Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

# Velvet antler removal from red deer

A thesis presented in partial fulfilment of the degree of

**Doctor of Philosophy** 

in Veterinary Medicine

at

Massey University Manawatu, New Zealand

> Pania Flint 2012

For my family

My fiancé, Paul Chambers and my wonderful children, Malina, Niko, Jessica, Robert, William and Thomas.

#### **Short Abstract**

Antlers are unique structures specific to deer that are cast and regrown annually. The harvest of velvet antler is a surgical procedure requiring analgesia and haemostasis. Analgesia is provided by lignocaine ring blocks around the base of the antler in adult stags or compression analgesia with a rubber (*Natur*O<sup>TM</sup>) ring in yearlings. Stags are often sedated with xylazine for velvet removal. This thesis covers four topics requiring refinement or further understanding: variability in local analgesia using lignocaine; the efficacy or noxiousness of *Natur*O<sup>TM</sup> rings; lignocaine residues in antlers; and post- xylazine stag death.

In a video observational study of antler harvest on commercial velvet farms, successful analgesia was characterised by fewer "gaps" in the ring block, greater volume of lignocaine per antler, greater time taken to inject lignocaine, greater number of injections, lower stag age, and other stag factors. There was significant confounding in the data and multiple variables are likely to be important.

In a series of studies investigating the use of *Natur*O<sup>TM</sup> rings, the rings prevented behavioural responses and partially prevented EEG responses to antler removal. On application of the *Natur*O<sup>TM</sup> ring, some animals demonstrated behavioural and EEG responses indicative of noxious sensation. *Natur*O<sup>TM</sup> rings provide analgesia during antler removal and may cause discomfort on application.

It is believed that lignocaine reaches the antler via the blood stream, resulting in tissue residues. Blood flow to the antler was investigated using acrylic models, fluorescent microspheres and cineangiography. Different tourniquet types were tested to determine whether they could prevent lignocaine residues. The arterial supply to the antler is elaborate and tourniquets minimised arterial flow at tensions less than 2kg but did not prevent lignocaine residues.

Occasionally, stags die within 48 hours of sedation with xylazine. In an epizoological survey, this was not related to drug or environmental factors. Reactions involved either acute respiratory distress or delayed death with severe pulmonary oedema. During observations of yearlings sedated with xylazine most demonstrated hypoxaemia that could be reversed by oxygen supplementation or yohimbine administration.

This work provides indicators of how best practice in velvet antler removal may be improved.

## Acknowledgements

Many people have helped with the academic and technical work of this thesis

Firstly to my supervisors:

- Associate Professor Craig Johnson
- Professor Peter Wilson

Thank you also goes to the many others who have given funding, advice or technical assistance for the various experiments:

- Velvet Antler Research New Zealand Funding for all the experiments in this thesis.
- Professor Ralph Ball Physics advice for tourniquet development
- Dr. Paul Chambers Expert academic advice, anaesthesia
- Martin Chesterfield Deer husbandry and handling
- Steven Denby Mechanical engineering tourniquet development
- Merv Foot Engineering
- Theresa Hardisty Fluorescent microsphere extraction and anaesthetic assistant
- Dr. Colin Mackintosh Academic advice and support
- Atieh Magmoddam Video data analysis
- Dr. Jo Murrell Expert advice on anaesthesia and EEG
- Sheryl Mitchinson Laboratory assistance and equipment
- Geoff Purchas Deer husbandry and handling, equipment
- Dr. Murray Woodbury Academic and technical advice and assistance
- Many deer farmers Access to farm and animals, participation in experiments and surveys.
- Many Veterinarians Data collection and survey participation
- BVSc undergraduate students
- My family Enduring patience and support

### **Ethics Approval**

The experiments contained in this thesis have received approval from the Massey University Human Ethics Committee and the Massey University Animal Ethics Committee.

## **Contents**

| Short Abstract                                            |                                                                      |
|-----------------------------------------------------------|----------------------------------------------------------------------|
| Acknowledgements                                          | vi                                                                   |
| Ethics Approval                                           | vi                                                                   |
| List of abbreviations                                     |                                                                      |
| List of figures                                           | xii                                                                  |
| List of tables                                            | xxi:                                                                 |
| Long Abstract                                             | xxxvi                                                                |
| Chapter 1: General introduction                           |                                                                      |
| Note to reader                                            |                                                                      |
|                                                           |                                                                      |
| *                                                         | Zealand4                                                             |
|                                                           | ssessment and prevention                                             |
| <u> </u>                                                  | lvet antler analgesia                                                |
| _                                                         | ler                                                                  |
| _                                                         |                                                                      |
|                                                           |                                                                      |
| Summary                                                   |                                                                      |
| Chapter 2: Lignocaine ring block fo                       | or velvet analgesia61                                                |
| The effect of ring block method on l                      | oehaviour during velvet antler cutting in adult stags63              |
| Chapter 3: <i>Natur</i> O <sup>TM</sup> ring              | 158                                                                  |
| 1                                                         |                                                                      |
| Introduction                                              | 160                                                                  |
|                                                           |                                                                      |
| 3.1 Effects of a <i>Natur</i> O <sup>TM</sup> ring or lig | mocaine ring block on the EEG response to cutting                    |
| the velvet antler of one-year-old red                     | deer under general anaesthesia162                                    |
|                                                           |                                                                      |
| 3.2: Effects of lignocaine and <i>Natur</i> (             | O™ rings on the behaviour of one-year-stags during                   |
| velvet antler removal                                     |                                                                      |
|                                                           |                                                                      |
|                                                           | ine or <i>Natur</i> O™ rings on the EEG spectrum of                  |
| anaesthetised one-year-old red deer.                      | 200                                                                  |
|                                                           |                                                                      |
|                                                           | nocaine ring blocks and/or <i>Natur</i> O <sup>TM</sup> rings on the |
| behaviour of one-year-stags                               |                                                                      |
| D: : (C! 2                                                | 22                                                                   |
| Discussion of Chapter 3                                   | 322                                                                  |

| Chapter 4: Antler blood flow, lignocaine residues and tourniquets                                                                               | 352 |
|-------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Introduction                                                                                                                                    | 355 |
| 4.1 Fluorescent microspheres to determine the effect of tourniquet tension on antler blood flow in adult red deer                               | 356 |
| 4.2 Angiography/cinefluoroscopy to explore antler circulation and determine the effect of tourniquet tension on antler blood flow in adult deer | 378 |
| 4.3 Observations of the effects of a tourniquet on haemorrhage during velvet antler removal in adult stags                                      | 388 |
| 4.4 Tourniquet prototype development                                                                                                            | 392 |
| 4.5: Field testing of tourniquet models stage 1                                                                                                 | 399 |
| 4.6: Studies on the anatomy and arterial pattern of the pedicle and antler using acrylic arterial casting and antler pedicle sections           | 408 |
| 4.7 Lignocaine residues from harvested velvet when different tourniquets were used for haemostasis                                              | 416 |
| Discussion of Chapter 44                                                                                                                        | 435 |
| Chapter 5: The effects of xylazine in red deer                                                                                                  | 140 |
| Introduction 4                                                                                                                                  | 442 |
| 5.1 Case report surveys of stag deaths post sedation with xylazine and xylazine combinations                                                    | 443 |
| 5.2 Hypoxaemia in weaner red deer following sedation with xylazine4                                                                             | 471 |
| 5.3 Observation of yearling stags sedated with "Fentazin" (fentanyl-azaperone-xylazine combination) and given oxygen during sedation            | 490 |
| 5.4 Observation of adult stags sedated with low dose xylazine and remaining standing during velvet antler removal                               | 194 |
| Discussion of Chapter 54                                                                                                                        | 496 |
| Chapter 6: General discussion and future research5                                                                                              | 602 |
| References                                                                                                                                      | 514 |

## List of abbreviations

|                  |                                                                    | Chapter     |
|------------------|--------------------------------------------------------------------|-------------|
| ACVM             | Agricultural Compounds and Veterinary Medicines                    | 1,5         |
| AP               | Antlerogenic Periosteum                                            | 1           |
| AWAC             | Animal Welfare Advisory Committee                                  | 1           |
| BW               | bodyweight                                                         | 1           |
| C                | Close-up video recordings                                          | 2           |
| cAMP             | Cyclic adenosine monophosphate                                     | 1           |
| CI               | Confidence interval (statistics)                                   | 2           |
| CNS              | Central Nervous System                                             | 1           |
| coeff            | coefficient                                                        | 2           |
| CSF              | Cerebrospinal fluid                                                | 1           |
| DINZ             | Deer Industry New Zealand                                          | 1           |
| DMA              | di-methyl-analine, also refered to as 2,6-DMA, DMHA or 2,6-xylid-  | 1,4         |
|                  | ine                                                                |             |
| DRU              | Massey University Deer Research Unit                               | 3,4,5       |
| EA               | Electroanalgesia                                                   | 1           |
| EEG              | Electroenchephalography, electroencephalogram                      | 1,3         |
| ESR              | Environmental Science and Research (Laboratory)                    | 1           |
| ET               | End Tidal (as in ET halothane concentration)                       | 3           |
| F50              | Median Frequency (of a power-frequency EEG spectrum)               | 3           |
| F95              | 95% Spectral Edge Frequency (of a power-frequency EEG spectrum)    | 3           |
| faf              | Focal Analysis File                                                | 3           |
| fl               | Flinch: Small sharp movement                                       | 2           |
| fmf              | Focal Master File                                                  | 3           |
| FMRC             | Fluorescent Microsphere Resource Centre                            | 4           |
| GCMS             | Gas Chromatography Mass Spectrometry                               | 1,4         |
| gdf              | Global definition file                                             | 3           |
| GLP              | Good Laboratory Practice                                           | 4           |
| HA               | Head Arch: Movement of the head and poll so that the nose moves    | 2           |
|                  | backwards and the poll moves upwards                               |             |
| HCO <sub>3</sub> | Bicarbonate (Blood gas analysis)                                   | 5           |
| HD               | Head down: Downwards displacement of the head                      | 2           |
| HPLC             | High Performance Liquid Chromatography                             | 4           |
| HR               | Head rotate                                                        | 2           |
| IARC             | International Agency for Research on Cancer                        | 4           |
| IJEACCM          | Interim Joint Expert Advisory Committee on Complementary Medicines | 1           |
| IV               | Intravenous                                                        | 1           |
| IVRA             | Intravenous regional anaesthesia                                   | 1           |
| LC-MS            | Liquid Chromatography-Mass Spectrometry                            | 1,4         |
| TC 1410          | Enquia Chiomatography wrass opechomeny                             | <b>⊥</b> ,¬ |

