STUDIES OF LOCAL ANAESTHETICS FOR VELVET ANTLER ANALGESIA

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ABSTRACT

Velvet antler removal on commercial deer farms is the elective surgical amputation of a live, vascular and innervated tissue. This is ethically defensible only if the well-being of the stag is not unacceptably compromised, particularly in relation to operative and post-operative pain. Those removing velvet antlers are ethically bound to employ "best practice" analgesic techniques. Previous studies have shown that the local anaesthetic lignocaine HCL (2%) is most effective using the high dose ring block around the antler pedicle.

The antlers from deer at the Massey University Deer Research Unit or a commercial deer farm in the Pohangina Valley, Manawatu were used for these analgesia onset and duration studies. The onset of analgesia was determined by electrically stimulating the velvet antler at 15-second intervals until behavioural responses ceased. Duration of analgesia was determined using a novel remote electrical stimulus, which registered the return of sensation in the antlers using behavioural responses. All treatments were administered in a ring block at dose rate of 1 ml/cm pedicle circumference.

In Study 1(a), the onset times of analgesia after application of one of three treatments (2% lignocaine hydrochloride (L), 2% lignocaine hydrochloride with 8.4% sodium bicarbonate (LBC) and 0.5% commercially available bupivacaine (BC) were determined in antlers of 21 rising 1-year-old red, and wapiti hybrid stags. Each antler received two treatments (28 antlers per treatment). The mean onset time was 31(SE± 7.0) seconds for L, 21(SE±2.5) seconds for LBC and 48(SE±12.7) seconds for BC. The difference in mean onset between BC and LBC was significant ($p=0.0225$).

In Study 1(b), antlers of 26 stags 2 years-of-age and older were allocated either L or LBC. Mean time of onset of analgesia for L was 31(SE±5.94) seconds and LBC was 36(SE±5.95) seconds. There was no significant difference between the two treatments.

Duration of analgesia in Study 1(a) was measured in eight antlers given L and nine antlers given BC. The mean duration for L was 88(SE±7.7) minutes, and for BC 273(SE±19) minutes ($p<0.001$).

Antlers of 39 rising 1-year old stags were used for Study 2. The onset and duration of analgesia were compared following two combinations of L and a novel formulation of bupivacaine (BN). In study 2(a), "high"(HLBN) (1.5% L and 0.5% BN) and "low"(LLBN) (1.0% L and 0.25% BN) concentrations of a mixture of lignocaine and bupivacaine were investigated for onset of analgesia on 25 antlers each. The mean time for onset of analgesia for HLBN was 37(SE±4.4) seconds and for LLBN, 55(SE ±8.8) seconds (p=0.049). The mean duration (n=10 antlers/treatment) for HLBN was 406(SE ±28.9) minutes compared with 333(SE± 25.2) minutes for LLBN (p=0.041).

In Study 2(b) antlers were treated with either 2% mepivacaine HCl (M), 1.5% mepivacaine/1.5% lignocaine (ML), 1.5% mepivacaine/0.5% bupivacaine (BN) (MB) or 0.5% bupivacaine (BN) (n=7/treatment). Mean onset times were 30(SE ±12.3) seconds for M, 30(SE ±6.6) seconds for MB, 34(SE ±7.8) seconds for ML, and 86(SE ±37.3) seconds for BN. There was no significant difference in mean onset times. Duration of the four treatments (n=7 antlers/treatment) was 271(SE± 26) minutes for M, 221(SE ±19) minutes for ML, 421(SE ±41) minutes for MB, and 461(SE ±37) minutes for BN. There were differences in duration between treatments with bupivacaine and those without (p=<0.0001). The mean duration of analgesia following the novel bupivacaine formulation was significantly longer than that for the commercial formulation (p=0.001).

In study 3, each step in the velvet antler removal procedure was timed and recorded. When both antlers on stags were given local anaesthetics together (n=16 antlers/treatment), the mean time between completion of the first ring block to the nick test on the first antler was 72 (range 52-151) seconds, while the mean time between completion of the second ring block and the nick test on the second antler was 70 (range 61-183) seconds. When only one antler was given a ring block, the time between completion of the block and the nick test was 42 (range 25-40) seconds. This study showed that the time interval between injection of a high dose ring block and application of the nick test by the experienced operator would rarely be less than 60 seconds when both antlers are treated together. Velvet antler removal can therefore be undertaken in a continuous sequence of activity eliminating the necessity of a wait time if 2% lignocaine is used at 1ml/cm antler pedicle circumference.

In anticipation that studies of postoperative pain control will be needed in the future, a pilot trial testing one proposed method was undertaken. Fifteen 2-year old stags were given the tranquilliser, azaperone, to test whether it reduced the confounding effects of handling stress on plasma cortisol concentrations. In addition, nine were given the non-steroidal inflammatory drug, flunixin meglumine, after velvet antler removal. Plasma cortisol concentrations were elevated in both groups. No significant difference was detected between the means of the control and NSAID treated groups over 5 hours.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BB	0.5% Bupivacaine BOMAC formulation
BC	0.5% Bupivacaine commercial formulation (Marcaine)
BP	Blood pressure
cm	Centimetre
DOM	Date of manufacture
EA	Electronic analgesia
et al.	Et altera
HLBB	High concentration lignocaine/bupivacaine (BOMAC) formulation
HPA	Hypothalamic-pituitary-adrenocortical axis
HR	Heart rate
i/m	Intramuscular
i/v	Intravenous
kg	Kilogram
L	2 % Lignocaine HCL
l	Litre
LAN	Long acting neuroleptic
LBC	2% Lignocaine HCL with 8.4% sodium bicarbonate
LLBB	Low concentration lignocaine/bupivacaine (BOMAC) formulation
M	2 % Mepivacaine HCL
MB	Mepivacaine/bupivacaine (BOMAC) formulation
ML	Mepivacaine/lignocaine formulation
ml	Millilitre
NAWAC	National Animal Welfare Advisory Committee
ng	Nanogram
nmol	Nanomol
NSAID	Non-steroidal anti-inflammatory drug
NVSB	National Velveting Standards Body
PABA	Para-aminobenzoic acid
QA	Quality assurance
S.E.	Standard Error
s/c	Subcutaneous
µg	Microgram
VARNZ	Velvet Antler New Zealand
ZPTA	Zuclopenthixol acetate

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Antlers have intrigued mankind throughout history. This fascination is illustrated in ancient cave paintings of antlers and their value as hunting trophies. Antlers are used in Asian medicine and their phenomenal growth rate has interested scientists in recent years (Anon., 1999b; Haigh and Hudson, 1993).

Antler growth is bone growth (Goss, 1983). Among mammals, the Cervid antler is the only organ composed of bone regenerated by any warm-blooded vertebrate. Nowhere else in the animal kingdom does an animal tolerate the retention of dead bone (Haigh and Hudson, 1993).

When the antler starts to grow, it is called velvet antler. This is a densely vascularised and richly innervated tissue (Adams, 1979). It is given this name because it is covered with unique hair follicles, which makes it look like velvet. Each follicle has a sebaceous gland that secretes an oily substance (sebum) into the shaft of the hair follicle when they are fully differentiated. It is this sebum that accounts for the shiny appearance of the velvet (Goss, 1983).

Velvet antler has been removed from various species of the deer family for at least 2,000 years and it is considered a valuable component of Asian medicines (Fennessy, 1991; Goss, 1983; Haigh and Hudson, 1993; Kong and But, 1985; Luick, 1983a; Rennie, 1982; Sunwoo and Sim, 2001; Suttie and Haines, 1998a). It is used as an elixir for general strengthening of the body and bones, and improving the tone and function of muscles. Velvet antler is also used to treat specific diseases and ailments (Luick, 1983b).

Deer in North America, Russia, China, Korea and neighbouring countries are farmed primarily for velvet antler production. In Europe, deer are farmed, with few exceptions, only for venison (Hudson, 2001). In New Zealand, Australia and New Caledonia both velvet and venison are produced from deer. While deer have been farmed in China, Russia and Korea for centuries for velvet antler production, New Zealand, North America and Australia have only recently become regular suppliers of this product (Lee and Chang, 1985; Hudson, 2001). Korea is the major market for

New Zealand velvet and New Zealand is the major world velvet producer, supplying 65% of the total market (Loza, 2001).

The removal of velvet antler from farmed deer on an annual basis raises unique moral and ethical questions, as velvet antler is living tissue that is removed from a live animal (Fennessy, 1991; Goss, 1983; Haigh and Hudson, 1993; Wilson, 1989; Wilson et al., 2001). Stags are reared specifically for velvet antler removal and this would not occur if it were not economically viable (Wilson et al., 2001). A more detailed discussion of the animal welfare implications of velvet antler removal is presented in a later section of this review.

The surgical act of velvet removal is painful (Matthews and Cook, 1991) and the use of analgesics is mandatory in many countries (Wilson et al., 2001). Considerable income is generated from the sale of velvet antler as a medicinal and nutritional product to Asian countries and North America, but there are concerns worldwide regarding the animal welfare implications of this form of farming. Thus, the deer industry in New Zealand has funded research into ensuring that velvet antler removal is humane (Weilburg, 1996).

It is not my intent in this thesis to make a judgement on the ethics of deer farming for the purpose of velvet antler removal. The premise of this thesis is that provided the welfare of the animal is optimal, the farming of animals by mankind is ethically defensible (Wilson et al., 2001). The removal of velvet antler is legal in New Zealand and is permitted only under strict compliance with the "Code of Recommendations and Minimum Standards for the Welfare of Deer during Removal of Antlers" (Anon., 1999b).

In this thesis, systems that may improve the welfare for stags undergoing velvet removal are evaluated. This introductory chapter will review the history of velvet antler use, the anatomy and physiology of antler, describe techniques for antler removal, and address post-operative pain and its evaluation and control. Farm animal welfare, in general, and as it pertains to deer, especially in regards to the removal of velvet antler will also be reviewed.

1.2 Deer welfare

1.2.1 General concepts on farm animal welfare

It is widely accepted in western societies that the welfare of an animal should be the first consideration of livestock production and humane aspects must override any economic consideration (Killorn and Heath, 1993). Criticism raised by animal welfarists regarding the quality of life of farm animals has in recent years, generated significant political and economic pressure on the livestock industries of some countries to introduce changes to, or discontinue, certain management practices (Hurnik, 1988).

Values relating to the care and welfare of animals are complex and constantly evolving (Stewart and Laing, 1999a). The changes in these values may possibly advance animal welfare by improving certain farm management practices.

A full understanding of animal welfare issues is essential for the deer industry in reinforcing its marketing into the future. This section briefly addresses animal welfare in general and specific issues relating to the intensive farming of deer, especially those involving velvet antler removal.

1.2.1.1 Defining animal welfare

The term "welfare" has been defined in a number of ways. In 1965 after an increase in controversial issues pertaining to intensive farming, the British Government set up a parliamentary inquiry conducted by Professor F.W.R Brambell (Blackmore, 1990; Blackshaw, 1991). The Brambell Committee concluded that welfare was: " . . .*a broad concept that embraces both the physical and mental well being of the animal.*"

Broom (1988) was more specific in his definition: "*the state of the individual as regards its attempts to cope with its environment*" and which includes feelings and health (Broom, 2001). Feelings of deer, however, will not be discussed here, as there is insufficient knowledge and understanding about this aspect of animal welfare or animal cognition. Welfare is a characteristic of an individual at the time of observation or measurement, to be assessed in an objective way using measurements of different welfare indicators (Table 1.1) (Broom, 2000). Welfare of the individual can range from very good to very poor (Broom, 2000).

Table 1.1 Measures of Welfare (From Broom, 2000)

Physiological indicators of pleasure
Behavioural indicators of pleasure
Extent to which strongly preferred behaviours can be shown
Variety of normal behaviours shown or suppressed
Extent to which normal physiological processes and anatomical development are possible
Extent of behavioural aversion shown
Physiological attempts to cope
Immunosuppression
Disease prevalence
Behavioural attempts to cope
Behaviour pathology
Brain changes, e.g. those indicating self narcotization
Body damage prevalence
Reduced ability to grow or breed
Reduced life expectancy

The term "well-being" is often used to denote the state of the animal itself instead of the broader sociological concept of "animal welfare" (Swanson, 1994). Hurnik (1988) uses the term "well-being" in his definition: " . . . *Animal well-being is a state or condition of physical and psychological harmony between the organism and its surroundings*". Pre-and post -operative care, pain recognition and management are critical to animal well-being (Swanson, 1994), and that concept has particular relevance to the removal of velvet antler, the topic of this thesis. However, while most authors seem to have a general understanding of what welfare or well-being means without defining it precisely (Weilburg, 1996), it is now widely accepted that good welfare or well-being is achieved when there is an absence of unacceptable compromise in the five domains listed in Table 1.2. These are commonly referred to as the "Five Freedoms".

Table 1.2 The five domains of animal welfare (Mellor, 1999)

DOMAIN	HOW COMPROMISE IS MINIMISED
NUTRITION	PROVISION OF FOOD AND WATER AND PREVENTION OF MALNUTRITION
ENVIRONMENT	PROVISION OF AN APPROPRIATE ENVIRONMENT
HEALTH	PREVENTION OF OR RAPID DIAGNOSIS AND TREATMENT OF DISEASE, INJURY AND FUNCTIONAL IMPAIRMENT
BEHAVIOUR	PROVISION FOR BEHAVIOURAL AND INTERACTIVE NEEDS
MENTAL	PREVENTION OF MENTAL AND PHYSICAL SUFFERING

It is accepted, however, that some compromise to these domains is likely at some stage of an animal's life (Wilson et al., 2001). Those authors discuss that concept in relation to the removal of velvet antlers, balancing potential or real negative impacts against positives, from a welfare and production perspective. Other species examples include electro-ejaculation in sheep, which is aversive and stressing, but is necessary to confirm a diagnosis of *Brucella ovis* (Stafford et al., 1996). Likewise, routine management practices such as drenching and administering vaccines may be stressful, but are done with the animal's optimum welfare in mind, with the aim of preventing disease conditions that if contracted, would compromise the welfare of the animal more than the act of drenching or vaccination. The acceptability of various handling practices depends on the balance between the amount of pain, stress or lack of behavioural stimulation experienced by the animal and the degree of subsequent benefits to both animals and humans (Matthews, 1992).

1.2.1.2 Animal welfare in an agricultural context

Technological progress and application of new scientific discoveries in agriculture during the last few decades has significantly increased the productivity of animals. The welfare status of agricultural animals is important, not only because of a well-established association between improved welfare and productivity (Haigh, 1998), but because serious questions have been raised with regard to the cost that farm animals are expected to bear in modern husbandry systems (Hurnik, 1988). A clear trend has emerged with regard to husbandry procedures that cause injury, as more emphasis is being placed on evaluating and minimising the pain and distress caused by such procedures (Mellor and Stafford, 1999). Opinion-shapers in the livestock industries rank animal welfare as one of three major challenges confronting

agriculture in the 21st century, along with environmental issues and food-safety concerns (Rollin, 1995).

Indeed, increasingly articulate concern by consumers, agricultural producers and their organisations in other countries may eventually result in non-tariff trade barriers, thus restricting access to markets (Matthews, 1992). Therefore, animal welfare is not solely a scientific issue. Politics, philosophy, ethics and aesthetics also influence society's expectations concerning the use, care and treatment of animals (Swanson, 1994). Since animal welfare is of such interest to so many people, animal welfare science should provide the information necessary to enable the attainment of a scientific, political and social consensus (Stafford and Mellor, 1993) by encompassing a multi-disciplinary approach to assess and define animal welfare in farm animals (Swanson, 1994).

1.2.1.3 Measurement of animal welfare

All criteria used to assess welfare rely on demonstrating some evidence of change (Barnett and Hemsworth, 1990). Stress is a biological response with no defined aetiology or prognosis. It is elicited when an individual perceives a threat to its homeostasis (Moberg, 2000). Physiological changes associated with the stress response have been widely used as indicators of welfare in the belief that as stress increases, welfare decreases (Broom, 1988; Fraser and Broom, 1998; Moberg, 1985).

The effects of stress can be measured by monitoring the changes that occur in the endocrine system and in various physiological functions of an animal (Moberg, 2000). Physical stressors include extremes of temperature, restraint, transport, surgery, and exposure to novel sounds, sights, odours, tastes or noxious chemicals (Griffin and Thomson, 1991).

Changes in behaviour have also been used as indicators of change in welfare (Broom, 1988) and, according to Broom (2000), are the most obvious in showing that some aspects of the situation may be aversive. The animal may stop moving forward, freeze, back off, run away or vocalise. The behaviours animals show during stress are performed to help the animal deal with the stress. The types of responses are often specific for a particular type of stressor, making it unlikely that there is a "general response" to a stressor (Rushen, 2000). Behavioural stressors can include

overcrowding, capture of wild animals, hierarchical challenge, exposure to unfamiliar surroundings, or sensory deprivation through isolation (Griffin and Thomson, 1991).

An adequate understanding of the complexity and integration of physiological and behavioural responses to stress requires a number of variables be measured (Cook et al., 2000). However, all physiological parameters that have been measured may be influenced by handling and restraint, by individual animal variation and by the degree of tameness of the subject (Haigh et al., 2001) and by the act of monitoring itself (Moberg, 2000). Difficulties of interpretation can be a major limitation to welfare research (Barnett and Hemsworth, 1990).

1.2.2 Deer welfare measurements

1.2.2.1 Physiological measurements used to assess deer welfare

1.2.2.1.1 Heart rate

Heart rate has been used by several researchers as a primary physiological indicator of pain and stress in deer while undergoing various procedures such as velvet antler removal, transport, yarding, handling and ear tagging (Cook et al., 2000; Grigor et al., 1998a; Matthews and Cook, 1991; Pollard et al., 1993b; Pollard et al., 1991; Price et al., 1993). However, as with hormone levels, there are factors other than stress that can have equally large or greater effect on the heart rate of an animal, such as physical or digestive activity (Rushen, 1991).

In Table 1.3, from Weilburg (1996), several authors and their findings have been summarised from a review of the literature.

Table 1.3 Heart rates of red deer under a variety of conditions as reported in the literature

Author (s)	No/Sex/Age	Sampling	Values (beats/min)	Activity or Treatment
Espmark & Langvatn, 1985	15/m&f/calves	r/h	170 mean	Less than 2 days old; lying
			155 mean	7 days old
			50-60	Approach of human
Pollard et al. 1991	9/m/yearling	r/h	110 mean	Visual isolation
Price et al. 1993	?/f/adult	r/h	58	Lying
			69	Standing
			87	Walking
			110	Alert, standing neck up
			120	Trotting
Matthews & Cook, 1991	7/m/yearling	r/h	83 mean	Local
			105	Local & velvet

Author (s)	No/Sex/Age	Sampling	Values (beats/min)	Activity or Treatment
			189	Velvet without local
			167	Ear tagging
Matthews et al. 1994	18/m/2yrs	r/h	~70-90	At pasture
			~70-135	In yard & restraint & local
			~80-145	In yard, restraint, local & velvet
Carragher et al. 1997	36/m/2 yrs	r/h	55 mean	Before handling
			111 mean	Yarding
			~ 90	Drafting
			~ 81	Handling
			~ 69	Post-handling

r/h: remote on harness; local: application of local anaesthetic; velvet: removal of velvet antler;
no: number; m: male; f: female; yrs: years; ~: approximately; ?; not reported;

Carragher et al. (1997) studied the effects of yarding and handling procedures on stress responses of red deer stags. Behaviour, heart rate (HR) and blood parameters were monitored to define the stress responses to three procedures involving yarding, handling and restraint. Heart rate increased because of the handling treatments, with increases following "Draft" and "Crush" being greater than after the "Pen" treatment (Carragher et al., 1997). The "Draft" treatment required a handler to twice enter the pen and move the animals around. The "Crush" procedure required animals to be drafted from the group and restrained in a pneumatic drop-floor crush for 2 minutes. The "Pen" treatment group was left undisturbed in an indoor pen for 12 minutes.

Since any type of activity causes a change in heart rate, the response to stressors may be confounded by activity patterns (Carragher et al., 1997). During the period when the different treatments were being applied, the HR of the "Pen" group was significantly lower than the other "Draft" and "Crush" groups. This may be due to the lesser degree of physical activity and psychological stress involved (Price et al., 1993). Carragher et al. (1997) found that the HR from the "Crush" animals did not increase as much as they expected because their HR was already elevated, which may be due to the movement required to enter the crush or struggling once in the crush or from isolation from the herd (Price et al., 1993). These authors, however, suggest that there may have been limitations with their HR recording technique as it recorded at 1-minute intervals, while other authors (Pollard et al., 1993b) used systems that signalled every heartbeat. Upon return to the pasture, the HRs of the "Pen" and "Draft" deer took 40 minutes to return to pre-handling levels, whereas the "Crush" group took 20 minutes longer. The authors could not conclude whether this

prolonged recovery was due to residual affects associated with the "Crush" procedure or because of activity differences between the groups once they returned to pasture. Carragher et al. (1997) observed that the treatment HR levels of the "Draft" and "Crush" were similar to those reported by others in Table 1.3 with values ranging from 90-110 beats/min.

While the heart rate levels of the restrained and penned deer were approximately twice as high as those measured in deer at pasture, the authors concluded they were not particularly high or prolonged relative to other procedures. Matthews and Cook (1991) monitored HRs of deer restrained in a crush while undergoing several manipulations. Their results suggest that some treatments were aversive. A HR of 167 beats/min was measured during ear tagging, but dropped to 102 beats/min after 4 minutes. One group of stags was velveted without a local anaesthetic and the HR reached 189 beats/min and was 133 beats/min 4 minutes post-velveting (Matthews and Cook, 1991) compared to 93 beats/min for velvet antler removal under local anaesthetic applied using a ring block. In a later study, HR was recorded for at least 3.5 hours following velvet antler removal (Matthews et al., 1994). In that study, HR was monitored in deer while in the yard, during restraint (~70-135 beats/min), and given a local anaesthetic and in another group undergoing the same manipulations plus velveting (~80-145 beats/min). There were no major differences between these two groups though HR and ECG responses were disturbed during and shortly after velveting. Heart rate measurements in these studies suggested that velvet removal under physical restraint with local anaesthesia resulted in a similar level of physiological stress as that measured in non-velveted deer subjected to physical restraint only (Matthews et al., 1994). However, Pollard et al. (1991) concluded that antler removal was more aversive than restraint only, as there was an increase in heart rate prior to removal of the second antler, in contrast to a decrease in the heart rate for stags receiving restraint only.

1.2.2.1.2 Plasma cortisol concentration levels

Cortisol is the primary glucocorticoid in most mammals. An important function of glucocorticoids is to curtail the HPA (hypothalamic-pituitary-adrenal) axis through negative feedback inhibition (Matteri et al., 2000). However, corticosteroid hormones secreted in response to stress have the potential to increase an animal's susceptibility to infectious disease by suppressing the immune system (Thomson et

al., 1994). Cortisol concentration-time curves derived from repeated blood sampling are a major tool for quantifying an animal's response to stress (Mellor et al., 2000). Quantitative tools for characterising this response include numerical representation (response duration, peak height) and statistical evaluation of concentration-time curves to detect within-group deviations and between groups differences (Mellor et al., 2000). There is no single numerical factor that adequately defines a stress response, and as the complexity of the response increases, then the likelihood of it being represented by a single number decreases (Mellor et al., 2000). Additionally, the assays used to determine plasma cortisol levels differ in how the levels are evaluated and in the measurements used, thus making it difficult to compare plasma cortisol levels between studies (Table 1.4).

A number of authors have investigated plasma cortisol levels in various deer species (Table 1.4). Weilburg (1996) summarised many of these investigations. The data from 1996 are from Weilburg (1996), while the more recent data are summarised from the original papers.

