The Royal Agricultural College

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An investigation into the possibility of Self-medication using common herbs in captive wolves (*Canis lupus)*

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Declaration of Authors Rights

I declare that this dissertation embodies the results of my own research or advanced studies and that it has been composed by myself. Where appropriate, I have made acknowledgement to the work of others.

Signed:

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Date 1/5/2008

Abstract

For millennia humans have used medicinal plants in the treatment of disease and injury, scientists from various disciplines are currently studying the possibility that different species of animal may also use plants as 'medicines'. So far this form of self-medication has only been studied in a few species of primate, there has, to date, been no research carried out on the possibility of self medication within any species of the canid family. As the largest member of the canid family, the wolf is known to be both a predator and a carnivore; this study looks at the possibility of captive wolves taking an interest in common medicinal herbs, with the possibility of these herbs being used for the purpose of self-medication.

Three packs of three wolves were involved in this study, all of which live at the UK Wolf Conservation Trust, Nr Reading, UK. Wolf proof planters were constructed to protect the herbs so that they could be accurately measured whilst allowing the wolves' access to a proportion of the plant. The herbs were placed in each enclosure for one week, one at a time, in a random order. The herbs dimensions were taken when planted and again at the end of the week before the plants were changed. The differences in these measurements were used to gauge the consumption of each herb by each pack of wolves.

The results showed that there was a significant interest in the herbs provided ($\boldsymbol{P} = 0.001$, $\boldsymbol{df} = 23$), with the greatest interest shown in the herb rosemary. There was no significant difference in the interest shown by the three different packs ($\boldsymbol{P} = 0.964$, $\boldsymbol{df} = 26$), there

was also no significant difference in the interest shown by the wolves during the first and second exposures of the herbs *(**P** = 0.444, **df** = 24).*

The significant findings of this research show that the wolves were interested in the herbs placed in their enclosures and that this interest was maintained throughout the study. Unfortunately it has not been possible, through this study to say whether this interest is as a result of the wolves making use of the medicinal properties of the herbs, or just showing a general interest in a new addition to their environment.

Acknowledgements

I would like to acknowledge and thank the following for their assistance through the process of this dissertation; Dr Rachael Foy (Wiltshire College, Lackham Campus), Dr Helen Moreton, John Nixon (Royal Agricultural College), for their guidance and advice on different aspects of this investigation. I would also like to thank Owain Wilford (Wiltshire College, Lackham) for producing the planters used in this dissertation.

I would like to thank the staff and volunteers of the UK Wolf Conservation Trust for their encouragement, support and assistance during this study; in particular thanks must be given to Toni Shelbourne (Education Officer) for the initial idea and support during the study and to Clive Readings (Wolf Keeper) for his assistance in placing the commissioned planters and allowing me regular access to the wolf enclosures, sometimes at short notice so that I could measure and plant the herbs. I must also thank Whitehall Garden Centre, (Lacock; Wiltshire) for the generous discount they gave me on the many herbs that I purchased from them for the purpose of this study.

I would also like to thank my wonderful husband, Elliot Hughes for putting up with me throughout the course of my degree, and my mother Kim Hunte for all the proof reading and her encouragement and support.

Contents

List of Figures

List of Plates

List of Tables

Chapter 1

Introduction

1.0 Introduction

1.1 The issue and its context

The Gray Wolf (*Canis lupus)* is the largest member of the canid family; a species which was once found throughout the northern hemisphere. In the many areas of the world where they still remain they are at the top of the food chain. The wolf is a widely known predator and carnivore although recently; in studies looking at diet through the examination of wolf scats; berries, grasses and other plant matter have been unexplainably found (Carbyn et al, 1995). There are two possible options for this discovery, the plant matter may have been picked up unintentionally whilst gorging on a kill, or it was eaten intentionally for a reason, possibly as a form of self medication.

It is through the process of natural selection that a range of behavioural strategies exist which enable all living organisms to cope with the range of health threats that come from injury, poisons, and other pathogens (Engel, 2002; Hart, 1990). Early in the co-evolution of relationships between plants and animals some species of arthropod began to utilise the plants chemical defences to protect themselves from both predators and parasites. Chimpanzees (*Pan troglodytes*) and Gorillas *(Gorilla gorilla)* have both been known to swallow and excrete whole leaves; it is thought that this habit is a physical means of purging internal parasites. (Huffman, 2003) The way that animals regulate their health through diet is a subject which is little understood and in which there has been little research conducted although it could have many implications in the captive management of many species.

Past research into animal nutrition has shown that when choosing what to eat animals will look for nutrients and avoid toxins; in 1978 scientist D. Janzen became one of the first people to propose that animals may use plant toxins to control internal parasites as a form of self-medication; he described how the Asiatic rhinoceros (*Didermocerus sumatrensis*) eat large quantities of tannin rich bark from the mangrove *Ceriops candoleana*; he concluded that the tannins, which can cause digestive upset, were able to help in the control of intestinal parasites such as worms; through the tannins ability to bind proteins they were able to bind to the worms and carry them out of the body.

For millennia humans have been using plants for medicinal purposes; the range of species used and their scope for healing is vast. The science of phytotherapy studies the use of herbal remedies to treat the sick and covers everything from powerful medicinal plants such as Digitalis and Belladonna, to plants with gentle actions such as chamomile or mint and many others. The plants that have gentle actions are no less effective than the powerful varieties, as a rule the gentle plants do not have any toxic effects and can therefore be taken safely over an extended period of time (Weiss, 1994). Zoopharmacognosy is a term coined by Dr Eloy Rodriguez in 1993; a biochemist and professor at Cornell University. He described the process by which animals self-medicate by selecting and utilizing plants, soils and insects to treat and prevent disease. Is it possible that our human ancestors learnt to use plants for their medicinal properties from watching the animals that lived around them?

It is the purpose of this study to investigate the possibility of self-medication using common herbs in captive wolves. All investigations were carried out at the UK Wolf Conservation Trust near Reading and looked at the interest shown by three packs, each containing three wolves, in a selection of herbs over a set period of time. This investigation will aim to compare the amount of interest shown in each herb, if any and the length of time interest is shown, as observed by staff and volunteers. The findings from this study will be used to assess the interest shown in medicinal herbs by the wolves, which may encourage future research in the area of zoopharmacognosy in both wild and captive wolves.

1.2 Aims and Objectives

1.2.1 Aim

The aim of this study is to investigate the possibility of captive wolves using common medicinal herbs in self medication.

1.2.2 Hypothesis

1° Ho – The wolves will show an equal amount of interest in the herbs provided and the interest will remain constant throughout the period of time the herbs are in the enclosures.

2° Ho – There will be no significant difference between the amount of each herb consumed by the wolves.

1.2.3 Objectives

Primary fieldwork will form the majority of this study and will be based upon the measurements taken from each herb before and after they have been planted in the wolves enclosures. Secondary information obtained from valid sources will also be used to provide background material. In order to successfully achieve the aim of this study, a series of objectives have been established:

- Design and build wolf proof planters so that the chosen herbs can be placed in the enclosures with the wolves only having limited access, thereby allowing the plant to remain in the planter so that the required measurements could be taken.

- Collect photographic evidence of the damage caused to the herbs by the wolves over the period of time in the enclosure.

- Obtain significant findings to achieve the aim of the study.

Chapter 2

Literature Review

2.0 Literature Review

2.1 The Wolf

Historically the Gray Wolf (*Canis lupus*) could be found throughout the Northern hemisphere. The habitat of the wolf includes the high Arctic, tundra, taiga, forests, plains, deserts and virtually every ecological niche which could provide sufficient prey for its existence (Busch, 1998). They are generally pack animals, working together as a family unit when hunting and looking after the young. Packs size can range from four to seven individuals; the alpha pair is at the centre of the pack and are the only ones to breed, the other pack members tend to be brothers or sisters of the alpha pair and offspring from the alpha pair (Mech, 2003).

The term "carnivore" refers to all species that obtain the most or all of its nutrients from other animals, either through predation or scavenging (Allen et al, 1997). The feeding habits of most canids have rather more breadth than this as their diets can also include a variety of fruits and other plants parts (Allen et al, 1997). Wolves are flexible, opportunistic predators; studies that have looked at wolf diet through scat analysis and stomach content analysis have indicated that the major part of the wolf's diet consists of ungulate prey; these include deer, moose, elk, caribou and wild boar depending on what prey is available in their habitat, although it has been recorded that they will eat anything from a mouse to a moose (Mech, 2003).

Wolves are flexible in their choice of prey which can be largely season dependent. In the Jasper National Park, USA, wolves have been recorded switching from adult mule deer to their calves in the spring; it has also been observed that some wolves will turn to hunting mice and ground squirrels when the caribou migrate north for the summer (Busch, 1998) It is important to realise that wolves are not only hunters, but scavengers as well. Wolves have been recorded eating from carcasses of moose which, upon closer inspection, had died natural deaths. In some European countries wolves have also been seen scavenging from human rubbish bags (Busch, 1998).

Even though wolves acquire a large percentage of their food from prey, they are not exclusive meat eaters. Scat analysis has found microscopic remnants of invertebrates such as earthworms and grass-hoppers, seeds and other vegetation. In southern areas of Eurasia greater amounts of plant material have been found in wolf scats; this could be as a direct result of the greater availability of fruit trees when compared to scats analysed from North America. Radio collared wolves in the lowlands of Italy have been monitored as they moved through mature vineyards (Ciucci & Peterson, 2003). It was considered that this consumption of fruit may provide vitamins for the wolves during the summer months, as even in North America it is not uncommon to find seeds from raspberries and blueberries in wolf scats (Mech, 2003). Cherries, apples, figs, plums, grapes, melon and watermelon have also been reported in wolf scats (Ciucci & Peterson, 2003). The question remains do wolves consume these miscellaneous foods during the short periods when they are abundant and their usual choice of prey is unavailable or is there some other reason?