| LPS               | Lipopolysaccharide                                                   | 5         |
|-------------------|----------------------------------------------------------------------|-----------|
| MAF               | Ministry of Agriculture and Forestry, New Zealand Government.        | 1         |
| MEGX              | Monoethylglycinexylidide                                             | 1         |
| min               | minutes                                                              | 2         |
| mL                | millilitres                                                          | 1,2,3,4,5 |
| mm                | millimetres                                                          | 2         |
| mmHg              | millimetres of mercury - a unit of pressure, e.g. blood pressure     | 5         |
| MPL               |                                                                      | 4         |
|                   | el, Maximum Residual Level, MPRL Maximum Permissible Residue         |           |
|                   | Level)                                                               |           |
| MRI               | Magnetic Resonance Image                                             | 1         |
| NAWAC             | National Animal Welfare Advisory Committee                           | 1         |
| NDLR              | Negative diagnostic likelihood ratio                                 | 2         |
| NPV               | Negative predictive value                                            | 2         |
| NSAID             | Non-steroidal anti-infammatory drug                                  | 1         |
| NumVelv-          | Number of stags velveted per year : The average number of stags that | 2         |
| Year              | an operator velvets per year                                         |           |
| NVSB              | National Velveting Standards Body                                    | 1         |
| NZDFA             | New Zealand Deer Farmers Association                                 | 1         |
| NZFSA             | New Zealand Food Safety Authority                                    | 1,4       |
| NZVA              | New Zealand Veterinary Association                                   | 1         |
| OR                | Odds Ratio (statistics)                                              | 2         |
| p                 | probability, statistics (as in p-value)                              | 2,3,4,5   |
| PaCO <sub>2</sub> | Arterial carbon dioxide pressure                                     | 5         |
| PaO <sub>2</sub>  | Arterial oxygen pressure                                             | 5         |
| PDLR              | Positive diagnostic likelihood ratio                                 | 2         |
| pН                | pH. A measure of acidity/alkalinity                                  | 5         |
| PIM               | Pulmonary Intravascular Macrophage                                   | 1,5       |
| PPV               | Positive predictive value                                            | 2         |
| RNZSPCA           | Royal New Zealand Society for the Prevention of Cruelty to Animals   | 1         |
| TCM               | Traditional Chinese Medicine                                         | 1         |
| TNF               | Tumour Necrosis Factor (as in TNF-alpha)                             | 5         |
| TP                | Total Power                                                          | 3         |
| $SaO_2$           | Arterial haemoglobin oxygen saturation calculated from blood gas     | 5         |
|                   | analysis                                                             |           |
| SD                | Standard deviation (statistics)                                      | 2         |
| sec               | seconds                                                              | 2         |
| sl                | slight: A small movement of the head                                 | 2         |
| $SpO_2$           | Haemoglobin oxygen saturation (%)                                    | 5         |
| stdev             | standard deviation (statistics)                                      | 2         |
| TCO <sub>2</sub>  | Total carbon dioxide (arterial blood gas analysis)                   | 5         |
| TNF               | Tumour Necrosis Factor                                               | 5         |

| USFDA   | United States Food and Drug Association                                                           | 1 |
|---------|---------------------------------------------------------------------------------------------------|---|
| vax     | Vaccination. As in vaccination gun                                                                | 2 |
| Vol     | Volume, usually in millitres                                                                      | 2 |
| vs      | versus                                                                                            |   |
| W       | Wide angle video recordings                                                                       | 2 |
| YrsVelv | Year velveting - The number of year the operator has been personally velveting stags              | 2 |
| ZT      | Zygomaticotemporal. Injection site for lignocaine for antler analgesia, below the zygomatic arch. | 2 |

# List of figures

| Figure 1.1.1 Annual antler growth cycle in seasonal red deer                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 8  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 1.1.2 Schematic illustration of deer antler growth and mineralisation                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 8  |
| Figure 1.1.3 Vascularisation of the antler pedicle showing the approximate distribution of the arterial supply (red) and venous drainage (blue) following descriptions by Adams (1979) and Suttie et al (1985)                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 0. |
| Figure 1.1.4 Innervation of the antler pedicle showing the approximate course of the zygomaticotemporal (yellow), infratrochlear (green) and auriculopalpebral (blue) nerves and their branches following descriptions by Adams (1979) and Woodbury and Haigh (1996).                                                                                                                                                                                                                                                                                                                                                                                                                      | .1 |
| Figure 1.2.1 (next page). Diagrammatic illustration of the predicted pain conducting, recognition and response pathway to noxious stimulation of the velvet antler (shown in red text and outlines). This begins with noxious stimulation of the antler (red and yellow star), followed by afferent nerve conduction, cerebral processing, efferent nerve conduction and outcome responses. Also shown are the points at which the function of the pathway can be assessed (green text and boxes). The advantages and disadvantages of each method of pain pathway function assessment are shown. The blue text and boxes show points at which the pain pathway can potentially be blocked | .3 |
| Figure 1.2.2 Molecular structure of lignocaine 2-(diethylamino)-N-(2,6-dimethylphenyl) ethanamide                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 7  |
| Figure 1.2.3 (left) Mechanism of action of Lignocaine. Pictures courtesy of Dr. Paul Chambers, Massey University                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 8  |
| Figure 1.2.4 Recommended sites for placement of local anaesthetic to block nerve conduction from the velvet antler and provide analgesia for velvet antler removal. Local nerve sites, as recommended by Adams (1979) are shown as blue shaded circles with an additional site to block the auriculopalpebral nerve recommended by Woodbury and Haigh (1996) in green and the "Ring block" is shown in pink shaded circles                                                                                                                                                                                                                                                                 | 0  |
| Figure 1.4.1 Breakdown pathway of lidocaine in humans shown in black and proposed activation pathways of 2,6-xylidine to potentially carcinogenic compounds shown in blue.  From Nelson et al, 1977                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 4  |
| Figure 1.5.1 Chemical structure of xylazine (N-(2,6-Dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | -7 |
| Figure 2.1 Setup of cameras for filming analgesic application and efficacy. a) View from the wide-angle video recorder showing the author holding the hand-held video recorder filming close up of the farmer administering the local anaesthetic. b) View from close-up video c) Author measuring the "pedicle circumference" to determine "dose" per cm of pedicle d) Close up view of pedicle measurement of 12cm. e) Author writing details onto the recording sheet                                                                                                                                                                                                                   | 56 |
| Figure 2.2 Copy of the original recording sheet used for recording details for local anaes-                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |    |

| thetic application and efficacy study. Note that only some of these details (indicated in bold) were recorded on this sheet as the rest of the information was entered directly onto excel spreadsheet from video recordings at a later time.                                                                                                                                                                                                                                                                                      | 67  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 2.3 Flow diagram for categorisation of each movement for an animal during restraint and manipulation for velvet antler removal                                                                                                                                                                                                                                                                                                                                                                                              | 71  |
| Figure 2.4a Distal-Proximal view of transverse section through antler or pedicle showing regions for recording volume and dose of local anaesthetic, presence of "gaps" and place being cut when a withdrawal response was seen                                                                                                                                                                                                                                                                                                    | 76  |
| Figure 2.4b Diagram showing high, medium and low level for placement of local anaesthetic ring blocks for velvet removal                                                                                                                                                                                                                                                                                                                                                                                                           | 76  |
| Figure 2.5 Correlation matrix of independent and outcome variables for determining which factors affect the likelihood of a withdrawal response during velvet antler cutting                                                                                                                                                                                                                                                                                                                                                       | 88  |
| Figure 2.6 Proportion of stags that did or did not show a withdrawal response during cutting each antler on 16 different farms ranked from the lowest percent withdrawal responses (best) to highest percent withdrawal responses (worst)                                                                                                                                                                                                                                                                                          | 101 |
| Figure 2.7 Proportion of stags that did or did not show a withdrawal response during cutting each antler for 23 different operators ranked from the lowest percent withdrawal responses (best) to highest percent withdrawal responses (worst)                                                                                                                                                                                                                                                                                     | 101 |
| Figure 2.8 Scatterplots of the proportion of stags that showed a withdrawal response during velvet antler cutting versus number of years the operator has been velveting (a), Number of stags velveted per year (b) and total number of stags velveted (Number velveted per year x number of years velveting) (c).                                                                                                                                                                                                                 | 102 |
| Figure 2.9 Scatterplot of stag age versus pedicle "circumference" showing antlers that the stag showed a withdrawal response during cutting (red) and antlers that the stag did not show a withdrawal response during cutting (green)                                                                                                                                                                                                                                                                                              | 105 |
| Figure 2.10 Scatterplots of the time taken to inject local anaesthetic around each antler for antlers for which the stag did or did not demonstrate a withdrawal response during removal (top), operators who were grouped as good, moderate or poor "Skill" (middle) and by operator "Rank" from best to worst (bottom). Lines indicate mean and standard deviation in the upper two graphs and the lower graph also shows the pearson $r^2$ coefficient and p value for likelihood that the slope is equal to zero.              | 109 |
| Figure 2.11 Scatterplots of the time waited after injection of local anaesthetic and before antler removal for antlers for which the stag did or did not demonstrate a withdrawal response during removal (top), operators who were grouped as good, moderate or poor "Skill" (middle) and by operator "Rank" from best to worst (bottom). Lines indicate mean and standard deviation in the upper two graphs and the lower graph also shows the pearson r² coefficient and p value for likelihood that the slope is equal to zero | 110 |
| Figure 2.12 Percent of antlers for which the stag did (red) or did not (green) show a with-drawal response during antler cutting. Antlers were grouped be either left or right antler, first or second antler to be cut or antler closest to the operator (near) or furthest away from the operator (far)                                                                                                                                                                                                                          | 112 |

| Figure 2.13 Number of injections of lignocaine administered around the pedicle of each antler by farmers and vets to provide analgesia for velvet removal from mature stags on commercial farms                                                                                                                                                                                                                                                                                                                       |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 2.14 Volume of lignocaine injected around each antler pedicle by farmers and veterinarians to provide analgesia for velvet removal from mature stags on commercial farms11.                                                                                                                                                                                                                                                                                                                                    |
| Figure 2.15 Schematic diagrams showing the volume of lignocaine injected in each antler "Region" (as shown in Figure 2.4a) by farmers and veterinarians to provide analgesia for velvet antler removal from mature stags on commercial farms                                                                                                                                                                                                                                                                          |
| Figure 2.16 "Dose" of lignocaine injected around the antler pedicle by farmers and veterinarians for providing analysesia for velvet removal from adult stags on commercial farms. (Measured in mL per cm of pedicle "Circumference")                                                                                                                                                                                                                                                                                 |
| Figure 2.17 Schematic diagram showing the "Dose" (mL per cm "circumference") of lignocaine injected at each antler "Region" (as shown in Figure 2.4a) by farmers and veterinarians to provide analysesia for velvet antler removal from mature stags on commercial farms.                                                                                                                                                                                                                                             |
| Figure 2.18 Number of antler "Regions" (Fig 2.4a) that had a "Gap" ("GapsTOT") in the lignocaine ring block when administered by farmers and veterinarians to provide analgesia for velvet removal from adult stags on commercial farms                                                                                                                                                                                                                                                                               |
| Figure 2.19 Stacked bar graph showing the proportion (percent) of stags that showed a withdrawal response (red) and did not show a withdrawal response (green) for antlers that had 0, 1, 2, 3, 4, 5, and 6 or more "Regions" with "Gaps" (Figure 2.4a and 2.21) in the local anaesthetic ring block around the pedicle. The number of antlers in each group is shown above the bars                                                                                                                                  |
| Figure 2.20 Schematic diagram showing the proportion of antlers that had a "gap" in the lignocaine ring block for each of the eight antler "Regions" (as shown in figures 2.4 and 2.21) when administered by farmers and veterinarians for analgesia for antler removal from adult stags on commercial farms.                                                                                                                                                                                                         |
| Figure 2.21 Dorsal view of pedicle cross sections showing the "Regions" designated "lateral side" ("Regions" 1, 8, 7, 6 and 5, and "Regions" designated "medial", ("Regions" 2, 3 and 4) for the purposes of analysing the effect of "gaps" in the lignocaine ring block on the "lateral" or "medial" side of the pedicle on the behavioural response of stags during removal of the same antler                                                                                                                      |
| Figure 2.22 Stacked bar charts showing the proportion of antlers for which the stag showed a withdrawal response (red) or did not show a withdrawal response (green) during velvet antler removal for antlers that had different numbers of "Regions with Gaps" on the "lateral" aspect of the antler (top chart) or "medial" aspect of the antler (lower chart) "Regions" are as shown in Fig 2.21. Note that the lateral aspect was divided into 5 "Regions" whereas the medial aspect was divided into 3 "Regions" |
| Figure 2.23 "Technique" model showing the effect of adding significant variables related to ring block technique on the difference between outcome groups "Withdrawal vs No Withdrawal" (top row of graphs); "good", "moderate" and "poor" operator "Skill" (middle row of graphs) and operator "Rank" (1 = fewest withdrawal responses to 23 = most                                                                                                                                                                  |