Table 1.4 Summary of means and/or ranges of cortisol levels measured under a range of situations as reported in the literature

Author	Species	No/Sex/Age	Sampling	Values	Treatment
Brelurut, 1991	Red deer	20/m&f/8 months	vp	53 (48-59) ng/ml	Semi-wild, transport for 3 hours
				32 (9-52)	Farmed, transport for 48 hours
Bubenik & Bartos, 1993	Red deer	7/m/2,5 years	can	<2 µg/100 ml	Sedated Xylazine/Ketamine 1-2mg/kg, in summer
	Fallow deer	8/m/yearling	can	2 µg/100 ml	Sedated Xylazine/Ketamine 1-2mg/kg, in summer
Bubenik et al., 1983	White-tailed deer	2/m/adult	can	0.8-5.2 ng/ml	Sedated Xylazine in summer
				3.8-13.4 ng/ml	In winter
Goddard et al., 1994	Red deer	20/f/adult	vp	207-332 nmol/l	Wild derived hinds, restraint
		40/m&f/calves		54 nmol/l	Farmed, restraint manually
				97 nmol/l	Wild derived, restraint manually
Hastings et al., 1992	Chinese water deer	36/?/?	as	9.1-17.3 nmol/l	Culled by gunshot
Jones & Price 1992	Fallow deer	?/m & f/?	vp	~15-180 ng/ml	Captured park animals
Jago & Matthews, 1994	Red deer	18/f/mixed age	as	18.6 ng/ml mean	Transport 80 km, stunned with captive bolt & slaughter
				15.6 ng/ml mean	Transport 230 km slaughter

Author	Species	No/Sex/Age	Sampling	Values	Treatment
				10.5 ng/ml mean	Transport 380 km slaughter
Matthews & Cook, 1991	Red deer	18/m/adult	cn	35-65 ng/ml mean	Restraint in crush Nov./Dec.
				26-32 ng/ml mean	Sedated Xylazine 0.9 mg/kg & local & velveted
				65 ng/ml mean	Local & velveted
		6/m/adult		35 ng/ml mean	Hard antler removal, late Feb.
Matthews et al., 1994	Red deer	18/m/2 yrs	re	11 ng/ml mean	On paddock free moving
				50-70 ng/ml mean	In yard, restraint, local or local & velvet
Smith & Dobson, 1990	Red deer	20/m/15-20 months	as	29.9 ± 7.0 ng/ml mean	Transport for 25 minutes & slaughter
		33/m/15-20 months		20.9 ± 5.3 ng/ml mean	Transport for 2 hours & slaughter
		10/m/15-20 months		5.7 ± 3.7 ng/ml mean	Culled by gunshot in field
Carragher et al., 1997	Red deer	36/m/2 yrs	re	5.4 ng/ml mean	All groups at pasture
				30 ng/ml mean	All 3 groups during yarding and handling
				23 ng/ml mean	Pen group - Post-handling
				30 ng/ml mean	Draft group - Post-handling
				46 ng/ml mean	Crush group - Post-handling
Grigor et al., 1998a	Red deer	60/m,f/yearling	vp	16.7 nmol/l mean	Pre-Journey: Stationary
				19.0 nmol/l mean	Pre-Journey: Transported
				25.3 nmol/l mean	Post-Journey: Stationary
				49.0 nmol/l mean	Post-Journey: Transported
				23.9 nmol/l mean	Post-Recovery: Stationary
				24.0 nmol/l mean	Post-Recovery: Transported
Grigor et al., 1998b	Red deer	24/f/yearlings	vp	2 Hr and 6 Hr Journey (nmol/l means)	
				33.5 41.9	Pre-Journey: Straight Road
				35.2 36.1	Pre-Journey: Winding Road
				100.6 88.4	Post-Journey: Straight Road
				123.8 112.9	Post-Journey: Winding Road
				32.8 33.5	Post-Recovery Straight Road
				37.4 30.8	Post-Recovery Winding Road
Pollard et	Red deer	58/m/15 mos	as	28 ng/ml mean	DSP culled

Author	Species	No/Sex/Age	Sampling	Values	Treatment
al., 2000				5.6 ng/ml mean	PAD culled
Read et al., 2000	Wapiti	23/m/1-2 yrs	vp	118 nmol/l mean	Day 1 - Pooled data
				98 nmol/l mean	Day 2 - Given ZPTA
				140 nmol/l mean	Day 2 - Given saline
				93 nmol/l mean	Day 4 - Given ZPTA
				136 nmol/l mean	Day 4 - Given saline

vp:venepuncture; as: after slaughter; can: cannulation; re: remote; local: application of local anaesthetic; velvet: removal of velvet antler; no: number; m: male; f: female; yrs: years; ?:not reported; DSP: deer slaughter premise; PAD: paddock culled; ZPTA: Zuclopenthixol acetate (a long-acting neuroleptic)
A conversion rate for cortisol is: 1 ng/ml = 0.1 µg/100ml = 2.76 nmol/L and 1 nmol/L = 0.363 ng/ml = 0.0363 µg/100 ml

Carragher et al. (1997) investigated the effects of yarding and handling on the cortisol responses of red deer stags. They used remote blood sampling devices to eliminate confounding effects from stress associated with manual blood sampling techniques. The plasma cortisol levels were low (5.4 ng/ml), as in other studies where remote blood sampling devices were employed (Ingram et al., 1994; Matthews et al., 1994). The "Crush" procedure, described in 1.2.2.1.1, resulted in greater plasma cortisol (46 ng/ml post-handling) than either the "Pen" or "Draft" procedures (23ng/ml and 30 ng/ml post-handling) (Carragher et al., 1997). This suggested that while the "Pen" treatment and the "Draft" treatment caused some stress, the process of restraining an animal, as in the "Crush" treatment, appeared to be a significant additional stressor. However, the authors suggested that the "Crush" procedure employed in this study did not maximally stimulate the adrenal cortex since the animals were restrained for 2 minutes. In a previous study by Matthews et al. (1994), cortisol levels of 60-70 ng/ml were obtained, but the deer were restrained for a period of 1.5 hours. These data highlight the difficulties when quantitatively comparing studies of cortisol.

The effects of vehicular motion during transport produced a significantly larger increase in cortisol concentrations compared with stationary confinement (Grigor et al., 1998a). Deer, in either groups of 5 or 10 animals, were loaded onto a livestock transporter for 3 hours during which they were either transported or remained stationary. Part of this study compared vehicular motion to confinement alone. Pre-journey measurements were 16.7 nmol/l for the stationary treatment and 19.0 nmol/l for the transported treatment. Immediately after the journey, the transported deer

had mean plasma cortisol readings of 49.0 nmol/l while the stationary deer had a reading of 25.3 nmol/l. Post-recovery of the transported deer and the stationary deer had reductions to 23.9 nmol/l and 24.0 nmol/l, respectively. It was suggested that the act of loading and being confined in a transporter was stressful for the deer. However, as the various measures used in this study tended to return to pre-treatment levels shortly after unloading, it suggests that the welfare of the deer was not compromised (Grigor et al., 1998a).

In another study, Grigor et al. (1998b) measured the effects of road type and journey time on red deer hinds. They compared the effects of straight roads to windy ones during two time frames (2 hours and 6 hours). There was a significant increase in cortisol when transported, which may suggest that it was a stressful experience. However, there was no difference found between road types and there was an indication that the deer became habituated to transport over time. After the 2-hour journey, deer had cortisol levels of 100.6 nmol/l for a straight road and123.8 nmol/l for the winding road. After the 6-hour journey the deer cortisol levels of 88.4 and 112.9 nmol/l, for straight and curving roads respectively. As in the previous study, the increase in cortisol level was short lived and quickly returned to pre-journey levels, suggesting that the deer's welfare was not significantly compromised by transportation (Grigor et al., 1998b).

Pollard et al. (2000) compared blood variables of paddock shot deer to deer slaughtered commercially. The mean cortisol value for the deer slaughter premise (DSP) deer was substantially higher at 28 ng/ml than the paddock culled deer (5.6 ng/ml) (Pollard et al., 2000). This value for paddock-culled deer was similar to that of a study by Smith and Dobson (1990) where the measurement was 5.7 ng/ml. The moderate levels seen in the DSP deer suggest they were only moderately stressed at the time of slaughter (Pollard et al., 2000). In Table 1.4 there are cortisol values obtained under normal farming procedures, which are relatively high compared to the DSP values.

Long-acting neuroleptics (LANs) have become a valuable tool for decreasing capture and handling stress in many species (Read et al., 2000). Those authors evaluated Zuclopenthixol acetate (ZPTA) for its ability to decrease stress and activity in wild wapiti. The control deer were given saline. ZPTA treated deer had plasma cortisol concentrations of 98 nmol/l and 93 nmol/l, on Day 2 and Day 4 post

treatment, respectively. Cortisol in the deer receiving the saline treatments measured 140 nmol/l and 136 nmol/l on Day 2 and Day 4 of the study. Thus, the use of ZPTA was effective in decreasing the cortisol response and the animals were easier to handle. It was also effective within several hours of administration, and its effects were measurable, but waning after 72 hours. While habituation to handling occurred with all animals, the ZPTA treated deer responded sooner (Read et al., 2000). The authors suggested that this drug has great potential to reduce stress in intensively farmed and managed species, such as deer.

1.2.2.2 Behavioural measurements of deer welfare

Behaviour is part of the general functioning of an animal and is a component of the various systems that are part of an animal's life, such as osmoregulation, predator avoidance or reproduction (Fraser and Broom, 1998). As with observation of physiological changes, certain changes in behaviour have been used as indicators of animal welfare (Broom, 1988). Some measurements include: the intensity, duration and frequency of startle responses and defensive or flight reaction; the time required for normal activity to resume after stress; and increases in the frequencies of aggression, stereotypies and apathetic or unresponsive behaviours (Cook et al., 2000). Changes in behaviour have been used as an indication of pain in deer. An increase in various activities such as head shaking, ear flicking, grooming, aggression and jumping after velveting suggested post-operative pain in deer (Pollard et al., 1993a; Pollard et al., 1992; Pollard et al., 1991). Fence pacing has also been exhibited during or after various management practices (Diverio et al., 1993; Pollard et al., 1993b). Withdrawal from the stimulus of a surgical cut has been used to evaluate the effectiveness of analgesia of the antler (Wilson et al., 2000a; Wilson et al., 1999b).

However, many non-specific responses observed after treatments may be subjective and so should be interpreted with caution (Matthews and Cook, 1991; Matthews et al., 1990; Pollard et al., 1992; Pollard et al., 1991). Wilson et al. (1999a, 1999b) reported that some stags not responding to an electrical stimulus after application of local anaesthetic responded to either a wood cut test or a nick test. The wood cut test consisted of sawing a block of wood that was held next to the velvet antler, while the nick test consisted of applying the saw to the antler to test for a response. The response of these stags could have been due to the vibration and/or activity

around the head region (Wilson et al., 1999b). One stag receiving the "high" dose ring block responded to the nick test at 4 minutes after application of the local anaesthetic, but as it displayed a behaviour score of 1, it was possibly a non-specific response (Wilson et al., 1999b). However, if it were a non-specific response rather than a painful stimulus, "best practice" would mandate that cutting cease immediately and a wait time be observed prior to continuation of velvet antler removal, so that the welfare of the stag is not compromised.

Some behaviour patterns may be normal in relation to a farming environment, but abnormal in relation to wild animals (Goddard and Matthews, 1997). However, whether or not such behaviour is necessarily indicative of compromised welfare could be open for debate.

1.2.3 Deer management practices that may compromise welfare

1.2.3.1 Yarding and handling

Animals, such as deer or antelope, which have excitable and flighty temperaments, frequently sustain injuries when handled for veterinary procedures (Grandin, 2000b). Injuries were the most common animal health problem in farmed red deer in a study of 15 red deer farms over a 2-year period (Audige et al., 2001). These species have intense flight responses which enable them to flee from predators, but in a farmed environment there are often obstacles such as fences obstructing flight paths. Tame animals that are accustomed to frequent handling and close contact with people are usually less stressed by restraint and handling than animals that seldom see people (Grandin, 1997). That is, activities that are stressful and compromise the welfare of certain individuals may not be similarly stressful in animals which have become accustomed to handling. The handling, however, must not be physically rough or aversive as this will have a major impact on their welfare leading to increased stress, considerable production losses and make handling more difficult and, possibly, dangerous for handlers and animals (Rushen et al., 1999).

Management of the health and production of farmed red deer requires periodic mustering of the animals into yards (Pollard and Littlejohn, 1996). This is thought to be stressful to deer as there may be an inability to avoid each other during the confinement period (Pollard and Littlejohn, 1996). These authors studied the effect of pen size on the behaviour of stags. Activities such as head butting, chases and

stepping were measured during spring and summer. Based on these measurements it was concluded that small pens were favoured in spring and larger pens in summer. However, it was also concluded that in the larger pens, the deer were able to maintain greater inter-individual distances and there was reduced pacing and head movements regardless of the season (Pollard and Littlejohn, 1996).

1.2.3.2 Restraint

Most deer require restraint for procedures requiring close handling, especially removal of antlers and artificial insemination (Matthews, 2000). In several studies, both physiological and behavioural measures showed restraint in a handling device, or crush, to be more aversive than transportation, separation from other deer, and nearness to humans (Carragher et al., 1997; Grigor et al., 1998c; Pollard et al., 1993b; Waas et al., 1997).

In a preference test deer were able to choose between a restraining device, which they could walk through or one where they would be restrained. It took progressively longer for them to enter the side where they would be restrained (Grigor et al., 1998c; Pollard et al., 1993c). This latency to enter the raceway and the length of time taken to move down it showed that restraint was the most aversive of a range of treatments that were introduced in that study (Grigor et al., 1998c).

1.2.3.3 Transport

The transportation of livestock represents a prime concern from both welfare and economic perspectives (Rollin, 1995). The transport-slaughter process is controversial for all farmed species because of the wide range of stressful situations that animals are exposed to, and because death is the endpoint (Matthews, 1992). Transportation of livestock is commonly in view of the public, thereby increasing their concern and ultimately spurring research into welfare of animals during transport (Knowles and Wariss, 2000).

The welfare problems associated with transportation pervade the whole process, from poor handling and management on the farm, to actual transit conditions and, finally, treatment received once arriving at their destination. In the United States of America, the losses stemming from transportation of cattle such as bruising, injuries and stress-related diseases are extremely high. Bruising alone annually costs that industry US$22 million (Rollin, 1995).

Transportation of deer may result in their being exposed to stressors that elicit behavioural and physiological responses that compromise their welfare (Grigor et al., 1997; Grigor et al., 1998b; Grigor et al., 1998a; Grigor et al., 1998c). The factors researched in those studies included effects of sex, group size, space allowance, vehicular motion, road-type and journey time.

A review by Weeks (2000) on the transport of deer has concluded that the magnitude of behavioural and physiological responses of deer to certain aspects of handling and transport are similar to those of other ruminants. Their welfare does not appear to be compromised despite their relatively recent domestication, as they do habituate to humans, flight distances reduce with familiarity and the effects of most of the stressors are short-lived. However, because of their flighty nature they require specialised handling facilities and equipment (Weeks, 2000).

Weeks (2000) states that there have been no scientific studies conducted of responses of deer to transport during actual commercial journeys. It is suggested that a fully loaded vehicle driven by a commercial driver would provide a unique physical and social environment that cannot be consistently duplicated or replicated under experimental conditions, especially if there is only partial loading (Weeks, 2000). However, studies by Jago et al. (1997) and Waas et al. (1997) measured behavioural and physiological responses during experimental journeys that may be relevant to a commercial situation, but similar observations during commercial journeys need to confirm this (Weeks, 2000). Thermal exchanges during transit of deer were also another issue raised by Weeks (2000), as these have not been specifically investigated for temperature, wind speed or humidity in regards to deer under experimental or commercial conditions. Adult ruminants are more likely to experience heat stress rather than cold. In New Zealand, the Animal Welfare Advisory Committee (1994, 1996) recommends that stock should not be transported when ambient temperatures exceed 30°C. These recommendations suggest water-cooling by hosing to reduce heat stress, and the provision of drinking water at least every 12 hours.

Therefore, a number of scientific studies undertaken in this area suggest that the welfare of deer is not compromised while they are being transported under proper conditions.

1.2.4 Velvet antler removal and deer welfare

The removal of velvet antler, which is a live and innervated tissue, from farmed deer on a sustainable annual basis, raises unique and interesting moral, ethical and legal issues (Fennessy, 1991; Goss, 1983; Haigh and Hudson, 1993; Wilson, 1989; Wilson et al., 2001). Most velvet antler for human consumption is removed from live animals. The justification for antler removal is rarely questioned in Asian countries, but open to debate in the West. Farming deer for velvet requires ethically acceptable and legal practices (Fennessy, 1991; Haigh and Hudson, 1993; Wilson, 1989; Wilson et al., 2001) because it involves some form of restraint of the stag followed by the act of cutting off the living antler (Haigh et al., 2001).

The welfare issues relevant to the removal of velvet antler have been reviewed in depth by Wilson (1989), English (1991) and Wilson et al (2001). In this section, the main issues will be highlighted.

1.2.4.1 Prevention of animal injury

Deer establish dominance hierarchies within a group. When deer are kept close together, as in a farming environment, the stresses of handling, yarding and transport often manifest as aggression toward other deer. Therefore, confinement reduces the ability of a subordinate stag to avoid this antagonistic behaviour, especially during the rut. Antlered stags inflict more serious injuries on other animals than de-antlered stags (Wilson, 1989; Wilson et al., 2001). The removal of antlers, therefore, may be seen as beneficial to the welfare of subordinate farmed deer.

Prevention of self-injury is another area of animal safety. The velvet antler, since it is living tissue, is sensitive and fragile and can be easily damaged or broken, even under optimum farming conditions. This injury has the possibility of inflicting severe pain and stress to the individual (Wilson, 1989; Wilson et al., 2001). Therefore, it may be argued that removal of antlers is beneficial to the affected individual under farming conditions.

Occasionally antlered stags lock together when fighting, and this has been observed as a cause of death in wild populations (Wilson, 1989; Wilson et al., 2001). Stags in hard antler are also more prone to entanglement in fences. Accidents such as these are eliminated by the removal of antlers and, thereby, benefiting the stags' welfare. Additionally, when a stag is in the rut sedation by a tranquilliser dart in a

paddock situation may be the only available option for the humane removal of hard antler (Griffiths, 1996).

1.2.4.2 Human safety

An antlered stag has the capability of inflicting more serious injury on a person than does a non-antlered stag (Wilson, 1989; Wilson et al., 2001). If management procedures such as drenching, vaccinating and treating diseases need to be undertaken, particularly while a stag is in the rut, there is less risk involved for the handler if the stag is non-antlered and the animal is, thus, more likely to receive treatment beneficial to its welfare.

1.2.4.3 Human benefit

The economic benefit of velvet antler removal to the producer and all parties in the value-added chain to the consumer is undisputed in all deer farming countries, regardless of whether velvet antler removal is permitted or not. The deer velvet industry in New Zealand has contributed up to $60 million annually to the economy and has provided significant employment. In New Zealand today, the farmed deer population comprises approximately 2 million deer and an estimated 470 tonnes of velvet is produced. Based on varying degrees of herd health, by 2004, the farmed deer population could reach 3.5 million and the amount of velvet produced could reach 660 tonnes, an increase of 40% (Loza, 2001).

The benefit of velvet antler to humans as a medical/health product was until recently, regarded solely as a traditional Asian phenomena of unproven value (Wilson, 1989; Wilson et al., 2001). Now in Western cultures there is a strong trend toward the adoption of "traditional" remedies as alternatives to, and/or to complement conventional Western medicine. There is also renewed interest in velvet antler for performance enhancement and a range of other medical uses (Suttie and Haines, 1998a; Suttie and Haines, 1998b). Although the benefits of velvet have been regarded with scepticism in Western cultures, controlled clinical and other studies should be able to demonstrate its value, if indeed they exist (Section 1.5). Therefore, Western countries where velvet removal is currently prohibited may reassess their position on velvet antler production.

1.2.4.4 Expression of normal behaviour

Hummels are naturally antlerless red deer stags. While the absence of antlers may be of genetic origin, hummels are able to produce offspring with normal antlers and exhibit normal behaviours along with physiological functions (Goss, 1983). Observations of hummels, combined with experience of farming of stags with antlers removed, demonstrates they are still able to express the same behaviours and, thus, the absence of antlers does not disadvantage the stag (Wilson, 1989; Wilson et al., 2001). However, battles between hard-antlered stags are normal behaviour. Therefore, expression of normal behaviour must be weighed against the risk of injury (Haigh and Hudson, 1993), particularly when confined by fences that close the route of escape for the subordinate or beaten stag.

1.2.5 Velvet antler removal

Because of concerns for the welfare of the deer, and probably because of scepticism of the medicinal value of velvet, in the United Kingdom and a number of other countries, antlers may only be removed when hard and no longer innervated (Wilson, 1989; Wilson et al., 2001). Indeed, the UK Farm Animal Welfare Council concluded that analgesia of the antlers could not be guaranteed in a commercial farm environment, and that was the major reason for them advising that the practice should not be permitted. However, this may pose a greater risk to the handler. In contrast to the situation in the UK, in New Zealand the Code of Recommendations and Minimum Standards for the Welfare of Deer during the Removal of Antlers (Anon., 1992) states that for both animal and human safety during the process of removal, the most appropriate time for antler removal is when in velvet. Not only does this Code address the animal and human safety issue for the removal of velvet antler, it also addresses a more controversial issue. It acknowledges that velvet antler is of economic value to the producer and an important overseas income for New Zealand, and therefore, must be removed at the appropriate time. These two different perspectives illustrate that, while the desirability of antler removal is rarely questioned in a farming environment where stags are contained, the timing of removal is. The NZ Code has been revised under the new Animal Welfare Act of 1999, which provides legal recognition for a Code of Welfare to be derived from the former Code of Recommendations and Minimum Standards.

While not every country where velvet antler removal is permitted has such stringent regulations, a universally accepted standard would be desirable for the animals' best interests (Wilson et al., 2001). The ideal method of velveting stags would provide the following elements (Haigh et al., 2001):

- Minimum stress/distress management and yarding system
- Minimal animal handling time
- Rapid onset of analgesia in all treated stags
- Hygienic surgical technique
- Control of post-operative bleeding
- Post-operative stag health and welfare
- Post-surgical analgesia
- Residue-free product
- Reasonable or low costs
- Accessibility to suitably qualified farmers

1.2.5.1 Velvet antler analgesia methodologies

Anaesthesia is a state in which sensations are not perceived (Brander et al., 1991). Drugs may be administered to a patient either parenterally (injection, inhalation or topical application), or enterally (oral dosage). Of the parenteral routes, subcutaneous (s/c) injection administered drugs have an advantage as their absorption rate is relatively slow and even, which provides for a sustained effect (Brander et al., 1991; Strichartz and Covino, 1990).

Currently, only local anaesthetics are permitted in New Zealand for the provision of analgesia during velvet antler removal from adult stags. Rubber rings, causing "compression analgesia," have been approved for use in yearling stags during velvet antler removal (Matthews et al., 1999; Matthews and Suttie, 2001). "Electro-analgesia" is another technique for analgesia that has been investigated (Haigh et al., 2001; Matthews et al., 1999b).

1.2.5.2 Local anaesthesia

1.2.5.2.1 Local anaesthetic drugs

While general anaesthetics achieve analgesia by rendering the patient unconscious and, therefore, insensible to pain, local anaesthesia simply prevents neural transmission, and thus sensation, regardless of the animal's state of consciousness (Brander et al., 1991; Matteson, 2000). True local anaesthetics are agents with reversible effects, and they interfere with the ability of peripheral nerves to function at drug concentrations below those at which they exert other actions (Brander et al., 1991; Matteson, 2000).