One family of plants which commonly appear in wolf scats throughout North America and Eurasia, with a 14 – 43 % frequency, are grasses from the family *Geraminae*. It has been acknowledged that this grass possibly acts as a scour or inducement to vomit, ridding the intestine of parasites or the stomach of long guard hairs that may delay the passage of food through the gut, or it could just be a source of vitamins (Ciucci & Peterson, 2003). This could be the only documented possibility of self-medication (Zoopharmacognosy) in wolves; however there are a large range of diseases that wolves are susceptible to, some of which could be treated through the use of medicinal herbs or plants. These diseases include both endo and ectoparasites and a range of viral, bacterial and fungal infections such as canine distemper, leptospirosis and blastomycosis (Kreeger, 2003).

Limited research has been carried out into the area of self –medication in certain species of primate. These studies have shown that primates will use certain plant species to aid the control of parasite infection as well as to provide relief from gastrointestinal upsets; it was found that Chimpanzees, Gorillas and Bonobos (*Pan paniscus*) all choose the same plant species or species from the same genera for similar illnesses suggesting common criteria in medicinal plant selection. With the exception of these few observations no research has been specifically carried out into the possibility of self-medication by wolves or any other large carnivores. This testimony is supported by the following: Dr David Mech, Wolf Biologist; Denise Taylor, Director, UK Wolf Conservation Trust; Cindy Engel, Author of Wild Health; Jeremy Heft, Wildlife Biologist; Pat Goodman,

Wolf Park USA and Jess Edberg, International Wolf Centre. Their replies can be found in full in Appendix I.

2.2 Zoopharmacognosy

Scientists from various disciplines are currently exploring the possibility that many species use plants, soils, insects and fungi as 'medicines' in ways that guard against future illness (preventative medicine) and / or relieve unpleasant symptoms caused by illness (curative / therapeutic medicine). The study of self-medication is not based on the assumption that animals possess an innate ability to know what's good for them. In most cases self-medication could be motivated by a desire to reduce unpleasant sensations that could be caused by illness. The term 'zoopharmacognosy' was coined to describe the process by which animals select and use specific plants that contain medicinal properties for the treatment and prevention of disease (Rodriguez & Wrangham, 1993).

Wild animals invariably obtain their nutrient requirements, regulate their ingestion of toxins and even self-medicate. It is suggested that, while size and general morphology dictates the major percentage of an animal's diet, the ability to select a diet is learnt. Animals can learn to distinguish nutritious foods from those that are less beneficial or even toxic through the possible positive and negative consequences of ingestion (Foley et al, 2003)

Rumours of wild chimpanzees practicing self-medication in Gombe National Park, Tanzania, have persisted for several decades. It was reported back in the 1970's that they

were using certain leaves in 'non-nutritional' ways , over the following decades, it was established that the chimpanzees were swallowing the leaves whole as mechanical scours to help expel intestinal worms (Wrangham, 1995). To date, experts have documented 30 plant species whose hairy leaves are "swallowed whole", not just by chimpanzees but bonobos and eastern lowland gorillas (Biser, 1998). In 1987, in the Mahale Mountains, another aspect of chimpanzee self-medication was observed and documented by scientists during the rainy season. Michael Huffman of Kyoto University had been following a small group of chimpanzees through forest for several weeks, one day it was noticed that one of the females was unwell. She separated herself from the group and spent most of the day asleep, during the afternoon she was seen feeding from a small shrub, *Vernonia amygdalina* also know as bitter leaf, a toxic plant and certainly not usual food for chimpanzees. What made this observation significant was a comment by local guide Mohamedi Seifu, who explained that the plant was often used by local people to treat schistosomiasis, amoebic dysentery and other intestinal parasites and infections. Sure enough within 24 hours the female chimpanzee's condition had improved and she was feeding normally again with the other members of her group (Grange, 2003). Primates in captivity have also been observed eating plants with medicinal properties that grow naturally or that have been provided for them in their enclosures (Cousins, 2007).

2.3 Medicinal Plants

Plants synthesise many defensive compounds to protect themselves from disease and predators; these compounds are bioactive and can be medicinal, toxic or intoxicating depending on the circumstances. Many insects are pharmacophagous; that is they eat

non- nutritious substances which may serve as 'drugs'. A benefit gained by insects, through the consumption of plant toxins, is protection from predators, fungi, bacteria and parasites. For example when infested with internal parasites the woolly caterpillar switches to eating highly toxic hemlock which increases its chances of surviving a normally lethal parasite infestation (Karban & English-Loeb, 1997). A further example of self-medication through the ingestion of plants can be seen in the mountain gorilla. The sweet red fruits of the *Aframomum angustifolium*, a form of wild ginger, are favoured by the gorillas. They contain no toxic substances; however, according to biologist John Berry at Cornell University, the fruits' antimicrobial properties can temporarily damage the micro-organisms found in the gorilla's gut, causing digestive upset (Biser, 1998)

It is not just through ingestion that animals use the properties of plants to maintain their health, for example. The male European Starlings, *Sturnus vulgaris* in North America have been known to line their nests with specific highly aromatic plants; when these plants are removed from nests the chicks appear to suffer from greater levels of mite infestations than those with the plants remaining (Clark & Mason, 1988). The preferred plants contain both monoterpenes and sesquiterpenes which have been discovered to be harmful to bacteria, mites and lice under laboratory conditions (Clark & Mason, 1985).

The white-faced capuchin monkey (*Cebus capucinus*) has also been studied for its use of certain species of citrus plants in another form of possible self-medication. In the early 1990's anthropologist Mary Baker from the University of California witnessed the

monkeys breaking open fruits from certain species of citrus plant and rubbing the pulp and juice into their fur. They also tore stems, leaves and seed pods from *Clematis dioica, Piper marginatum and Sloanea terniflorastems*, respectively; they mixed these pungent plants with saliva before vigorously rubbing them in. Later research showed that plants from those genera were used by local people to treat skin irritations or repel insects. Examination of the plants revealed that they contained secondary compounds with healing and insect repellent characteristics (Biser, 1998).

As already mentioned self-medication is not exclusively restricted to the use of plants, the use of soil in self-medication has also been documented.

2.4 Soil Use as Medication

Many species of mammals, birds, reptiles and even insects, all over the world, eat soil. Known as 'geophagy', this behaviour has long been assumed to be an attempt to rectify mineral deficiencies in their diets (Duquette & Johns, 1991). However evidence suggests that this is not always the case. It has become apparent that the clay content is often the most important ingredient of selected soils. In Venezuela free-ranging cattle have been seen digging and licking at clay subsoil's (Kruelen, 1985). Chimpanzees, giraffes, elephants and rhinoceroses eat regular mouthfuls of clay rich soil from termite mounds, whilst gorillas mine clay rich volcanic rock from under the exposed roots of ancient trees (Houston et al, 2000).

Clay is both an efficient binding agent and an effective deactivator, allowing toxins from the diet or pathogens to be effectively controlled. In the rain forests of New Guinea, parrots, pigeons and crows have been observed eating recently exposed soil at the site of a new landslide; when analysed it was found that the newly uncovered soil had a high clay content. Only eight of the one hundred and forty species that flocked to the landslide area were seen eating soil; these species were identified as being herbivorous. It was suggested that these species were taking advantage of the newly disturbed earth and selecting soil that contain the right properties to bind and deactivate the toxins found in the plants they feed upon (Diamond, 1998).

2.5 Medicinal Herbs used in this Study

Virtually all cultures have, throughout history, used a variety of plants or materials taken from plants in the prevention and treatment of disease. Evidence of the beneficial effects in these medicinal herbs can be seen in their continued use. Medicinal herbs have played a vital role in the development of modern medicine (Fisher et al, 1999). With the current popularity of traditional herbal supplements, coupled with recent scientific legitimacy in the use of some herbs, more and more people are turning back to herbal treatments for both themselves and their animals. Medicinal herbs are used by both animals and humans with the apparent prophylactic effects of reducing the chance of severe illness from pathogens or parasites in the future. Medicinal herbs with anti-inflammatory, antimicrobial, immunomodulatory and/or analgesic properties are used in a therapeutic way to treat inflammatory conditions and acute infections (Hart, 2004).

The medicinal herbs used in this study have specific properties which are known to have potential benefit to humans; the species used include Rosemary (*Rosmarinus officinalis)*, Thyme *(Thymus vulgaris)*, Spearmint (*Mentha spicata)* and Fennel (*Foeniculum vulgare*).

2.5.1 Rosemary (*Rosmarinus officinalis)*

Plate 1.Rosemary

Rosemary is a hardy, bushy perennial shrub with aromatic, evergreen leaves which are long and thin and pale-blue flowers around the stem. Rosemary can grow to between 1-2 meters tall and can be found growing wild throughout the Mediterranean. Both the leaves and flowers can be used for their properties (Bremness, 1991). Rosemary is a tonic, astringent, restorative herb that relaxes spasm and increases the rate of perspiration, while stimulating the liver and gall bladder. Rosemary is considered to improve digestion and circulation and controls pathogenic organisms. It has antibacterial, antifungal, antiviral, spasmolytic, antioxidant, smooth muscle modulating, analgesic, venotonic, as well as anti-inflammatory properties. It is high in easily assimilable calcium, which can benefit the entire nervous system. When taken internally it can be used to treat dyspeptic complaints, flatulence, to stimulate appetite and the secretion of gastric juices. It is also used as a treatment for rheumatism and circulatory problems. (Weiss, 1994)

2.5.2 Thyme *(Thymus vulgaris)*

Plate 2 Thyme

Thyme is a hardy, bushy perennial shrub, with aromatic, evergreen leaves which are small and round. In bush form it can grown to a maximum height of 38cm. Different varieties of thyme can be found growing wild throughout Europe. Both the leaves and flowering tops are used for their properties (Bremness, 1991). Thyme is important as a parasiticide for intestinal worms. It is a warming herb that is astringent, aromatic, anti-septic, and anti-fungal. It helps to improve digestion, relax spasms and controls coughing. When taken internally it can be used to treat respiratory disorders such as bronchitis, excess bronchial mucus, asthma and laryngitis. It can also be of benefit in the treatment of diarrhea, chronic gastritis and lack of appetite (Weiss, 1994)

2.5.3 Spearmint *(Mentha spicata)*

Plate 3 Mint

Spearmint is a hardy herbaceous perennial; it has sweetly scented, bright green leaves and bears white, pink or lilac flowers on cylindrical spikes. Some species can grow to one meter in height. It grows wild in temperate areas of Europe, Asia and Africa. The whole plant can be used for its properties (Bremness, 1991). Spearmint is a decongestant, cooling, fragrant and bitter herb that is anti-spasmodic, diaphoretic, digestive, antiseptic and slightly anesthetic. It is an aromatic herb that improves digestion and relieves spasms, whilst

having stimulant properties. Spearmint can also be used to treat indigestion, gas, colic and some upper respiratory tract infections (Weiss, 1994).