| withdrawal responses) in the lower row of graphs                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 2.24 "Effects" model investigating aspects of lignocaine injection technique and stag factors to separate antlers for which the stag that did (red) or did not (green) show a withdrawal response during velvet antler cutting when velvet removal was undertaken by farmers and veterinarians on commercial farms.                                                                                                                                                                                                              |
| Figure 2.25 Scatterplot of the number of injections per antler versus time to inject around each antler when local anaesthetic was injected in a ring block for velvet antler removal. 14                                                                                                                                                                                                                                                                                                                                               |
| Figure 2.26 Scatterplot of the number of injections per antler versus total volume injected around each antler when local anaesthetic was injected in a ring block for velvet antler removal.                                                                                                                                                                                                                                                                                                                                           |
| Figure 2.27 Trend between "dose" and proportion of antlers for which the stag showed a withdrawal response within each 0.25mL/cm pedicle circumference grouping. Proportion withdrawal response was the number of antlers for which the stag showed a withdrawal response divided by the total number of antlers injected with that "dose" range15                                                                                                                                                                                      |
| Figure 2.28 Schematic diagram of the interactions between different factors and their effect on the analgesic efficacy of the ring block as determined by the absence of a withdrawal response. Factors are broadly grouped into those associated with the farm, the animal and the injection technique (operator "Skill"). There is overlap shown between these groupings. Arrows indicate factors that have an influence on each other and the size of the arrows indicated the importance of these influences                        |
| Figure 3.1.1 Anaesthetic procedure and monitoring equipment set-up for EEG recording during velvet antler removal. Top left: Placement of 20 gauge catheter in the facial vein. Top right, intubation standing in the crush by manually advancing the hand and tube through the dental arcade, lower: Maintenance on halothane in oxygen with an out-of-circuit rebreathing system.                                                                                                                                                     |
| Figure 3.1.2 Recording sheet for details of anaesthetic drugs and monitoring during general anaesthesia for recording the electroencephalogram during velvet antler cutting for deer that had been previously treated with either a lignocaine ring block, a NaturO™ ring or no treatment.  16.                                                                                                                                                                                                                                         |
| Figure 3.1.3 Set-up of EEG recording equipment and leads for measuring the electroencephalogram of deer during velvet removal after treatment with either a lignocaine ring block, a NaturO™ ring or no treatment. Two channels of EEG were recorded, one from each cerebral hemisphere. A single earth lead was connected with one of these montages only. Each montage was connected to an individual breakout box which then fed into an amplifier. These were connected to a Powerlab and then to the laptop computer for recording |
| Figure 3.1.4 Diagram showing the positions for placement of the electrodes and connection                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| to the computer recording system for recording electroencephalogram waveforms in anaesthetised yearling red deer during velvet antler removal                                                                                                                                                                                                                                                                                                                                                                                           |
| Figure 3.1.5 Mean (± 95% CI) Respiratory rate, heart rate and end tidal halothane concentration recorded at five-minute intervals in deer under general anaesthesia for recording of electroencephalogram during velvet antler removal after treatment with either                                                                                                                                                                                                                                                                      |

| lignocaine(blue), a NaturO <sup>TM</sup> ring (green) or no treatment (blue). Observation times for each animals have been standardised to the time of cutting (time 0) rather than from induction of anaesthesia                                                                                                                                                                                                                                                                                                                                                                                        | .168 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Figure 3.1.6 (pages 172-173) Individual animal time series plots showing electroencephalogram recordings of raw TP, F50 and F95 for left and right channels (on the same graph) before and after cutting the antler following treatment with either lignocaine, a NaturO™ ring or no analgesia. One second averages shown                                                                                                                                                                                                                                                                                | .169 |
| Figure 3.1.7 (pages 174-175) Individual animal time series plots showing electroencephalogram recordings for TP, F50 and F95 for left and right cerebral hemispheres on the same graph before and after cutting the antler following treatment with lignocaine, a NaturO™ ring or no analgesia. Results are as a percentage of pre-cut baseline and averaged over 15 second epochs                                                                                                                                                                                                                       | .169 |
| Figure 3.1.8 Mean and 95% confidence interval of the mean) Total Power, F95 and F50 recorded from yearling stags under general anaesthesia before and after cutting antler after either no analgesia (Control), Lignocaine (LA) or an NaturO for analgesia. Data is plotted at percentage of baseline value for each deer. 100% = baseline and the dashed lines either side of 100% represent the 95% confidence interval of the baseline recordings.                                                                                                                                                    | .176 |
| Figure 3.3.1 (overleaf) Total power, median frequency and spectral edge frequency averaged over 15-second intervals for the period from five minutes before treatment and ten minutes after treatment with either lignocaine ring block at the base of both antler pedicles (a) or a NaturO™ ring to both antler pedicles (b). Each graph panel represents an individual animal and both left and right channels are shown on the same graph. Time is shown on the x-axis with the time of treatment indicated with a solid black line. The y-axis shows power for TP and frequency (Hz) for F50 and F95 | .203 |
| Figure 3.3.2 Moving average of 15 second means plotted every second for TP, F50 and F95 from encephalogram recording from yearling deer that were treated with either a lignocaine ring block (blue) or NaturO™ ring (green). Time is from -2 minutes to +5 minutes from treatment. Treatment time is indicated by the solid black line                                                                                                                                                                                                                                                                  | .207 |
| Figure 3.3.3 Mean TP, F50 and F95 of the electroencephalogram recorded from yearling deer that had either a lignocaine ring block administered (blue) or a NaturO™ ring applied (green) Error bars are 95% confidence intervals. Time of recording is from two minutes prior to treatment until five minutes after treatment and averaged over 15 second intervals. Arrow indicates the time of treatment. Significant differences are indicated above each graph ^ = significantly different from baseline within each group, * = significant difference between treatment groups                       | .208 |
| Figure 3.3.4 Mean TP, F50 and F95 of the electroencephalogram recorded from yearling deer that had either a lignocaine ring block administered (blue) or a NaturO™ ring applied (green). Error bars are 95% confidence intervals. Time of recording is from five minutes prior to treatment until ten minutes after treatment and averaged over one minute intervals. Arrow indicates the time of treatment. Significant differences are indicated above each graph ^ = significantly different from baseline within each group, * = significant difference between treatment groups                     | .209 |

| Figure 3.3.5 Individual animal 15-second means as a percent of baseline plotted on the same graph panel along with overall group means (thick black line) for Total power (a), F50 (b) and F95 (c) from five minutes before treatment until 10 minutes after treatment with lignocaine.                                                                                                                                                        |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 3.3.6 Individual animal 15-second means as a percent of baseline plotted on the same graph panel along with overall group means (thick black line) for Total power (a), F50 (b) and F95 (c) from five minutes before treatment until 10 minutes after treatment with NaturO™ rings                                                                                                                                                      |     |
| Figure 3.3.7 Schematic diagram showing significant changes from pre-treatment baseline values for F50, F95 and TP for each minute from 5 minutes before treatment to ten minutes after treatment with a lignocaine ring block around the base of the pedicle of yearling stags.                                                                                                                                                                |     |
| Figure 3.3.8 Schematic diagram showing significant changes from pre-treatment baseline values for F50, F95 and TP for each minute from 5 minutes before treatment to ten minutes after treatment with a NaturO™ ring around the base of the pedicle of yearling stags.                                                                                                                                                                         |     |
| Figure 3.4.1 Layout of the crush and observation pens for video recording the behaviour of yearling stags after the application of NaturO™ rings                                                                                                                                                                                                                                                                                               |     |
| Figure 3.4.2 Deer in the observation pen for videoing of behaviour during one hour after placement of local anaesthetic or NaturO <sup>TM</sup> rings. Each deer is marked with a different coloured collar and raddle. The velcro band has been removed from the antler of one deer to show placement of the NaturO <sup>TM</sup> ring                                                                                                        |     |
| Figure 3.4.3 Book to accompany JWatcher software                                                                                                                                                                                                                                                                                                                                                                                               | 224 |
| Figure 3.4.4 Interface for the JWatcher behavioural data collection software showing the global definition for the ethogram of behaviours and descriptions.                                                                                                                                                                                                                                                                                    | 226 |
| Figure 3.4.5 Histograms of the distribution of the proportion of time that yearling stags spent in the "Standing", "Walking" and "Walking backwards" behaviour states during one hour of video observation in a pen either before (pre) or after (post) treatment with either NaturO™ rings, lignocaine, no treatment or a combination of these. "Pre" treatment values are shown in dark fill and "Post" treatment values in light fill       | 233 |
| Figure 3.4.6 Histograms of the distribution of the proportion of time that yearling stags spent in the "Pacing", "Lying down" and "Circling" behaviour states during one hour of video observation in a pen either before (pre) or after (post) treatment with either NaturO <sup>TM</sup> rings, lignocaine, no treatment or a combination of these. "Pre" treatment values are shown in dark fill and "Post" treatment values in light fill. |     |
| Figure 3.4.7 Scatterplots of the proportion of time that yearling stags spent in whole body state behaviours "Pacing", "Walking" and "Walking backwards" during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with NaturO™ rings (NaturO, lignocaine ring blocks(Lignocaine) or no treatment (Control) or an NaturO on one antler and Lignocaine on the other antler (Both).         |     |
| Correlation analysis lines for each of the treatment groups are shown                                                                                                                                                                                                                                                                                                                                                                          | 237 |