According to Upson (1988), the properties of an ideal local anaesthetic are:

- Soluble in water, stable and near neutral pH
- Produce minimal irritation at site of injection
- Have a specific effect on sensory nerve endings and/or nerve fibres
- Have poor absorption from injection site thus providing longer local action and less drug entering systemic circulation
- Have minimal systemic toxicity
- Non-addictive
- Reversible
- The clinically important properties of the various local anaesthetics include potency, speed of onset, duration of analgesia, and differential sensory/motor blockade (Strichartz and Covino, 1990).
- Advantages of local anaesthetics include (Riebold et al., 1982):
- Minimal equipment required
- Economical
- Useful in minor surgical or diagnostic procedures
- Safe
- Easy to administer

Disadvantages of local anaesthetics when used without sedation include (Riebold et al., 1982):

- Patient movement during the procedure
- Increased risk to the surgeon

In addition, local or systemic reactions to the anaesthetic drug can occur and residues and metabolites may remain in meat-producing animals (Section 1.2.5.2.2).

Local anaesthetics are classified as belonging to the ester or amine groups based on their chemical structure (Duke, 2000a). The local anaesthetic base molecule is a tertiary amine separated from an unsaturated ring system by an intermediate chain. The chain contains either an ester or amide linkage (Strichartz and Covino, 1990). These linkages influence anaesthetic potency.

Lignocaine, bupivacaine and mepivacaine (all amine amides) are local anaesthetics used in veterinary medicine, with lignocaine being used most frequently for removal of velvet antler (Matthews et al., 1992; Wilson et al., 2000a; Wilson et al., 2000b; Wilson et al., 1999a; Wilson et al., 1999b).

Lignocaine hydrochloride is the most versatile and commonly used local anaesthetic, as it is potent and safe. It has a rapid onset of effect, and a moderate duration of action of approximately 1-2 hours (Covino, 1986; Strichartz and Covino, 1990).

Mepivacaine shares the potency of lignocaine but is less toxic. Its time to onset is similar to that of lignocaine, but its duration of effect is slightly longer (Brander et al., 1991; Covino, 1986; Strichartz and Covino, 1990). It also produces a profound depth of analgesia (Strichartz and Covino, 1990). It is not used, however, in obstetric anaesthesia as its metabolism is markedly prolonged in the foetus and newborn (Covino, 1986). Mepivacaine is used for specific nerve blocks, local infiltration and epidural anaesthesia and is commonly used in horses for intra-articular anaesthesia (Riebold et al., 1982). Unlike lignocaine, mepivacaine is not effective as a topical agent (Covino, 1986).

Bupivacaine is the most potent member of the amine amide group of local anaesthetics, having four times the potency of lignocaine and twice its duration of action, though it has a longer onset period (Brander et al., 1991; Covino, 1986; Jones, 1995). It is used for various regional anaesthetic procedures, including peripheral nerve blocks, extradural and spinal anaesthesia. It is used extensively in obstetric analgesia as it provides satisfactory pain relief for 2-3 hours, significantly decreasing the need for repeated injections in the parturient patient (Strichartz and Covino, 1990).

Mepivacaine and bupivacaine are related compounds with similar structures. In bupivacaine, a butyl group is added to the tertiary amine, while mepivacaine has a methyl group (Strichartz and Covino, 1990).

Epinephrine is a vasoconstrictor frequently included in local anaesthetic solutions to decrease the rate of absorption, thereby allowing more drug molecules to reach the nerve membrane and improve the depth and duration of analgesia (Strichartz and Covino, 1990). Lignocaine with epinephrine, however, is not recommended for velvet antler removal, as it is believed the vasoconstriction of the epinephrine can cause sufficient anoxia for *Clostridium septicum* spores to become active resulting in facial oedema and necrosis (Seifert, 1997).

1.2.5.2.2 Local anaesthetic drug and metabolite residues

The export markets for velvet antler have traditionally been Asian countries, which are relatively unregulated markets. The introduction of New Zealand velvet antler as a "nutraceutical" or "functional food" into the North American market has heightened its classification for export as a "food product" (Kamen, 2001; Sim and Sunwoo, 2001). If a "food product" contains drug residues, it is considered adulterated, and violates the United States Federal Drug and Cosmetic Act (Walsh et al., 2001).

A residue of a parent drug or chemical and its metabolites may accumulate and be deposited or stored within the cells, tissue or organs of an animal following the use of drugs and chemicals (Booth, 1988). Drug residue concentrations vary considerably from tissue to tissue. They are generally observed to be higher in tissues of storage, such as body fat or in organs that actively metabolise and excrete them (Booth, 1988).

The amine amide local anaesthetics, lignocaine, mepivacaine and bupivacaine, are metabolised by the liver, and in most species, the major urinary metabolite is the by-product 2,6-xylidine (also a metabolite of xylazine) (Walsh et al., 2001). This by-product was found to have carcinogenic activity in male and female rats, as there was a significant increase in the incidence of carcinomas of the nasal cavity and subcutaneous fibromas and fibrosarcomas (Chamberlain and Brynes, 1998).

The ester-linked local anaesthetics, tetracaine, procaine and chloroprocaine, are rarely used in veterinary medicine. These agents are primarily metabolised by

esterases in plasma, red blood cells and liver to para-aminobenzoic acid (PABA) (Walsh et al., 2001). PABA may induce allergic-type reactions in a small percentage of patients (Strichartz and Covino, 1990). The ester-linked agents, however, are broken down rapidly by plasma esterase activity and, therefore, the likelihood of finding drug residues is less (Walsh et al., 2001).

The ester-linked agents do not appear to be metabolised into 2,6-xylidine, which offers a potential advantage over the amine-linked agents. However, their slow onset and allergic potential may limit their use. Future manipulation of the physiochemical properties of the ester amides may lead to a re-evaluation of their usefulness (Walsh et al., 2001).

However, while residues may be of significance, there are no published reports of local anaesthetic residues in velvet antler.

1.2.5.3 "Electro-analgesia"

The potential for local anaesthetic drug residues in antler intended for health conscious consumers has been a concern to velvet antler producers and marketers (Loza, 2001; Walsh et al., 2001; Woodbury et al., 2001). This has increased the industry's interest in the use of non-chemical means of inducing analgesia (Matthews et al., 1999).

Electronic analgesia (EA) is used to provide pain relief in human dentistry (Haigh et al., 2001; Matthews et al., 1999; Matthews and Suttie, 2001; Woodbury et al., 2001). Burgio (1998) proposed a similar technique for the removal of velvet antler, using a modified device "Vet-EA" as an effective method of pain control. This technique has become widespread in the North American elk industry (Haigh et al., 2001; Matthews and Suttie, 2001; Woodbury et al., 2001).

Matthews et al. (1999) conducted a study using the "Vet-EA" device and found that it provided variable, non-repeatable pain relief during velvet antler removal, using behavioural observations. A further study by Woodbury et al. (2001) assessed electroanaesthesia for its effectiveness relative to lignocaine. In that study, deer were given zuclopenthixol acetate (ZPTA), a long acting tranquilliser, to remove background effects created by fear and stress on the physiological parameters measured. That drug proved to be very effective in decreasing stress response and activity during physical restraint (Read et al., 2000). By using the physiological

measures of heart rate (HR), blood pressure (BP) and cortisol levels, and behavioural responses, as indicators, those authors demonstrated that induction of "electroanalgesia" was painful and variably effective. There was a significant increase in heart rate, not only while antler was removed under supposed EA, but during the application of EA itself.

Compared with the repeatability and effectiveness achieved with local anaesthetics, EA was highly variable among animals and, in most cases, there was only a very mild degree of analgesia. The intensity and frequency of avoidance responses to applications of the EA have given cause for concern (Woodbury et al., 2001).

On the basis of published observations, one can conclude that, using current technology, "electroanalgesia" does not provide sufficient pain relief to be considered as an adequate alternative to the use of local anaesthetics for velvet antler removal (Haigh et al., 2001; Matthews et al., 1999; Matthews and Suttie, 2001; Woodbury et al., 2001).

1.2.5.4 Compression

There is a requirement for a simple, ethically acceptable and practical technique for analgesia during spiker velvet removal, as large numbers of animals are involved and because velvet must be removed prior to transport and slaughter (Matthews et al., 1999b; Matthews and Suttie, 2001). Compression induced analgesia by specified rubber rings is thought by some to fulfil this requirement (Matthews and Suttie, 2001).

Matthews and Suttie (2001) demonstrated that a specified rubber ring applied to the pedicles of antlers of yearling stags (spikers) induced analgesia within 60 minutes. The analgesia was measured by lack of behavioural response by the deer as the antler was cut off (Matthews and Suttie, 2001). This lack of behavioural response, however, is not necessarily a complete measurement of the effects of pain. Under natural conditions, an animal's behaviour is often related to helping the animal survive (Livingston, 1994). Deer are species that are subject to predation; therefore, the behaviour in response to pain, which is most likely to help them survive, is the behaviour that does not attract the attention of predators. Predators usually target an animal that demonstrates some abnormal behaviour, so the animal's best chance of

survival is to act normally, that is, not to show it is suffering pain (Livingston, 1994). The lack of behavioural response was questioned by Haigh et al. (2001).

Pain from tourniquet use was first studied in 1952 by Cole (Estebe et al., 2000), and to date there have been no data on the possible aversiveness of the technique in deer.

Full circumference compression of the antler pedicle, while it will pressure all sensory nerves that supply the antler, also creates ischaemia (Wilson pers. comm. 2001). This could cause ischaemic pain, which is used as a model for pain in research trials evaluating various methods of analgesia in humans.

Compression has been used to produce analgesia in other species. Various tourniquet methods have been used to induce acute or chronic compression of a peripheral nerve in animal models. In rats, there is evidence of expansion of the receptive field of noci-response neurones in response to tourniquet pain following the application of a pneumatic tourniquet to the thigh (Crews and Cahall, 1999). Nerve compression and ischaemia resulted in block of input to low threshold mechanoreceptor neurons having receptor fields distal to the tourniquet cuff, but there was an increase in spontaneous activity and expansion of the receptor fields of high threshold noci-responsive neurons located proximal to the tourniquet. Expansion of the receptive fields of the nociceptors proximal to the tourniquet may explain the mechanism of tourniquet-related pain (Crews and Cahall, 1999).

It is difficult, however, to control the precise location and pressure on the nerve with a tourniquet (Rempel et al., 1999). Metal spring clips, compression clamps and tubes of various materials have been used for compression, and while it was possible to obtain a graded compression, it was not possible to measure or control the applied extraneural pressure (Rempel et al., 1999). It is necessary that repeatability and reproducibility be achievable for any technique designed to produce analgesia. However, these studies used soft tissue, which may be different to the skin over the bone anatomy of the pedicle. It is, therefore, likely that repeatability of compression about the pedicle could be greater than when deeper softer tissues are studied, as in the above research.

In another study of tourniquet pain, twenty human volunteers were evaluated for pain using different cuff widths and pressures. All subjects experienced tourniquet pain in each of the tests and the pain from the compression increased as the rest of

the arm became desensitised (Estebe et al., 2000). Narrow diameter bands were more painful than broader bands.

There is also concern that there is a potential for altered antler growth in years following the use of rubber ring compression (Haigh et al., 2001). Matthews et al. (1999b) and Matthews and Suttie (2001) proposed that this technique could be utilised on deer that are to be retained for velveting in subsequent years without unduly affecting commercially relevant velvet production. In that study, they compared 2-year-old stags, which had been velveted as spikers with either local anaesthetic or ring compression. There was a decrease in velvet weight that approached significance (P=0.053), with the compression treated deer having antler weights of 0.57 kg versus 0.67 kg for those treated with anaesthetic (Haigh et al., 2001; Matthews et al., 1999b; Matthews and Suttie, 2001).

While compression could possibly be suitable for velvet removal in spikers prior to transport, research needs to be undertaken to determine whether the technique is aversive in deer. Additionally, further research needs to be undertaken in older stags not only to evaluate the possible aversiveness, but also to confirm analgesia effectiveness and evaluate the significance compression may have on shape, length and weight of the velvet antler in succeeding years.

Application of specified rubber rings for antler analgesia in 1-year-old stags only has been approved by NAWAC and compliance standards for this technique are described in the "Velvet Removal Programme" manual (NVSB, 1998). However, there are no data to show that application of this procedure *per se* is pain-free. If the application of the rubber ring is painful, then it does not fulfil one of the important criteria for an analgesic. Clearly, further study of this technique is required.

1.2.6 Operative and post-operative pain control

Pain plays an important role in survival. It signals impending or actual tissue damage and may help an animal avoid harm (Gaynor, 1999). In a protective role after serious injury, it can prevent movement that would cause further trauma, and aids in convalescence. However, prolonged immobility can be detrimental to body functions. Physiological responses to pain, which include elevation in plasma ACTH, cortisol, anti-diuretic hormone (ADH) and catecholamines can result in a general catabolic state with muscle protein catabolism, lipolysis, water and sodium

retention and potassium secretion. A prolonged stress response due to pain can decrease the rate of healing, and can result in serious complications and even death (Gaynor, 1999).

Surgery is painful. The pain begins when an incision is made and tissue damage activates nociceptors present in skin and underlying tissues. When activated, the nerve fibres transmit information to the spinal cord and on to the brain, resulting in the sensation of pain (Roberge and McEwen, 1998). These authors described the Gate Control Theory of Pain. This theory involves three spinal cord systems that transmit nerve impulses when the skin is stimulated. These include the cells of the substantia gelatinosa in the dorsal horn, the dorsal column fibres that project toward the brain and the first central transmission cells in the dorsal horn. The neural mechanisms in the dorsal horns of the spinal cord act like a gate by increasing or stopping the flow of nerve impulses from peripheral fibres to the spinal cord cells that project on to the brain.

Pain that results from tissue injury is initiated by activation of peripheral small fibre terminals that keep the gate in a slightly open position. Stimulation of large fibre terminals tends to close the gate (Roberge and McEwen, 1998).

The veterinary care of agricultural animals has historically been directed to restoring their productivity or killing them if restoration were not possible. Keeping them free of pain was not a concern (Rollin, 1997). Merillat, the author of a 1905 textbook on veterinary surgery, lamented the failure of veterinarians to use anaesthesia, except for occasional canine practitioners, whose clients attached more than economic value to their animals. The first textbook of veterinary anaesthesia published in the United States of America was not until 1973 and control of pain was not listed as a reason for anaesthesia (Rollin, 1997).

It was not until the early 1990s that post-surgical anaesthesia was taken seriously in research, and has now become normal procedure in veterinary companion animal practice (Rollin, 1997). Pain control in large animal medicine has been a recent phenomenon and is an issue that has not been fully addressed in velvet antler removal of deer.

Pre-emptive analgesia, administering an analgesic before onset of surgical trauma, is a strategy used to reduce post-surgical pain and analgesic requirement (Hellyer,

1997; Raffe, 1997). Post-operative pain typically follows a predictable course, peaking within 6 to 24 hours following surgery and waning progressively thereafter (Hansen, 1997). In human surgery, patients randomly assigned to a saline placebo for pain control had post-operative subcutaneous oxygen partial pressures (tissue-oxygen tension) that were significantly less than patients given lignocaine for post-operative pain treatment. The incidence of surgical-wound infection is highly correlated with tissue oxygen partial pressure (Akca et al., 1999). Proper prevention of post-operative pain will reduce the incidence of surgical wound infection and reduce the time taken for post-operative recovery (Flecknell, 1997-1998; Matteson, 2000). Thus, providing effective post-operative pain relief should have a positive effect on the speed with which animals return to normality following surgical procedures (Flecknell, 1997-1998) and better fulfil one of the five domains of animal welfare, namely freedom from pain. This is particularly important in relation to deer, in terms of linking "animal friendly" systems for ensuring marketability of products (Wilson et al., 2001)

1.2.6.1 Non-steroidal anti-inflammatory drugs (NSAIDs)

Allergy, anaphylaxis and inflammation are the body's response to stimuli that are perceived as foreign (Brander et al., 1991). These stimuli include bacteria, their toxins, internal and external parasites, trauma and necrosis of tissue (Brander et al., 1991; Upson, 1988).

Inflammation is the circulatory and cellular reaction of the body's tissues to these stimuli. The objective of inflammation is to provide body defence mechanisms against injury and insult by destroying, removing or neutralizing the action of these injurious agents and then to repair the damage and restore the tissues to normal (Brander et al., 1991; Upson, 1988). The primary signs of inflammation are redness, heat, swelling, pain and loss of function. Initially, an inflammatory reaction is protective, but may proceed to be injurious to the insulted tissue or even the entire animal (Upson, 1988).

Prostaglandins are in a class of chemical messengers known as eicosanoids that play a significant role in many physiological processes (Hadley, 1996). These chemical mediators are involved in the mechanism that brings about inflammation and other pathological responses in the cells (Brander et al., 1991; Hadley, 1996; Upson, 1988).

Non-steroidal, anti-inflammatory drugs (NSAIDs, also known as Prostaglandin Synthesis Inhibitors), block the synthesis of prostaglandin, thereby decreasing the inflammatory response and the pain often associated with it (Brander et al., 1991; Frandson and Spurgeon, 1992; Hadley, 1996; Upson, 1988). They are acidic drugs and are extensively protein bound, which allows them to persist at active concentrations in tissues when plasma concentrations have already fallen (Brander et al., 1991). These drugs act predominately at the site of the painful stimulus rather than in the spinal cord or brain, as opiates do (Hosking and Welchew, 1985).

There are several NSAIDs available for use in veterinary medicine. None is licensed for use in deer. The experiment in Chapter 6 set out as a pilot evaluation of possible methods for evaluating post-operative pain using an NSAID, flunixin meglumine. This NSAID was chosen as the test product for this pilot study since it is a commonly used product in large animals.

Flunixin meglumine is a highly substituted nicotinic acid that is significantly analgesic and inhibits the biosynthesis of certain prostaglandins. As with other NSAIDs, it has anti-inflammatory, antipyretic and analgesic properties. The onset of action for Flunixin is 2 hours, with a peak time of 12-16 hours, while its length of duration may extend for 24-36 hours (Upson, 1988). Flunixin has a plasma elimination half-life of 1.6-2.1 hours in horses, 8 hours in cattle and 3.7 hours in dogs and is excreted in urine (The Merck Veterinary Manual, 1998; Upson, 1988). Flunixin has been used for its analgesic properties in horses and cattle. In horses it is used to alleviate colic as it reduces the intestinal smooth muscle spasms, and in cattle and sheep it blunts the inflammatory process and the associated tissue damage with mastitis (Brander et al., 1991; Fthenakis, 2000).

1.2.7 Conclusion on welfare and velvet antler removal

Increasing social interest in farm animal welfare will be a strong and continuous impetus for the best possible reputation and future marketability of animal products (Hurnik, 1988) such as velvet antler. Welfare is the biggest single issue the deer industry faces in developing and protecting a market for deer velvet in the West. The practice of velvet removal is an issue in some European markets because it is not permitted and local deer farmers frequently raise this contention in the media against the importation of venison (Loza, 2001). Globally there is an emerging consensus that some human benefits are not worth any amount of animal suffering (Rollin,

1995; Rollin, 2000). Therefore, the concept of continuous improvement must be adopted by deer industries worldwide if welfare concerns are to be allayed (Wilson et al., 2001) since economic benefit will no longer outweigh animal welfare considerations (Rollin, 2000).

1.3 Antler anatomy and physiology

The anatomy and physiology of the Cervid antler has been extensively reviewed in the literature (See Goss 1983; Brown 1992; Fennessy and Drew 1985; Bubenik 1990). The following section will comprise a general overview of this subject.

1.3.1 Introduction

Antlers are deciduous appendages that are grown and cast annually (Goss, 1983). Except in reindeer (*Rangifer tarandus*), only male deer develop antlers despite the presence of primordial tissue in both sexes. Of the 42 species of deer, males of only two species do not have antlers, the Musk deer and the Chinese water deer (Bubenik, 1982; Bubenik, 1990a; Goss, 1983). To regenerate antlers, the mature skin cells from the rim of the pedicle must first re-differentiate into primitive embryonic cells. The presence of embryonic stem cells in growing antlers of adult animals is unique among mammals (Bubenik, 2001).

There are three phases to the complete development of red deer antlers. Antler growth takes 102-115 days, antler ossification takes 30-40 days and the shedding of the velvet skin continues for 6-22 days.

1.3.2 Pedicle initiation

Antlers grow from pedicles, which are permanent outgrowths of the frontal bones and prerequisites for normal antler growth. Unlike antlers, the development of the pedicle happens only once. The pedicle develops from a specialised periosteum of the frontal bone (inductive periosteum) which is thicker than periosteum elsewhere on the body (Bubenik, 1990a; Goss, 1983; Haigh and Hudson, 1993). The term "inductive" periosteum, however, may be a poor choice of terminology as the periosteum is not an inducer in itself, but may induce "dermal periosteal ossification" leading to the pedicle development (Suttie and Bubenik, 1992).

Pedicle development usually begins between the ages of six to nine months, and is a secondary sexual characteristic as the deer approaches puberty (Li et al., 1993). The early stages of pedicle growth are difficult to pinpoint because they are hidden by

the skin and hair over the frontal bone. In wapiti and red deer, there is microscopic evidence of pedicle development during foetal life (Haigh and Hudson, 1993). Once pedicle development has begun, however, it continues until it reaches a height of several centimetres (Goss, 1983). It is covered by the skin and hair of the scalp. Once the pedicle reaches this height, the skin changes in appearance and texture, giving it a "velvety" appearance. The antler pedicle, while containing blood vessels, nerves and connective tissue, has neither joints nor skeletal muscle (Goss, 1983).

1.3.3 Velvet skin and growth

The skin covering the growing antler is a special type of integument (Goss, 1983). It is the only tissue in the body to develop hair follicles as it regenerates, but these lack erector pili muscles (Haigh and Hudson, 1993). It is from these hair follicles that the velvet antler gets its name. Velvet antlers are the fastest growing mammalian tissue; in elk (Goss, 1983) and in reindeer, the maximal elongation is around 2 cm. per day. This is phenomenal as this includes the growth of multiple tissues, such as skin, cartilage, bone, blood vessels and nerves (Bubenik, 2001).

The velvet skin is separated from the growing antler by undifferentiated connective tissue and the blood supply is present in this layer. There is a gradient of progressive tissue differentiation from the tip toward the base of the antler (Haigh and Hudson, 1993). Located at the tip is a zone of fibroblasts that change to successive layers of cartilage, calcified cartilage and then bone. The growth point is from the tip of the antler and not the base.

The first pair of antlers are usually unbranched spikes, hence the name "spikers" given to yearling stags. They then undergo an annual cycle of calcification, skin shedding, casting and regeneration. The pedicle increases in diameter from the annual deposition of concentric rings of bone.

1.3.4 Antler innervation

Innervation of the developing pedicle is mainly from the infratrochlear and zygomaticotemporal branches of the trigeminal nerve (Adams, 1979; Kirk and Adams, 1980). The zygomaticotemporal nerve leaves the caudal margin of the zygomatic process and innervates the caudal and lateral features of the pedicle. The infratrochlear nerve emerges from the dorsal rim of the orbit and innervates the rostral and medial facets (Adams, 1979; Kirk and Adams, 1980). Additionally, it

has been confirmed that red deer, wapiti and fallow deer have a third branch from the auriculopalpebral nerve, which is a branch from the seventh cranial nerve (both a sensory and motor nerve) that can supply the medial antler (Woodbury and Haigh, 1996). It is thought that 20% of red deer possess this type of pedicle innervation (Suttie et al., 1994).

Nerves and hormones regulate antler growth. Parasympathetic nerves are involved in the regulation of antler shape and size, but not in the cycle of antler regeneration. It was observed that if antlers were unilaterally neurectomised, they resulted in being smaller and less branched than antlers on the control side (Suttie and Bubenik, 1992).

1.3.5 Blood supply of antlers

Deer antlers are the only external mammalian structures in which temperature equals that of the deep body. This is due to the copious blood flow to the growing tips, which makes the velvet antler warm to the touch (Goss, 1983). It is interpreted that this blood flow is a mechanism that ensures elevated temperatures conducive to the rapid proliferation of cells in a structure that grows so rapidly it reaches full dimensions in a few short months (Goss, 1983). Antler growth even surpasses the expansion of the most rapidly growing cancers (Goss, 1995).