2.5.4 Fennel (*Foeniculum vulgare*)

Plate 4 Fennel

Fennel is a tall hardy herbaceous perennial; it is an aromatic herb with a sweet licorice taste. It forms clumps, with deep roots, hollow stems and glossy, feathery leaves. Tiny, dull, yellow flowers appear in umbels in the summer, followed by oval brown seeds. It grows wild throughout the Mediterranean area where it can grow up to 2.1 meters in height. The leaves, stem, roots and seeds are all used for medicinal purposes (Bremness, 1991). Fennel is a diuretic herb that relieves digestive problems, increases lactation, relaxes spasms and reduces inflammation with expectorant, carminative and aromatic properties. When taken internally it can be used as an antispasmodic, diuretic, pain and fever – reducer and has antimicrobial properties. Its mildly estrogenic effect can have a calming effect on the bronchial tubes. Fennel can also be used to aid digestion and relive indigestion and gas (Weiss, 1994)

Chapter 3

Methodology

3.0 Methodology

3.1 The Study Subjects and Site

The animals used in this study are all captive, socialised wolves kept at the UK Wolf Conservation Trust near Reading, Berkshire.

3.1.1 The UK Wolf Conservation Trust

The UK Wolf Conservation Trust was formed in 1995 by the late Roger Palmer. The Trust aims to dispel the many myths and misconceptions that surround wolves. In order to do this the Trust has nine captive ambassador wolves, all of which have been socialised as cubs to allow them to accept humans. It is because the wolves are socialised that they regularly visit schools, shows and attend seminars which in turn helps the Trust achieve its aims. Roger's vision was to use the funds raised in the UK to help keep wolves in the wild throughout the world especially throughout Europe. Although Britain lost its wild wolves in the 1700's, there are many British people who care passionately about wildlife and our lost indigenous species and in turn are keen to help support wolves in other countries (UKWCT, 2007).

The aims of the Trust are:

- To enhance the conservation, scientific knowledge and public awareness of wolves and their habitats.

- To provide opportunities for both ethological research and for people to interact with wolves.
- To improve the chances of survival for wolves in the wild.
- To run education programmes for schools, conservation and other organisations.

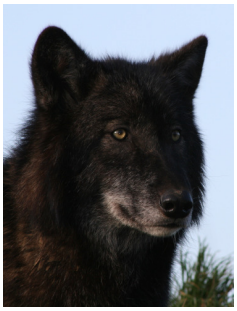
The Trust has many strong links and partnerships throughout the world with different groups and organisations working together to help both communities and wildlife to live happily alongside each other. This is especially important for wolves as they have been and continue to be persecuted in many countries. Ensuring a happy co-existence between humans and wolves is the key to ensuring their long term survival in the wild (UKWCT, 2007).

3.1.2 The Wolves

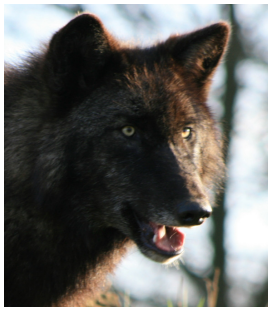
All nine wolves from the UK Wolf Conservation Trust are involved in this study; they are kept in three packs, each containing three wolves, one male and two females.

The Mackenzie Pack: This is the youngest pack of wolves kept at the Trust; the pack members are alpha male Torak, alpha female Mai and beta female Mosi. All three were born in April 2006 and hand reared at the trust from the age of 3 weeks. Mosi and Mai are full sisters born at Dartmoor Zoological Park, whilst Torak was born at the Anglian Wolf Society. This is the only group to have no known health issues during the course of this study (UKWCT, 2007). The plates on the next page show the three pack members.

(Plate 5, Torak)



(Plate 6, Mai)



(Plate7, Mosi)

The European Pack: This pack consists of three siblings, alpha male Alba, alpha female Latea and beta female Lunca; all three were born at the Trust on the 3rd May 1999 and are of special significance to the UK Wolf Conservation Trust as they were the first European wolves to be successfully born and reared to adulthood in the UK since their extinction in the mid 1700's. This group has some specific health issues; apart from their age, which if they were wild wolves would be considered old; alpha male Alba is lucky to be alive after a freak accident in 2005 threatened his life. He was found paralysed by volunteers after running into a tree; x-rays and scans revealed that he had fractured one of the vertebrae in his neck. It was decided to give Alba a chance of recovery; as he is a socialised wolf it was possible to administer his medication and carry out physiotherapy without causing him too much stress. This would not have been possible with a wild wolf or even most captive wolves. After nursing him through the initial injury and shock his recovery continued slowly from being completely paralysed to being able to walk 'crab like', to walking with only a slight twist. He has retained his position at the top of the pack and in 2007 started swimming therapy to strengthen his muscles and improve his mobility still more. As a result of his injury Alba suffers from the early onset of arthritis. This is just one type of illness that could be potentially successfully treated through the

use of medicinal herbs (UKWCT, 2007). The plates below show the three pack members.

(Plate 8 Alba)

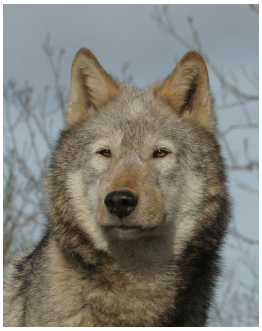
(Plate 9 Latea)

(Plate 10 Lunca)

The North American Pack: This is the oldest pack of wolves at the Trust. It is made up of alpha male Kodiak, alpha female Duma and beta female Dakota. Kodiak is the oldest wolf at the Trust; he was born in April 1994 at Woburn Safari Park in Bedfordshire. Kodiak's pack mates are his younger sisters Duma and Dakota, both of whom were born in May 1998 also at Woburn Safari Park. This group have the most medical conditions, being the oldest wolves at the Trust. Kodiak suffers from arthritis in his pelvis and back legs; on top of this he also suffers from a skin condition which results in boils appearing on his back; these boils, during the summer especially, are susceptible to fly strike. Dakota has suffered from cancer for the last 12 months, she is currently being treated with steroids and a range of complementary herbal therapies; so far she has outlasted most domestic dogs diagnosed with the same condition (UKWCT, 2007). Plates showing the wolves from this pack can be seen on the following page.

(Plate 11 Kodiak)

(Plate 12 Duma)



(Plate 13 Dakota)

3.1.3 The Site

The wolves live in three, natural enclosures which are approximately 2 acres in size, at the UK Wolf Conservation Trust, Butlers Farm, Beenham near Reading. Each enclosure is laid to grass with a range of native trees and bushes planted (as seen in plate 14 below), these included apple and pear trees. Wild flowers are also encouraged to grow in the enclosures which allow them to be as natural as possible. An aerial map of the site can be seen in Appendix II

Plate 14 Torak in one of the Trust's enclosures

3.2 The Herbs

Before this study could be carried out, a list of possible herbs was submitted to the UK Wolf Conservation Trust to be checked by Herbal Vet Nick Thompson who regularly sees the Trusts wolves.

3.2.1 Herb Planters

In order to carry out this study, planters needed to be designed that would provide for the needs of the herbs and be wolf-proof so that the herbs could be protected enough for the wolves to have access but not destroy them completely. Planters were designed to be deep enough to accommodate the plants that were to be accessible to the wolves and a control plant of the same species to be planted out of reach of the wolves. Photographs below can be seen below showing a planter under construction (plate 15) and situated in one of the enclosures (plate 16).

Plate 15 Planter under construction

Plate16 Planter in-situ in one of the enclosures

The planters were constructed from second hand water troughs formally used by a livery yard in paddocks for horses, the ball cock etc were removed from the troughs and holes drilled in the bottom to allow drainage. The lids were constructed to fit the troughs by Technician Owain Wilford from Wiltshire College, Lackham; the mesh allows the plants to grow through so that the foliage and tips are accessible to the wolves, but prevents the wolves from pulling or digging up the herbs (see plate 17 below). The lids are secured to the troughs with pins which prevent the lids from being lifted off unintentionally. The planters were placed in the holding areas of each enclosure so that the wolves could easily be shut out allowing safe access to the planters when measuring and changing the herbs. Two weeks was allowed between introducing the planters and the start of this study to give the wolves to opportunity to get used to having an unusual object in their enclosure.

(Plate 17 A wolf proof lid for the planter

As already stated, the herbs chosen for use in this study include Rosemary (*Rosmarinus officinalis)*, Thyme *(Thymus vulgaris)*, Spearmint *(Mentha spicata)* and Fennel

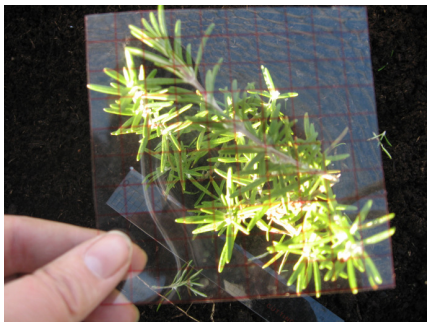
(*Foeniculum vulgare*). These herbs were chosen because of their hardy properties, which was necessary as this study took place during the winter months. Through writing to garden centres and nurseries close to Wiltshire College Lackham, near Tidworth, Wiltshire and close to Beenham near Reading, Berkshire; support was received from Whitehall Garden Centre, Lacock, Wiltshire in funding the cost of the plants. A copy of the letter sent can be found in Appendix III

3.3 Method

This study was conducted using the following method. The data collection took place over a period of 10 weeks. Two plants of each herb were planted in the specially designed planter for 1 week. One plant was to be accessible to the wolves whilst the other was planted at a lower level to prevent the wolves gaining access, this lower plant was to be used as a control to measure any possible impact on the plants other than the wolves, for example frost. Plate 18 below shows the two levels in which the herbs were planted.