| Figure 3.4.8 Scatterplots of the proportion of time that yearling stags spent in either "Standing" (top graph) or "Active" (lower graph) behaviour during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with NaturO™ rings (NaturO), lignocaine ring blocks(Lignocaine) or no treatment (Control) or an NaturO on one antler and Lignocaine on the other antler (Both). Correlation analysis lines for each of the treatment groups are shown                                       | 238 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 3.4.9 Individual value data plots of the proportion of time that one-year-old male deer spent in different whole body state behaviours: "Standing", "Walking" and "Pacing" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.                                                                                                                                                                   | 240 |
| Figure 3.4.10 Individual value data plots of the proportion of time that one-year-old male deer spent in different whole body state behaviours: "Walking backwards", "Circling" and "Lying down" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle                                                                                                                                                     | 241 |
| Figure 3.4.11 Individual data plot of the difference ("post" - "pre") in the proportion of time that one-year-old male deer spent in different whole body state behaviours during one hour of video recording after treatment with either NaturO™ rings, lignocaine ring blocks or no treatment around the antler pedicle compared to no treatment                                                                                                                                                                                            | 242 |
| Figure 3.4.12 Time series plot of the mean proportion of time spent in four different whole body states during each 10 minute period of one hour of video observation in pens. Separate data are shown for "pre" and "post" treatment recordings. Means are for all deer across all treatment groups. Significant differences between "pre" and "post" treatment recordings are shown with an asterix.                                                                                                                                        | 244 |
| Figure 3.4.13 Histograms of the distribution of the proportion of time that one-year-old deer spent in the "Head straight" (top graph), "Head left" (centre graph) and "Head right" (lower graph) behaviour state during 60 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately.                                                                                                                                                                                                                        | 246 |
| Figure 3.4.14 Histograms of the distribution of the proportion of time that one-year-old deer spent in the "Head high" (top graph), and "Head low" (lower graph) behaviour state during 60 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately.                                                                                                                                                                                                                                                         | 247 |
| Figure 3.4.15 Scatterplots of the proportion of time that yearling stags spent in head position state behaviours "Head Straight", "Head Left" and "Head Right", "Head High" and "Head Low" during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with one of 10 treatment combinations of NaturO <sup>TM</sup> rings (NaturO), lignocaine ring blocks(Lignocaine) or no treatment (Control) on either antler. Correlation analysis lines for each of the treatment groups are shown. | 248 |
| Figure 3.4.16 Individual value data plots of the proportion of time that one-year-old male deer spent in different head position state behaviours "Head straight", "Head left" and                                                                                                                                                                                                                                                                                                                                                            |     |

| with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treat-<br>ment on the antler pedicle.                                                                                                                                                                                                                                                                                                                                          | .250 |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Figure 3.4.17 Individual value data plots of the proportion of time that one-year-old male deer spent in different head position state behaviours "Head high" and "Head low" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.                                                                                        | .251 |
| Figure 3.4.18 Individual data plot of the difference ("post" - "pre") in the proportion of time that one-year-old male deer spent in different head position state behaviours during one hour of video recording after treatment with either NaturO™ rings, lignocaine ring blocks or no treatment around the antler pedicle compared to no treatment                                                                                                         | .252 |
| Figure 3.4.19 Time-series plot showing the proportion of time spent in each head position state for each 10 minute period of 60 minutes of video observation of deer in pens either without "pre" or following "post" treatment with NaturO™ rings, lignocaine ring blocks, no treatment or combinations of treatment.                                                                                                                                        | .253 |
| Figure 3.4.20 Histograms of the frequency distribution of the number of times one-year-old male deer put their heads in the "Head straight" (top graph), "Head left" (middle graph) and "Head right (lower graph) position during 30 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately.                                                                                                                               | .256 |
| Figure 3.4.21 Histograms of the frequency distribution of the number of times one-year-old male deer put their heads in the "Head straight" (top graph), "Head left" (middle graph) and "Head right (lower graph) position during 30 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately.                                                                                                                               | .257 |
| Figure 3.4.22 Scatterplots of the number of time that yearling stags demonstrated each head position state behaviour "Head Straight", "Head Left" and "Head Right", "Head High" and "Head Low" during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with one of 10 treatment combinations of NaturO™ rings (NaturO), lignocaine ring blocks(Lignocaine) or no treatment (Control) on either antler. | .259 |
| Figure 3.4.23 Individual value data plots of the number of times that one-year-old male deer demonstrated different head position behaviours "Head straight", "Head left" and "Head right" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.                                                                          | .261 |
| Figure 3.4.24 Individual value data plots of the number of times that one-year-old male deer demonstrated different head position behaviours "Head straight", "Head left" and "Head right" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.                                                                          | .262 |
| Figure 3.4.25 Individual data plot of the difference ("post" - "pre") in the number of times that one-year-old male deer demonstrated different head position behaviours during                                                                                                                                                                                                                                                                               |      |

| one hour of video recording after treatment with either NaturO™ rings, lignocaine ring blocks or no treatment around the antler pedicle compared to no treatment2                                                                                                                                                                                                                                                                                                                                                 | 263 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Figure 3.4.26 Time-series plot showing the mean number of time that one-year-old deer put their heads into different head positions during each 10 minute period of 60 minutes of video observation of deer in pens either without "pre" or following "post" treatment with NaturO <sup>TM</sup> rings, lignocaine ring blocks, no treatment or combinations of treatment. All treatment groups are combined.                                                                                                     | 265 |
| Figure 3.4.27 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different event behaviours "Look up" (top graph), "Nose to ground" (middle graph) and "Lick walls" (lower graph) during 60 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately. All treatment groups are combined.                                                                                                                                        | 267 |
| Figure 3.4.28 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different event behaviours "Flick tongue" (top graph), "Flick ears" (middle graph) and "Shake" (lower graph) during 60 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately. All treatment groups are combined.                                                                                                                                            | 269 |
| Figure 3.4.29 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different event behaviours "Groom self" (top graph), "Groom other" (middle graph) and "Scratch" (lower graph) during 60 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately. All treatment groups are combined.                                                                                                                                           | 270 |
| Figure 3.4.30 Scatterplots of the number of time that yearling stags demonstrated the event behaviours "Look up" (top graph), "Nose to ground" (middle graph)and "Lick the walls" (lower graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO <sup>TM</sup> rings (NaturO), lignocaine ring blocks(Lignocaine) no treatment (Control) or a NaturO <sup>TM</sup> ring on one antler and lignocaine on the other antler (Both). | 273 |
| Figure 3.4.31 Scatterplots of the number of time that yearling stags demonstrated the event behaviours "Flick tongue" (top left graph), "Flick tongue" (top right graph) and "Shake" (lower graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO™ rings (NaturO), lignocaine ring blocks(Lignocaine) no treatment (Control) or a NaturO™ ring on one antler and lignocaine on the other antler (Both).                        | 274 |
| Figure 3.4.32 Scatterplots of the number of time that yearling stags demonstrated the event behaviours "Groom self" (top graph), "Groom other" (middle graph)and "Scratch" (lower graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO™ rings (NaturO), lignocaine ring blocks(Lignocaine) no treatment (Control) or a NaturO™ ring on one antler and lignocaine on the other antler (Both).                                  | 275 |
| Figure 3.4.33 Individual value data plots of the number of times that one-year-old male deer demonstrated different event behaviours "Look up", "Nose to ground" and "Lick                                                                                                                                                                                                                                                                                                                                        |     |

| the walls' during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 77 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 3.4.34 Individual value data plots of the number of times that one-year-old male deer demonstrated the different event behaviours: "Flick tongue", "Flick ears" and "Shake" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.   2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 78 |
| Figure 3.4.35 Individual value data plots of the number of times that one-year-old male deer demonstrated the different event behaviours "Groom self", "Groom other" and "Scratch" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 79 |
| Figure 3.4.36 Individual data plot of the difference ("post" - "pre") in the number of times that one-year-old male deer demonstrated different non-specific event behaviours during one hour of video recording after treatment with either NaturO™ rings, lignocaine ring blocks or no treatment around the antler pedicle compared to no treatment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 82 |
| Figure 3.4.37 Time-series plot showing the mean number of time that one-year-old deer demonstrated different non-specific event behaviours during each 10 minute period of 60 minutes of video observation of deer in pens either without "pre" or following "post" treatment with NaturO™ rings, lignocaine ring blocks, no treatment or combinations of treatment. All treatment groups are combined                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 86 |
| Figure 3.4.38 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different specific head related event behaviours "Rotate left" (top graph), "Rotate right" (middle graph) and "Turn head 360°" (lower graph) during 60 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately. All treatment groups are combined.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 90 |
| Figure 3.4.39 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different specific head related event behaviours "Arch down" (top graph), "Arch up" (middle graph) and "Press head" (lower graph) during 60 minutes of video observation in a pen. "Pre" and "post" treatment values shown separately. All treatment groups are combined.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 91 |
| Figure 3.4.40 Scatterplots of the number of time that yearling stags demonstrated the specific head related event behaviours "Rotate left" (left graph), "Rotate right" (middle graph) and "Turn head 3600" (right graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO <sup>TM</sup> rings (NaturO), lignocaine ring blocks(Lignocaine) no treatment (Control) or a NaturO <sup>TM</sup> ring on one antler and lignocaine on the other antler (Both). All axis are the same scale 2000 or the same sc | 94 |
| Figure 3.4.41 Scatterplots of the number of time that yearling stags demonstrated the specific head related event behaviours "Arch down" (top graph), "Arch up" (lower left graph) and "Press head" (lower right graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO <sup>TM</sup> rings                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |    |

| (NaturO), lignocaine ring blocks(Lignocaine) no treatment (Control) or a NaturO™ ring on one antler and lignocaine on the other antler (Both)295                                                                                                                                                                                                                                                                                                                                                | 5 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| Figure 3.4.42 Individual value data plots of the number of times that one-year-old male deer demonstrated the different head position behaviours: "Rotate left", "Rotate right" and "Turn head 360" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle                                                                                                    | 8 |
| Figure 3.4.43 Individual value data plots of the number of times that one-year-old male deer demonstrated the different head position behaviours "Arch down", "Arch up" and "Press head" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle. ————————————————————————————————————                                                                         | 9 |
| Figure 3.4.44 Individual data plot of the difference ("post" - "pre") in the number of times that one-year-old male deer demonstrated different specific head related event behaviours during one hour of video recording after treatment with either No treatment (Control), NaturO <sup>TM</sup> rings, lignocaine ring blocks or a NaturO <sup>TM</sup> ring on one antler and lignocaine ring block on the other (both).                                                                    | 1 |
| Figure 3.4.45 Time series plots of the total number of times each specific head related behaviour was seen across all animals during each ten minute interval from the start of video observations in pens. "Pre" treatment observations are shown with a solid line and "post" treatment with a dashed line                                                                                                                                                                                    | 6 |
| Figure 3.4.46 Time series plots of the number of times that each deer in each treatment group demonstrated "Specific head related behaviours" during each ten minute interval of one hour of observation "post" treatment with either control (black), NaturO™ rings (green), lignocaine (blue) or NaturO on one antler and lignocaine on the other ("both") (pink). Lines of corresponding colour indicate the mean for each group although the data are not normally distributed              | 7 |
| Figure 3.4.47 Histogram of the number of times that deer demonstrated the specific head related behaviours that were combined to make the "Head Twist" behaviour during one hour of video recording before ("pre") and after ("post") treatment with either no treatment, lignocaine ring blocks, NaturO <sup>TM</sup> rings or a combination of these. All treatment groups are combined. "Pre" and "post" values are shown separately309                                                      | 9 |
| Figure 3.4.48 Scatterplot of the number of times that yearling stags demonstrated the specific head related event behaviours combined as "Twist head" during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO <sup>TM</sup> rings (NaturO), lignocaine ring blocks(Lignocaine) no treatment (Control) or a NaturO <sup>TM</sup> ring on one antler and lignocaine on the other antler (Both). Axis are the same scale | 0 |
| Figure 3.4.49 Individual value data plots of the number of times that one-year-old male deer demonstrated different head position behaviours combined as "Head twist" during one hour of video recording before "pre" or after "post" treatment with a combination of either NaturO <sup>TM</sup> rings, Lignocaine ring blocks, no treatment (control) or a lignocaine                                                                                                                         |   |