The arterial blood supply to growing antlers is provided mostly by the superficial temporal artery (a branch of the external carotid artery), which divides extensively in the pedicle. The branches first run under the velvet skin and then penetrate the antler bone cortex (Suttie, 1985). The external jugular vein in deer drains blood from the whole head area, except the internal organs (mainly brain tissues). This means the jugular blood contains the venous blood from the antlers (Bubenik, 1990c). Antler arteries are similar to arteries of the umbilical cord in that they share a common transient life span (Goss, 1983). As antler calcification progresses, arterial supply and venous drainage diminish.

1.3.6 Antler structure

Whereas the frontal bone is compact, the pedicle is composed of spongy bone (Goss, 1983). This spongy bone is interspersed with numerous blood vessels, and it comprises most of the shaft of the growing antler. The trabeculae of the spongy bone, under the antlerogenic periosteum, push up under the scalp, which makes the

growing pedicle visible. As highly vascularised cartilaginous trabeculae are formed on top, ossification advances distally where the cartilage is replaced by bone (Goss, 1983).

The process of ossification takes place in conjunction with calcification of the cartilage. Osteoblasts align themselves along the degenerating calcified cartilage trabeculae and mediate the transformation into bone (Goss, 1983). The most intense mineralisation occurs at the tips and the antler base, which is eventually sealed by a compact bony plug from the living pedicle. Calcification is completed shortly before the rut (Haigh and Hudson, 1993).

1.3.7 Velvet skin shedding

Hardening of the antler bone involves restriction of the vascular channels and occurs when testosterone levels in the blood are increasing. This results in ischaemic necrosis of the velvet skin and gives the skin a shrivelled appearance. The velvet skin is then shed, leaving a mature antler composed of dead solid bone (Bubenik, 1990a; Bubenik, 1990b; Bubenik, 2001; Goss, 1983). However, recent studies on fallow deer hard antler revealed living bone with regions of living osteocytes, osteoblasts and even early stages of trabecular microcallus formation, thus indicating a continuous bone remodelling up to 3 weeks prior to antler casting (Rolf et al., 2001). In white-tailed deer the presence of a viable blood supply in hard antlers was confirmed by the existence of fluorescent dye injected intravenously three months after velvet shedding (Bubenik, 1999).

1.3.8 Antler casting

The base of the mature antler consists of compact bone without the spongy bone core and is bound to the pedicle by many small spicules. There is a line of future separation indicated by a narrow band of minute blood vessels (Goss, 1983).

Antler casting has been attributed to the reabsorption of bone around the Haversian canals, because of osteoclastic activity. This results in the separation of the old antler from the pedicle, as the spicules of bone connecting the two are broken (Goss, 1983)

1.3.9 Control of annual growth cycles

Antler growth cycles are closely related to sexual cycles in stags (Haigh and Hudson, 1993). In temperate species, the seasonal production of antlers is primarily

triggered by photoperiodic changes, which influence the pineal gland to produce melatonin. Melatonin, in turn, modifies the secretion of prolactin, luteinizing hormone and testosterone, the sexual hormones involved in the regulation of the antler cycle (Bubenik, 1990a; Bubenik, 1990b; Bubenik, 2001; Goss, 1983).

New antler growth is usually initiated in the spring, after the hard antlers are cast and when concentrations of testosterone are low. When testosterone levels rise, the shedding of velvet and the mineralisation of antlers occur (Bubenik, 2001; Suttie and Bubenik, 1992). Therefore, pedicle initiation is caused by increased plasma levels of testosterone stimulated by increasing luteinizing hormone (LH) pulse frequency, and testosterone is stimulatory for pedicle growth but not necessarily so for velvet antler growth (Suttie et al., 1991). Besides steroids, many growth factors, vitamins and enzymes have also been detected in growing antlers (Suttie and Bubenik, 1992).

1.3.10 Function of antlers

The function of antlers continues to cause considerable speculation (Haigh and Hudson, 1993). The most important primary uses are the display of social status, weaponry and as pheromone dissipaters. Secondary uses include such things as back scratchers and tools for knocking down fruit (Bubenik, 1982; Goss, 1983; Haigh and Hudson, 1993). Antlers are used as sparring tools to establish rank order or occasionally for attack on predators, which can include humans. Most conflicts, however, are resolved by a series of ritualised displays that include parallel walking and displaying of antlers. If a fight does occur it is usually between stags of similar size and because display alone is not enough to resolve the dispute (Bubenik, 1982; Haigh and Hudson, 1993).

Although antlers provide a visible expression of dominance, other social and individual factors are of similar importance. Antlerless stags will successfully defend a harem and breed. However, it is essential that stags with antlers not be in the same paddock as antlerless stags (Bubenik, 1982; Haigh and Hudson, 1993).

The stag with the largest antlers is usually dominant, but threatening and intimidating behaviour patterns also contribute to assertion of dominance. There is evidence that hinds in oestrus will seek out the stags with the largest antlers,

indicating a male-female interaction dependent on antlers in addition to the well-known male-male interaction (Bubenik, 1982; Haigh and Hudson, 1993).

Antlers are also functional for nonsexual and social pursuits. When an older stag loses his antlers (mature stags cast antlers before younger stags), he also loses his dominance in the hierarchy. The younger stags will temporarily assert social dominance until their antlers are cast, and then normal hierarchy is re-established (Bubenik, 1982; Haigh and Hudson, 1993).

1.4 History of Velvet Antler Use

In the Chinese culture deer, especially spotted deer, have been recognised as lucky animals that bring health and longevity. A deer always accompanies their god of longevity, symbolising the role of a deer as a mascot of medicinal values (Kong and But, 1985).

The earliest record of the medicinal application of deer products was found on a silk scroll excavated from a Han Tomb from Mawangtui in Changsha County, Hunan Province, China, with a burial date of 168 B.C (Kong and But, 1985). The scroll chronicled several medical treatises, including a section on prescriptions for 52 diseases. Three of these prescriptions included deer antlers, venison and glue prepared from deer antlers (Putman, 1988). More systematic recording of the medicinal virtues of deer parts was compiled in a series of Chinese herbals, collectively known as *pênts'ao* and dating from AD 200 (Putman, 1988). Among the 25 deer parts registered in *pênts'ao* are: velvet, antler, antler glue, bone, bone marrow, spinal cord, penis and testes, venison, headmeat, head glue, sinew, blood, tooth, shank, skin, fat, brain, semen, thyroid gland, meconium, foetus, undigested milk and bone of lower limb (Kong and But, 1985). The claimed cures range from treatment of a general malaise to more specific symptoms relating to consumptive diseases, impotence, spermatorrhea, lumbago, abnormal menstruation, infertility, carbuncles, dermatitis, traumatic injury, goitre, apoplexy, epilepsy, diabetes and fever (Putman, 1988).

The *Pharmacopoeia of the People's Republic of China* (1977) adopted a more cautious stance, registering only four deer parts (antler, antler glue, residue of antler glue and velvet) and recognising their functions only in the treatment of lumbago, gonalgia, mastitis, ecchymosis, carbuncles, tuberculosis in bones and joints,

impotence, spermatorrhea, metrorrhagia, frequent urination, 'wet dreams', vertigo and anaemia (Putman, 1988). However, a compilation of the prescriptions of over-the-counter drugs used in China showed that deer parts were found in 48 tonics, 23 drugs for the treatment of gynecopathy, three for rheumatism and one each for gastro-intestinal problems and cardiovascular problems (Kong and But, 1985). Deer parts used in these over-the-counter preparations include velvet antler, antler, antler glue, residue of antler glue, sinew, foetus, penis and testis, venison, bone, and tail (Kong and But, 1985).

The basic philosophy of traditional Oriental medicine differs from the principal underlying Western medicine in that it aims to promote health in the whole person, rather than treat specific ailments (Rennie, 1982). The use of velvet in Asian medicine is based in the concepts of Yin and Yang, the two primordial cosmic forces (Haigh and Hudson, 1993). Yin represents the passive, descending, feminine force. Yang is the active, bright, ascending, masculine force. Good health is stated to require the harmonious balance of these forces. Velvet is Yang and shifts the balance in this direction when Yin forces predominate. Therefore, it is considered a general tonic and body strengthener and, as such, is considered useful for a variety of ailments (Rennie, 1982). In fact, Yoon (1989) is noted for saying that 70% of velvet users in his clinic are children (Fennessy, 1991). The fact that traditional medicines have prospered in Asia, often alongside and complementary to science based Western medicine, is of great relevance (Suttie and Haines, 1998a) as it has led to interest from both the general public and medical professions of Western countries to increase their investigations into the claims of velvet's medicinal benefits (Fennessy, 1989).

1.5 Pharmacological properties of velvet antler

Currently, there is an unprecedented scientific interest in velvet antler. Much is prompted by the unique biology of antlers, which provides a model for investigation of other biological systems, such as bone and cartilage growth and nerve regeneration (Wilson et al., 2001). There is significant research underway to understand the composition of velvet antler and to evaluate its effectiveness in human medicine and health, as used in many Asian cultures, as an alternative to modern pharmaceuticals and as a dietary supplement for health and performance (Suttie and Haines, 1998b).

Of the approximately 5 million captive deer worldwide, more than half supply the velvet market (Hudson, 2001). In New Zealand, Australia and North America the human use of velvet is becoming widespread and velvet antler products are now commercially available in health food and pharmacy outlets in many Western countries (Wilson et al., 2001).

Velvet antler, when removed at the appropriate stage, is an actively growing cartilage-type tissue and does not have uniform composition. In traditional Oriental medicine the different parts have different uses (Fennessy, 1989; Fennessy, 1991; Sim and Sunwoo, 2001). The tip is known as the wax piece and is the colour and texture of honey. The next section is the blood piece, followed by a piece with a honeycomb appearance, and the base, or bone piece (Fennessy, 1991). The upper two sections are used as preventative medicines (tonics) in children and old people, while the middle portion (honeycomb) is used in the treatment of arthritis and osteomyelitis (bone and joint-related ailments), and the lower part is a benefit to older people subject to calcium deficiency, such as osteoporosis (Fennessy, 1989).

The growing velvet antler is composed of two major tissues, cartilage and bone (Sunwoo and Sim, 2001), and a number of different cell types including fibroblasts, chondroblasts, chondrocytes, osteoblasts and osteocytes (Banks and Newbry, 1982). There are wide chemical variations inherent in the growing velvet antlers (Sim and Sunwoo, 2001). Seven essential amino acids have been found in different parts of the velvet antlers of both sika and red deer. Total amino acids, essential amino acids and phospholipids increase and calcium and phosphorous contents decrease towards the top of the antlers (Chen, 1998). Amino acids and fatty acids are mostly contained in the tip section, which is the growth centre of the antler (Sunwoo et al., 1995). Concentrations of uronic acid, sulphated glycosaminoglycan and sialic acid gradually decrease downward. The tip section has the highest proportions of tyrosine and isoleucine and lowest proportions of glycine and alanine. Linolenic acid is found in the tip section only.

The macromolecules that make up the matrix are polysaccharide glycosaminoglycans linked to protein in forms of proteoglycans and fibrous proteins such as collagen, elastin and fibronectin. Their function is to bind cells and tissues and to influence the transportation of local chemical mediators, neurotransmitters,

hormones, steroidal derivatives and inorganic ions that play a significant role in antler growth and development (Sunwoo and Sim, 2001).

In Asian cultures there have been traditional claims and clinical reports showing that velvet antler contains biologically active ingredients to improve human health (Sim and Sunwoo, 2001; Sunwoo and Sim, 2000). Until recently, studies mainly originated from the former Soviet Union, Korea, China, Hong Kong and Japan (Fennessy, 1991). The pharmacological effects of velvet antler found in many of these studies are presented in Table 1.5.

Table 1.5 Pharmacological effects of velvet antler (From Fennessy, 1989, cited by Haigh and Hudson, 1993)

EFFECT	SUBJECT
GONADOTROPHIC EFFECTS	IMMATURE MICE, RATS AND CHICKENS
HAEMATOPOIETIC EFFECTS	RATS, RABBITS, HUMANS
HYPOTENSIVE EFFECTS	CATS, RABBITS
PROTECTION AGAINST SHOCK/STRESS	RATS, HUMANS
RECOVERY FROM LIVER DAMAGE	RATS
STIMULATION OF GROWTH	CHICKENS
RETARDATION OF AGING	MICE
RECOVERY FROM WHIPLASH INJURY	RATS, RABBITS, HUMANS

There have been a number of studies into the health properties of velvet antler: stimulation of growth in experimental animals (Sunwoo et al., 1995), its anti-inflammatory effect (Sunwoo and Sim, 2001), its tendency to increase high density lipoprotein in blood and the increase in erythropoietic activity in rats (Sunwoo and Sim, 2000). Sunwoo and Sim (2001) have also reviewed studies by other researchers, showing effects such as: decrease of cholesterol content on the liver tissue of rabbit (Yong 1964), decrease of blood pressure (Yudin and Dobryakov 1974), effects of liver detoxification and anti-oxidation (Choi et al. 1979), and boosting of the immune system by increasing number of T and B lymphocyte and natural killer cells in mice (Ko and Song, 1986).

The complex chemical composition of antler tissues provides an abundance of many other compounds, such as proteoglycans, phospholipids, growth factors and enzymes. In the future, these might be used in the treatment of osteoarthritis, degenerative diseases of cartilage and tendons, skin burns, bone fractures and immune disorders (Bubenik, 2001).

Additionally, scientific research is currently being conducted, in many laboratories and research centres, on the pharmacological effect of velvet antlers to justify their usage as a functional food or dietary supplement (Sunwoo and Sim, 2001). The Velvet Antler Research New Zealand (VARNZ) research programme, originally set up to evaluate velvet antler for the traditional Korean market, has been gradually repositioned to provide predominate support for the emerging North American dietary supplement market (Suttie et al., 2000). Product efficacy was tested by these authors in two different studies. In the first study it was determined that velvet powder aided in increasing muscular endurance and isokinetic strength. The second study was a skeletal muscle trial to determine if deer antler products have an enhancing role in human athletic performance by preventing ultrastructural muscle damage and/or enhancing repair. While there was no significant evidence that supplementation enhanced muscle repair at an ultrastructural level, serum creatine kinase was significantly lower 96 hours post exercise in the group supplemented with velvet powder. Muscle soreness in this group returned to normal level 24 hours earlier than the other groups. However, neither study was subject to statistical analysis.

VARNZ has also tested product safety and found that it is unlikely for any toxicological risks to occur in the doses commonly consumed by humans (Suttie et al., 2000). Information generated from velvet antler research is most crucial to substantiate velvet antlers as nutraceuticals or medicinal foods acceptable in the West (Sim and Sunwoo, 2001).

1.6 Purpose of this thesis

This thesis describes research extending previous studies of local anaesthetic agents for the abolition of pain during velvet antler removal from stags, and has also investigated the potential of local anaesthetics for control of post-operative pain.

The studies reported in this thesis explore a number of elements with the aim of assisting the deer farming industry to formulate a better velveting standard for improving the level of welfare of stags.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Introduction

In this thesis, there are a number of techniques common to several studies. These common procedures are described in this chapter. The techniques that are specific to individual experiments will be described in relevant chapters.

2.2 Animals, handling and restraint

Velvet antler removal was the subject of this research. Rising 1-year old stags and older stags were studied when there was sufficient antler (250 mm) to allow application of the electrical test apparatus and/or the velvet antler was ready for removal. Experiments were performed on the Massey University Deer Research Unit or a commercial deer farm in the Manawatu region.

2.2.1 Massey University Deer Research Unit

Deer at the Massey University Deer Research Unit were grazed on pastures of rye grass and white clover. They were yarded immediately prior to any trial.

Deer were restrained in a padded, pneumatic handling device (Nu-mac Crush) with the head resting in a padded cradle and with feet off the ground. Ropes were placed behind the antler pedicles and across the nose to limit head movement and to prevent self-injury during the procedures (Figure 2.1).

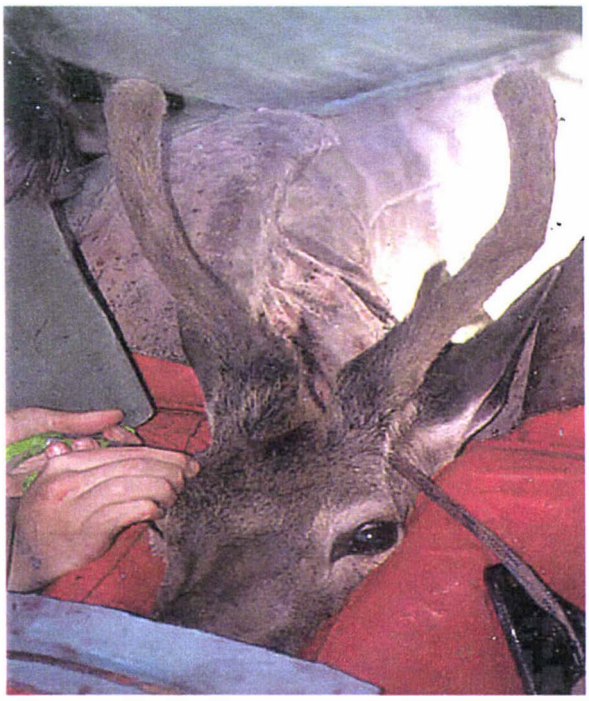

Figure 2.1 Spiker restrained in handling device while receiving local anaesthetic by way of the "high" dose ring block

After research manipulations, the deer were released into a yard or a dark room for further evaluation. Once the day's work was completed, the deer were returned to pasture.

2.2.1.1 Rising 1-year old stags on Massey University Deer Research Unit

Antlers of 21 rising 1-year-old red stags and wapiti hybrid stags were used to determine onset and duration of three local anaesthetic treatments described in Chapter 3.

2.2.1.2 Rising 2-year old stags on Massey University Deer Research Unit

Seventeen rising 2-year-old red stags were used in a pilot trial to evaluate a methodology to remove the effect of background stress factors that confound observations of post-velveting behaviour and physiological measurements (Chapter 6). This technique was used to determine if post-operative pain could be alleviated through the administration of a non-steroidal-anti-inflammatory drug (NSAID) after velvet antler removal.

2.2.2 Manawatu commercial deer farm

The deer were grazed on a commercial deer farm in the Pohangina Valley. They were grazed on conventional rye grass/white clover pastures and yarded prior to any experiments.

The pneumatic hydraulic foam-padded handling device, commonly known as a "workroom", allowed the feet of the deer to rest on the ground. This device permitted the head to be accessible for the procedures (Figure 2.2).

Figure 2.2 Stag receiving local anaesthetic at the commercial deer farm via the "high" dose ring block

After the manipulations in the restraining device were completed, and depending on the study, the deer were either held in darkened pens for additional monitoring or returned to pasture.

2.2.2.1 Rising 1-year old stags on commercial deer farm

Antlers of 39 rising 1-year old stags were used in two studies of analgesia (Chapter 5). The first study (n=25) evaluated two concentrations of a lignocaine/bupivacaine mixture. The second study (n=14) evaluated the onset and duration of analgesia of four treatments, two of which were novel combinations of local anaesthetics.

2.2.2.2 Mixed-age red stags on commercial deer farm

Antlers of 52 mixed-age stags were used in two studies (Chapters 3 and 4). The first trial (n=26) compared onset of analgesia by two local anaesthetic combinations for velvet antler removal. The second study (n=26) measured the time sequencing of procedures in velvet antler removal using two local anaesthetics.

2.3 Electrical stimulation test for determining onset of analgesia in the velvet antler

To determine the onset of analgesia, a clip connected to a Grass Stimulator (Figure 2.3) (Grass Instruments, Quincy, MA, USA) and containing two electrodes, was placed on the antler approximately 5 cm distal to the pedicle junction, maintaining complete contact with the skin (Figure 2.4). Electrical stimulation by the Grass Stimulator is a non-invasive, repeatable and relatively benign procedure originally described by Matthews et al. (1992) and Matthews et al. (2001) to assess velvet sensitivity and employed in further studies described by Wilson et al. (1999a, 1999b, 2000).

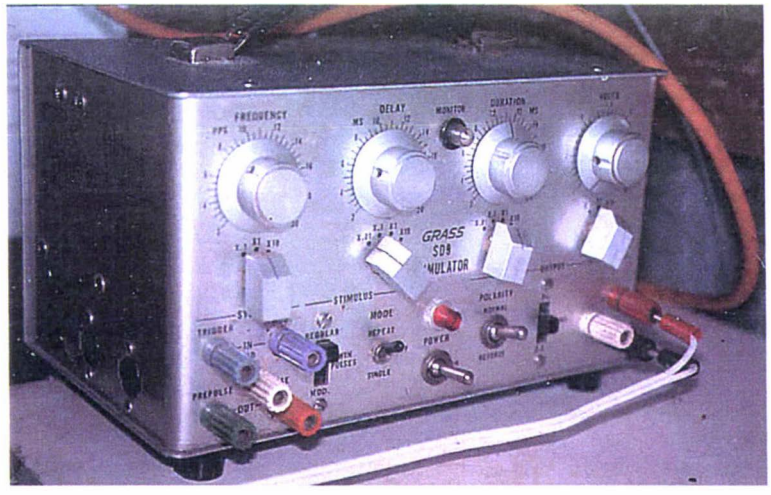

Figure 2.3 Grass Stimulator

A baseline response to the electrical stimulus was obtained prior to administering the local anaesthetic. The Grass Stimulator emitted a voltage calibrated to a range of 10-75 V. The voltage delivered was based on a scale of 1-10. The baseline response was determined by increasing the voltage until the deer responded.

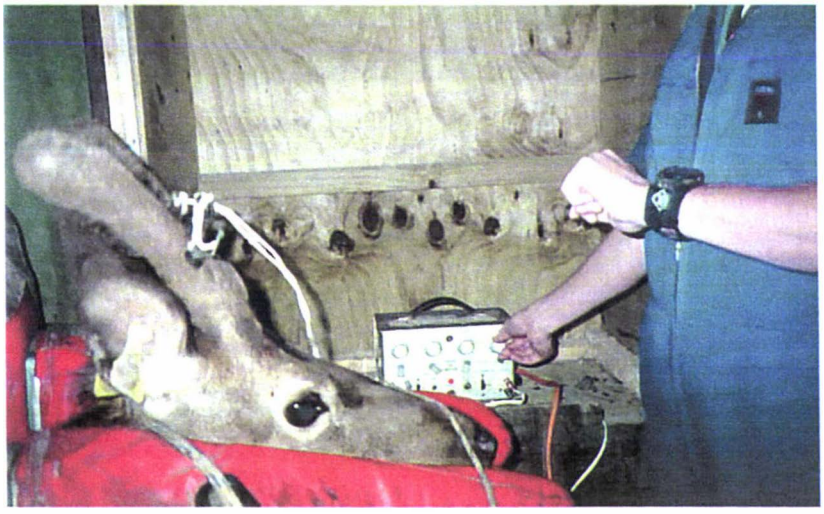

Figure 2.4 Application of stimulus to a spiker at the Massey University Deer Research Unit

Once the animal responded to the electrical stimulus, the stimulation was stopped and the voltage recorded. This background assessment ensured that the antler had sensation prior to administration of local anaesthetic. The behavioural response to the electrical impulse was any sudden withdrawal response with an abrupt shaking or tossing of the head. The few animals that did not respond to the initial stimulus were excluded from further study.

After the local anaesthetic was administered, using a "high" dose ring block at 1 ml/cm of antler pedicle (Wilson et al., 2000b; Wilson et al., 1999a; Wilson et al., 1999b), the electrical stimulus was applied at 15-second intervals until the animal failed to exhibit a response at the highest voltage. Testing did not exceed 4 minutes. This differed from previous studies (Wilson et al., 2000a; Wilson et al., 1999a) which used a wait time after injection of local anaesthetic of 1 minute rather than 15 seconds used in these studies and continued for 8 minutes.

Additionally, because of the increased frequency of stimulation a pilot assessment was conducted to test for habituation to the electrical stimulus using the same methods as above, but without a local anaesthetic (Chapter 3). The results were recorded and the deer were then released.