(Plate 18 - 2 levels inside the planter

The maximum height, width and total area were measured as the herbs were planted and again, before the herbs were taken out, after a period of one week. The area of the plants was measured using a 100 cm^2 grid made from clear plastic; this was placed on the top of the plants and the area measured through the plastic grid (see plate 19 below) for an example of the plant area being measured) a copy of the 100cm^2 grid can be found in Appendix IV



(Plate 19)

The herbs were assigned to the packs randomly so that weather damage could be ruled out of the results. A second control was used in the form of grass taken from the wolves own enclosures, the aim of this was to rule out the novelty factor of plants appearing in the planters. A record book was provided in the volunteer room at the Trust so that a record could be kept of any noticed interactions between the wolves and the herbs.

Each pack was exposed to each herb for one week; it took a period of 5 weeks for each pack to have access to each herb plus the grass control. Once each pack of wolves had seen the herbs the first time, they were all placed in the enclosures for a second time repeating the experiment, still in a random order. The wolves were locked out of their holding areas each time a new herb was placed in the planters; there were two reasons for

this; the first was to allow safe access to the planters whilst the herbs were being measured and changed, the second reason was to keep the wolves away from the planters for a set period of time after the herbs had been changed; this was to allow the initial interest shown by the wolves in the activity in their enclosure to wear off before allowing them access to the herbs. This time period was for one hour and was standard for each pack and each time the herbs were changed. A copy of the results table used can be seen below.

(© = control plant) Measurements taken in cm and cm2 (for area)			Start of week			End of week			Difference		
Date	**Pack**	**Plant**	**Height**	**Width**	**Area**	**Height**	**Width**	**Area**	**Height**	**Width**	**Area**
	1 - North Americans	Rosemary									
	1 - North Americans	Rosemary ©									
	2 - Europeans	Thyme									
	2 - Europeans	Thyme ©									
	3 - M, M & T	Mint									
	3 - M, M & T	Mint ©									

(Table 1 = Results were recorded in a table similar to this)

Photographs showing the measurement of the herbs and some of the behaviour shown by the wolves towards the herbs can be found on the disc in Appendix VIII.

3.4 Analysis of Data

The measurements recorded in the designed results table were used to work out the difference between the start and end measurements; it was these differences that were used to evaluate the interest shown by the wolves in the herbs. Evaluations were also carried out on the data looking for differences between the first and second exposures,

and for differences between the different packs and their preferences for different herbs. The observations recorded by staff and volunteers at the trust have also been used in the evaluation of this data. Graphs and pie charts have been used to graphically show the results of this study.

3.4.1 Statistical Analysis

On completion of the data collection the results were analysed using an ANOVA (Analysis of Variance) statistical analysis computer programme. This test is used when comparing three or more treatments / samples. Using this test avoids the possibility of producing false negatives which may be possible if a standard T-test was used. The ANOVA will be used to identify the significant differences or similarities in the relationships between the results.

The primary analysis was to look at the interest shown in the herbs provided; in particular looking at the level of interest shown in the herbs in comparison with the grass control.

The secondary analysis looked at the differences between the packs and their interest levels in the herbs; it also looked for any differences between the wolves' first and second exposures of the herbs to see if any interest shown by the wolves the first time they were exposed to the herbs was maintained during the second exposure.

Chapter 4

Results

4.0 Results

The differences in the measurements taken from each of the herbs were used to construct the figures and tables within this chapter; a full set of results is available in Appendix V.

Each pack of wolves was randomly given a different herb each week over a period of five weeks; this was then repeated over an additional five weeks in order to compare any difference in the consumption of the herbs. A bar chart showing the full set of results can be seen in figure 1. The chart shows that in each pack and in both repeats that the most popular herb was Rosemary. This is confirmed when the average consumption for each herb, for each pack is worked out. These results can be seen in figure 2. where the mean consumption of Rosemary can be seen to be much greater than the consumption of the other herbs.

Figure 2. also shows the interest in the different species of herb by each pack and this is illustrated in a clearer format in figures 3a, 3b and 3c. These pie charts clearly show that the North American pack showed the most interest in all four species of herb, whilst the European pack showed interest in three out of the four species. The Mackenzie pack showed the least interest in the herbs, choosing to consume part of only two out of the four species of herb offered.

The values used to conduct the ANOVA statistical tests are illustrated in table 2. To ensure clarity each pack was given the following numbers.

Pack 1 North American

Pack 2 European

Pack 3 Mackenzie

There was very little interest shown in the grass which was transplanted from the wolves own enclosures into the planters. There was also very little difference in the before and after measurements of the control plants, planted in the lower level of the trough.

The results of the ANOVA statistical test showed that there is no statistical difference in the amount of herbs consumed by the three different packs *(**P** = 0.964, **df** = 26)*, there is also no statistical difference in the results of the first and second exposures *(**P** = 0.444, **df** = 24)*. Pie charts showing the differences in the first and second exposures can be seen in figure 4a and 4b.

There was however a significant difference shown in the amount of herbs consumed *(**P** = 0.001, **df** = 23),* with rosemary being consumed by a significantly greater amount than the other three.

Through observations noted by staff and volunteers at the trust, it has been noticed that the wolves only take interest in the herbs for a period of 2 days after they were planted. A full record of these observations can be found in Appendix VI

Exposure	Pack number	Herb	Height	Width	Area
1	1	Fennel	7	0	3
1	2	Fennel	4	0	0
1	3	Fennel	0	0	0
1	1	Grass	6	0	0
1	2	Grass	2	0	0
1	3	Grass	1	0	0
1	1	Mint	2	4	6
1	2	Mint	1	5	11
1	3	Mint	0	0.5	0
1	1	Rosemary	18	7	56
1	2	Rosemary	17.5	12.5	39
1	3	Rosemary	20	8.5	58
1	1	Thyme	1.5	1.5	23
1	2	Thyme	0.5	0	0
1	3	Thyme	4.5	0	0
2	1	Fennel	1	0	0
2	2	Fennel	1	1	3
2	3	Fennel	1	0	0
2	1	Grass	0.5	0	0
2	2	Grass	0.5	0	0
2	3	Grass	0.5	0	0
2	1	Mint	1.5	0	0
2	2	Mint	0.5	0	0
2	3	Mint	1.5	0	0
2	1	Rosemary	14	11.5	50
2	2	Rosemary	13	7	61
2	3	Rosemary	15	5.5	52
2	1	Thyme	1	0	0
2	2	Thyme	0.5	0	0
2	3	Thyme	3.5	1	5