| ring block on one antler and NaturO™ ring on the other (both)31                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 3.4.50 Individual data plot of the "difference" ("post" - "pre") in the number of times that one-year-old male deer demonstrate specific head related event behaviours combined to give a "Head twist" behaviour score during one hour of video recording after treatment with either No treatment (Control), NaturO <sup>TM</sup> rings, lignocaine ring blocks or a NaturO <sup>TM</sup> ring on one antler and lignocaine ring block on the other (both)31                                                                                                                                                                                                                                                                                             |
| Figure 3.4.51 Time series plots of the frequency of combined specific head related behaviours as "Head twist". Top graph: Total number of times that "Head twist" was observed in one year old male deer in all treatment groups during each 10 minute interval after the start of video recording for one hour without (pre) and after treatment (post). Middle and lower graphs: Number of times that each deer in each treatment group demonstrated "Head twist" during each ten minute interval during the "pre" treatment period (middle graph) and "post" treatment period (lower graph). Treatment was either control (black), NaturO <sup>TM</sup> rings (green), lignocaine (blue) or NaturO on one antler and lignocaine on the other ("both") (pink). |
| Figure 3.5.1 Number of times that individual yearling male deer demonstrated the combination of specific head related behaviour "Rotate left", "Rotate right", "Rotate head 360°", "Arch down", "Arch up" and "Press head" summated as "Head twist" during one hour of video observations. Deer were from two treatment groups that had a Velcro band around the pedicle during the "pre" treatment period and had a Velcro band and the addition of either a lignocaine ring block or NaturO <sup>TM</sup> rings and a lignocaine ring block applied to both antlers during the "post" treatment period. The diff is the difference (post-pre) in frequency for individual deer                                                                                 |
| Figure 3.5.2 Total Power, Median Frequency and Spectral edge frequency recorded from a single channel of EEG in an anaesthetised rising one-year-old male deer that was being treated with a NaturO™ ring. The figure shows actual one second values from five minutes prior to treatment until ten minutes after treatment. Time of treatment is indicated by the black line                                                                                                                                                                                                                                                                                                                                                                                    |
| Figure 3.5.3 Frequency-Power-Time spectrum of the fast-fourier transformed EEG wave-<br>form in a single deer during velvet antler removal under general anasethesia. The deer<br>was not given any analgesia prior to cutting the antler                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Figure 3.5.4 Full spectral array for the fast-fourier transformed electroencephalogram from a rising one-year-old male deer (Deer 3, channel 2) that was administered a lignocaine ring block around the base of the pedicle at time zero. Time is shown on the x axis from 5 minutes before treatment until 10 minutes after treatment. Treatment is marked with the white arrow. Frequency in shown on the z axis with low frequency (1Hz) at the rear of the graph and high frequency (30Hz) at the front of the graph and power is shown on the y axis.                                                                                                                                                                                                      |
| Figure 4.1.1 Photograph of the eight mature stags used in a study using fluorescent microspheres to determine the relative blood flow to the antlers with tourniquets of different tension applied to the antler pedicle                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Figure 4.1.2 (above) Experimental tourniquet used to determine the effect of tourniquet                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

| tension around the antler pedicle of mature stags on the relative blood flow to the antler as determined using fluorescent microspheres, showing design and features (upper picture) and fitting onto the antler (lower picture above)                                                                                                                                                                                                                                                                                                                                                                           | .359 |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Figure 4.1.3 Fitting of the experimental tourniquet used to determine the effect of tourniquet tension around the antler pedicle of mature stags on the relative blood flow to the antler as determined using fluorescent microspheres, showing complete overlap of cord around the antler pedicle.                                                                                                                                                                                                                                                                                                              | .359 |
| Figure 4.1.4 Surgical site prepared for cannulation of the left carotid artery showing exposed artery for injection of fluorescent microspheres to determine the effect of a tourniquet applied at different tensions around the antler pedicle on the relative blood flow to the antler of mature stags.                                                                                                                                                                                                                                                                                                        | .360 |
| Figure 4.1.5 Fluorescent blood flow determination colour kit #2 showing the seven different colours in 10mL bottles as used to determine the effect of tourniquets applied at different tensions around the antler pedicle on relative blood flow to the antler in mature stags.                                                                                                                                                                                                                                                                                                                                 | .362 |
| Figure 4.1.6 Injection of 2mL of yellow fluorescent microspheres into a cannula placed in the left ventricle of an anaesthetised stag to determine the effect of tourniquets at different tensions applied to the antler pedicle of mature stags on the relative blood flow to the antler                                                                                                                                                                                                                                                                                                                        | .362 |
| Figure 4.1.7 Set-up of negative pressure filtration system used to recover and count fluorescent microspheres from antler tissue from stags that had been injected with fluorescent microspheres while a tourniquet was applied to the antler pedicle at different tensions. Running water was used to create vacuum pressure in the collecting flask (top photograph). A 10 micron PTFE filter is placed on the stand to trap 15 micron microspheres (middle photograph). Filter papers from each filtration cut in half and mounted on glass slides for microsphere counting are shown in the lower photograph | .366 |
| Figure 4.1.8 Example of the tally sheet used to quantify the number of fluorescent microspheres that were recovered following filtration of digested antler from stags that had been injected with different coloured microspheres with a tourniquet applied to the antler pedicle at different tensions.                                                                                                                                                                                                                                                                                                        | .368 |
| Figure 4.1.9 (right) Photograph of fluorescent microspheres recovered from antlers of stags that had been injected with microspheres of different colours with a tourniquet applied to the antler pedicle at different tensions.                                                                                                                                                                                                                                                                                                                                                                                 | .368 |
| Figure 4.1.10 Systolic blood pressure recorded at the time of each injection of fluorescent microsheres in anaesthetised adult stags that had fluorescent microspheres injection while a tourniquet was applied to the antler pedicle at different tensions                                                                                                                                                                                                                                                                                                                                                      | .368 |
| Figure 4.1.11 Relative number of fluorescent microspheres recovered from antlers that had a tourniquet applied to the antler pedicle at different tensions during injection of different coloured fluorescent microspheres. The relative recovery rate is the number of microspheres recovered from the treatment antler compared to the control antler and compared to the treatment antler when no tourniquet was applied (zero tension)                                                                                                                                                                       | .371 |
| Figure 4.1.12 Relative number of fluorescent microspheres recovered from antlers that had                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |      |

| a tourniquet applied to the antler pedicle at different tensions during injection of dif-<br>ferent coloured fluorescent microspheres compared to systolic blood pressure recorded<br>at the time of injection.                                                                                                                                                                                                           | 2  |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 4.1.13 Relative (treatment/control) recovery rate of fluorescent microspheres from deer antlers for each consecutive injection event following intracardiac injection of 6 different colours of 15µm polystyrene fluorescent microspheres. The first injection was the control (no tourniquet) and results are not shown here, subsequent injections were made after a tourniquet was placed on the antler pedicle | 2  |
| Figure 4.2.1 Mature stag positioned for fluoroscopic examination of the velvet antler after injection of contrast iodine without or with a tourniquet applied to the antler pedicle.  Anaesthetised stag in the Massey University teaching hospital with the Philips BV  Libra digital C arm fluoroscope receiving plate positioned against the antler                                                                    | '9 |
| Figure 4.2.2 Pania Flint and Radiographer Angela Hartman observe the real time fluoroscopy videos with an anaesthetised stag on the table.                                                                                                                                                                                                                                                                                | 9  |
| Figure 4.2.3 Still image captured from fluoroscopic study of the velvet antler of a mature stag after injection of contrast iodine into the carotid artery on the same side. Picture is of the arterial pattern at the level of the pedicle and lower antler                                                                                                                                                              | 1  |
| Figure 4.4.1 Examples of tourniquets used in the field. Clockwise from top left: Rubber band, Car inner tubing (inset fastening pin); Solid rubber cord (inset fastened with a haemostat) advocated by NVSB; Rope attached to the crush on both sides; Bungy tie down                                                                                                                                                     | 4  |
| Figure 4.4.2 Examples of tourniquets that were developed during the trial. 1. Friction barrel; 2, pneumatic tube; 3, cable tie with tension device; 4, rope and cleat; 5 spring clamp and cord; 6 spring clamp with cam cleat; 7, as for 6 with cleat repositioned for line of force; 8, as for 7 with 4mmm cord; 9, as for 7 with solid rubber O ring cord                                                               | 5  |
| Figure 4.5.1 Number of adult stags with each haemorrhage score recorded during cutting the velvet antler during the first stage of tourniquet prototype testing in the field40                                                                                                                                                                                                                                            | 1  |
| Figure 4.6.1 Photographs of an acrylic cast of the arterial supply from the carotid artery to the antlers and head of a mature male red deer                                                                                                                                                                                                                                                                              | 0  |
| Figure 4.6.2 Transverse section cut through the antler pedicle of adult stag heads at the level of tourniquet placement showing outline of the skin and location of bony core within the pedicle.                                                                                                                                                                                                                         | 2  |
| Figure 4.6.3 outline trace of sections in figure 4.6.2 made using Adobe Illustrator live trace.  Showing the outer skin profile                                                                                                                                                                                                                                                                                           | 3  |
| Figure 4.6.4 Larger view of the transverse section of an antler pedicle of a mature red deer stag (Antler 7, Fig 4.6.1) and relief outline showing the outer skin and the outline of the bone of the pedicle. Numerous blood vessels are present in the dermis and subdermis and a trabecular pattern is evident in the centre of the pedicle bone                                                                        | 4  |
| Figure 4.7.1 Lignocaine concentration (mg/kg of antler) from antlers of adult stags after velveting using different tourniquets for haemostasis. The range and distribution of all values (5 data points excluded due to tourniquet malfunction) are shown. The graph on                                                                                                                                                  |    |

| the left shows linear values and illustrates the skewed spread of the data. The graph on the right shows data on a log10 scale with an approximately normal distribution4                                                                                                                                                      | 125             |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| Figure 4.7.2 Lignocaine in antler harvested from mature stags for each different tourniquet type used for haemostasis. Individual values and median are shown for each tourniquet type. Dotted line represents the MPL of 0.1mg/kg. Note y-axis is log 10 scale4                                                               | 126             |
| Figure 4.7.3 Lignocaine residue in velvet antlers of mature stags harvested when a tourniquet was applied either wrapped around each pedicle once (S) or twice (D), showing individual values and median                                                                                                                       | 127             |
| Figure 4.7.4 Lignocaine in velvet harvested from stags of different ages showing individual values and median. (Note y-axis is log10 scale)                                                                                                                                                                                    | 127             |
| Figure 4.7.5 Antler lignocaine concentrations from adult stags that were velveted under 3 different dose rates of intramuscular xylazine: Nil, 0.25mg/kg and 0.6mg/kg. Dose rates are approximate. Data are shown on a log10 scale. Line is at the median for each group and the MPL of 0.1mg/kg is shown as a dotted line     | 128             |
| Figure 4.7.6 Lignocaine residue in velvet from stags injected with either 8 to 10 mL or 11 to 20 mL of local anaesthetic in a ring block around the base of the pedicle below the tourniquet                                                                                                                                   | 128             |
| Figure 4.7.7 Scatterplot of the time waited between injecting local anaesthetic and velveting vs lignocaine residue measured in the same antler                                                                                                                                                                                | 129             |
| Figure 4.7.8 Lignocaine residues measured from velvet antlers from mature stags that were graded with different haemorrhage scores during antler cutting. Line is at the median and interquartile range. y-axis is log10 scale                                                                                                 | 129             |
| Figure 5.1.1 Location of farms that reported stag deaths following sedation to the NVSB or Massey University (this thesis) between 2002 and 2006                                                                                                                                                                               | <del>1</del> 59 |
| Figure 5.1.2 Dose rate (mg/kg bodyweight) of xylazine administered to stags that did and did not die after sedation as reported in case report data collection forms returned to the NVSB and Massey University from 2002 to 2006                                                                                              | 169             |
| Figure 5.2.1 Heart rate of rising one year old red deer recorded before sedation and during sedation with xylazine or detomidine. Numbers above each time interval indicate the number of animals from which recordings were made at each time. Lines and error bars are the mean and 95% confidence interval of the mean      | 175             |
| Figure 5.2.2 Respiratory rate of rising one year old red deer recorded before sedation and during sedation with xylazine or detomidine. Numbers above each time interval indicate the number of animals from which recordings were made at each time. Lines and error bars are the mean and 95% confience interval of the mean | 175             |
| Figure 5.2.3 (above) SpO <sub>2</sub> measured using a pulse oximeter in rising one year old deer prior to and at various time intervals during sedation with xylazine and after reversal of sedation. Line is at the mean and error bars are 95% CI of the mean                                                               | 177             |
| Figure 5.2.4 (left) Minimum SpO <sub>2</sub> measured using a pulse oximeter in rising one year old deer during sedation with xylazine. Line is at the mean and error bars are 95% CI of                                                                                                                                       |                 |