2.4 Determination of duration of analgesia with remote controlled electrical stimulation

2.4.1 Equipment

For analgesia duration studies a remote stimulator, The Beagler (Tri-tronics, Tucson, Arizona, USA), was used to monitor return of sensation in the antlers (Figure 2.5). This was a novel technique, not previously used with deer. The Beagler is a device for training three dogs simultaneously. It delivers electrical stimuli to the animal via two pronged electrodes which contact with the skin. Stimuli are triggered from a remote control unit programmed for three separate frequencies, with each frequency having a corresponding electrode.

Figure 2.5 "The Beagler" from Tritronics. Three channel remote dog-training device. Electrode on left was attached to the antler

The Beagler had five stimulus intensity levels of varying voltage and duration. Preliminary observations were made on non-anaesthetised antlers to determine the appropriate method of monitoring for response to the stimulus. Level 1 was applied initially and, if no response was obtained, the levels were increased until there was a response. It was determined that when lower settings were used, the number of

responding animals varied. Therefore, the "Beagler" was set at grades 4 and 5 as these elicited clear responses from all deer.

The voltage for grades 4 and 5 was 1400V and had pulse duration was 258ms and 528ms, respectively. The Beagler was equipped with a safety feature which allowed for repeated stimuli to only occur for up to 5-seconds.

An assessment of habituation to pain using the Beagler without local anaesthetic was performed prior to the actual study. The deer had the remote electrodes attached to the antlers as above, and were stimulated and observed every 30 minutes over a 4-hour period.

2.4.2 Fixed-application remote triggering

Prior to administration of analgesia, the electrode was attached to the antler with colour-coded surgical tape to match one of the three transmission frequencies (Figure 2.6). Contact was ensured when a baseline reading was attained (Section 2.3). After administration of the local anaesthetic and analgesia onset, and when the recordings described in Section 2.3 were completed, the deer were released into an open deer yard or a paddock (Figure 2.7). Here, in view of the operator, increasingly intense stimuli were triggered with the remote control at 15-minute intervals until a response was observed. The reaction exhibited by the deer to the stimulus involved a very vigorous, repeatable and characteristic withdrawal movement with a jerk of the head and shoulder favouring the antler with the electrode. This movement was simultaneous with the operation of the remote. Once a response occurred, the time was recorded and the testing ceased. The electrodes were manually removed shortly after and the deer were returned to pasture.

Figure 2.6 Remote aimed at electrode attached to antler to test for analgesia

Figure 2.7 Deer with electrodes attached in amongst the rest of the herd.

2.4.3 Multiple-manual application of electrodes

In Chapter 5 the analgesia duration protocol was revised. The drugs used in this study had longer periods of duration (7-8 hours), and it was observed that the pressure from the taped electrodes caused ischaemic de-sensitisation of the antler. The two prongs on the electrode also caused damage to the velvet antler such that after the research was completed, small tines grew from the damaged points.

The deer, usually in mobs of five animals, were, therefore, kept in darkened pens. One person would walk amongst the deer, place the electrode on the side of the antler, and then advise the second person to trigger the remote when ready. The response of the stag was the same as when the electrode was taped to the antler as described in Section 2.4.2. Up to 15 deer could be monitored for duration at one time using this manual method.

2.5 Local anaesthetics

The local anaesthetics used in these studies were:

- Lignocaine hydrochloride 2% (L), ("Bomacaine", Bomac Laboratories Ltd., Batch 01728, DOM 8/2000, EXP 8/2003).
- Lignocaine hydrochloride 2% with 8.4% sodium bicarbonate (LBC), (David Bull Laboratories, Victoria, Australia, Batch J028627, DOM 7/99, EXP 10/2001).
- Bupivacaine hydrochloride 0.5% (BC), ("Marcaine", Astra Pharmaceuticals Pty Ltd., NSW, Australia, Batch 208854, EXP 4/2002). This commercial formulation was used for the studies in Chapters 3 and 4, and is abbreviated as BC (bupivacaine commercial).
- Mepivacaine hydrochloride 2% (M), (Nature Vet Pty Ltd., Agnes Banks, NSW, Batch G040, EXP 6/2002)

Drug combinations specifically formulated for veterinary investigation were:

- 0.5% bupivacaine (BN), (A novel Bomac formulation manufactured by the Pharmacy School, University for Otago). This novel formulation of bupivacaine was used in the studies in Chapter 5 and is referred to as BN (bupivacaine novel).
- 1.5% mepivacaine and 0.5% bupivacaine (MB), (DOM 7/01/2001, MedNZ, Medicine E&D). BN was the bupivacaine used in this combination.
- 1.5% mepivacaine and 1.5% lignocaine (ML), (DOM 7/01/2001, MedNZ, Medicine E&D)
- "High" (1.5% and 0.5%) (HLBN) and "low" (1% and 0.25%) (LLBN) concentrations (Chapter 5) of lignocaine hydrochloride and bupivacaine hydrochloride, respectively (DOM 24/10/2000, Med NZ Medicines).

2.6 Administration of local anaesthetics

Pedicle circumference measurements were taken by placing a nylon cord about the pedicle below the velvet antler and reading the length against a measuring tape to determine the dosage required for the local anaesthetics. The dosage of 1 ml/cm of pedicle circumference was given as a ring block (Wilson et al., 1999a). The local

anaesthetics were administered from a 500 ml flexipak using a "Vaximate" (Instrument Supplies, Hamilton, NZ) set at 2 ml. with a 1"x 20 gauge sterile needle.

2.7 Antler removal procedure

Antlers of rising 2-years and older stags were subjected to both electrical testing and removal, or removal alone as the analgesic test stimulus. Once onset of analgesia was determined with the electrical stimulus, a rubber tourniquet was wound around the base of each pedicle in a figure-8 to prevent arterial haemorrhage. Using a medium tooth meat saw, a "nick" test was made against the lateral border of the velvet antler as an initial analgesia test stimulus (Wilson et al., 2000a). This involved gently rubbing the saw blade across the lateral border of the velvet antler approximately 1 cm above the antler/pedicle junction. If a response occurred, a 30-second wait was employed and the reaction recorded. If there was no response, the antlers were removed starting from the site of the nick test and sawing in a medial direction. After the tourniquets were removed, the deer were released into the pasture.

2.8 Data management

All data were recorded on prepared sheets (Appendix 3) and then transferred to spreadsheets in Microsoft Excel.

2.9 Statistical analysis

The data were analysed on GraphPad Software, Prism 3.0. To determine the statistics for onset a Survival Analysis (Logrank test) was used along with a Paired-t test for data on Day 1 and Day 2 administration of lignocaine with sodium bicarbonate. All data for duration was analysed using one-way ANOVA, Paired-t test, Bartlett's test for equal variances, and a Tukey's multiple comparison test. A two-way ANOVA (SAS 8.2) was used to analyse cortisol concentrations over time.

CHAPTER 3

STUDIES OF ANTLER ANALGESIA AFTER LIGNOCAINE HCL, LIGNOCAINE HCL WITH SODIUM BICARCBONATE AND BUPIVACAINE HCL

3. 1 Introduction

For humane reasons, it is essential that dependable local anaesthetic agents and techniques be available for the removal of velvet antler from stags. The criteria for all local anaesthetic agents used for this purpose are outlined in Section 1.2.5.2.1. Local anaesthesia should be reliable, highly repeatable, simple to apply, have a rapid onset and long duration of effect and be affordable. Injection of 2% lignocaine hydrochloride by a "high" dose ring block pattern fulfils a number of these requirements (Wilson et al., 2000a; Wilson et al., 1999a; Wilson et al., 1999b) but does not have a long duration of activity (Covino, 1986; Strichartz and Covino, 1990).

The time until the onset of analgesia (wait time) is a significant factor for the deer farmer and veterinarian. The required wait time after administration of local anaesthetic was 4 minutes (NZ Velvet Removal Programme, NVSB). Thus, the deer farmer is faced with unoccupied wait time, which has discouraged the use of physical restraint devices and local anaesthetic and encouraged chemical restraint such as xylazine.

Alleviating post-operative pain is now normal procedure in companion animal medicine (Duke, 2000a; Gaynor, 1999; Hellyer, 1999). With changing attitudes and reassessment of traditionally accepted outdoor farming routines (Matthews, 1992), post-operative pain control is becoming a more common practice in farm animal medicine. Post-operative pain control after velvet antler removal, however, is not currently practised. As the deer industry moves to export product into more animal welfare-sensitive markets that insist upon "animal-friendly, whole farming systems", it is necessary to address the issue of post-operative pain (Wilson et al., 2001). Long acting local anaesthetics may play a role in this respect.

The local anaesthetic, 2% lignocaine hydrochloride, is the most commonly used local anaesthetic for velvet antler removal (Walsh et al., 2001). Lignocaine, which

was the first drug of the amine amide type used in clinical practice, has an inherent potency, rapid onset, moderate duration of action (1 to 2 hours for various regional anaesthetic procedures in dogs, cats and cattle) (Duke, 2000b; Jones, 1995) and topical anaesthetic activity. It is used for infiltration, peripheral nerve blocks and extradural anaesthesia (Covino, 1986).

The alkalinisation of 2% lignocaine hydrochloride with 8.4% sodium bicarbonate, immediately before injection, has been reported to result in a faster onset time in humans than lignocaine alone (Sinnott et al., 2000), but other research failed to show a significant clinical advantage (Chow et al., 1998). The addition of sodium bicarbonate increases the pH of lignocaine, which in turn increases the amount of drug present in the uncharged base form. It could be expected, therefore, that the rate of diffusion across the nerve sheath and nerve membrane would be faster and that the combination would accelerate the onset of analgesia (Covino, 1986). In humans, the addition of sodium bicarbonate to lignocaine decreases pain on injection (Richtsmeier and Hatcher, 1995).

Bupivacaine is a potent, long-acting, highly hydrophobic local anaesthetic agent that is more highly bound to serum proteins than the more hydrophilic lignocaine (Strichartz and Covino, 1990). This results in a lower dissociation from the receptor, thereby producing a prolonged effect (Duke, 2000a; Upson, 1988). It has about four times the potency, and a duration at least double that of lignocaine (Jones, 1995). Bupivacaine, while intermediate in terms of anaesthetic latency, is probably the most versatile of the long-acting local anaesthetics and is utilised for infiltration, peripheral nerve blockade, epidurals and spinals (Covino, 1980). Recovery from surgery may be improved when longer acting local anaesthetics such as bupivacaine are used (Hellyer, 1999).

The aim of these studies was to evaluate and compare the onset and duration of analgesia produced by lignocaine, lignocaine with sodium bicarbonate, and bupivacaine and to assess their potential in fulfilling the preferred criteria of a local anaesthetic in velvet antler removal in 1-year-old and adult stags.

3.2 Study 1. Onset and duration of lignocaine HCl, lignocaine HCl with sodium bicarbonate, and bupivacaine HCl in spikers

3.2.1 Materials and methods

3.2.1.1 Onset

The antlers of 21 spikers (1-year-old male deer) from the Massey University Deer Unit, as described in Section 2.2.1.1, were used to evaluate onset times of 2% lignocaine hydrochloride (L), 2% lignocaine hydrochloride with 8.4% sodium bicarbonate (LBC) and 0.5% bupivacaine (BC). Refer to Section 2.5 for brand names and manufacturers of these drugs.

Administration and dosages of the local anaesthetics are described in Section 2.3. Handling and restraint are described in Section 2.2.1. The application of the electrical stimulation test timed from the completion of the ring block is described in Section 2.6. Onset times were recorded from the time of completion of the ring block.

3.2.1.2 Stimulus habituation assessment for the onset study

Eight of the spikers were initially used to assess their potential to habituate to repeated high frequency use of the electrical stimulation device, prior to the analgesia studies. Their antlers, without local anaesthesia, were electrically stimulated every 15 seconds for 2 minutes and then every 30 seconds for a further 2 minutes, and voltages and deer responses recorded.

3.2.1.3 Onset of local anaesthetic effect

Each antler was allocated to two of the three treatments, allowing a minimum of 3 days to pass before the second treatment (in one case, a third treatment) to reduce a potential confounding effect of treatment order (Table 3.1). There were 28 replicates for each of the three treatments.

Table 3.1 Treatment order combinations on individual antlers from 21 deer (n=28 antlers/treatment) Lignocaine (L), Lignocaine with sodium bicarbonate (LBC), Bupivacaine (BC)

TREATMENT COMBINATION	DAY 1	N	DAY 2	N	DAY 3	N
1	L	7	BC	7		
2	L	7	LBC	7		
3	BC	6	LBC	6		
4	BC	7	L	7		
5	LBC	7	L	7		
6	LBC	7	BC	7		
7	BC*	1	BC	1	LBC	1

*Data not used because the antler did not receive the required dose of BC.

There were two antler substitutions for Day 2 LBC treatment. Deer 957 broke his left antler prior to the Day 2 LBC treatment. This antler was substituted with the left antler of deer 909. On Day 1 deer 937 was given BC on the left antler, but continued to respond to the electrical stimulus. Therefore, no data were recorded on that day for Deer 937. On the last day of the study, the left antler of 937 was again treated with BC. In order to achieve a balanced design, the right antler of 937, which had previously received its two treatments, was given LBC on "Day 3" (Table 3.1).

3.2.1.4 Duration

Duration of analgesia was monitored using the remote, electronic dog-training device (Beagler) described in Section 2.4.2. Seventeen antlers of 13 deer were used. There were nine replicates testing the bupivacaine (BC) and eight replicates forL. Selection was based on the first eight and nine antlers of sufficient length to accommodate the electrode. Only lignocaine and bupivacaine were compared, firstly, because only small numbers could be tested at one time, and secondly because early indications were that the onset with LBC was not more rapid than L suggesting it was unlikely to have practical application in the future.

3.2.1.5 Stimulus habituation assessment for the duration study

Four spikers were assessed without local anaesthesia for the potential habituation to an electrical stimulus over an extended period. The Beagler device was attached and stimulated every 15 minutes for 2 hours and then every 30 minutes until the completion of hour 4, as described in Section 2.4.1 and 2.4.2.

3.2.1.6 Statistical analysis

To determine if there was a significant difference in onset between the two treatment days (treatment order) and the duration of analgesia after lignocaine and Marcaine, a Paired t-test was used. The statistical test used to evaluate onset of analgesia was a Survival Analysis (Log rank test). GraphPad Software, the Prism 3.0 version was the software used to analyse the data.

3.2.2 Results

3.2.2.1 Onset

All deer assessed in the preliminary study for habituation responded to each electrical stimulus (Grass Stimulator) after the 15 and 30-second intervals for the 4-minute period, thus validating the use of this testing stimulus frequency (Table 3.2).

Table 3.2 The voltage at which deer responded to electrical stimulation over a 4-minute period. Time is in seconds and is recorded from Time 0. Voltage meter is recorded on a scale of 1 to 10, calibrated 10-75V

Animal	Baseline	15	30	45	60	75	90	105	120	150	180	210	240
914	4	4	3	4	4	4	4	4	4	3	4	4	3
918	2	4	7	8	3	4	4	5	5	4	4	4	6
926	4	6	4	6	4	3	4	7	6	9	9	6	7
935	5	6	5	4	4	4	6	8	8	8	8	7	6
939	7	7	6	4	7	7	6	5	6	6	7	6	7
947	2	2	2	4	3	3	3	3	3	3	3	3	3
950	6	3	3	3	3	3	3	4	3	3	3	3	3
962	4	3	4	4	4	3	3	5	3	3	3	3	3
Mean	4.3	4.4	4.3	4.7	4.0	3.9	4.1	5.1	4.4	4.9	5.1	4.5	4.4

Mean and ranges for onset times are presented in Table 3.3. Raw data are presented in Appendix Tables A1- A3.

There was no significant difference in onset time between Day 1 and Day 2 of L (P=0.9) or BC administration (P=0.15). Initially it appeared that there might have been a treatment order effect with LBC. When LBC was given as the treatment on Day 2, no stags reacted to the stimulus 15 seconds after administration (P=0.0055), while on Day 1, 43% (6/14) were desensitised in that time (Figure 3.1). . However, 6/14 deer received LBC as both a first and second treatment (on different antlers).

The results of a Paired t-test on these six deer resulted in a mean difference of 10 seconds (P=0.23). There was no significant difference between Day 1 and Day 2 LBC treatment for these six deer. These analyses are interpreted as eliminating a treatment order effect. Thus, in the absence of a treatment order effect, data from days 1 and 2 were combined for further analysis.

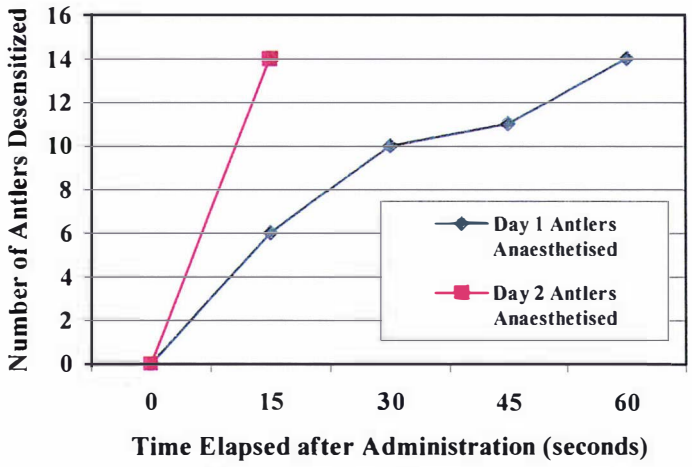

Figure 3.1 Deer not responding to electrical stimulation of anaesthetised antler over time when treated with LBC on Day 1 and Day 2 (n=14)

The mean onset times are printed in Table 3.3.

Table 3.3 Mean and range of onset times (seconds) of LBC, L and BC analgesia in spikers

	LBC	L	BC
N	28	28	28
MEAN	21	31	48
MINIMUM	15	15	15
MAXIMUM	60	195	255
S.E.M.	2.5	7.0	12.7

LBC produced a significantly faster mean onset than bupivacaine (P=0.023). There was no significant difference between lignocaine and bupivacaine (P=0.21) or lignocaine and LBC (P=0.22). The number of antlers desensitised at each time interval are presented in Figure 3.2. Seventy-nine percent (22/28) of antlers treated with LBC, 68% (19/28) given L and 57% (16/28) of antlers treated with BC were desensitised within 15 seconds. All LBC treated antlers were desensitised by 60

seconds. All antlers treated with L and BC were desensitised by 195 and 255 seconds post injection, respectively.

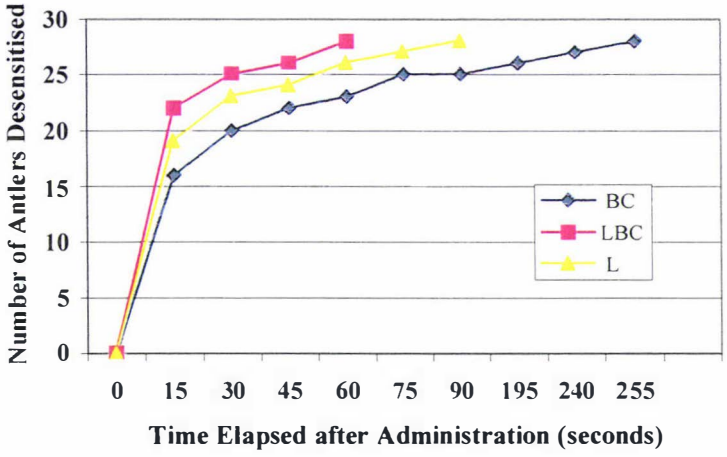

Figure 3.2 Number of antlers desensitised at each time interval after local anaesthetic administration (n=28)

3.2.2.3 Duration

All deer that were assessed in the pilot study for pain habituation over duration, responded to the electrical stimulation at each time interval (Table 3.4).

Table 3.4 Electrical stimulus setting required to elicit a behavioural response in stags with non-anaesthetised antlers over a 4 hour time period to evaluate whether habituation occurred. Time is in minutes.

Animal	0	15	30	45	60	75	90	105	120	150	180	210	240
916	4	4	4	4	4	4	4	4	4	4	4	4	4
908	5	3	3	3	3	3	3	3	3	3	3	3	3
937	4	4	4	4	4	4	4	4	3	4	3	4	4
957	5	5	5	4	5	5	5	4	5	4	4	4	4

The mean and range of duration times of the local anaesthetics are presented in Table 3.5, and the numbers of animals not responding at each time interval are in Figure 3.3. Commercial bupivacaine's induced analgesia lasted an average of 273 minutes (range195-300) while that of lignocaine averaged 88 minutes (range 60-120).

Bupivacaine produced analgesia of a significantly longer duration than lignocaine (Figure 3.3) according to the Paired t-test ($P<0.0001$).

Table 3.5 Mean and range of the duration (minutes) of lignocaine (L) and bupivacaine (BC) anaesthetics in spikers.

	L	BC
n	8	9
Mean	88	273
Minimum	60	195
Maximum	120	360
S.E.	7.7	19

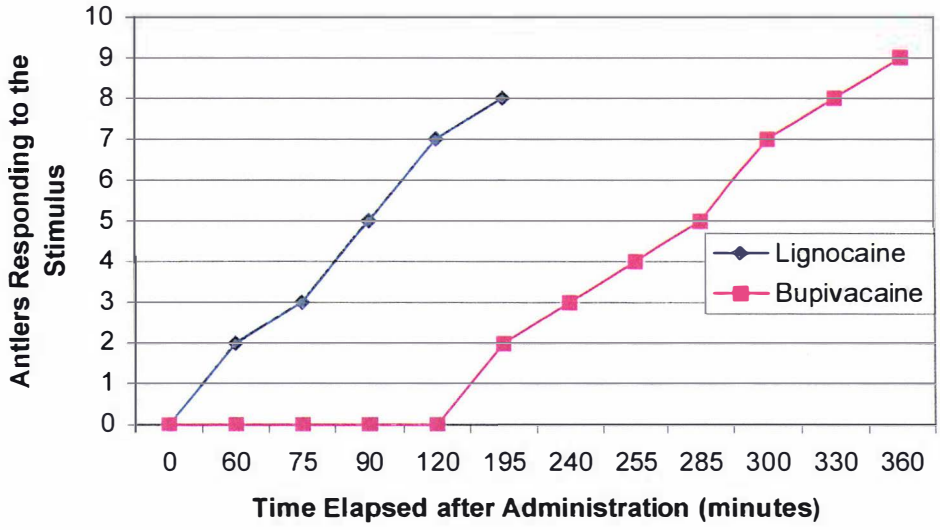

Figure 3.3 Duration of analgesia in antlers of spikers given L (n=8) and BC (n=9)

3.3 Study 2. An evaluation of analgesia induced by lignocaine HCL and lignocaine HCL with sodium bicarbonate for velvet antler removal from adult stags

3.3.1 Materials and methods

The antlers of 26 mixed-age stags (≥ 3 years old), from the commercial deer farm (Section 2.2.2.2), were allocated to one of two local anaesthetic treatment groups on the day of velvet antler removal. The antlers were of the optimum size according to the New Zealand Game Industry Board grading standard. The treatments, 2 % lignocaine HCL (L) and 2% lignocaine HCL with 8.4% sodium bicarbonate (LBC), were assigned randomly to the left antler first, with the right receiving the remaining

treatment. The effectiveness of analgesia was determined using electrical stimulation (Sections 2.3 and 2.7). When the electrical test showed analgesia (Section 2.3), the electrode was removed, a tourniquet applied and the nick test done (Section 2.7). If analgesia was confirmed with the nick test, the antler was removed and the time recorded. Since removal of velvet antler was the end point of further study, duration of analgesia was not measured. The second antler was treated only after completion of all procedures on the first.

3.3.2 Statistical analysis

The statistical test used for analysis of onset of analgesia was a Survival Analysis (Log Rank test). The software was GraphPad Prism 3.0.