Table 2 – Data used in ANOVA analysis

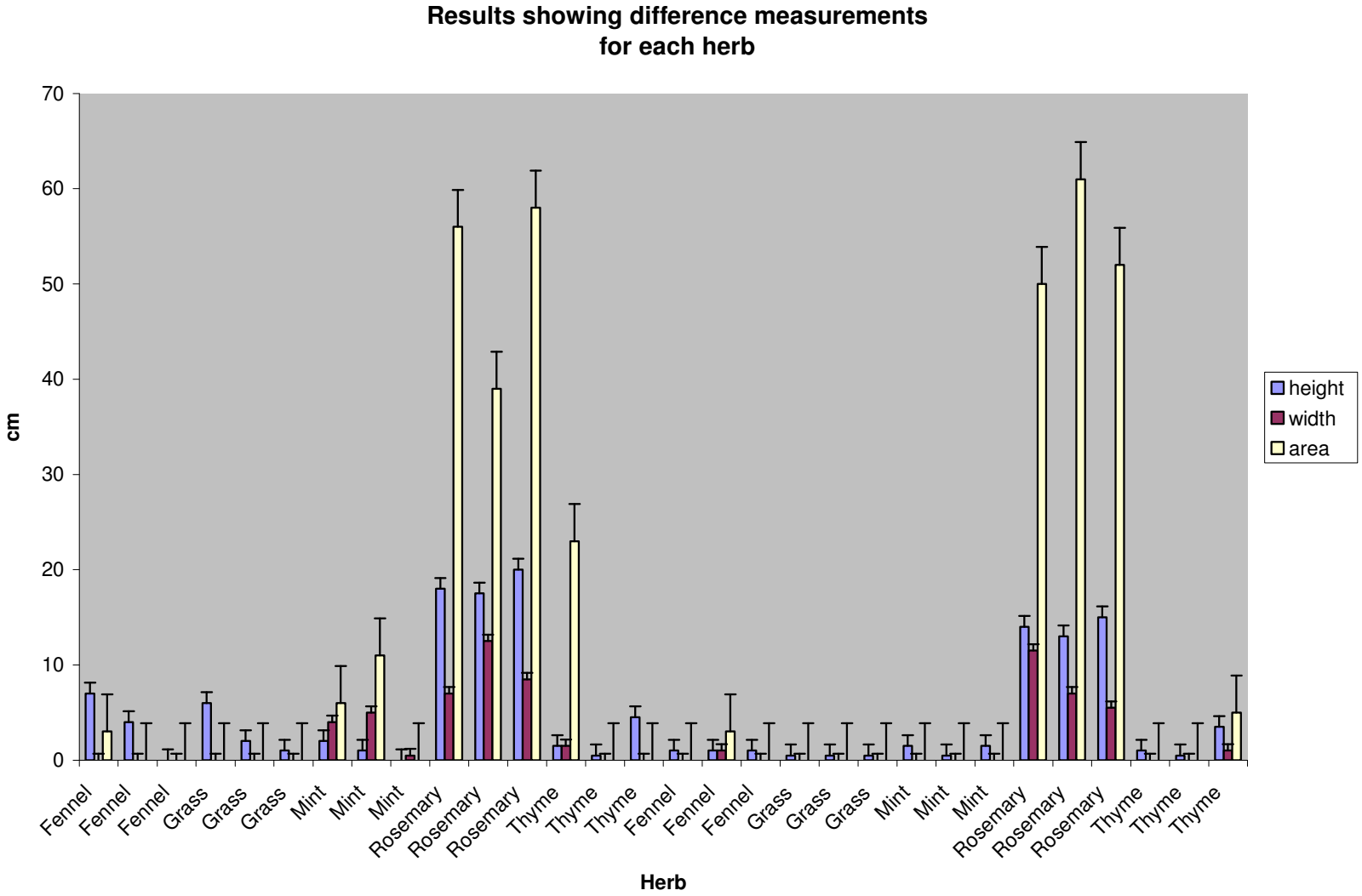


Figure 1. Full set of results

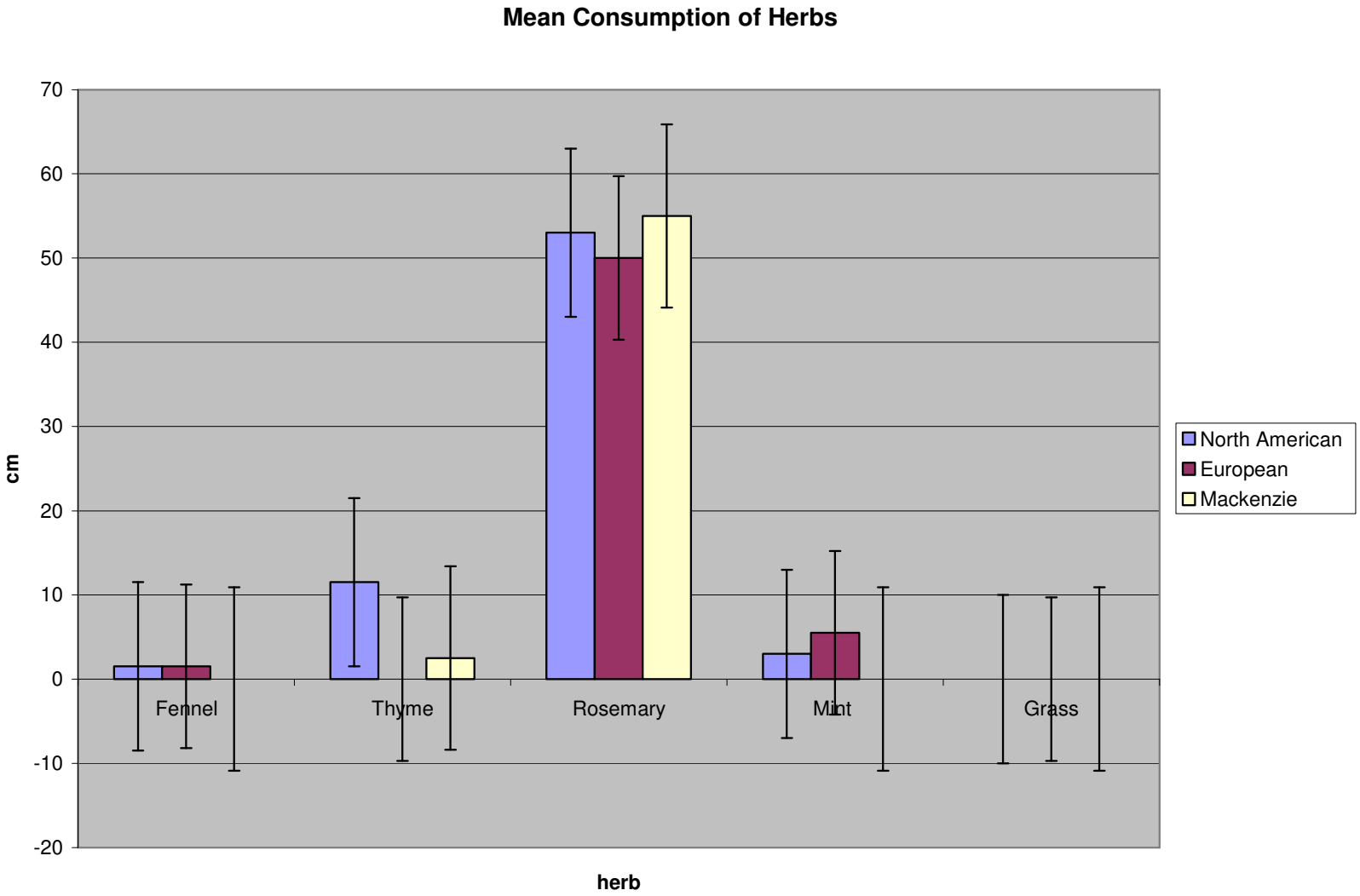


Figure 2: Mean consumption of herbs by each wolf pack

Figure 3a

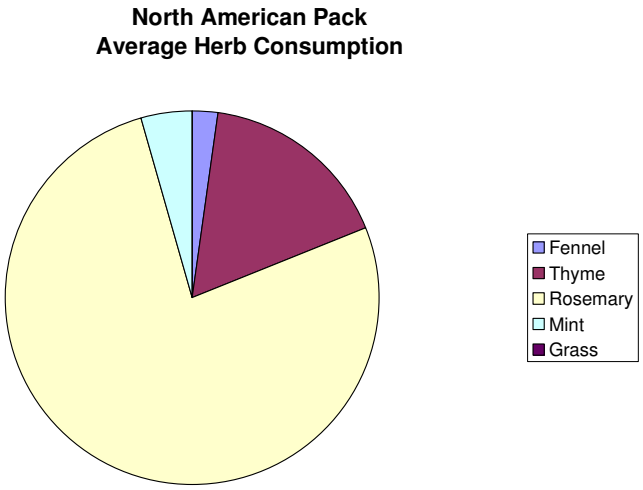


Figure 3b

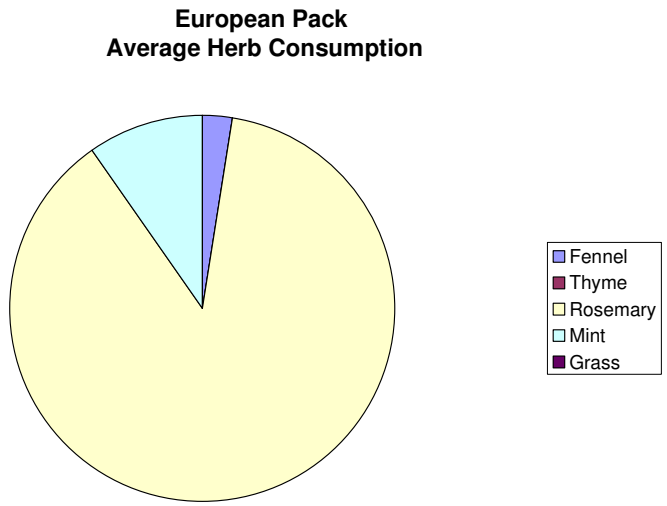
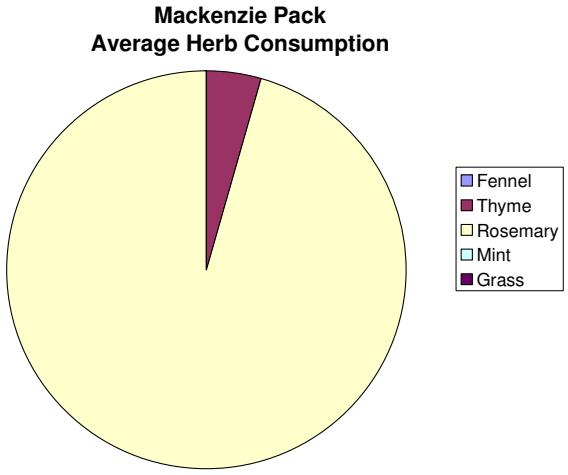
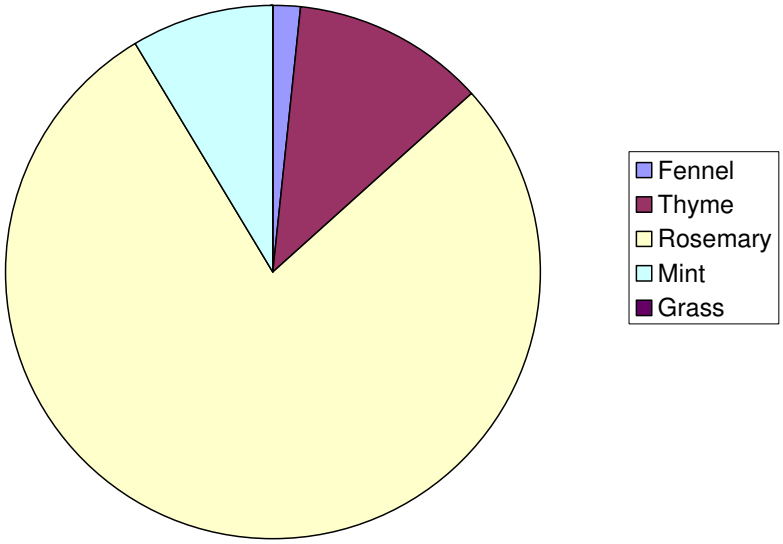


Figure 3c



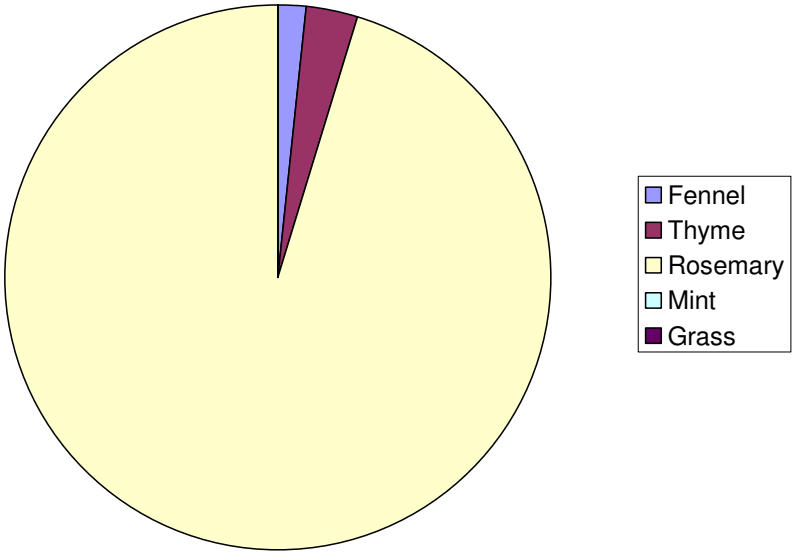
Exposure 1: Average consumption of herbs

Figure 4a



Exposure 2: Average consumption of herbs

Figure 4b



Chapter 5

Discussion

5.0 Discussion

Analysis of the results gathered during this study have shown that there is a significant interest in the herbs given to the wolves *(**P** = 0.001, **df** = 23*), there is no significant difference in the interest shown by the three packs of wolves *(**P** = 0.964, **df** = 26*), and there is also no significant difference between the first and second exposures of the herbs to the wolves (***P*** *= 0.444,* ***df*** *= 24*).

