog Repellent Study

This study is a part of Masters research project being carried out by Melissa Collins in the School of Science at the University of Waikato.

Project background

Anticoagulant rodenticides are used to control rodent populations worldwide. However, they can have measurable adverse effects on non-target species, particularly domestic dogs. Anticoagulant rodenticides are baited poisons that prevent blood clotting. Exposure to these poisons (i.e., via consumption) can lead to uncontrolled bleeding, weakness and in most cases death. This project aims to find a natural olfactory (smell) repellent that has the potential to deter dogs from anticoagulant rodenticides.

Research procedure

To participate in the trials your dog will be required to come to the University of Waikato on two separate occasions. In a test arena your dog will be presented with two bowls containing dog biscuits. One bowl will be treated with the selected repellent, and the other will be a control (i.e., no repellent present). Using video cameras each trial will be recorded. I will then analyse the footage, examining the dogs behaviours towards the repellent and non-repellent treated bowls.

You may stop your dog from taking part in the project at any time and you may also request that I withdraw their information. If you would like to know the findings of this project, please let me know and I will inform you of what we discover from this research.

This study has approval from the University of Waikato Animal Ethics Committee, and is supervised by Dr Clare Browne and Associate Professor Nick Ling from the School of Science at the University of Waikato.

Please use the contact details below if you have any further questions about any aspect of this project:

Melissa Collins
Masters student
School of Science
University of Waikato
Private Bag 3105
Hamilton 3240
missycollins816@gmail.com

Dr Clare Browne
MSc supervisor
School of Science
University of Waikato
Private Bag 3105
Hamilton 3240
clare.browne@waikato.ac.nz

Can your dog eat any food, including kibble (biscuits) and different kinds of meat products? **Yes / No**

If **no**, please explain briefly: _____

_____

_____

Is your dog comfortable with people getting near their food? **Yes / No**

E.g., if your dog has shown any aggression (freezing, growling, snarling, biting) around food, please select 'no'.

If **no**, please explain briefly: _____

_____

_____

Is your dog friendly towards other dogs? **Yes / No**

E.g., if your dog has shown any aggression or fear towards other dogs, please select 'no'.
(We will not necessarily have more than one dog at the testing facility at once. If we do, it will be with permission of all owners and the dogs will be kept separate.)

If **no**, please explain briefly: _____

_____

_____

Is your dog comfortable with unexpected/loud noises, such as beeping sounds? **Yes / No**

If **no**, please explain briefly: _____

_____

_____

Is your dog free of medical conditions that could be aggrevated by walking? **Yes / No**

E.g., if your dog has any joint or other problems that might be affected, please select 'no'.

If **no**, please explain briefly: _____

_____

_____

We want to make sure that all dogs enjoy participating in our research. If you answered "no" to any of these questions, this may indicate that your dog is not suitable for some of this research; however, it does not necessarily exclude them from taking part. A researcher will be in touch with you to discuss the information you have provided here. Thank you for taking the time to complete this form.

Please email this form to: missycollins816@gmail.com

DOG BEHAVIOUR RESEARCH
Participant Information Form

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Thank you for your interest in our dog behaviour research.

Please provide us with the following contact information:

Owner's name	
Mobile phone	
Home phone	
Email address	
Home address	
Most convenient days & times to drop off/collect your dog?	
How did you hear about this research?	

Please provide us with the following information about a nominated emergency contact person (in case we cannot contact you):

Nominated contact person	
Mobile phone	
Home phone	
Email address	
Home address	

Please provide us with the following information about your dog's normal veterinarian:

Normal vet clinic	
Normal veterinarian	
Clinic phone	
Clinic address	

Please provide us with the following information about your dog:

Dog's name	
Breed	
Date of birth	
Age	
Sex	
Are they de-sexed?	
Weight	
Colour & distinguishing features	
Fully vaccinated?	
When are their next vaccinations due?*	
Normal food type	
Normal meal times/amounts	
Favourite food type	
Any aggression around food?	
Allergies/illnesses	
Behaviour issues	
Other likes/dislikes (e.g., other dogs, being touched, being alone, noises, etc.)	

Thank you for taking the time to complete this form.

Please email this form to: [INSERT EMAIL HERE]

Appendix E: Rat Pilot Study

Introduction

The purpose of this pilot study was determine the methods and equipment for Chapter 3: Predator Odours and their Repellent Effects on Rats. The aim was to determine if the repellents that were most effective on dogs; lion, tiger and dog faeces, deterred rats from their food.

Methods

The method used in this pilot study similar the method described in section 2.2 of Chapter 2. The only differences were that in this pilot study (1) the rats were kept in their chambers over night for the first two nights of the experiment, (2) the rats test arenas were not covered with glass, (3) the rats were feed two different diets, (4) video data was not analysed.

Results/Discussion

The results from this section of the pilot study revealed that, regardless of the food type, the rats took similar quantities of food from the repellent and control feeding apparatus when tiger, lion and dog faeces were present. Thus, suggesting that they were not averse to these odours. The first group of rats (Rats 1-5; Figure 1 A) took marginally more food from the control food bowl when presented tiger and lion faeces, while they took less food from the control food bowl when presented dog faeces. The second group of rats (Rats 6-10; Figure 1 B) took marginally more from the control food bowl when presented tiger faeces, but less from the control food bowl when presented dog and lion faeces. Though no statistical analyses were performed on these data it is unlikely that there would be a statistical difference between the amount of food taken from the repellent food bowl and the amount of food taken from the control food bowl, for each repellent type.