3.3.3 Results

The mean onset times of analgesia and range using the electrical stimulation test are shown in Table 3.6. Raw data are presented in Tables A4 and A5. LBC had a mean onset time of 36 seconds and lignocaine had a mean onset time of 31 seconds (P=0.2878). Using LBC, onset of analgesia was observed in 20/26 antlers by 60 seconds; while 22/26 of lignocaine treated antlers were analgesic by 60 seconds (Figure 3.4).

Table 3.6 Mean and range of onset times (seconds) of analgesia following lignocaine (L) and lignocaine with sodium bicarbonate (LBC) in mixed-age stags, using the electrical stimulation test

	L	LBC
n	26	26
Mean	31	36
Minimum	15	15
Maximum	120	120
S.E.	5.94	5.95

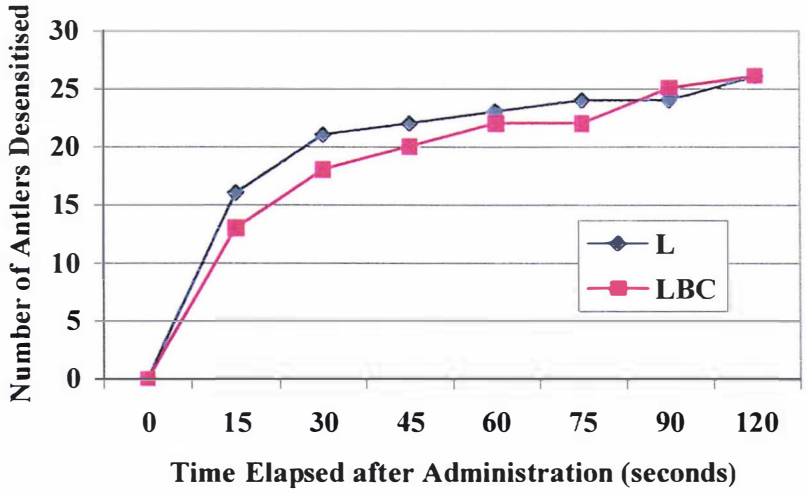

Figure 3.4 Number of antlers of mixed-age stags anaesthetised over time when treated with L (n=26) or LBC (n=26)

Mean and ranges of times for velvet antler removal are listed in Table 3.7. The minimum time for velvet antler removal was 31 seconds with a maximum of 159 seconds (Figure 3.5). The mean time for velvet antler removal was 55 seconds (range 31-159) for lignocaine treated antlers and 63 seconds (range 31-136) for antlers treated with LBC. Once analgesia was determined, 16/26 stags treated with LBC and 20/26 of the lignocaine treated deer had the velvet antler removed in less than 1 minute. There was no significant difference in the removal times between the two treatment groups (P=0.1885).

Table 3.7 Mean and ranges of velvet removal times (seconds) after completion of the ring block using L and LBC in mixed-age stags

	L	LBC
n	26	26
Mean	55	63
Minimum	31	31
Maximum	159	136
S.E.	6.00	5.96

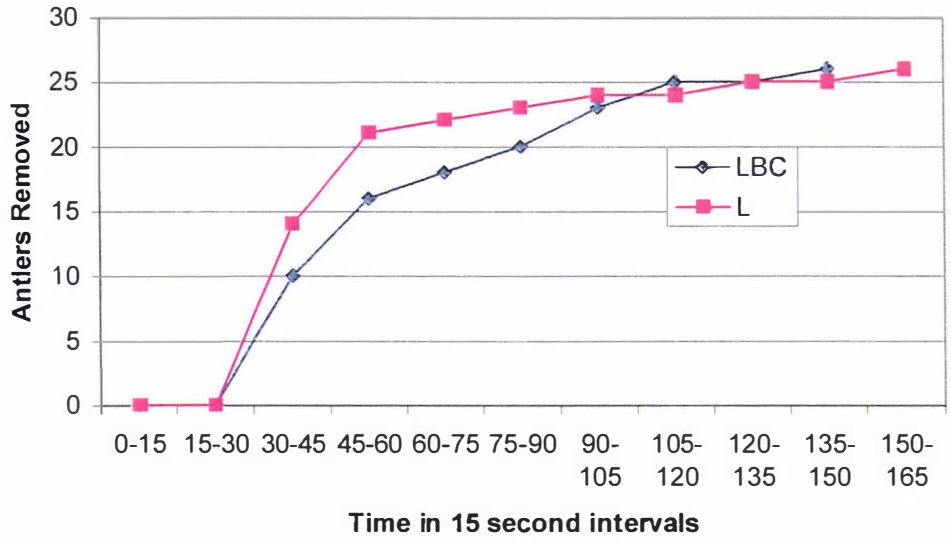

Figure 3.5 Removal times for antlers of adult stags after administration of L (n=26) and LBC (n=26)

3.4 Discussion

While lignocaine is routinely used for velvet antler removal, there are no reports to the authors' knowledge that either bupivacaine or lignocaine with sodium bicarbonate have been evaluated for this procedure. Evaluation of onset has been reported elsewhere (Matthews and Suttie, 2001; Wilson et al., 2000a; Wilson et al., 2000b), but this study is the first to report a novel method for assessing duration of analgesia.

Electronic dog collars have been used to train cattle to avoid certain portions of pasture and have been applied to coyotes to deter them from predation of sheep (Andelt et al., 1999). They have also been used as a determent to deer breeching electric fences (Wilson pers. comm. 2001). However, the use of an electronic dog-training device to test for return of sensation in velvet antler by remote triggering is a novel technique that is non-invasive, repeatable and should be less stressful for deer as there is no interaction with humans.

The 3-channel Beagler system employed could stimulate three different deer at one time, which limited the number of deer that could be studied simultaneously. The method of using the device was changed for a subsequent study and it was held

against the antler while the deer were in pens (multiple manual application), in which case 15 deer were assessed concurrently (Chapter 5).

The mean duration times in Table 3.4 are the average times at which the antlers returned to sensitivity with a 30-minute observation interval. The actual times would likely average 15 minutes earlier than the times reported assuming data was normally distributed. The same applies to the onset data recorded at 15-second intervals where the actual time would likely be 7.5 seconds less.

In previous studies, duration has been measured by returning the deer, at various intervals (5 minutes, 30 minutes, 2 hours and 24 hours post-ring block administration), to the physical restraining device and reapplying the electrical stimulation (Matthews et al., 1994; Matthews et al., 1992). Changes in responses of the stags to the stimuli were used to assess the efficacy and duration of the analgesic treatments. Scoring was measured by the intensity of the behaviour. Head movements alone were given a score of 1-2. A score of 3-7 involved head movements with neck and shoulder displacement and 8-9 (upper threshold stimuli) involved body displacement (Matthews et al., 1992). In the present study, there was no individual behaviour score for either determination of onset or cessation of duration. The variable was "yes" there was a response or "no" there was not a response to each electrical stimulus, thus combining scales used by Wilson et al. (1999). The use of a remote operated device has an advantage since returning the deer to the restraint for further stimulation involves additional yarding and handling, which has been shown to be aversive to deer (Carragher et al., 1997; Diverio et al., 1996a; Diverio et al., 1993)

Changes in behaviour have been used elsewhere as an indication of pain in deer. An increase in various activities such as head shaking, ear flicking, grooming, aggression and jumping after velveting suggested post-operative pain in deer (Pollard et al., 1993a; Pollard et al., 1992; Pollard et al., 1991). When evaluating xylazine and xylazine combinations for velvet antler removal, the tips of the antler were pinched using an Allis tissue forceps (Wilson et al., 1996a). In calves undergoing dehorning, duration of analgesia was tested using a needle-prick in the region of the horn buds (Petrie et al., 1995). In investigations of epidural, paravertebral and infiltration properties of local anaesthetics in dairy cattle, duration was determined by pricking the skin with a needle. Additionally, in the case of

epidural anaesthesia, the vulva was pinched with forceps and the response of the tail muscles was noted (Link and Smith, 1956).

In the present study, it was essential to evaluate whether deer would become refractory to electrical stimulation tests at 15-second intervals. Previous studies used 1-minute intervals (Wilson et al., 2000a; Wilson et al., 2000b; Wilson et al., 1999b). It was noted that all deer responded at each 15 and 30-second interval over the 4-minute time period for the onset study, and all deer responded at each 15 and 30-minute interval over the 4 hours for the duration study and, therefore, did not habituate to the stimulus.

In this study, lignocaine had a more rapid onset than previously reported when administered via a high dose ring block. This was because in previous studies (Wilson et al., 2000a; Wilson et al., 1999a; Wilson et al., 1999b), analgesia was tested with electrical stimulation at 1 minute intervals after administering the local anaesthetic. That was the first published data critically evaluating time to effectiveness of local anaesthetics and showed that few animals responded to electrical stimulus following the "medium" and "high" dose ring blocks after 1 minute (Wilson et al., 1999a). For the present study, the electrical test was applied at 15 seconds intervals post-injection because the hypothesis was that the addition of bicarbonate to lignocaine would accelerate the onset of analgesia.

In a high proportion of animals (19/28), onset of analgesia, using 2% lignocaine, was as rapid as 15 seconds, yet in other animals analgesia onset took as long as 195 seconds. Furthermore, duration varied from 1 to 2 hours with lignocaine. While commercial bupivacaine produced analgesia of a significantly longer duration than lignocaine, the duration range times still varied from 195 to 360 minutes. The differences in the onset and duration of analgesia may be due in part to the placement of injection. This can influence the rate of diffusion and vascular absorption of the local anaesthetics (Strichartz and Covino, 1990). For example, if bupivacaine is given to humans by an intrathecal injection, directly into the cerebral spinal fluid, onset is almost immediate. When administered as subcutaneous (s/c) injectionanalgesia will occur within 5 minutes and will persist for 3 to 4 hours (Strichartz and Covino, 1990). In calves given bupivacaine, in a nerve block resulting from injections around each corneal nerve midway along the lateral edge of the frontal bone crest, prior to undergoing de-horning, analgesia lasted for

approximately 4 hours (McMeekan et al., 1998a; McMeekan et al., 1998b). However, if it is administered for a brachial plexus blockade in humans, the onset time is approximately 20 to 30 minutes, while the duration may average 10 hours (Strichartz and Covino, 1990).

The three local anaesthetic treatments (L, LBC, BC) were administered subcutaneously to the base of the antler pedicle. Onset of local anaesthetic is both volume and concentration dependent (Covino, 1986; Strichartz and Covino, 1990). The method of using high concentration and high volume of local anaesthesia in a small area is why the high dose ring block is a more effective method in velvet antler removal. Its application results in more rapid and reliable analgesia (Wilson et al., 2000a; Wilson et al., 2000b; Wilson et al., 1999a; Wilson et al., 1999b).

These experiments have shown that bupivacaine has a significantly slower onset of analgesia than LBC. While the mean onset time for lignocaine was 31 seconds and bupivacaine was 48 seconds, there was large variation between animals. In Study 1, two operators administered the local anaesthetic. This raises the issue of operator variation, or reproducibility, of ring block application. The onset time was recorded from the completion of the block. If a different operator is slower in administering the block, it allows more time for the local anaesthetic to diffuse until completion of the ring block. This could be due in part to where in the tissue the local anaesthetic is deposited in relation to antler innervation. While infusion of a local anaesthetic will proceed beyond the needle tip, it is probable that there is a relationship between pedicle circumference, needle length and numbers of injection sites, allowing gaps in analgesia between injection sites (Wilson et al., 1999a). Some deer moved more than others, adding to the difficulty of injecting the analgesics. It is possible that uniformity of administration will vary at critical points. In addition, the site of administration in relation to the location of nerves could result in a different onset time.

Failure to obtain anaesthesia in some cases may be due to faulty techniques (Link and Smith, 1956). A pre-requisite for work comparing injection sites is a reproducible method (Shafford et al., 2001; Wilson et al., 2000b). However, Hingson, who has administered local anaesthetics to thousands of human patients, is cited (Link and Smith, 1956) as reporting: "*We have been intrigued (in our prolonged continuous conduction anaesthesias) that a given patient who has on*

occasion had two or three times the average adequate conduction anaesthesia has failed to obtain anaesthesia. Even though such a phenomenon may on occasion be due to inaccurate positioning of the needle, we have proved they occur just as frequently in the presence of perfect technique. In such cases the mere substitution of another anaesthetic agent has produced prompt and adequate anaesthesia." Therefore, even if a technique is reproducible and repeatable, there is still the possibility of variability among individual stags.

The question of individual stag variation prompted comparison of data from this study with data of Wilson et al. (2000) from 1999, which used the same stags in the previous velvet removal season. Data for deer evaluated in both studies were compared. This was done to see if individual deer followed the same pattern each year for onset of analgesia (Table 3.8). There appears to be no relationship between the times of onset between years. However, the study for 1999 tested analgesia at 60-second intervals, while the current study tested at 15-second intervals. Therefore, deer desensitised in 1999 at 60-seconds, may have actually been desensitised at 15-seconds, 30-seconds or 45-seconds. Further research needs to be done on the trends of individual deer over a period of years to determine if onset of analgesia is related to animal variation or variation in technique.

Table 3.8 Time of onset of analgesia (seconds) on stags studied in consecutive years

	Year 1 (1999)	Year 2 (2000)
Animal ID		
319	60	15
30	60	15
139	60	15
322	120	15
303	120	15
364	60	15
323	60	15
309	60	30
357	60	30
944	120	75
918	120	120
195	60	120

All 28 antlers of the spikers in Study 1 receiving LBC were anaesthetised within 60 seconds. However, when LBC was the treatment for Day 2 (n=14), onset of analgesia occurred in all deer within 15 seconds. This raised the possibility of a confounding effect from treatment order. Local anaesthetic agents can produce systemic reactions, especially when given in high doses or repeated doses (Riebold et al., 1982). In the walls of lymphatics and capillaries, there are small openings at the junctions of some of the endothelial cells, which make up the vessels. Even mild trauma causes an increase in the number of such open junctions and leads to a great increase in the permeability of the vessels. This tissue damage is due to the direct effect of the needle, to the distension and disruption caused by the injected substance and the composition of the injected substance (Baggot, 1977; MacDiarmid, 1983). However, Wilson et al. (2000) looked at treatment order effect and found none. LBC was not utilised in that study. In this study, while a t-test showed a difference for all deer receiving LBC on Day 1 and/or Day 2, a treatment order effect was discounted because there was no difference related to previous treatment for LBC treated antlers, or with the other two local anaesthetics. The six deer given LBC on both Days 1 and Day 2 showed no significant difference between them for the two treatment days, and therefore treatment order effect was unlikely to be a factor. For duration observations in Study 1, only lignocaine and bupivacaine were compared. Bupivacaine was observed in nine antlers, while duration of lignocaine was observed in eight. The small number was chosen because differences between lignocaine and bupivacaine were considered likely to be large and this was verified by the results. The range of duration times corresponded to the published information regarding these two drugs in humans (Covino, 1980; Covino, 1986).

In Study 2 the surgical removal of velvet antler using lignocaine alone, when one antler was studied at a time, was achieved in less than 60 seconds on average. This is of significance to the farmer as a long wait time for onset of analgesia has been a deterrent in using physical restraint with local anaesthetics versus chemical restraint with a local anaesthetic. This prompted the timing study reported in Chapter 4.

The mean onset time of lignocaine for both spikers (Study 1) and adult stags (Study 2) was 31 seconds. Mean onset of LBC in the adult stags was 36 seconds, whereas, in the spikers the onset time for LBC averaged 20 seconds. A t-test was done to determine if there was a significant difference between the two groups when LBC

was administered. There was a significant difference (P=0.0222). There was also a difference in the maximum time for onset. The spikers were all anaesthetised by 60 seconds, but the adults were all anaesthetised only by 120 seconds. The difference could be due to age as younger animals may absorb some drugs more rapidly from subcutaneous sites than older animals (MacDiarmid, 1983). It has been suggested that this may be due to an increase in thickness of subcutaneous tissue with age, as well as to changes in the composition of subcutaneous fat tissues (MacDiarmid, 1983). This would lead to less elasticity in adults, thus making it more difficult to place the local anaesthetic. The operators in this study observed that it is easier to administer a LA ring block to spikers than to older stags.

3.5 Conclusion

These studies, using 15-second intervals when applying the electrical stimulus, have shown onset of analgesia occurring more rapidly than previously believed and reported.

In spikers, all were desensitised within 60 seconds when receiving LBC. The antlers of the mature stags were all desensitised by 2 minutes after LBC injection. This difference may be attributed to the thicker subcutaneous tissue and for anatomical differences in the older stags.

- The new technique of monitoring duration of analgesia was effective. It is non-invasive and repeatable.
- Bupivacaine (BC formulation) is effective and has a slow onset (mean of 48 seconds compared to 31 seconds for lignocaine).
- Lignocaine has a shorter duration, while bupivacaine has a long duration.
- Addition of sodium bicarbonate had no significant effect on increasing the rate of onset of analgesia.

The practical applications of the results are:

- Antler removal can be performed more rapidly than currently permitted with a mandatory 4-minute wait time.
- Long duration of bupivacaine may have a role in managing post-operative pain.

- This study defined the parameters of bupivacaine that could be used for future studies of post-operative pain control.
- There is repeatability and consistency of onset with previous studies, but variation within stags, and also within and between operators may be important.
- The detection of rapid onset with lignocaine and LBC allows for quicker removal of the velvet antler than previously thought.

Further studies undertaken or proposed as a result of this study are:

- The rapid onset of local anaesthesia prompted an evaluation of the timing sequence of the procedures for the removal of velvet antler (Chapter 4). It was thought that once both antlers received the "high" dose ring block and the tourniquet was applied, there would be no response to the nick test, thus velvet antler removal could proceed without unoccupied wait time for the deer farmer and no welfare cost for the stag.
- Assessment of combinations of short and long duration local anaesthetics (Chapter 5).
- Further investigate repeatability and reproducibility between stags over consecutive years and, also, between age groups.
- Research new methodologies for determining if there is post-operative pain after velvet antler removal and ways to address it (Chapter 6).

CHAPTER 4

THE TIMING OF LOCAL ANAESTHETIC ADMINISTRATION AND VELVET ANTLER REMOVAL

4.1 Introduction

Veterinarians and farmers responsible for stags during velvet antler removal have an ethical obligation to ensure that only the most reliable and repeatable methods of analgesia be used (Wilson, 1989; Wilson et al., 2001).

The National Velveting Standards Body of New Zealand (NVSB, 1998) in its "Manual and Support System for Deer Farmer Removal of Velvet Antler" defined a minimum mandatory 4-minute time limit between administering the local anaesthetic and velvet antler removal. This 4-minute delay discourages many farmers from using physical restraint and local anaesthetic alone. Failure to wait for the local anaesthetic to become effective is a contributing factor to non-compliance with the standard, as shown by audit (Wilson pers. comm. 2001). Further, restraint in a handling device *per se* is a source of stress to deer (Matthews et al., 1990) and it is important that confinement time be kept to a minimum, and so currently, the majority of deer farmers opt for chemical rather than physical restraint of deer prior to velvet antler removal. Many deer farmers find it more time efficient to chemically immobilise several animals at one time with a sedative, i.e. xylazine or xylazine in combination with other drugs, then administer the local anaesthetic, then remove the velvet in sequence, obviating wait times (Wilson, pers. comm. 2001).

Xylazine is used extensively as a sedative in ruminants, but with normal doses ruminal movements are abolished and this can lead to tympany and ruminal distension (Brander et al., 1991). In cattle, xylazine causes hyperglycemia, a decrease in plasma insulin and hematocrit, and an increase of blood urea nitrogen (Riebold et al., 1982). More serious, however, are deaths attributed to the administration of xylazine in deer. Walker (1989) reported a death rate of 0.17%. According to Walker and Middleberg (1988) "It is our feeling we are dealing with some form of delayed hypersensitivity as a consequence of xylazine administration, probably to a product of xylazine metabolism. Deaths during the period of 12 to 24 hours post-velveting do not support an anaphylactic type reaction to xylazine *per se*,

but perhaps to a metabolic product or a carrier agent or metabolic product of such a carrier agent." Xylazine produces hypoxemia in ruminants and severe hypoxemia could be linked with mortality (Caulkett, pers. comm. 2001).

Xylazine is a basic lipophilic drug and is concentrated in the lungs. In stags that are hypersensitive to xylazine, systemic vascular permeability is increased and this contributes to airway oedema. Death occurs from 1 to 3 hours to several days after velveting (Mackintosh and Cross, 1989). Additionally, xylazine of itself provides insufficient analgesia for velvet antler removal (Wilson et al., 1996a; Wilson et al., 1996b) and must be supplemented with local anaesthetic.

Thus, while xylazine provides a humane method of sedation, it is not without its side effects, causing death, or possibly, sub-clinical distress in some stags.

As the deer industry moves to promote a healthier product, free of chemicals, it is desirable to restrict or eliminate the use of chemicals. In the United States, xylazine is not licensed for use in food animals (Riebold et al., 1982), and the Federal Drug Administration is concerned because xylazine's metabolite, 2,6-xylidine, has been shown to be carcinogenic in rats (Walsh et al., 2001). This issue is of particular importance as the New Zealand velvet producer is targeting the United States health food market's demand for velvet antler (Loza, 2001). This will highlight the need to reduce chemical restraint within the industry and the search for alternative methods of restraint during velvet antler removal (Walsh et al., 2001).

The velvet antler removal process must be done with the welfare of stags foremost in mind. However, no studies have reported on the timing of events related to velvet antler removal. In Chapter 3 the onset of action of local anaesthetic was shown to be more rapid than previously believed. Local anaesthetic was effective in many stags within 15 seconds of administration while it was effective in most stags within 1 minute.

The purpose of this study was to determine whether velvet antler removal could be undertaken in a continuous sequence of activity from the initial administration of local anaesthetic to velvet antler removal without the necessity of a wait time.

4.2 Methods and materials

The study evaluated the time involved in each step of velvet antler removal. A veterinarian with significant clinical and research experience in the techniques

involved, Peter Wilson, performed all the procedures and identified when he began and completed each step. A timekeeper called out the times, which were entered onto a data sheet by a recorder. The sequence and procedures timed (described in Chapter 2) were as follows:

- Time = 0. Start of first antler ring block.
- Finish of the first antler ring block
- Start and finish of the second antler ring block
- Completion of tourniquet application
- Application of a nick test for the first antler
- Completion of removal of the first antler
- Application of a nick test for the second antler
- Completion of removal of the second antler

These steps were planned as a continuous, uninterrupted sequence. However, if a stag responded to the nick test (Section 2.7), a wait of 30 seconds was applied. These 30-second waits were repeated until there was no response to the nick test. Then velvet antler removal was resumed.

Twenty-six mixed-age stags from the commercial deer farm, described in Section 2.2.2.2, were used. Local anaesthetic administration and velvet antler removal procedures are described in Section 2.6 and 2.7.

Sixteen deer had both antlers removed on the same day. They received either lignocaine (L, n=8) or the commercial formulation of bupivacaine (BC, n=8), also used in Chapter 3. See Section 2.5 for drug information. The initial antler was randomly chosen.

Since both antlers do not always mature at the same rate, 14 antlers from 10 deer were removed on separate occasions. Treatment (L or BC; n=7) was randomly assigned to each individual antler (Table 4.2).

4.3 Results

The means and ranges of times recorded for stags that had both antlers removed are shown in Table 4.1 and raw data are presented in Appendix Tables A8 and A9. No deer that had both antlers removed on the same occasion using lignocaine responded to the nick test on either the first or second antler.