The North American wolves showed the most interest in the herbs provided, consuming a percentage of each herb and in particular thyme and rosemary. Being the oldest pack at the UK Wolf Conservation Trust, this result was unexpected as it could be considered that it would be more usual for younger animals to be more adventurous in what they try, this study has shown the opposite with the youngest pack taking interest in the least species of herbs. The European pack showed a greater interest in the mint that the North American pack but an equal amount of interest in the fennel. The Mackenzie pack meanwhile only showed interest in the rosemary and the thyme.

Having no significant difference in interest between the packs could link to the reasoning behind the area of zoopharmacognosy, as suggested by Rodriguez and Wrangham in 1993, in that animals will experiment with different plants motivated by the desire to reduce the symptoms of disease or injury that they may possess. The wolves could, in the course of this experiment, have been sampling the herbs provided, possibly motivated by scent, to better understand the new editions to their enclosures. This is enforced by the

observations made by the staff and volunteers from the UK Wolf Conservation Trust as the wolves were only seen interacting with the herbs during the first two days after the herbs were planted in the enclosure; there were no observations made during the latter half of the week.

The lack of a significant difference between the first and second exposures shows that the wolves maintained their interest in the herbs throughout the experiment, and that it wasn't just an initial curiosity in a new feature in their enclosures. The lack of a difference in the measurements taken from the control herbs confirms that the difference in the herbs accessible by the wolves was caused by the animals themselves and not by other factors such as snow or frost.

It is also interesting to note that there was very little interest taken in the grass that was planted in the herb troughs from their own enclosures; this shows that it was the herbs themselves that attracted the wolves and not the fact that they were in the trough planters, ruling out the novelty factor.

Unfortunately, due to the lack of previous research on self-medication in wolves or any other large carnivore; or research into the use of the medicinal herbs given to the wolves during this study, on animals in general; it is not possible to link the wolves' preferences for the herbs with their current medical conditions. Although as previously noted medicinal plants have been used by humans and animals similarly, this has been seen in a study carried out by Michael Huffman in 1987 on chimpanzees in the Mahale Mountains.

This study experienced some limitations; the first being the time of year during which the study was carried out. The winter months meant that only hardy evergreen species could be successfully used as those with a less hardy nature tend to die back during the winter months making them unsuitable for this study. The second problem is that the planters took longer to make than anticipated, this in turn delayed the start of the experiment. The wolves were the cause of the third limitation as they occasionally uprooted the herbs dropping them on their sides, which put them out of reach for the rest of the week until the herbs were measured and changed.

Chapter 6

Conclusion

6.0 Conclusion

The significant findings of this research allow the null hypothesis to be rejected and the alternative hypothesis to be accepted. It can be seen that the wolves showed a significant amount of interest in the herbs placed in their enclosures; and in one herb in particular. Each pack showed a similar amount of interest in the herbs and there was no significant difference between the first and second exposures of the herbs to the wolves.

Whether the interest shown is as a result of the wolves making use of the medicinal properties of the herbs, or just showing a general interest in a new addition to their environment would take further investigation. It may be possible in the future to use medicinal plants in the enrichment of captive carnivores in a similar way as to how it is currently used with some species of captive primate.

The planters that were designed and produced were successful, as they remained fully intact and offered the plants sufficient protection whilst at the same time allowing the wolves' limited access. Photographic evidence of the wolves' impact on the herbs can be seen in the photos on the disk in appendix VIII.

The interest shown by the wolves in the herbs provided, over the period of this study, demonstrates that, although they are on the whole carnivores, they will investigate new plants that enter their environment. It is also possible that one of the reasons for the interest shown could be as a result of trial and error behaviour by the wolves to work out

the possible benefits of the new plants. That it was the older wolves that showed the most interest in the breadth of herbs offered was an interesting aspect as, because of age and health problems, they potentially had the most to gain. The outcome of this research confirms the aim of this investigation which was to show that there is a possibility that wolves could use common herbs to self medicate.

Chapter 7

Evaluation and Further Research

7.0 Evaluation and Further Research

7.1 Evaluation

This study, on the whole, achieved its main aim and has provided original evidence that it may be possible that large carnivores such as wolves do use medicinal plants in self-medication. The methodology adopted is unique to this study and has proven to be an effective way of measuring the consumption of each herb by the three wolf packs. The documentation of the measurements from each herb was time consuming and gaining access to the enclosures could be difficult if the wolves were not locked out in advance. The photographs have documented some of the interest shown by the wolves, although their behaviour towards the herbs, other than the occasional observation, was not recorded so it is not known to what extent each member of the three different packs showed interest in the herbs, only the total group interest is recorded.

This is a relatively small study, on a small sample of captive wolves, over a short period of time, using only a tiny selection of the world's medicinal plants. A long term study would provide the opportunity to investigate these findings further; it is only through such a study that evidence can be gathered to confirm whether there is a connection between the plants that are provided and the wolves that choose to consume them.

7.2 Further Research

With zoopharmacognosy being a relatively new area of research, there are many opportunities to both extend this study further or to carry out research into self-

medication carried out by wild wolves or other carnivores. Should further research be carried out it would need to be on the basis of a long term study if it was to be successful. In previous studies looking at wolf diet, scientists have noted the presence of grass, seed and other plant matter. Any future research into self-medication on either captive or wild wolves should include scat analysis to look at the amount of plant matter ingested, particularly if, as in this study, specific plants are provided in a controlled manner. Consideration should also be given to the possibility of carrying out a behaviour study, particularly with captive wolves into the interest shown in the herbs.

This and possible further research could change the way carnivores, particularly non-obligate carnivores are kept in captivity; these studies could impact on the types of enrichment provided for such animals, possibly giving zoos and other animal collections the opportunity, in the future, to provide specific plants on the basis that the animals will use them in self-medication when needed.

Chapter 8

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Appendix I

Replies to Research Enquiry

The following e-mail was sent to biologists and herbalists from all over the world, the replies that were received can be seen below:

Dear (name)
I was wondering if you could help, I am a mature student in the UK studying for a Bachelor of Science with Hons in Animal Science. For my dissertation I have been looking at the possibility of self medication in large carnivores (particularly wolves). This study has been carried out at the UK Wolf Conservation Trust where I work, and has shown some interesting results with the wolves showing significantly more interest in one herb over the others.

I am looking for any background research on self-medication in large carnivores, there has been a small amount carried out on different species of primates, but as it is a relatively new area of research there is very little that I have found.

Have you heard of any such research? Or made any notes during your studies on plant matter during scat analysis? Any help you can offer would be very much appreciated. Even if it is to say that you have heard of no such research as this would back up my findings for my literature review.

Thank you for taking the time to read this email and thank you in advance for you reply.

Yours

Vicky Allison-Hughes
UK Wolf Conservation Trust

Hi Victoria,

I can't think of anything off hand, and could not find anything in Wolves, Behavior, Ecology, and Conservation, edited by Mech and Boitani.

The closest I can come is the way wolves, like dogs, will occasionally gobble grass and then vomit, which I presume they do to deliberately get rid of something in their stomachs.

I forwarded your e-mail to three other people who may be able to come up with other species if not specifically wolves. I told them you would probably prefer animals other than primates.

Pat Goodmann
Wolf Park

Vicky,

Sorry, I've not seen anything like this in wolves, and I know of no literature on it.

Dave
www.davemech.org

Dear Vicky,

I tapped my resource for wolf research, Dave Mech, and he mentioned he already responded to you. Have tried to contact two others knowledgeable in wolf behavior and

was not able to find any papers or documentation of this behavior aside from casual observation of our captive wolves eating grass and vomiting when they have a large bone or obstruction in their stomach. I'm sorry I cannot be of more help.

Sincerely,

Jess Edberg
Information Services Director

Teaching the world about wolves.
1396 Highway 169, Ely, MN 55731
http://www.wolf.org

Hi Vicky
Thanks for contacting me. Just a rushed reply as I am out the office today...
I'm glad you are doing research on wolf self-medication. I have heard of no other published research.
I do assume geophagy is commonplace in all mammals but again it is not widely published.
I guess you will have to deal with claims that the captive wolves are not getting a balanced diet and are therefore showing abnormal eating patterns (e.g. pica) but I am confident that the consumption of soils is part of an adaptive strategy for digestive and nutritive balance in the wild. The best review I came across was Kreulen et al 1985 Lick use by large herbivores: a review of the benefits and banes of soil consumption. Mam. Rev 15 pp 107-123. When you look at the impact of soil consumption it becomes apparent why herbivores are not the only group to need it.