It is noted by the researcher that the rats were given two different food types; group one (rats 1-5) received Diet 86 (Figure 1 A), while group two received Specialty foods (Figure 1 B). Evidently from the results (Figure 1) the rats preferred the

latter food type (Specialty Food) in comparison to the former food type (Diet 86). Thus, the latter food type (Specialty Foods) was used in Chapter three.

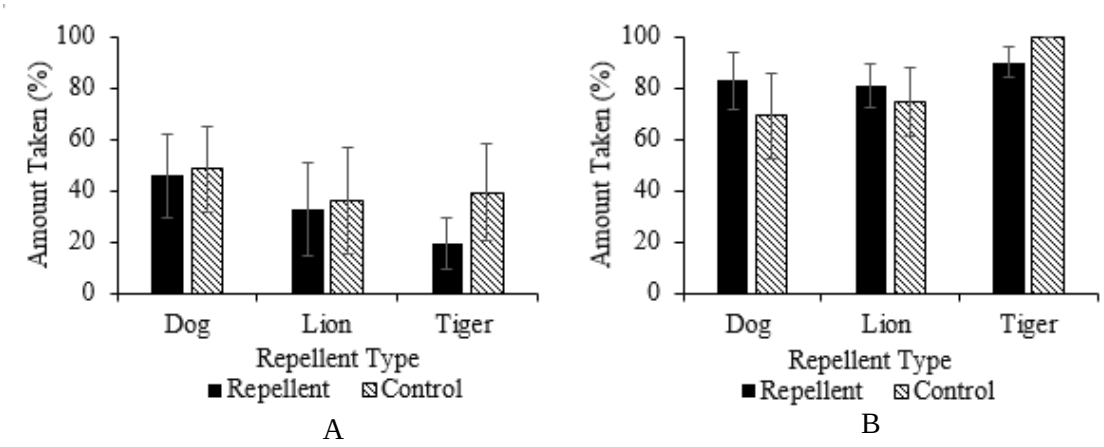

Figure 1: Average (± S.E.M) amount of food taken by the rats when presented dog, lion and tiger faeces. Rats 1-5 feed diet 86 (A); Rats 6-10 feed Specialty Foods (B).

Appendix F: Exotic Animals Diet

Table 1: Zoo Keepers information sheet for Hamadryas baboons (*Papio hamadryas*)

Collectors Name	**Name of Species**	**Date of Collection**	**Time of Collection**	**Time of Refrigeration**	**Time/day of freezing**	**Sex (if possible)**	**Age (if possible)**	**Diet of species prior to collection**	**Additional Notes**
Sam Roberston	Hamadryas Baboons	27/03/18	9:30 am 12:00 pm 4:00 pm	10:30 am 12:30 pm 4:30 pm	-	Male and Female	All ages	Apples, Oranges, Carrot, Kumara, Courgette, Green beans, Celery, Cabbage, Eggs, Seeds	
Sam Roberston	Hamadryas Baboons	23/01/18	9:30 am	10:00 am	1:00 pm	Male and Female	All ages	Apples, Oranges, Carrot, Kumara, Courgette, Green beans, Celery, Cabbage, Eggs, rolled oats, Omnivore pellets, Sprouted mung Beans, Chickpeas	

Table 2: Zoo Keepers information sheet for Sumatran tigers (*Panthera tigris*)

Collectors Name	Name of Species	Date of Collection	Time of Collection	Time of Refrigeration	Time/day of freezing	Sex (if possible)	Age (if possible)	Diet of species prior to collection	Additional Notes
-	Tiger	-	-	-	-	-	-	Chicken, Horse, Beef, Venison, Goat, Rabbit	
Note: forms were lost and no information on the date and time of collection or freezing was supplied.									

Table 3: Zoo Keepers information sheet for African lions (*Panthera leo*)

Collectors Name	Name of Species	Date of Collection	Time of Collection	Time of Refrigeration	Time/day of freezing	Sex (if possible)	Age (if possible)	Diet of species prior to collection
Ellie S	African Lion	04/01/18	8:15 am	-	04/01/18	Female	19 16	Rabbit
Ellie S	African Lion	05/01/18	8:15 am 10:00 am	-	05/01/18	Female	19 16	Beef
Emma	African Lion	06/01/18	8:10 am 12:05 pm	-	06/01/18 8:10 am 12:05 pm	Female	19 16	Venison
Renny	African Lion	07/01/18	7:30 am	-	07/01/18	Female	19 16	Venison
Kristin	African Lion	08/01/18	9:05 am	9:07 am	09/01/18 9:30 am	Female	19 16	Horse Chicken
Joanna	African Lion	09/01/18	7:25 am	7:28 am	09/01/18	Female	19 16	Horse Chicken
Joanna	African Lion	10/01/18	7:40 am	7:45 am	10/01/18 7:45 am	Female	19 16	Horse
Karen	African Lion	11/11/18	7:20 am	7:40 am		Female	19 16	Horse
Sadun	African Lion	13/01/18	2:10 pm	2:30 pm	13/01/18 2:35 pm	Female	19 16	Horse
Ellie	African Lion	23/01/18	8:30 am	9:00 am	1:30 pm	Female	19 16	Horse/Chicken
Helen	African Lion	24/01/18	8:47	8:47 am	9:50 am	Female	19 16	Beef/ Horse
L B	African Lion	28/01/18	8:00 am	-	8:10 am	Female	19 16	Beef/Wallaby

Note: Forms for second collection of faeces were not given back to the researcher.