As it was the timing of the procedures *per se* that was being measured, the data for lignocaine and bupivacaine were combined up until the removal of the antlers, when local anaesthetic type would have influenced the observations. Thus, observations to this point are from 16 stags. Note that data in Table 4.1 are presented separately for both treatments. The mean time for completion of the first ring block was 22 seconds (range10-55). The mean starting time for the second ring block was 25 seconds (range 11-64). The second ring block was completed at a mean of 53 seconds (range 20-168). Tourniquet application was completed at a mean time of 85 seconds (range 52-198). The mean time, from completion of the first antler ring block until the first nick test, was 72 seconds (range 52-151). Removal of the first antler given lignocaine was completed at a mean time of 85 seconds (range 73-120) and for bupivacaine, the mean was 140 seconds (range 86-337) from the start of the first ring block (Time = 0). From the completion of the second antler ring block to the second antler nick test, the mean time was 70 seconds (range 61-183). The mean time for the second nick test was 123 seconds (range 81-351). For lignocaine treated deer the mean time for removal of the second antler was 104 seconds (range 87-150). The mean time for removal of the second antler treated with bupivacaine was 167 seconds (range 117-390). These times were calculated from the start of timing. Of the deer treated with bupivacaine, three responded to the first nick test and three responded to the second nick test, thus requiring a wait time, which increased the mean.

Table 4.1 Mean times and ranges (in seconds) for the sequence of procedures for antler removal when both antlers were removed using either lignocaine (L) or bupivacaine (BC).

	Local Anaesthetic Treatment	
	L	BC
No. of Deer	8	8
Completion of 1st Ring Block	19 (10-55)	25 (10-52)
Start of 2nd Ring Block	22 (12-64)	28 (11-57)
Completion of 2nd Ring Block	42 (20-81)	63 (33-168)
Tourniquet Application	69 (52-107)	101 (68-198)
1st Nick Test	77 (62-116)	111 (80-206)
Deer Responding	0	3
1st Antler Removal	85 (73-120)	140 (86-337)
2nd Nick Test	92 (81-124)	153 (107-351)
Deer Responding	0	3
2nd Antler Removal	104 (87-150)	167 (117-390)

The data for single antler removal data from lignocaine and bupivacaine deer are shown in Table 4.2 and the raw data are presented in Appendix 2. Note that data from L and BC treatments were combined up to the nick test for results. The mean time for completion of the ring block was 21 seconds (range 10-65). Application of the tourniquet was completed at a mean time of 48 seconds (range 25-91). The mean nick test time was 63 seconds (range 35-105), which was 42 seconds (range 25-40) from completion of the ring block. This is 27 seconds shorter than for the dual antler removal. The removal of the antler from lignocaine treated stags had a mean time of 69 seconds (range 58-108) and for removal with bupivacaine, the mean time was 100 seconds (range 71-135) (from Time = 0). Two deer treated with lignocaine responded to the nick test and three deer given bupivacaine responded to the nick test.

Table 4.2 Mean times and ranges (seconds) for the sequence procedure for removal of a single antler when using either lignocaine (L) or bupivacaine (BC).

	Local Anaesthetic Treatment	
	L	BC
No. of Antlers	7	7
Completion of Ring Block	18 (10-25)	24 (13-65)
Tourniquet Application	43 (36-52)	53 (25-91)
Nick Test	50 (44-57)	76 (35-105)
Deer Responding	2	3
Antler Removal	69 (58-108)	100 (71-135)

4.4 Discussion

This study confirmed that the 4-minute wait time required by the NVSB's "Velvet Removal Programme" was unnecessarily long. Indeed, these results demonstrated that no wait time was needed for stags when both antlers were to be removed using lignocaine if the normal time sequence of events during velvet antler removal was followed. When only 1 antler was being removed using lignocaine, the time between the injection of the local anaesthetic and the nick test was reduced, and 2/7 stags responded, thus a 30-second unoccupied wait time was required to ensure analgesia.

In previous studies, the efficacy of local anaesthetic was tested using electrical stimulation of the velvet antler at 1 minute intervals after injection of the local anaesthetic(Wilson et al., 2000a; Wilson et al., 1999a; Wilson et al., 1999b). They

showed that few animals responded to the electrical stimulus following the "high" dose ring block. The studies in Chapter 3 tested the efficacy of the local anaesthetic at 15-second interval tests using electrical stimulation. In a high proportion of animals, onset of analgesia with lignocaine was as rapid as 15 seconds. Therefore, if the right dose is used and local anaesthetic is properly administered then no unoccupied wait time is necessary.

The timing of the sequence of events when both velvet antlers were removed on the same occasion has shown it is unlikely that the time interval between injection of a high dose ring block and antler removal can be much less than 60 seconds, even for experienced operators. In this study, when lignocaine was used, all antlers were desensitised. In the studies in Chapter 3, however, it has been shown that the onset of local anaesthetic may be delayed for some antlers. As the welfare of the stag must not be compromised in these situations, applying the nick test carefully, waiting and reapplying the nick test is necessary for those few stags that respond. When bupivacaine (BC) was used, some stags responded to the nick test. Therefore, an additional wait time was needed since bupivacaine has a longer onset time. This is consistent with the earlier study in Chapter 3. Thus, in most cases the velvet antler can be removed without a wait time when lignocaine is used.

When only one antler was removed, two of seven stags treated with lignocaine responded to the nick test, because the time between completion of the block and the nick test was shorter. The deer which had the tourniquet applied at 52 seconds responded to the first nick test at 57 seconds and to a second at 68 seconds. The antler was not removed until 108 seconds. The second deer received the ring block in the minimum time of 10 seconds and the tourniquet was applied by 34 seconds. It responded to the nick test at 44 seconds but was analgesic at 83 seconds and the antler was removed at 87 seconds.

Three of seven stags treated with bupivacaine for single antler removal responded to the nick test. One stag had the tourniquet applied at 34 seconds, responded to the nick test at 42 seconds and had the antler removed at 74 seconds. A second deer had the tourniquet applied at 25 seconds, and responded to the nick test at 35 seconds and again at 74 seconds. The antler was removed at 111 seconds. Finally, the third deer had its tourniquet in place at 34 seconds, but did not receive the nick test until

87 seconds post-injection of local anaesthetic. The antler was removed at 135 seconds.

When only a single antler is to be removed, the issue is raised as to whether a 60-second wait time should be applied after the local anaesthetic injection as occurs when both antlers are removed on the same occasion. When both antlers are removed, there is a longer time- period between completion of injection and when the first nick test is administered (88 seconds vs. 32 seconds), thus, allowing more time for onset of analgesia. However, in 9/14 deer with a single antler for removal local anaesthetic was effective at the time of the first nick test, and so it appears that an extended mandatory wait time may not be necessary as long as the 30-second wait time is applied if there is a positive response to the nick test.

Additionally, there is considerable variation amongst deer (See also Chapter 3). One of the deer receiving lignocaine, a local anaesthetic with faster onset, had the maximum time for removal in this part of the study at 108 seconds. This deer also had the tourniquet applied at 52 seconds, which was the maximum time for application. However, the removal of the antler occurred in less than 2 minutes and so would not burden the deer farmer with additional unoccupied time.

Time taken for infusion of the ring block varied amongst stags because of pedicle size, movement by the stags and characteristics of the subcutaneous tissue. In some stags the skin and/or subcutaneous tissue is thick and fibrous, making local anaesthetic injection difficult, and therefore more time-consuming. For one stag treated with bupivacaine, it took 6 minutes and 30 seconds from the first ring block until removal of the second antler. The operator had difficulty injecting the local anaesthetic as the skin and subcutaneous tissue over the pedicle was very tight, and the stag moved often.

An incidental observation during this study was lacrimation in two stags when they were injected with bupivacaine. The skin covering the pedicle on these two animals was very tight and thick. While this variable was not measured in our study, it may be valuable to include this observation in later studies.

4.5 Conclusion

This study has confirmed and supported previous data suggesting that the 4-minute wait time, prescribed by the National Velveting Standards Body, is not necessary.

Antlers can be removed without unoccupied time after administration of the local anaesthetic lignocaine in a "high" dose ring block. It is proposed that the welfare of the few stags which are not analgesic when nick tested is not compromised, provided a further 30-second wait time is allowed for a stag responding to the nick test (Cruz et al., 1997; Wilson et al., 2000a; Wilson et al., 1999a). It is recommended that the NVSB should incorporate these findings into its compliance standards for velvet antler removal.

It is suggested that further studies investigate why there is such variation between stags in local anaesthetic application times, as the administration of a local anaesthetic needs to be both repeatable and reproducible.

4.6 Postscript

As a result of this study, combined with those of Chapter 3 and Wilson et al. (1999a, 1999b, 2000), the NVSB has now (September 2001) altered compliance standards with the Velvet Removal Programme to permit a 1-minute wait time provided the "high" dose ring block is applied.

CHAPTER 5

INVESTIGATION OF NOVEL LOCAL ANAESTHETIC COMBINATIONS FOR VELVET ANTLER REMOVAL

5.1 Introduction

No single anaesthetic agent is perfect and no single agent can treat all types of pain, although each agent may have distinct advantages or disadvantages when compared to another (Raffa, 2001). Lack of an ideal anaesthetic drug has led veterinarians to devise drug combinations that induce good-quality anaesthesia with minimal risk to the patient (Hubbell, 1994). "Fentazin", a combination of xylazine, fentanyl citrate and azaperone (Parnell Laboratories, NZ), is an example of a sedative cocktail used in deer (Wilson et al., 1996a; Wilson et al., 1996b).

Under certain conditions, the use of a combination of analgesics may result in improved outcomes compared with those resulting from the use of a single component drug. Summation is when two or more drugs, administered together give a response which is simply the sum of their effects individually (Brander et al., 1991). A combination is most effective, however, when the individual agents act synergistically; that is, the actual response is greater than explainable on the basis of simple summation (Brander et al., 1991; Raffa, 2001; Upson, 1988).

The use of local anaesthetic mixtures for regional analgesia has become relatively popular in recent years (Raffa, 2001; Strichartz and Covino, 1990). For example, a mixture of lignocaine and bupivacaine has been administered via a ring block to provide intra-and post-operative analgesia for feline onychectomy or tenectomy (Matteson, 2000). In epidural anaesthesia in dogs, a combination of bupivacaine and lignocaine produced a shorter time to loss of inter-digital reflex than bupivacaine alone and longer analgesia than lignocaine alone (Cruz et al., 1997). Sheep given intra-articular lignocaine/bupivacaine injections, as a pre-emptive analgesic protocol in joint surgery, had significantly lower post-operative pain scores than the control group (Shafford et al., 2001). In people undergoing lower abdominal surgery, a combination of lignocaine and bupivacaine has been reported to produce longer post-operative analgesia with lower post-operative pain scores than lignocaine alone (Rodriguez et al., 1998).

Children receiving a caudal block for inguinal herniorraphy were given mepivacaine, bupivacaine or a bupivacaine/mepivacaine mixture to assess post-operative analgesia (Hashizume et al., 2001). The patients receiving either the bupivacaine or mepivacaine/bupivacaine combination required no additional post-operative analgesic within the first 24 hours compared with the group receiving only mepivacaine. In the mepivacaine group, 4/20 patients required analgesics post-operatively (Hashizume et al., 2001).

The basis for the practice of combining local anaesthetics is to compensate for short duration of action of certain agents, such as lignocaine, and the long latency of other agents, such as bupivacaine (Raffa, 2001; Strichartz and Covino, 1990).

This chapter describes two studies that evaluated local anaesthetic combinations for rate of onset and duration of analgesia. The local anaesthetic formulations were assessed for pain control during velvet antler removal and, potentially, for post-operative pain. The first study investigated two concentrations of a lignocaine/bupivacaine mixture. The second evaluated mixtures of mepivacaine, lignocaine and bupivacaine.

5.2 Study 1. "High" and "low" concentrations of lignocaine hydrochloride and bupivacaine hydrochloride combinations

5.2.1 Methods and materials

5.2.1.1 Experimental procedures

Antlers of 25 rising 1-year-old red deer stags from a commercial deer farm (Section 2.2.2.1) were used to compare "high" and "low" concentrations of lignocaine (L) and bupivacaine (BN, a novel bupivacaine formulation) for rate of onset (n=25/treatment) and duration (n=10/treatment) of velvet antler analgesia. The 10 deer were selected based on length of the velvet antler. The antlers had to be long enough for the application of the remote stimulator. Each antler of each stag was randomly allocated one of the treatments.

Unlike the studies in Chapters 3 and 4 that used a commercial formulation of bupivacaine (BC), the bupivacaine used in this chapter for both studies was formulated by Bomac specifically for our research. The two concentrations were:

- "High" concentration (HLBN): 1.5% lignocaine hydrochloride and 0.5% bupivacaine hydrochloride.
- "Low" concentration (LLBN): 1% lignocaine hydrochloride and 0.25% bupivacaine hydrochloride.

Refer to Section 2.5 for manufacturing details. These combinations were randomly assigned to the left antler first, with the right antler receiving the alternate treatment. Application and dose rate were as described in Section 2.6.

Onset of analgesia was determined as described in Section 2.3. Deer were then moved to darkened pens where duration of analgesia was monitored as described in Section 2.4.3.

5.2.1.2 Statistical analysis

The onset data were analysed using a Survival Log rank test. A Paired T-test was used to analyse the duration data. Both tests were run on GraphPad Prism 3.0 software.

5.2.2 Results

5.2.2.1 Onset of analgesia

Summary data for the onset of analgesia are presented in Table 5.1 and number of antlers desentised at each time interval are in Figure 5.1. The "high" concentration formula (HLBN) had a significantly shorter mean onset time of 37 seconds (range 15-90) than the "low" concentration formula (LLBN), which had a mean onset time of 55 seconds (range 15-210) (P=0.049).

Table 5.1 Mean and range of local anaesthetic analgesia onset times (seconds) for "high" (HLBN) and "low" (LLBN) concentration lignocaine/bupivacaine formulations

	Concentration	
	"High"	"Low"
n	25	25
Mean	37	55
Minimum	15	15
Maximum	90	210
S.E.	4.42	8.8

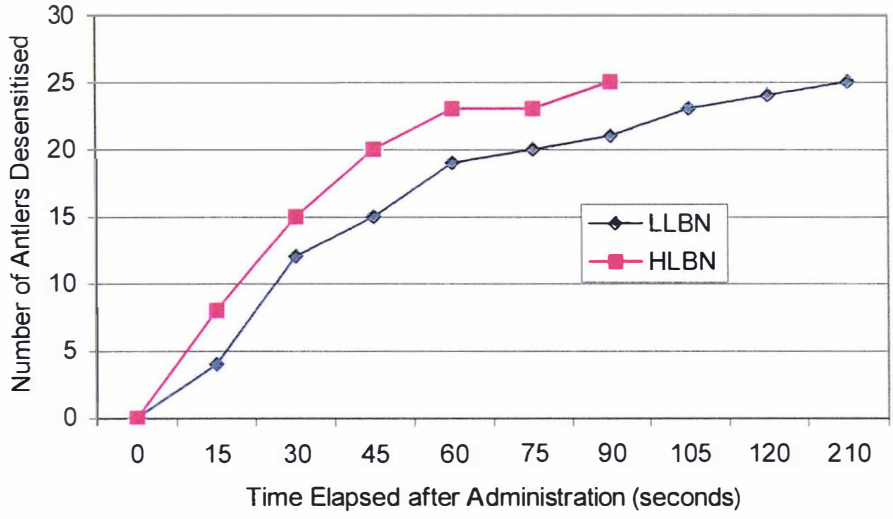

Figure 5.1 Number of antlers desensitised at each time interval after administration of LLBN (n=25) and HLBN (n=25).

5.2.2.2 Duration of analgesia

Summary data for duration are presented in Table 5.2 and individual duration times for stags are listed in Table 5.3. The mean duration time of analgesia for HLBN was 406 minutes (range 290-535), which was significantly longer than that for LLBN which was 333 minutes (range 240-475) (P=0.041).

Table 5.2 Mean and range of analgesia duration times (minutes) for "high"(HLBN) and "low"(LLBN) concentration lignocaine/bupivacaine formulation.

	Concentration	
	"High"	"Low"
n	10	10
Mean	406	333
Minimum	290	240
Maximum	535	475
S.E.	28.9	25.2

Table 5.3 Time (minutes) for antlers of spikers to return to sensation after administration of local anaesthetic treatments HLBN (n=10) and LLBN (n=10).

	Treatment	
Time	HLBN	LLBN
240		1
245		2
255		3
270		4
290	1	
300	2	
320		5
330	3	
350	4	6
370	5	
375		7
395		8
400		9
405	6	
475		10
480	7	
485	8	
515	9	
535	10	

5.3 Study 2. Comparison of Mepivacaine, Bupivacaine, Mepivacaine and Bupivacaine, and Mepivacaine and Lignocaine combinations for velvet antler analgesia

5.3.1 Methods and materials

5.3.1.1 Experimental procedures

Antlers of 14 yearling stags from the commercial deer farm were used in this study (Section 2.2.2.1). Local anaesthetics and their manufactures for this study were described in Section 2.5. Application and dose rates of local anaesthetics were described in Section 2.6. Onset and duration times were measured as specified in Sections 2.3 and 2.4. The four treatments administered in this study were mepivacaine (M), mepivacaine and novel bupivacaine (MB), novel bupivacaine (BN), and mepivacaine and lignocaine (ML).

The deer were randomly allocated to two groups and each deer was randomly assigned two drug treatments, such that the stag received a different treatment on each antler. Seven antlers were subjected to each treatment. Thus, a deer received

either mepivacaine (M) on one antler and the mepivacaine/bupivacaine (MB) combination on the other or bupivacaine (BN) on one antler and the mepivacaine/lignocaine (ML) combination on the other. For onset of M, only six antlers were available because stag 430 was stressed and failed to respond to the Grass Stimulator. He did respond to the remote stimulator, however, and was used for the duration study only.

The first antler treated was determined by the way the stag stood once it entered the restraining device. The treatment was randomly selected for the antler nearer the operator with the further antler receiving the second treatment.

5.3.1.2 Statistical analysis

Analgesia onset was analysed using Survival Log-rank test. A one-way analysis of variance (ANOVA) and a Tukey's multiple comparison test were used to determine if there were significant differences in the duration times of the treatments. The data were analysed using GraphPad Prism 3.0 software.

5.3.2 Results

5.3.2.1 Onset of analgesia

Summary data are presented in Table 5.4 and individual onset times for anaesthesia are in Figure 5.2. Mean onset time for mepivacaine (M) was 30 seconds (range 15-75). The combination of mepivacaine/lignocaine (ML) had a mean onset time of 34 seconds (range 15-75). For the novel BOMAC formulation of bupivacaine (BN), the mean onset time was 86 seconds (range 15-300), and for the mepivacaine/bupivacaine (MB) combination, the mean onset time was 30 seconds (range 15-60). There was no significant difference in mean onset times.

Table 5.4 Mean and ranges of onset of analgesia (seconds) following mepivacaine (M), bupivacaine (BN) and mixtures of lignocaine/mepivacaine (ML) and mepivacaine/bupivacaine (MB)

	Treatment			
	M	**ML**	**BN**	**MB**
n	6	7	7	7
Mean	30	34	86	30
Minimum	15	15	15	15
Maximum	75	75	300	60
S.E.	12.3	7.8	37.3	6.6

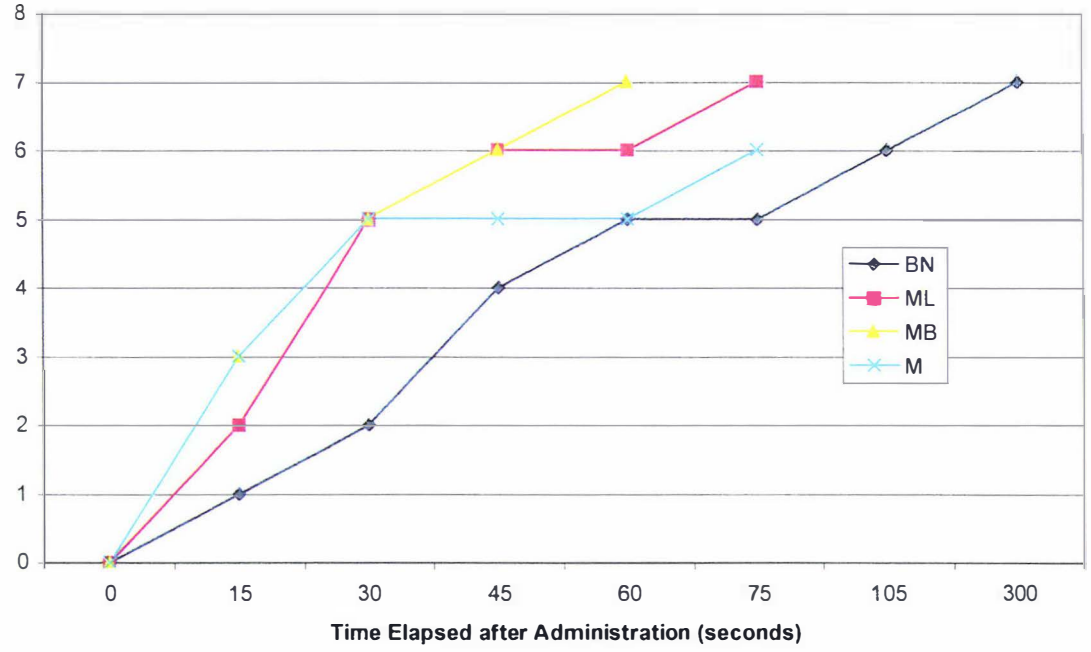

Figure 5.2 Number of antlers of spikers anaesthetised over time when treated with BN (n=7) ML (n=7), MB (n=7) and M (n=6).

5.3.2.2 Duration of analgesia

Summary data for duration of analgesia are presented in Table 5.5 and individual duration times for each stag are in Table 5.6. Mepivacaine (M) had a mean duration of 271 minutes (range 180-375). The mepivacaine/lignocaine (ML) combination had a mean duration of 221 minutes (range 120-270). The mean duration for the novel bupivacaine (BN) was 461 minutes (range 335-570), and for the mepivacaine/bupivacaine (MB) combination, the mean duration was 421 minutes (range 310-570). According to the ANOVA, there was a statistical difference in duration between the treatments that had novel bupivacaine (BN), and those that did not (P=0.0001).

Table 5.5 **Mean and ranges for duration of analgesia (minutes) following mepivacaine (M), bupivacaine (BN), and mixtures of mepivacaine/lignocaine (ML) and mepivacaine/bupivacaine (MB)**

	Treatment			
	M	**ML**	**BN**	**MB**
n	7	7	7	7
Mean	271	221	461	421
Minimum	180	120	335	310
Maximum	375	270	570	570
S.E.	26	19	37	41

Table 5.6 **Time (minutes) for antlers of spikers to return to sensation after administration of local anaesthetic treatments M (n=7), ML (n=7), MB (n=7), and BN (n=7).**

	Treatments			
Time	M	ML	MB	BN
120		1		
180	1			
190	2	2		
230		3		
240		4		
245		5		
255		6		
260	3			
270		7		
285	4			
300	5			
310	6		1	
320			2	
330			3	
335				1
350				2
375	7			
405			4	
430				3
450				4
465			5	
540				5
550			6	
555				6
570			7	7

5.4 Discussion

In Study 1, data for duration of analgesia after lignocaine/bupivacaine combinations suggested a synergistic effect when compared with duration of commercial bupivacaine (BC) alone, described in Chapter 3. The mean times for duration of "high" lignocaine/bupivacaine (HLBN) and "low" lignocaine/bupivacaine (LLBN) were 406 minutes and 333 minutes, respectively, compared with the mean time for commercial bupivacaine (BC) from Chapter 3 of 273 minutes. Lignocaine alone had a duration of 88 minutes (Chapter 3). The maximum duration for BC was 360 minutes, which was only 27 minutes longer than the mean duration of LLBN.

The mean onset time for HLBN was 37 seconds and for LLBN it was 55 seconds, compared with onset of L, which was 31 seconds, and BC, which was 48 seconds (Chapter 3). Because of onset and duration times, there appeared to be the possibility of synergism in both concentrations of lignocaine/bupivacaine. However, it was HLBN that showed the greater likelihood of synergy and, therefore, more advantageous for velvet antler removal.