| the mean. Level that an animal is considered to be hypoxaemic and oxygen should be provided is shown at a dotted line                                                                                                                                                                | ,        |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Figure 5.2.5 (this and the following page) Arterial blood gas analysis from the auricular artery of yearling deer 5 minutes after sedation with either xylazine or detomidine480                                                                                                     | )        |
| Figure 5.2.6 Haematology values from eight rising one-year-old deer before (pre) and 5 minutes after (during) sedation with intravenous xylazine. Dashed lines indicate upper and lower reference values from unsedated red deer from Cross et al (1989) and Wilson and Pauli (1982) | <b>4</b> |
| Figure 5.3.1 Yearling stag sedated with fentazin and strapped into a specially designed cradle for CT scanning of meat to bone ratio. The stag is connected to an anaesthetic machine supplying oxygen being monitored for oxyhaemoglobin saturation using a pulse oximeter          | )        |
| Figure 5.3.2 SpO2 readings from a pulse oximeter showing haemoglobin oxygen saturation levels of stags that had been sedated with Fentazin for CT scanning and either supplemented with oxygen or not supplemented with oxygen during CT. Mean and 95% CI of the mean are shown      | <u>!</u> |

## List of tables

| Table 1.4.1 Copy of an exert from the Schedule one of the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards 201141                                                                                                                                                                                                                           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Table 1.5.1 Products registered with the ACVM in 2011 that contain xylazine and are labelled for use in deer                                                                                                                                                                                                                                                             |
| Table 2.1 Farm ID, location, operator and number of stags per operator for on-farm observations of local anaesthetic technique and behaviour outcome during velveting65                                                                                                                                                                                                  |
| Table 2.2 Behaviours observed during initial video observation of stags during velveting when a potentially painful stimulus was and was not being applied                                                                                                                                                                                                               |
| Table 2.3 Order of observations for recording behaviour outcome and injection technique to determine factors that contribute to the likelihood of a withdrawal response during antler cutting in adult stags on commercial farms                                                                                                                                         |
| Table 2.4 Criteria for subjective assessment of withdrawal reaction to the nick test or antler cutting of adult stags velveted by farmers and veterinarians on farms                                                                                                                                                                                                     |
| 70                                                                                                                                                                                                                                                                                                                                                                       |
| Table 2.5 Description of "grade" "behaviours" assigned to "movements" during application of tourniquet through to cutting of the second antler of adult stags                                                                                                                                                                                                            |
| Table 2.6 Description of specific "behaviours" recorded during application of tourniquet through to cutting of the second antler in adult stags on commercial farms70                                                                                                                                                                                                    |
| Table 2.7 Definitions of variables recorded or calculated to determine which factors affect the likelihood of a withdrawal response during velvet antler cutting in adult stags74                                                                                                                                                                                        |
| Table 2.7 continued                                                                                                                                                                                                                                                                                                                                                      |
| Table 2.8 Number of movements that included each behaviour type during different phases of velvet antler removal from tourniquet application to antler cutting. Data is broken down into movements that occurred during or between potential noxious stimuli and movements that occurred during antler cutting that were or were not classified as a withdrawal response |
| Table 2.9 Retrospective diagnostic test parameters of "behaviours" for determining whether a "movement" occurred during a potentially noxious stimulus (tourniquet pull, injecting local anaesthetic or antler cutting) or at times when a stimulus was not being applied84                                                                                              |
| Table 2.10 Diagnostic test parameters of "behaviours" for determining whether a "movement" occurred during antler cutting when a withdrawal response occurred or during antler cutting without a withdrawal response.                                                                                                                                                    |
| Table 2.11 Mean, Standard Deviation and 95% confidence interval of the mean of variables analysed from adult stags that did and did not show a withdrawal response during antler cutting and t-test result for comparison of the means between stags that did and did not show a withdrawal response.                                                                    |

| Table 2.12 Mean, standard deviation and p-values from t-test result of variables analysed to determine which factors were different between the techniques of good operators (<20% withdrawal during antler cutting), moderate operators (20 to 50% withdrawal during antler cutting) and poor operators (>50% withdrawal during antler cutting) | 94 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 2.13 Summary of logistic regression results for determining which independent variables affect the likelihood of a withdrawal response during velvet antler cutting in adult stags.                                                                                                                                                        | 98 |
| Table 2.14 Different types of injection devices used by farmers and veterinarians on commercial farms for injecting lignocaine to provide analgesia for velvet removal from adult stags.                                                                                                                                                         | 03 |
| Table 2.15 Different length needles used by farmers and veterinarians on commercial farms to inject lignocaine to provide analgesia for velvet removal from adult stags                                                                                                                                                                          | 03 |
| Table 2.16 Different Needle gauges used by farmers and veterinarians on commercial farms for injecting lignocaine to provide analgesia for velvet antler removal from adult stags10                                                                                                                                                              | 03 |
| Table 2.17 Age distribution of stags that were observed for behaviour during velvet antler removal. The proportion of stags in each age group that demonstrated a withdrawal response during antler cutting is shown                                                                                                                             | 05 |
| Table 2.18 Number of antlers removed from stags that were sedated with low dose xylazine for velvet removal and the proportion of sedated and non-sedated stags that demonstrated a withdrawal response during antler removal.                                                                                                                   | 07 |
| Table 2.19 Number of antlers removed in the primary antler growth or regrowth stage and the proportion of stags that showed a withdrawal response during antler removal. The average age of stags in primary growth and regrowth is also shown                                                                                                   | 07 |
| Table 2.20 Number of antlers that were removed from stags with their head restrained or not restrained and the proportion of stags that showed a withdrawal response                                                                                                                                                                             | 07 |
| Table 2.21 Mean, maximum and minimum volume of local anaesthetic injected by farmers and veterinarians in each of the eight "Regions" around the antler pedicle (as shown in Figure 2.4a) for velvet removal from adult stags on commercial farms                                                                                                | 16 |
| Table 2.22 Number (and percent) of antlers that had a "Gap" in the local anaesthetic ring block in each of the eight "Regions" around the pedicle (Figures 2.4a, 2.21). Of the antlers with a "Gap" in each specified "Region", the percent that showed a withdrawal response during cutting is shown                                            | 24 |
| Table 2.23 Number of antlers that were removed by farmers and veterinarians on commercial farms from stags that were sedated or not sedated in different age groups ( 2years old, 3 years old and 4 years and older) and the percent withdrawal response during antler cutting for each group                                                    | 45 |
| Table 2.24 Number of antlers injected by farmers and veterinarians on commercial farms with different dose (mL/cm pedicle circumference) ranges of lignocaine and the proportion of these for which the stag showed a withdrawal response during velvet antler cutting.                                                                          | 51 |

| Table 3.1.1 Summary of visual inspection of individual EEG profiles of deer during antler removal following lignocaine (Lig), NaturO™ ring (NO) or no analgesia (Con) to determine whether they were suitable for further analysis                                                                                                                                                                                                                                                                                                                                                                                                                    | 74             |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| Table 3.1.2 Statistical summary of EEG changes in TP, F50 and F95 as a percent of baseline, averaged over 15 second epochs before and after cutting the velvet antler of yearling stags provided with either lignocaine, a NaturO™ ring or no analgesia prior to cutting                                                                                                                                                                                                                                                                                                                                                                              | 30             |
| Table 3.1.3 p-values generated from t-tests for comparison of equal means of EEG data for Total Power, Median Frequency and 95% spectral edge frequency data recorded during cutting the antlers of anaesthetised stags following analgesia treatment with either lignocaine, a NaturO™ ring or no analgesia                                                                                                                                                                                                                                                                                                                                          | 33             |
| Table 3.2.1 Description of behaviours recorded during cutting the antlers of yearling stags that had either subcutaneous lignocaine or a Natur $O^{\text{TM}}$ ring applied for analgesia19                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 93             |
| Table 3.2.2 Behaviours recorded from individual yearling stags during antler removal following application of either subcutaneous lAnocaine or a NaturO™ ring. Outcome shown as either withdrawal, no response or not determinable. Farm of orAin M=Massey, B= brought in                                                                                                                                                                                                                                                                                                                                                                             | 94             |
| Table 3.2.2 cont                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | €              |
| Table 3.2.2 cont                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <del>)</del> 6 |
| Table 3.2.2 cont                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <del>)</del> 7 |
| Table 3.2.3 Criteria for determining outcome classifications from reactions during velvet removal from yearling stags                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 97             |
| Table 3.2.4 Requirements for classification of a withdrawal response to velvet antler cutting in yearling stags                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 97             |
| Table 3.2.5 Proportion of observed response (number of animals, n, in parenthesise) and 95%CI (in parenthesise below) that were classified as either a withdrawal response, no response or not determinable (ND) behavioural outcomes during antler cutting after either subcutaneous lignocaine or a NaturO™ ring for analgesia. Deer were sourced either on the Massey farm or brought in as shown                                                                                                                                                                                                                                                  | 98             |
| Table 3.2.6 Statistical test results for equal means in predicted behavioural outcome during antler cutting of yearling stags from two different sources that were treated with either lignocaine local anaesthetic (LA) or a NaturO™ ring (NO) for analgesia                                                                                                                                                                                                                                                                                                                                                                                         | 99             |
| Table 3.3.1 Summary of Two-way ANOVAs for effects of time, treatment and interaction on TP, F50 and F95 from electroencephalogram recordings from yearling male deer that were treated with either a lignocaine ring block at the base of the antler or a NaturO™ ring. Calculations are shown for averages taken over each minute from five minutes prior to treatment until ten minutes after treatment and for average taken over each 15 second interval from 2 minutes prior to treatment until five minutes after treatment. p-value is the probability that these results would have occurred if treatment, time or interaction had no effect. |                |