As for seeking out particular herbs, I know of no published research on wolves in that area either. There is (as I say in my book) a thin line between diet selection and self-

medication - if any at all. There is no doubt no line for the wolf itself. In order to establish self-medication in wolves, you would do well to fit the published requirements as outlined by Huffman...

The closest material published is perhaps DaSilveria cited in my book and elsewhere showing that the red maned wolf consumes Loberia or Wolf's fruit which keeps kidney worms under control. Velozia 1969 1: 58-60.

If I can be of any further help, just ask.
Best wishes
Cindy Engel

Hi Vicky,
I am sorry to report that I have never heard of any such research on wolves or any other carnivore. Sounds like you have an interesting topic. I would enjoy viewing your results when you are complete. Sorry I could not be of assistance.
Best of luck,
Jeremy Heft

Appendix II

Aerial Map

Appendix II

Site Layout of the UK Wolf Conservation Trust

Enclosure Boundary = ————

UK Wolf Conservation Trust Boundary = ————

Appendix III

Copy of Letter

Date: as postmark

Mrs Victoria Allison-Hughes
99 Sidbury Circular Road
Tidworth
Wiltshire
SP9 7HD
Tel: 01980 846771

The Manager
Nursery / Garden Centre Name
Address

Dear Sir / Madam

I am about to start my final year of BSc Animal Science and Management at Wiltshire College, Lackham. For my final year research project and dissertation, I am investigating the behaviour of wolves with regard to how they might use plants (herbs) to self medicate. Using three wolf packs at the UK Wolf Conservation Trust at Beenham nr Reading; I intend to plant the following herbs in each of their enclosures and monitor how the wolves respond to their presence. The decision on which plants to use was made in consultation with an animal herbalist.

Herbs:

Thyme	Fennel
Rosemary	Mint

As a garden centre close to (college / Tidworth / UK Wolf Conservation Trust at Beenham); I am contacting you to ask whether you would consider sponsoring my research project by supplying 5 each of the herbs listed above. They would need to be fairly mature plants of a reasonable size in order to have an impact and to be able to withstand the initial interest the wolves will undoubtedly show.

In return for your sponsorship, you will receive acknowledgement of your support in the form of a sign placed beside the enclosures at the Trust (please see information leaflet enclosed) and gratitude expressed within my dissertation; which you will receive a copy of.

I do hope that you will be able to help. Should you need any further information or want to discuss my request further, please do not hesitate to contact me.

I look forward to hearing from you

Yours truly,

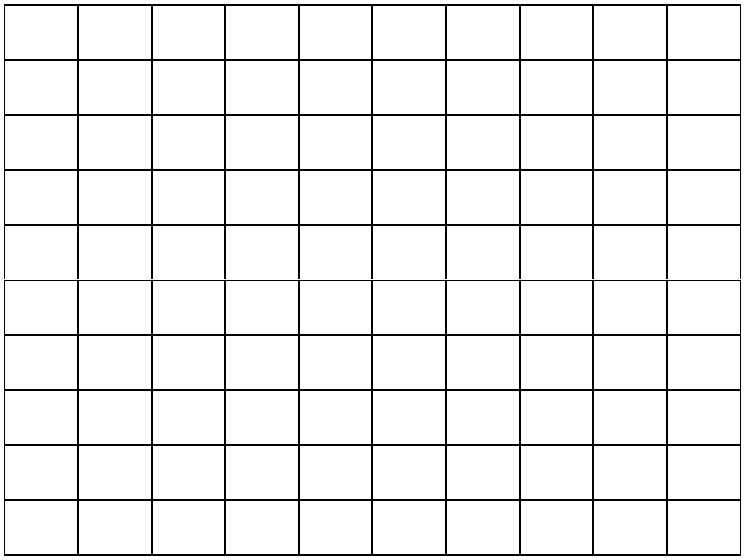
Victoria Allison-Hughes
Email: victoria.allison-hughes@ntlworld.com

Appendix IIII

100cm² Grid

Appendix IV

A copy of the clear 100cm² grid used to measure the area of each herb can be found below.



Appendix V

Results

© = control plant, Measurments taken in cm and cm2 (for area)

			start of week			end of week			Difference		
Date (Exposure 1)	Enclosure	Plant	Height	Width	Area	Height	Width	Area	Height	Width	Area
11/01/2008	1 - North Americans	Rosemary	26	14	70	8	7	14	18	7	56
	1 - North Americans	Rosemary ©	26	12	68	26	12	68	0	0	0
	2 - Europeans	Thyme	13.5	15	88	13	15	88	0.5	0	0
	2 - Europeans	Thyme ©	14.5	14.5	100	14.5	14	100	0	0.5	0
	3 - M, M & T	Mint	15.5	11.5	30	15.5	11	30	0	0.5	0
	3 - M, M & T	Mint ©	9	12	59	9	12	59	0	0	0
30/01/2008	1 - North Americans	Fennel	29	4	55	22	4	52	7	0	3
	1 - North Americans	Fennel ©	15	4	55	15	4	55	0	0	0
	2 - Europeans	Mint	16	16	63	15	11	52	1	5	11
	2 - Europeans	Mint ©	15.5	11	58	15.5	11	58	0	0	0
	3 - M, M & T	Rosemary	31	10.5	69	11	2	11	20	8.5	58
	3 - M, M & T	Rosemary ©	23	11	69	23	11	69	0	0	0
06/02/2008	1 - North Americans	Grass	22	18	100	16	18	100	6	0	0
	1 - North Americans	Grass ©	22	18	100	22	18	100	0	0	0
	2 - Europeans	Fennel	14	4	52	10	4	52	4	0	0
	2 - Europeans	Fennel ©	13	7	45	12.5	4	45	0.5	3	0
	3 - M, M & T	Thyme	16.5	11.5	89	12	11.5	89	4.5	0	0
	3 - M, M & T	Thyme ©	16.5	14.5	90	16.5	14.5	90	2	0	0
13/02/2008	1 - North Americans	Mint	17	19	57	15	14	51	2	4	6
	1 - North Americans	Mint ©	8.5	11	51	8.5	11	51	0	0	0
	2 - Europeans	Grass	23	19	100	21	19	100	2	0	0
	2 - Europeans	Grass ©	16	13	100	16	13	100	0	0	0
	3 - M, M & T	Grass	14	16	100	13	16	100	1	0	0
	3 - M, M & T	Grass ©	11	16	100	11	16	100	0	0	0
20/02/2008	1 - North Americans	Thyme	14.5	14	88	13	12.5	65	1.5	1.5	23
	1 - North Americans	Thyme ©	12	13.5	82	12	13.5	82	0	0	0
	2 - Europeans	Rosemary	28.5	13	43	11	0.5	4	17.5	12.5	39
	2 - Europeans	Rosemary ©	22	10	52	22	10	52	0	0	0
	3 - M, M & T	Fennel	18	8	72	18	8	72	0	0	0
	3 - M, M & T	Fennel ©	14.5	14	47	14.5	14	47	0	0	0

© = control plant, Measurments taken in cm and cm2 (for area)

			start of week			end of week			Difference		
Date (Exposure 2)	Enclosure	Plant	Height	Width	Area	Height	Width	Area	Height	Width	Area
27/02/2008	1 - North Americans	Rosemary	24	12	53	10	0.5	3	14	11.5	50
	1 - North Americans	Rosemary ©	21	8	47	21	8	47	0	0	0
	2 - Europeans	Thyme	15	12	85	14.5	12	85	0.5	0	0
	2 - Europeans	Thyme ©	14	11	83	14	11	83	0	0	0
	3 - M, M & T	Mint	18	10	72	16.5	10	72	1.5	0	0
	3 - M, M & T	Mint ©	5	6	46	5	6	46	0	0	0
05/03/2008	1 - North Americans	Fennel	17	8	71	16	8	71	1	0	0
	1 - North Americans	Fennel ©	15	13	63	15	13	63	0	0	0
	2 - Europeans	Mint	15	7	64	14.5	7	64	0.5	0	0
	2 - Europeans	Mint ©	10	4	56	10	4	56	0	0	0
	3 - M, M & T	Rosemary	23	6	53	8	0.5	1	15	5.5	52
	3 - M, M & T	Rosemary ©	19	5	47	19	5	47	0	0	0
12/03/2008	1 - North Americans	Grass	14	10	100	13.5	10	100	0.5	0	0
	1 - North Americans	Grass ©	11	9	100	11	9	100	0	0	0
	2 - Europeans	Fennel	16	9	71	15	8	68	1	1	3
	2 - Europeans	Fennel ©	15	12	64	15	12	64	0	0	0
	3 - M, M & T	Thyme	16	11	78	14.5	10	73	3.5	1	5
	3 - M, M & T	Thyme ©	14	9.5	69	14	9.5	69	0	0	0
19/03/2008	1 - North Americans	Mint	11	9	67	9.5	9	67	1.5	0	0
	1 - North Americans	Mint ©	6	6	59	6	6	59	0	0	0
	2 - Europeans	Rosemary	22	8	63	9	1	2	13	7	61
	2 - Europeans	Rosemary ©	17	6	58	17	6	58	0	0	0
	3 - M, M & T	Grass	13	12	100	12.5	12	100	0.5	0	0
	3 - M, M & T	Grass ©	9	16	100	9	16	100	0	0	0
26/03/2008	1 - North Americans	Thyme	15	9	73	14	9	73	1	0	0
	1 - North Americans	Thyme ©	12	6.5	67	12	6.5	67	0	0	0
	2 - Europeans	Grass	14	11	100	13.5	11	100	0.5	0	0
	2 - Europeans	Grass ©	9.5	14	100	9.5	14	100	0	0	0
	3 - M, M & T	Fennel	14	11	67	13	11	67	1	0	0
	3 - M, M & T	Fennel ©	12.5	9	59	12.5	9	59	0	0	0

Appendix VI

Staff and Volunteer Observations

Appendix VII

The following observations were made by staff and volunteers at the UK Wolf Conservation Trust of the behaviour shown by the wolves towards the herbs. Unfortunately this record is not complete for the entire period due to staff holidays and periods during which the trust was particularly busy with visitors such as school holidays.