| 95% spectral edge frequency from electroencephalogram recordings from deer that were treated with either a lignocaine ring block or a NaturO <sup>™</sup> ring. Difference shown is Lignocaine - NaturO. Comparisons are for each one minute interval from 5 mins prior to treatment until 10 mins after treatment                                                                                                                                                                                                                                                                            | .210 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Table 3.3.3 Post ANOVA Bonferroni comparisons of Total Power, Median Frequency and 95% spectral edge frequency from electroencephalogram recordings from deer that were treated with either a lignocaine ring block or a NaturO™ ring. Difference shown is Lignocaine - NaturO.Comparisons are for each fifteen second interval from 5 mins prior to treatment until 10 mins after treatment                                                                                                                                                                                                  | .211 |
| Table 3.3.4 Summary of t-tests performed to compare post treatment measurements with pre-treatment baseline measurements of Total Power, Median Frequency and 95% spectral edge frequency from electroencephaolgram recording from rising one-year-old male deer that were treated with either a ligncaine ring block at the base of the antler or a NaturO™ ring. Tests were done on averages for each one minute time period from five minutes prior to treatment until ten minutes post treatment. Lignocaine and O Ring results are shown side by side for each of the variables measured | .212 |
| Table 3.3.5 Summary of t-tests performed to compare post treatment measurements with pre-treatment baseline measurements of Total Power, Median Frequency and 95% spectral edge frequency from electroencephaolgram recording from rising one-year-old male deer that were treated with either a lignocaine ring block at the base of the antler or a NaturO™ ring. Tests were done on averages for each 15 second time period from two minutes prior to treatment until five minutes post treatment. Lignocaine and O Ring results are shown side by side for each of the variables measured | .214 |
| Table 3.4.1 Treatment groups and descriptions for observations of the effects of NaturO™ rings on the behaviour of yearling stags                                                                                                                                                                                                                                                                                                                                                                                                                                                             |      |
| Table 3.4.2 Behaviours included in the Global Definition, Focal Master, Analysis and Statistical Ethograms                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | .227 |
| Table 3.4.3 Correlation analyses, goodness of fit (Pearson $R^2$ ) and p-value for test for significant difference from zero for the proportion of time spent in whole body state behaviours of deer without(pre) and with (post) treatment with NaturO <sup>TM</sup> rings, lignocaine ring blocks or control. or both (NaturO on one antler, Lignocaine on other antler)                                                                                                                                                                                                                    | .239 |
| Table 3.4.4 Number of animals that showed a 50% "increase" or "decrease" in the proportion of time spent in each whole body state behaviour when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes                                                                                                                                                                                                                                                                             | .243 |
| Table 3.4.5 Correlation analyses, goodness of fit (Pearson R²) and p-value for test for significant difference from zero for the proportion of time spent in head position state behaviours of deer without(pre) and with (post) treatment with NaturO™ rings, lignocaine ring blocks or control                                                                                                                                                                                                                                                                                              | .247 |
| Table 3.4.6 Number of animals that showed a 50% "increase" or "decrease" in the proportion of time spent in each head position state behaviour when post treatment recordings                                                                                                                                                                                                                                                                                                                                                                                                                 |      |

| were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes25                                                                                                                                                                                                                                                                    | 4 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| Table 3.4.7 Correlation analyses, goodness of fit (Pearson R², Spearman R) and p-value for test for significant difference from zero for the number of times that deer demonstrated head position behaviours without (pre) versus with (post) treatment with NaturO™ rings, lignocaine ring blocks or control. or both (NaturO on one antler, Lignocaine on other antler)                                   | 0 |
| Table 3.4.8 Number of animals that showed a double "increase" or halve "decrease" in the number of times they put their heads into each head position when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes                                                                                 | 4 |
| Table 3.4.9 Correlation analyses, goodness of fit (Spearman R) and p-value for test for significant difference from zero for the number of times that deer demonstrated event behaviours without (pre) versus with (post) treatment with NaturO™ rings, lignocaine ring blocks or control. or both (NaturO on one antler, Lignocaine on other antler)276                                                    | 6 |
| Table 3.4.10 Number of animals that showed a double "increase" or halve "decrease" in the frequency of the behaviours "Look up", "Nose to ground", "lick the walls", "Flick tongue", and "Flick ears" when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes.                                | 4 |
| Table 3.4.11 Correlation analyses, goodness of fit (Spearman R) and p-value for test for significant difference from zero for the number of times that deer demonstrated specific head related event behaviours without (pre) versus with (post) treatment with NaturO™ rings, lignocaine ring blocks or control. or both (NaturO on one antler, Lignocaine on other antler)                                | 6 |
| Table 3.4.12 Number of animals that showed an 50% "increase" or "decrease" in the frequency of the behaviour "Rotate left" when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes                                                                                                            | 3 |
| Table 3.4.13 Statistical Summary of the data distribution and statistical tests applied to data collected relating to behaviours observed of one-year-old male deer during video observation for one hour without treatment (pre) or with treatment (post) with no treatment (control), NaturO™ rings, Lignocaine ring blocks or a NaturO™ ring on one antler and lignocaine ring block on the other (both) | 6 |
| Table 3.5.1 Mean number of times that deer demonstrated the behaviour "Flick ears" during one hour of video observation for each video tape of a pen of 5 deer                                                                                                                                                                                                                                              | 6 |
| Table 3.5.2 Number of animals that showed an EEG response to cutting the antler after treatment with either a NaturO™ ring, lignocaine ring block or no treatment (control). Superscript letters indicate significant differences between treatment groups                                                                                                                                                  | 0 |
| Table 4.1.1 Details of stags used for fluorescent microsphere study including bodyweight (where recorded, pedicle circumference, antler weights, estimated and actual velvet harvest dates                                                                                                                                                                                                                  | 7 |

| Table 4.1.2 Excitation and Emission wavelengths for fluorescence of different coloured Fluospheres in the Blood Flow Determination Kit as specified by the manufacturer (Molecular Probes).                                                                                                                                                                                                                                                                                         | .362 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Table 4.1.3 Fluorescent microsphere counts from stags that were injected with different coloured fluorescent microspheres while a tourniquet was applied to the antler pedicle at different tensions. Table shows deer ID, tourniquet tension, order of treatment, systolic blood pressure at time of injection, absolute counts from left (control) and right (treatment) antler, and the relative proportion recovery of each colour in the treatment antler compared to control. | .369 |
| Table 4.1.4 Relative fluorescent microsphere recovery from antlers from stags that had been injected with different colours of fluorescent microspheres while a tourniquet was applied to the antler pedicle at different tensions. The number is compared to the control antler and zero tourniquet tension.                                                                                                                                                                       | .370 |
| Table 4.2.1 Deer angiography results showing record number, radiographic view, blood pressure and time during recording, site of injection,                                                                                                                                                                                                                                                                                                                                         | .382 |
| observations during injection and notes recorded.                                                                                                                                                                                                                                                                                                                                                                                                                                   | 382  |
| Table 4.3 Details of velvet removal and observations of haemorrhage during velvet harvest and after release and reapplication of a tourniquet in 2 stags restrained in a hydraulic crush                                                                                                                                                                                                                                                                                            | .389 |
| Table 4.5.1 Mean and range of factors and comments recorded during the first stage of tourniquet prototype testing on adult stags during velvet antler removal                                                                                                                                                                                                                                                                                                                      | .400 |
| Table 4.5.2 Details of observations during testing of two prototype tourniquets on farms during normal velvet harvest in adult stags four years and older                                                                                                                                                                                                                                                                                                                           | .403 |
| Table 4.7.1 Photos and descriptions of tourniquets used in the final testing stage in the attempted development of a standardised reliable tourniquet for prevention of lignocaine residues in harvested antler from mature stags. Lignocaine residues recorded from antlers harvested with each of the tourniquets in place are shown                                                                                                                                              | .420 |
| Table 4.7.2 Groupings of tourniquet type for statistical comparison of lignocaine residues. For each tourniquet type, the models that were used are shown along with the number of antlers tested for lignocaine for each tourniquet type and a description of the similar features of the models in each group.                                                                                                                                                                    | .423 |
| Table 4.7.3 Descriptive statistics for overall lignocaine residue values from antlers harvested after application of different tourniquet models                                                                                                                                                                                                                                                                                                                                    | .425 |
| Table 4.7.4 Descriptive statistics on lignocaine residue in velvet antler (mg/kg) harvested using six different types of tourniquet                                                                                                                                                                                                                                                                                                                                                 | .426 |
| Table 4.7.5 Results from duplicate samples sent to AsureQuality laboratory on separate occasions                                                                                                                                                                                                                                                                                                                                                                                    | .433 |
| Table 4.8 Results of lignocaine tests from different studies including those from this thesis                                                                                                                                                                                                                                                                                                                                                                                       | 436  |
| Table 5.1.1 Number of post-xylazine stag deaths reported and data collection forms returned from 12 farms that participated in a post-xyalzine stag death survey. Also shown                                                                                                                                                                                                                                                                                                        |      |

| is the number of cases for which each farmer submitted samples for histopathology446                                                                                                                                                                                                                                                |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Table 5.1.2 Location of farms, number of deaths and velvet herd size for farms that completed case report survey forms relating to post xylazine stag deaths                                                                                                                                                                        |
| Table 5.1.3 Yarding conditions on farms that returned post sedation stag death case report forms (at least one stag death occurred in these environments). Yard and pen size and number, flooring and lighting conditions and number of animals per yard or pen are shown.                                                          |
| Table 5.1.4 Weather conditions, temperature, wind conditions, velveting time and time of death reported by farmers for each velveting episode for which a form was returned. Multiple velveting episodes were recorded on most farms. Episodes where deaths did and did not occur are shown on separate parts of the table          |
| Table 5.1.5 Farmer reports of stress during mustering or yarding for each velveting episode from farms that returned case report data collection forms relating to post sedation stag deaths. Episodes where deaths occurred are shown here and episodes when deaths not occur are shown on the next page                           |
| Table 5.1.6 Case report data relating to dose and route of administration of xylazine or xylazine combinations given to stags on farms that did and did not experience one or more stag deaths post sedation. Actual dose given to stags that died has also been calculated. 452                                                    |
| Table 5.1.7 Data relating to the brand and dose rate of reversal (alpha 2 antagonist) drug administered to stags after sedation with xylazine and xylazine combinations. For those that answered this question, the table also shows whether all stags in the mob were given reversal and whether any that died were given reversal |
| Table 5.1.8 Postmortem findings reported by the attending veterinarian or farmer in completing case report data collection forms relating the post sedation stag death. Only the significant pathological findings are listed                                                                                                       |
| Table 5.1.9 Histopathology findings from samples that were submitted to Massey University from stags that died after sedation during a case report study                                                                                                                                                                            |
| Table 5.1.10 Sources of information used to investigate the occurrence and risk factors associated with death in stags after being given xylazine for sedation                                                                                                                                                                      |
| Table 5.1.11 Number of stags velveted, number of stag deaths reported and annual incidence of post sedation death from 4 surveys                                                                                                                                                                                                    |
| Table 5.1.12 Location of farms and number of deaths recorded after sedating stags with xylazine from farms that reported post-xylazine deaths to the NVSB or this thesis from 2002 to 2006                                                                                                                                          |
| Table 5.1.13 Number of stags of each age that were reported to the NVSB and Massey University as dying after being sedated between 2002 and 2006. NS=not stated                                                                                                                                                                     |
| Table 5.1.14 Time after sedation that stags were reported as dying in case reports submitted to the NVSB and Massey University between 2002 and 2006                                                                                                                                                                                |
| Table 5.1.15 Gross pathological findings reported to the NVSB or Massey University from 2002 to 2006 from postmortem examination of stags that died after administration of                                                                                                                                                         |

| xylazine or xylazine combinations for sedation                                                                                                                                                                                                                                                                                                                                        | 464  |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Table 5.1.16 Significant histopathological findings from samples that were submitted from case reports returned to the NVSB and Massey University from 2002 to 2006 from stags that died after being sedated with xylazine or xylazine combinations                                                                                                                                   | .465 |
| Table 5.1.17 Diagnoses made based on clinical observations, postmortem examination or histopathology for the cause of death in stags that died after sedation with xylazine and had case reports submitted to the NVSB or Massey University                                                                                                                                           | .465 |
| Table 5.1.18 Number of occasions (episodes) that different formulations containing xylazine were used to sedate deer on farms that submitted case report survey forms to the NVSB or Massey University between 2002 and 2006. Also shown is the number of deaths reported in total and the number of deer that were sedated with each formulation where that information was provided | .467 |
| Table 5.2.1 Arterial blood gas analysis results from rising one year old red deer 5 minutes after intravenous injection of xylazine or detomidine.                                                                                                                                                                                                                                    | .479 |
| Table 5.2.2 Complete blood count results from rising one year old deer prior to (pre) and during sedation with intravenous xylazine as part of veterinary student teaching practical classes.                                                                                                                                                                                         | .483 |
| Table 5.2.3 TNF alpha results from rising one year old deer from samples taken prior to and 5 minutes after intravenous injection of 0.5mg/kg of xylazine                                                                                                                                                                                                                             | .483 |
| Table 5.3.1 Pulse oximeter readings from yearling stags that were sedated with Fentazin for CT scanning and provided with supplemental oxygen during CT. Readings after sedation and before starting oxygen (pre CT) and during CT with and without oxygen are shown.                                                                                                                 | .491 |
| Table 5.4 Pulse oximeter SpO <sub>2</sub> and heart rate readings from 5 adult red deer stags during standing sedation with xylazine for velvet removal                                                                                                                                                                                                                               |      |
|                                                                                                                                                                                                                                                                                                                                                                                       |      |

## Long Abstract

Antlers are unique structures specific to male deer that are grown and cast on an annual cycle and are unlike the horns of other ungulate species. During the growth phase, velvet antlers have a well developed neurological and vascular supply and are sensitive to pain. Antlers are usually removed from deer, for commercial and management reasons, during the velvet growth stage, when they are sensitive to pain and therefore analgesia must be provided. The removal of growing velvet antlers is a controlled surgical procedure that can only be carried out by a veterinarian or a farmer that has been certified by a veterinarian. There is considerable legislation surrounding the removal of antlers and the New Zealand Deer Industry has a desire to continue its right to harvest antlers and wishes to ensure that methods used are safe, effective and humane. The experiments in this thesis examine some areas of velvet antler removal that may be refined in order to improve the quality assurance of the procedure. These include analgesia with lignocaine and compression bands, drug residues and sedation.

Chapter one of this thesis provides background information on the anatomy and commercial harvest of antlers and introduces and discusses concepts and methods relevant to the experimental chapters (2 to 5) that follow. The general outline is as follows:

- Method for applying local anaesthetic ring blocks and determining a pain response Introduced in Chapter 1.2, experiment in Chapter 2.
- Use of compression with the NaturO<sup>TM</sup> ring for analgesia in yearling stags
   Introduced in Chapter 1.3, experiments in Chapter 3
- Lignocaine residues and the development of a tourniquet to prevent residues Introduced in Chapter 1.4, experiments in Chapter 4
- Post xylazine stag death and the effects of xylazine on blood oxygen levels Introduced in Chapter 1.5, experiments in Chapter 5

#### Lignocaine ring blocks

Under current practice in New Zealand, analgesia is provided by subcutaneous lignocaine injections in adult stags or *Natur*O<sup>TM</sup> ring compression bands in yearling stags. Experiments have shown that lignocaine injected in a high dose ring block around the base of the antler provides superior analgesia compared to blocking nerve trunks more proximally. Limited data from field observations of farmers applying lignocaine and removing velvet suggests that the achievement of analgesia in the field may be variable, with some operators achieving better results than others. There is a need to describe the best practice for applying lignocaine ring blocks to maximise the chances of analgesia for all stags on commercial farms. In order to do this a method for determining whether analgesia has been achieved needed to be developed. Previous methods have used a behaviour grading scale based on the amount of the stags body involved in the movement. This method may have limited sensitivity and/or specificity and there was a need to better define a

withdrawal response caused by pain sensation from antler that were cut with insufficient analgesia. Methods for assessing a pain response in animals are reviewed in Chapter 1.2, while Chapter 2 consists of an observational study of farmers and veterinarians harvesting velvet antler in the field. A detailed behaviour analysis was done to develop the criteria for determining a withdrawal response to antler removal. Using this behaviour scoring system, operators were ranked according to the proportion of stags that showed a withdrawal response during antler cutting. Variables associated with the farm, operator, animal and injection method were analysed to determine which had a significant effect on the likelihood of a withdrawal response during antler removal. "Flinch" had a higher sensitivity for a withdrawal response compared to previously described movement of the head, neck or body. "Flinch", "Head arch" "Head down" and "Head rotate" had greater specificity for a withdrawal response compared to previously described movements and a combination of these specific head-related movements is likely to be a more accurate predictor of failed analgesia than previously described behaviours. The overall withdrawal response during commercial antler harvest was 35% and ranged from 0 to 76% between operators. The most important factor influencing the likelihood of a withdrawal response was the presence or absence of visible "gaps" in the ring block, whereby injection sites did not overlap. Other variables that were important included the number of injections, total volume injected and injection time. Factors relating to the stag including stag age, sedation and stage of antler growth also appeared to have an effect on behaviour during antler removal although there was a high level of confounding in the data. An interaction model was drawn and recommendations made for best practice injection method.

#### NaturO<sup>TM</sup> ring

Compression analgesia by the use of NaturOTM rings is reviewed in Chapter 1.3 and four experiments relating to this topic are presented in Chapter 3. Compression analgesia has been investigated in deer and is currently legal in New Zealand for the removal of velvet from one-year-old stags using NaturO<sup>TM</sup>rings. This method of analgesia provides a solution to problems concerning drug residues in velvet antler and transport of yearling deer following velvet removal. Compression causes displacement of myelin sheaths and hypoxia of nerves, causing them to cease functioning within a time period that is related to the level of pressure applied by the compression device. There are limited studies on the efficacy of compression for preventing a pain response to velvet antler removal or on whether compression is itself noxious to the animal. Chapter 3 of this thesis details four experiments using electroencephalography (EEG) and behaviour to help determine whether NaturO<sup>TM</sup>rings prevent a response to antler removal and also to determine whether NaturO<sup>TM</sup>rings cause a pain response when they are applied to the animal. When antler were cut from anaesthetised stags that had a NaturO<sup>TM</sup>ring applied to the pedicle, the average EEG response was somewhere between that of stags that had been treated with lignocaine ring blocks and those that had no treatment. There was no difference in the behaviour of yearling stags during velvet antler removal that had been treated with either a NaturO<sup>TM</sup>ring or lignocaine ring block prior to antler removal. When comparing responses to application of NaturOTM rings and lignocaine ring blocks, there were significant differences in the EEG pattern following application of the different treatments and this was mostly due to changes in the lignocaine group. Overall, there was little effect on the mean EEG parameters in the NaturOTM ring treated stags but on an individual animal basis, seven out of 10 stags treated with NaturOTM rings showed some significant changes consistent with noxious somatic stimulus. Stags that were videoed for one hour following

application of one of a combination of *Natur*O<sup>TM</sup>rings, lignocaine or no treatment did not show differences in the majority of normal behaviours. Stags that were treated with *Natur*O<sup>TM</sup>rings had a higher frequency of some specific head related movements such as head rotating, turning head in circles, arching the head up or down and head pressing. Overall, the *Natur*O<sup>TM</sup>ring appears to offer analgesia during antler removal but may be perceived as noxious on application in some spikers but not others.

#### Lignocaine residues

Lignocaine use and safety is reviewed in Chapter 1.4 and the experiments relating to lignocaine residues are presented in Chapter 4. While compression analgesia ensures no residues are present in the antler, it is currently not an accepted form of analgesia for adult stags and lignocaine hydrochloride is used for analgesia in adult stags. One metabolic product of lignocaine, 2,6 dimethylanaline is classified as a potential carcinogen to humans after studies on the carcinogenic effects on rats. The maximum permissible level for lignocaine in velvet antler is set at 1mg/kg. This level is frequently exceeded in antler tissue so a method of preventing lignocaine residues was sought. It was believed that lignocaine reached the velvet antler via the blood stream and that a tourniquet placed prior to lignocaine injection would prevent lignocaine residues. Fluorescent microspheres, cineangiography and direct observations were used to determine the tourniquet tension required to minimise antler blood flow. In most cases tourniquets applied at a tension of 2kg or less prevented antler blood flow. Efforts to develop a consistent tourniquet for use in the field were unsuccessful at preventing lignocaine residues in the antler suggesting that either tourniquets are unable to fully prevent leakage of blood and lignocaine into the antler or that lignocaine is reaching the antler by another route.

#### **Xylazine reactions**

Stags often require sedation for velveting to ensure the safety of both the stag and the operator. The most commonly used drug for this is xylazine. Xylazine is prescribed and distributed to farmers that are licenced to harvest velvet from their own animals. Stags occasionally die up to 48 hours after administration of xylazine and this has generally been described as an anaphylactic or delayed anaphylactic reaction. Deaths are sporadic and variable from one year to another. Little is known about the factors that cause stag death following sedation with xylazine or the exact mechanism of the pathology. Chapter 5 of this thesis includes two main areas of research, an survey study or stag deaths and a series of experimental studies and observations of the hypoxaemic effects of xylazine in young deer. Survey data was limited and unable to identify specific risk factors although a number of potential causes were able to be excluded. Deaths were not related to any environmental factors including weather, temperature, time of day, facilities and method. The formulation and dose of drug administered was not a contributing factor, nor was the administration of the reversal agent, yohimbine. Deaths were sporadic in location and time, with some farms experiencing multiple deaths and others never experiencing a death. A limited number of postmortem samples were sent to Massey University for histopathology. The majority of these revealed pulmonary oedema with or without pulmonary haemorrhage. Yearling deer sedated with xylazine demonstrated marked hypoxaemia measured as reduced SpO2 and PaO2 levels. This

was reversed after administration of endotracheal oxygen or reversal with yohimbine. It is hypothesised that xylazine causes hypoxaemia and pulmonary oedema in nearly all deer and that in most cases this is transient but a population of deer experience severe hypoxaemia from which they do not recover. Supplemental oxygen is recommended for all deer given xylazine or xylazine combinations and susceptible stags should be identified and alternative methods of restraint used.

Chapter 6 identifies some potential future research options that may help to further refine the methods used for velvet removal making the procedure safer and more humane for the animals and more predictable for the assurance of the general public. Potential options include the development of an automated needle-free drug delivery device, the use of a different local anaesthetic agent that does not produce carcinogenic metabolites and the use of peripheral alpha-2 antagonist agents to mitigate the hypoxaemic effects of xylazine in deer. These methods could also be extended to use in other ruminant species.