Date	Wolf	Description of Behaviour
11/01/2008	Mosi	Sniffing, licking and chewing at the herb in the enclosure
11/01/2008	Torak	Sniffing, licking and chewing at the herb in the enclosure
11/01/2008	Kodiak	Trying to roll on planter
12/01/2008	Latea	Sniffing and Investigating
12/01/2008	Alba	Sniffing and investigating
30/01/2008	Mosi	Up rooted plant
30/01/2008	Mai	Tried to access up rooted plant – unsuccessful
30/01/2008	Duma	Sniffing, Licking
30/01/2008	Kodiak	Rubbing neck over plant then cocked his leg and urinated on it
04/02/2008	Euro's enclosure	Plant scorched, looks as if its been urinated on
06/02/2008	Torak	Licked and chewed plant
06/02/2008	Mai	Licked and chewed new plant
06/02/2008	Kodiak	Urinated on planter
06/02/2008	Mosi	Up rooted plant, moved away still chewing part of the plant
08/02/2008	Lunca	Sniffing around planter before Latea pushed her away
08/02/2008	Dakota	Sniffing and eating grass in planter
14/02/2008	Dakota	Sniffing plants and around planter
20/02/2008	Mosi	Tried to roll on plant unsuccessful

27/02/2008	Dakota	Walking past planter, stopped moved over to in sniffed plants, came away chewing part
28/02/2008	Duma	Sniffing
05/03/2008	Latea	Investigating plants
06/03/2008	Lunca	Investigating plants
19/03/2008	Kodiak	Defecated on planter!
20/03/2008	Torak	Sniffing, licking and chewing at the herb
26/03/2008	Mai	Investigating herbs
26/03/2008	Kodiak	Chewed then urinated on herb
27/03/2008	Alba	Investigated and chewed on herb

Appendix VII

ANOVA Statistics Analysis

GenStat Release 9.1 (PC/Windows XP) 01 May 2008 09:26:44

Registered to: Royal Agricultural College

GenStat Ninth Edition
GenStat Procedure Library Release PL17

```
  1  %CD '//filestore/users/jNixon'
  2  "Data taken from unsaved spreadsheet: New Data;1"
  3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
  4  READ [PRINT=*; SETNVALUES=yes] _stitle_
  7  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard
on: 1-May-2008 13:10:46

```
  8  DELETE [REDEFINE=yes] Exposure,Pack_number,herb,height,width,area
  9  UNITS [NVALUES=*]
 10  FACTOR [MODIFY=yes; NVALUES=30; LEVELS=2; REFERENCE=1] Exposure
 11  READ Exposure; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Exposure	30	0	2

```
 13  FACTOR [MODIFY=yes; NVALUES=30; LEVELS=3; REFERENCE=1] Pack_number
 14  READ Pack_number; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Pack_number	30	0	3

```
 16  FACTOR [MODIFY=yes; NVALUES=30; LEVELS=5;
LABELS=!t('Fennel','Grass','Mint',\
 17  'Rosemary','Thyme'); REFERENCE=1] herb
 18  READ herb; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
herb	30	0	5

```
 20  VARIATE [NVALUES=30] height
 21  READ height
```

Identifier	Minimum	Mean	Maximum	Values	Missing	
height	0.0000	4.667	20.00	30	0	Skew

```
 24  VARIATE [NVALUES=30] width
 25  READ width
```

Identifier	Minimum	Mean	Maximum	Values	Missing	
width	0.0000	2.167	12.50	30	0	Skew

```
 27  VARIATE [NVALUES=30] area
 28  READ area
```

Identifier	Minimum	Mean	Maximum	Values	Missing	
area	0.0000	12.23	61.00	30	0	Skew

```
  30
  31  "One-way design in randomized blocks"
  32  DELETE [REDEFINE=yes] _ibalance
  33  A2WAY [PRINT=aovtable,information,means; TREATMENTS=herb;
BLOCKS=Pack_number; FPROB=yes;\
  34   PSE=diff; PLOT=*; EXIT=_ibalance] area; SAVE=_a2save
```

Analysis of variance

Variate: area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pack_number stratum	2	36.87	18.43	0.52	
Pack_number.*Units* stratum					
herb	4	12337.87	3084.47	87.52	<.001
Residual	23	810.63	35.24		
Total	29	13185.37			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Pack_number 1 *units* 5	16.8	s.e.	5.2
Pack_number 2 *units* 4	-12.8	s.e.	5.2

Tables of means

Variate: area

Grand mean 12.2

herb	Fennel	Grass	Mint	Rosemary	Thyme
	1.0	0.0	2.8	52.7	4.7

Standard errors of differences of means

Table	herb
rep.	6
d.f.	23

s.e.d.	3.43

Least significant differences of means (5% level)

Table	herb
rep.	6
d.f.	23
l.s.d.	7.09

```
  45  SET [IN=*]
  51  "One-way design in randomized blocks"
  52  DELETE [REDEFINE=yes] _ibalance
  53  A2WAY [PRINT=aovtable,information,means; TREATMENTS=Pack_number;
BLOCKS=Exposure;\
  54   FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; EXIT=_ibalance]
area; SAVE=_a2save
```

Analysis of variance

Variate: area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Exposure stratum	1	20.8	20.8	0.04	
Exposure.*Units* stratum					
Pack_number	2	36.9	18.4	0.04	0.964
Residual	26	13127.7	504.9		
Total	29	13185.4			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Exposure 1 *units* 12	45.7	s.e.	20.9
Exposure 2 *units* 11	50.4	s.e.	20.9

Tables of means

Variate: area

Grand mean 12.2

Pack_number	1	2	3

13.8 11.4 11.5

Standard errors of differences of means

Table	Pack_number
rep.	10
d.f.	26
s.e.d.	10.05

Least significant differences of means (5% level)

Table	Pack_number
rep.	10
d.f.	26
l.s.d.	20.66

```
  55  SET [IN=*]
  61  "One-way design in randomized blocks"
  62  DELETE [REDEFINE=yes] _ibalance
  63  A2WAY [PRINT=aovtable,information,means; TREATMENTS=Exposure;
BLOCKS=herb; FPROB=yes;\
  64   PSE=diff,lsd; LSDLEVEL=5; PLOT=*; EXIT=_ibalance] area;
SAVE=_a2save
```

Analysis of variance

Variate: area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
herb stratum	4	12337.87	3084.47	89.55	
herb.*Units* stratum					
Exposure	1	20.83	20.83	0.60	0.444
Residual	24	826.67	34.44		
Total	29	13185.37			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

herb Rosemary *units* 2	-14.5	s.e. 5.2
herb Thyme *units* 1	17.5	s.e. 5.2

Tables of means

Variate: area

Grand mean 12.2

Exposure	1	2
	13.1	11.4

Standard errors of differences of means

Table	Exposure
rep.	15
d.f.	24
s.e.d.	2.14

Least significant differences of means (5% level)

Table	Exposure
rep.	15
d.f.	24
l.s.d.	4.42

```
  65  SET [IN=*]
  71  "One-way design in randomized blocks"
  72  DELETE [REDEFINE=yes] _ibalance
  73  A2WAY [PRINT=aovtable,information,means; TREATMENTS=herb;
BLOCKS=Exposure; FPROB=yes;\
  74   PSE=diff,lsd; LSDLEVEL=5; PLOT=*; EXIT=_ibalance] height;
SAVE=_a2save
```

Analysis of variance

Variate: height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Exposure stratum	1	30.000	30.000	8.83	
Exposure.*Units* stratum					
herb	4	1011.167	252.792	74.44	<.001
Residual	24	81.500	3.396		
Total	29	1122.667			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Exposure 1 *units* 1 3.67 s.e. 1.6

Tables of means

Variate: height

Grand mean 4.67

herb	Fennel	Grass	Mint	Rosemary	Thyme
	2.33	1.75	1.08	16.25	1.92

Standard errors of differences of means

Table	herb
rep.	6
d.f.	24
s.e.d.	1.064

Least significant differences of means (5% level)

Table	herb
rep.	6
d.f.	24
l.s.d.	2.196

```
  75  SET [IN=*]
  81  "One-way design in randomized blocks"
  82  DELETE [REDEFINE=yes] _ibalance
  83  A2WAY [PRINT=aovtable,information,means; TREATMENTS=Pack_number;
BLOCKS=Exposure;\
  84   FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; EXIT=_ibalance]
height; SAVE=_a2save
```

Analysis of variance

Variate: height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Exposure stratum	1	30.00	30.00	0.72	
Exposure.*Units* stratum					
Pack_number	2	7.22	3.61	0.09	0.917
Residual	26	1085.45	41.75		
Total	29	1122.67			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Exposure 1 *units* 12 14.3

Tables of means

Variate: height

Grand mean 4.7

Pack_number	1	2	3
	5.3	4.1	4.7

Standard errors of differences of means

Table	Pack_number
rep.	10
d.f.	26
s.e.d.	2.89

Least significant differences of means (5% level)

Table	Pack_number
rep.	10
d.f.	26
l.s.d.	5.94

```
  85  SET [IN=*]
  91  "One-way design in randomized blocks"
  92  DELETE [REDEFINE=yes] _ibalance
  93  A2WAY [PRINT=aovtable,information,means; TREATMENTS=Exposure;
BLOCKS=Pack_number;\
  94   FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; EXIT=_ibalance]
height; SAVE=_a2save
```

Analysis of variance

Variate: height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pack_number stratum	2	7.22	3.61	0.09	
Pack_number.*Units* stratum					
Exposure	1	30.00	30.00	0.72	0.404
Residual	26	1085.45	41.75		
Total	29	1122.67			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Pack_number 3 *units* 4 14.3

Tables of means

Variate: height

Grand mean 4.7

Exposure	1	2
	5.7	3.7

Standard errors of differences of means

Table	Exposure
rep.	15
d.f.	26
s.e.d.	2.36

Least significant differences of means (5% level)

Table	Exposure
rep.	15
d.f.	26
l.s.d.	4.85

```
95  SET [IN=*]
```