Study 2 showed that mepivacaine, mepivacaine/bupivacaine and mepivacaine/lignocaine had mean onset times of 30-34 seconds, similar to that of lignocaine (31 seconds) reported in Chapter 3. The mean onset for novel bupivacaine was 86 seconds, while commercial bupivacaine (Chapter 3) was 48 seconds. The difference in onset times of the two bupivacaines suggested a difference in formulations.

It was late in the velvet antler-growing season when the results of Study 1 became available, and there was only a limited number of deer available with suitable length antlers for Study 2. However, it was decided to proceed because the major differences of interest were of duration rather than onset of analgesia. Earlier studies (Study 1 of this chapter, and data in Chapter 3) indicated that seven antlers per treatment should be sufficient to show differences in duration. Observations of onset were included to gather preliminary data of trends rather than an expectation of achieving statistically significant results, based on numbers needed to show a difference in onset times from Study 1 and Chapter 3. No significant difference for onset times was observed.

Mepivacaine alone had a rapid onset and its duration was equivalent to that of BC (4.5 hours, Chapter 3). In clinical practice, bupivacaine has a slower onset of action than mepivacaine, but bupivacaine is thought to inhibit sensory nerve fibres more selectively than mepivacaine (Butterworth et al., 1998). The combination of mepivacaine and the novel bupivacaine (MB), however, had a mean duration of 7 hours. This suggests that the combination of local anaesthetics administered at appropriate dose rates gives an onset time equivalent to the most rapid component, and a duration equivalent to the longest acting component at concentrations evaluated.

The mean duration of analgesia following the novel bupivacaine (461 minutes) in Study 2 was significantly longer (P=0.001) than for commercial bupivacaine (273 minutes, Chapter 3), consistent with the observation from the onset study (Study 1, Chapter 5) that a difference exists between those two product formulations. These findings negated the hypothesis proposed in Study 1 that the action of lignocaine and bupivacaine was synergistic. There may be a difference in bioavailability between the bupivacaine formulations. Bioavailability is the quantification of the rate and extent of drug absorption (Baggot, 1977; MacDiarmid, 1983; Riviere, 1994; Tucker, 1986). Slight changes in formulation such as product particle size, pharmaceutical interactions involving binding, dissolution, or solubility interactions may have substantial impact on bioavailability (Riviere, 1994).

Further evaluation of drug mixtures for onset of analgesia is necessary using greater numbers of antlers if statistically significant results are to be achieved. The mepivacaine/bupivacaine mixture appears to be a promising, practical mixture for the removal of velvet antler. It has an onset time similar to that of lignocaine, yet a duration almost equal to that of novel bupivacaine. This combination would obviate any unoccupied wait time for the deer farmer using restraint, and at least potentially address post-operative pain after velvet antler removal.

Further evaluation of onset and duration should be done between the two formulations of bupivacaine, but greater numbers of deer would be necessary to achieve levels of variability low enough to distinguish between the small time differences observed.

Again, there is the recurring theme that onset and duration of analgesia are variable among deer as discussed in Chapter 3 and 4. Whether it is due to site of injection, pedicle circumference, location of antler innervation or operator variation and/or reproducibility is not known. It would be desirable, therefore, to evaluate these variables so that the velvet antler removal process and its timing would have a greater uniformity and predictability in the future.

5.5 Conclusions

- Time to onset of analgesia following lignocaine and mepivacaine appears similar.
- Mepivacaine and bupivacaine (both commercial and novel formulations) have significantly longer duration than lignocaine.
- Combinations of local analgesics have an onset generally equivalent to that of the most rapid acting components, and duration generally equivalent to that of the longer acting component, but this is dependent on the concentration of the active ingredient.
- The possibility that combinations of local analgesics may have a synergistic action could not be confirmed in this study.
- There appears to be a difference in the onset and duration of different formulations of bupivacaine.

Further research proposed:

- Besides evaluating different drug mixtures in a greater number of spikers, drug mixtures could be evaluated in adult stags to determine if there is a difference in onset and duration times.
- Compare both formulations of bupivacaine in a greater number of spikers and in adult stags undergoing velvet antler removal.
- Research should be undertaken to determine which concentrations and formulations of drug mixtures would provide the optimum onset and/or duration times for analgesia.

CHAPTER 6

PILOT EVALUATION OF A TRANQUILLISER TO DECREASE BACKGROUND PHYSIOLOGICAL RESPONSES, AS A MODEL FOR STUDY OF POST-VELVETING PAIN

6.1 Introduction

Red deer (*Cervus elaphus*) are a relatively novel farmed species and may be particularly susceptible to stress (Grigor et al., 1997). Thus, routine management practices, such as yarding, handling and restraint, elicit behavioural and physiological changes and stress responses (Matthews and Cook, 1991; Pollard et al., 1992; Pollard et al., 1991). Physiological changes have been measured using white blood cell parameters, heart rate, respiratory rate and cortisol concentrations in saliva or blood (Matthews and Cook, 1991; Matthews et al., 1990; Matthews et al., 1992).

One of the physiological variables used to assess stress levels is the activity of the hypothalamic-pituitary-adrenocortical (HPA) system, usually assessed by measuring plasma cortisol, which initiates metabolic and anti-inflammatory responses that promote healing (Mellor et al., 2000; Mellor and Stafford, 1999). Cortisol is the primary glucocorticoid in humans and most mammals, and maintaining a sufficient, yet not excessive, concentration of glucocorticoids is necessary to maintain homeostasis (Matteri et al., 2000). Thus, its release in response to stressors is an adaptive mechanism.

However, there has been difficulty in differentiating between the behavioural and physiological stress response resulting from the pain associated with velvet antler removal from the stress of handling and restraint alone (Matthews and Cook, 1991; Matthews et al., 1992; Pollard et al., 1991; Wilson et al., 1999b). Stress responses to handling and restraint may mask any effects of antler removal on hormonal parameters once hormones reach peak concentrations (Matthews and Cook, 1991).

While these parameters of behaviour, hormone concentrations and heart rate are used as indicators of pain, many other factors such as handling, restraint, fear and hunger, can affect these results (Rushen, 1991). Therefore, in this study it was hoped

that these confounding, environmental factors could be reduced so a more accurate assessment of post-operative pain over time could be determined.

Long-acting neuroleptics (LAN) are drugs that have become valuable tools for alleviating handling stress in many species and they allow for more accurate interpretation of hormonal and other physiological parameters, such as heart rate (HR) and blood pressure (BP) of animals experiencing stress (Read et al., 2000). Neuroleptics (tranquilliser-sedatives) are central nervous system (CNS) depressants that exert quietening, calming effects on animals, lessening anxiety and sometimes reducing fear and aggression in animal species with naturally vicious or nervous temperaments (Brander et al., 1991). Zuclopenthixol acetate (ZPTA) has been demonstrated to be useful in reducing handling stress in North American Elk (Read et al., 2000; Woodbury et al., 2001) and red deer, when combined with perphenazine enanthate (Diverio et al., 1996a; Diverio et al., 1996b; Diverio et al., 1993). By reducing the background stress effects of handling, restraint and experimental manipulation, ZPTA has shown that the effects of various procedures pertaining to antler analgesia and removal can be differentiated from those of handling and other procedures *per se* (Woodbury et al., 2001).

Zuclopenthixol acetate, however, is not licensed for use in animals in New Zealand and is expensive. Azaperone, a tranquilliser, was chosen as an alternative to test in this pilot study, because of its pharmacological properties, including lack of analgesic properties, lower cost, and availability.

Combining drugs of different classes and with different mechanisms of action is an established anaesthesia technique used to achieve a desired effect with a minimum of adverse side effects. This multimodal approach can be used to address an animal's pain and hasten its recovery (Hellyer, 1997). The combined use of local anaesthesia and a non-steroidal anti-inflammatory drug (NSAID) abolishes or reduces the pain-induced distress response to various husbandry procedures, such as dehorning in calves (Mellor and Stafford, 1999). Local anaesthesia blocks transmission of sensory input, including pain impulses, whereas NSAIDs prevent or significantly reduce the development of inflammatory pain after the local anaesthetic wears off.

In deer, there have been few studies on the duration of pain relief after the administration of any protocol designed to provide analgesia (Haigh et al., 2001). In one study assessing post-operative behavioural changes associated with the pain and distress of velvet antler removal, a systemic analgesic, acetyl salicylate, reduced many behavioural changes otherwise seen following velvet antler removal (Pollard et al., 1992). In a subsequent study, however, there were no significant differences between stags treated with systemic analgesics and those that were not (Pollard et al., 1994). However, there was a difference in the amount of local anaesthesia administered in the two studies, in Pollard et al. (1992) a ring block of only 5 ml of 2% lignocaine was used, while in Pollard et al. (1994) used 20-25 ml was used, and this may help explain the different responses to the systemic analgesic.

This pilot study to investigate the suitability of the tranquilliser, azaperone, was undertaken to assess its efficacy in reducing "background" stress effects on stags. A second aim was to determine if pain after velvet antler removal, and once the effect of the local anaesthetic had ceased, was of animal welfare concern, and whether it could be alleviated by the administration of non-steroidal anti-inflammatory drugs.

6.2 Materials and methods

6.2.1 Experimental procedures

Fifteen 2-year-old stags, with growing velvet antler at or near the optimum stage for commercial removal were studied over a three-day period (December 6 - 8, 2000) at the Massey University Deer Unit, as described in Section 2.2.2.1.

Prior to experimental procedures, all deer were given the tranquilliser, azaperone (Stresnil, Boehringer Ingelheim NZ, Ltd., Batch 98D22/885, Exp. 4/01), by intramuscular injection into the neck at a dose rate of 0.2mg/kg/body weight.

On Day 1 of the study, four deer were injected with azaperone and, about 30-minutes later, standard intravenous catheters were inserted into a jugular vein. They were glued and taped to the neck of the deer. When the deer were restrained for velvet antler removal in the pneumatic handling device (Section 2.2.1), some of the catheters became displaced when the deer struggled. On Day 2 the pre-velveting blood samples were obtained from six deer in the yard before physical restraint by jugular venipuncture using 20gauge 1"needles. The intravenous catheters were subsequently inserted when the deer were in the pneumatic restraining device. This

method was still difficult as the position of the deer in the device made it difficult to insert the catheter, it was more time consuming and there was still the tendency for the catheters to become loose if the animal struggled.

Velvet antler was removed from all deer according to National Velvet Standards Body "Velvet Removal Programme" (1998) using 2% lignocaine as the local anaesthetic in a "high" dose ring block (See Section 2.5 for manufacturer details and Section 2.6 for ring block administration). Of the 15 stags, nine stags were given a non-steroidal anti-inflammatory drug (NSAID), flunixin meglumine (Fluximine, BOMAC Laboratories, Batch 01194, DOM 4/2000, EXP 4/2002), immediately after velvet antler removal, while the other six deer acted as controls. Deer assigned the NSAID treatment were given the drug via the intravenous catheter at a dose rate of 2.2mg/kg of bodyweight immediately following velvet antler removal. All deer were then moved to adjacent dark rooms for further blood sampling.

Three blood samples were taken within the 15-minute period prior to velvet antler removal. After antler removal, blood samples were taken every 30 minutes for 4 hours and again 1 hour later at hour 5. There were 12 blood samples taken per deer. Blood samples were collected in an evacuated glass tube containing heparin and stored on ice. Once the last blood sample was taken, the catheters were removed and the deer returned to pasture.

Blood samples were centrifuged for 10 minutes at 3000 rpm and the plasma stored at -20°C until analysed by radioimmunoassays (Clinical Assays™ GammaCoat™ Cortisol ^{125} I Radioimmunoassay Kit, DiaSorin, Stillwater, Minnesota 55082-0285, U.S.A.). Each sample was assayed twice. The standard curve was based on cow plasma and used to estimate the concentration of cortisol in the plasma samples.

Since the cortisol assay results showed unexpectedly high baseline cortisol concentrations, on February 26, 2001 additional blood samples were taken from nine of the deer to re-measure baseline cortisol levels. The deer were handled and restrained only, as described in Section 2.2.1, and did not receive azaperone or flunixin meglumine. Single blood samples were taken via jugular venipuncture with a heparinised tube, after which the deer were released into the paddock. The blood samples were processed as above. Eight deer from the original study were re-tested, plus one deer, which belonged to the same mob, but had not been used in any of the

studies. Only nine deer could be tested, as the remainder were difficult to handle due to the onset of the mating season and consequent aggressive behaviour.

6.2.2 Statistical Analysis

A two-way ANOVA was used to analyse the cortisol concentrations between both treatments and over time. The software employed was the SAS 8.2 programme. To compare cortisol concentrations between deer at 15 minutes pre-velveting that had been given azaperone, and to deer approximately six-weeks later, a paired t-test was administered using Excel from Microsoft Office 2000 programme.

6.3 Results

The mean and range of cortisol concentrations are presented in Table 6.1 and Figure 6.1. Raw data are located in Appendix 4.

Both treatments elicited elevated plasma cortisol concentrations. As the deer were from the same population, pre-velveting data were pooled. The pre-velveting mean blood cortisol concentrations for both groups were 141, 137, and 134 nmol/l for -15, -10 and -5 minutes before restraint for velvet antler removal, respectively. Mean cortisol concentration for the Control group at 30 minutes post-velveting was 134 nmol/l and decreased to 87 nmol/l at 5 hours post-velveting. The mean cortisol concentrations for the NSAID treatment group was 131 nmol/l at 30 minutes post-velveting, and decreased over the 5 hours to 105 nmol/l. No significant difference was detected between the means of the control group and the NSAID treated group (Figure 6.1). However, there was a significant reduction in cortisol over the 5-hour period (P= 0.0001). This was the same for both groups.

Table 6.1 Plasma cortisol concentrations (nmol/l) for the control and NSAID treatment groups of stags from 15 minutes pre-velveting to 5 hours post- velveting. Time is in minutes

	Pre-velveting			Post-Velveting								
Time	-15	-10	-5	30	60	90	120	150	180	210	240	300
n=6 Control												
Mean	145	137	133	134	132	133	125	128	112	108	103	87
Minimum	96	98	92	99	96	104	109	100	77	85	73	57
Maximum	179	168	159	160	150	162	158	159	142	144	130	114
SE	13.5	10.9	12.4	8.5	8	8.7	7.6	8.7	8.8	8.1	9.1	8.7
n=9 NSAID												
Mean	137	136	135	131	124	126	132	132	122	121	113	105
Minimum	95	97	100	102	107	111	111	113	104	107	94	79
Maximum	174	170	185	156	167	149	162	169	144	179	184	154
SE	8.9	8.3	9.4	6.3	6.3	4.4	5.1	5.5	4.4	7.5	9.3	6.9

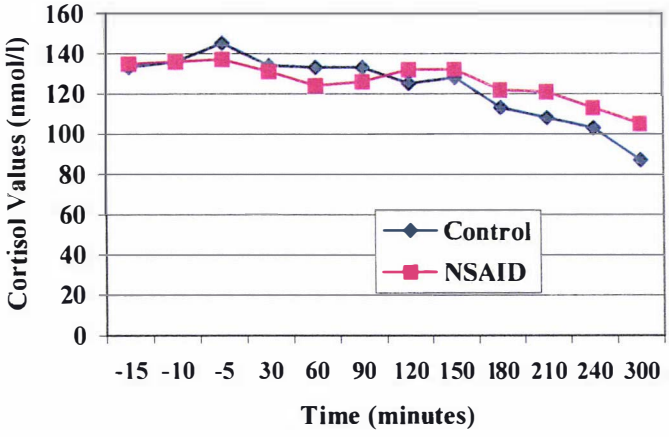

Figure 6.1 Mean cortisol concentrations (nmol/l) for control and NSAID treated stags from 15 minutes pre-velveting to 5 hours post- velveting.

Plasma cortisol concentrations in the deer re-sampled in February 2001 are presented in Table 6.2. The cortisol concentrations were significantly lower at this sampling (P=0.001) than in December 2000 when they had received azaperone.

Table 6.2 Plasma cortisol concentrations (nmol/l) from stags sampled in December after azaperone, and again 6 weeks later with physical restraint only

Animal ID	Sampled December, after azaperone	Sampled February, physical restraint only
826	163	34
827	174	110
833	95	15
839	145	78
842	113	70
861	152	85
864	132	45
866	96	59
Mean	**134**	**62**

6.4 Discussion

This was a pilot trial to study whether the use of the tranquilliser azaperone reduces plasma cortisol levels and which could then be measured in subsequent studies to differentiate between the stress caused by handling and velveting. It is apparent that cortisol levels are not reduced by azaperone administration; indeed the opposite was observed.

In this study, the data suggest there was no difference between the control group and the group receiving the non-steroidal anti-inflammatory drug. The most important observation was that both groups exhibited elevated concentrations of cortisol in the plasma samples despite using azaperone. The group receiving azaperone and the NSAID had a mean cortisol concentration of 126.17 nmol/l and the control group, receiving only azaperone, had a mean cortisol concentration of 123.17 nmol/l. The standard cortisol concentrations for tranquillised deer range from 6.31 to 27.08 nmol/l and for non-tranquillised deer from 6.50 to 71.66 nmol/l (Diverio et al., 1996a; Read et al., 2000).

Assessing pain in animals has been a difficult task and has relied on a combination of behavioural and physiological observations (Thierman et al., 1999). In calves and lambs, certain behavioural observations, such as tail flicking, vocalisation and changes in posture, have been proven indicators of pain (Graham et al., 1997; Molony and Kent, 1996; Molony et al., 1995). In red deer, removal of velvet antler

without anaesthetic is painful and the deer reacts with a moderate or strong response, but not when a local anaesthetic is applied prior to velvet antler removal (Matthews and Cook, 1991). Pollard et al. (1993, 1992, 1991) compared the behaviour of deer that were velveted and those that were not. There was a considerable difference in the behaviour of the two groups, but it was unclear whether the treated animals responded to post-operative pain or to the abrupt, reduction of weight to the head (Thierman et al., 1999). Quantifying post-operative pain relying solely on behaviour is difficult and can be subjective.

Physiological measurements are also used to assess and monitor pain. These measurements include blood pressure, temperature and various hormone concentrations (Thierman et al., 1999). Plasma cortisol levels were monitored on red deer both post-velveting and a control group and there were no differences between treatments (Matthews et al., 1994). It was shown that handling and restraint also increase hormone levels.

The heart rate is another useful indicator of stress or pain (Thierman et al., 1999). When a cattle prodder was used on calves their heart rate increased 1.5 times from the relaxed rate, while it increased 1.7 times when they were forced to climb a ramp (Rushen, 1991). In red deer, the average heart rate increased during velvet antler removal, but there were no differences between the control group and the treatment groups post-velveting (Matthews et al., 1992). Pollard et al. (1992) also compared the heart rates of stags on two consecutive days. The stags having their antlers removed on the first day showed an increase in heart rate and did so when they were handled on the second day without removal of the antlers. The deer from the control group showed a decrease in heart rate on the second day of handling.

Measuring stress by evaluating cortisol concentrations is considered the "classic" method (Read et al., 2000) and to simplify experimental procedures and minimise extraneous influences, was the only parameter chosen for this study.

In studies by Read et al. (2000) and Diverio et al. (1996a) ZPTA reduced the serum cortisol levels of elk and red deer to levels significantly lower than in control animals. Read et al. (2000) showed that 24 hours after drug administration, the mean cortisol concentration for the control group was 139.50 nmol/l and the ZPTA group had a cortisol concentration of 97.91 nmol/l ($P<0.05$). Thus, the use of ZPTA

in these animals was effective in decreasing their stress response and activity during physical restraint, and this has been sufficient to allow the demonstration of physiological differences in elk during and following "Electro-analgesia" or local anaesthesia for velvet antler removal (Haigh et al., 2001).

The present study took place over a 3-day period. The mob was brought in from the paddock and yarded daily. Once they arrived in the yard, the pre-selected deer were separated from the mob and placed in two small groups. Each group was kept in a darkened pen adjacent to the handling device. The remaining deer were returned to pasture. Yarding and separation from the main herd have been shown to cause stress in deer (Carragher et al., 1997; Grigor et al., 1998c; Matthews et al., 1990). It is possible that the high initial cortisol values seen in the present study were a reflection of the stress from yarding and handling. When deer are placed in different groups, there is re-establishment of rank amongst them. Bringing deer into the yards sooner to acclimatise them their surroundings may have resulted in lower pre-velveting cortisol levels.

Additionally, this mob may have exhibited anxiety due to pre-conditioning as they had previously undergone routine sedation for semen collection via electro-ejaculation. In sheep, this procedure was found to be an aversive experience (Stafford et al., 1996). However, when the stags were yarded for additional blood sampling in February, the cortisol values were significantly lower with a mean of 61.6 nmol/l (range 15-110 nmol/l), so the high plasma cortisol concentrations in December were unlikely to be due only to a pre-conditioned response.

An alternative to the method used in this study would be to yard the deer a day or two prior to velveting and surgically implant the IV and place a cap on the catheter. It is unlikely, however, that this would be successful as it was difficult keeping the catheters attached over the 5-6 hours needed for completion of the study. Another option would be to use a long acting tranquilliser and do all manipulations after the tranquilliser has taken effect.

The plasma cortisol result observed here raises the question of whether azaperone, itself, elevates cortisol concentrations in deer. It has been concluded that azaperone is a suitable method of sedation in Sambar deer for collection of samples for haematological analysis (Yang and Lee 1993). Azaperone has been most used

specifically and extensively in pigs. However, some neuroleptic drugs, such as octoclothepine or diazepam at 0.1/mg/kg, may control the external signs, i.e. the physical manifestation, but not the physiological manifestation of stress (Dvorak and Raszyk, 1975).

In young pigs given azaperone and then tested for discomfort under standard husbandry procedures, azaperone caused a mean rise of 52% in plasma corticosteroid concentrations (Dvorak and Raszyk, 1975). The saline-injected controls and the pigs injected with azaperone displayed equal values of corticosteroid concentrations even though the azaperone injected pigs behaved more calmly.

Azaperone administered to male goats prior to 2 hours of transport resulted in elevated plasma cortisol concentrations for approximately 60 minutes after transport. This was significantly longer than with goats not receiving azaperone (Sanhouri et al., 1992). However, azaperone had been given IV and could have potentially caused hypotension and, therefore, with the increase in heart rate there is the increase in cortisol.

Azaperone has been used to sedate antelope in Africa. After the animals were darted there appeared to be a very high level of anxiety, but because of the situation and location, it was not practical to take measurements at that time (Gradwell, 2001). Gradwell (2001) reported that there was no problem with azaperone's use in commercial pigs. After further enquiries about the product, however, Gradwell (pers. comm. 2001) reviewed the records and found that there were "quite a few deaths" in Vietnamese Pot Bellied Pigs over the past 5 years following routine procedures such as hoof trimming.

There was also the possibility that the time between administration of azaperone and insertion of the catheter for blood sampling was not of adequate duration. During the period when sedative drugs are taking effect, it is important that animals not be disturbed; all sensory stimulation should be minimised because an exaggerated arousal reaction is most likely at this time, especially with tranquilliser-sedatives (Brander et al., 1991). Therefore, it may be advisable to extend the time from administration of azaperone to the insertion of the catheters and removal of the velvet antler, if indeed this drug is to be effective for the purpose of this study. It

appears that using a LAN versus a tranquilliser, like azaperone, may be more feasible, as it would provide more suitable tranquillisation prior to yarding.

Cortisol concentrations for both treatment groups began declining approximately 180 minutes post velvet removal. This may have been due to the deer becoming habituated to the blood sampling over time, as there was little reaction to human contact by the end of the experiment and because of the relatively short half-life of azaperone. In pigs and horses, it has a duration time of 2-3 hours (Upson, 1988).

Another question would be whether azaperone minimises the analgesic effect flunixin meglumine may produce in stags. However, in studies of dehorning in calves, it was found that when a local anaesthetic and a NSAID where given simultaneously, it provided an effective way of alleviating post-operative pain (McMeekan et al., 1998b; McMeekan et al., 1999)

6.5 Conclusion

There was no detectable difference in mean plasma cortisol concentrations between treatments of azaperone and azaperone with flunixin meglumine.

6.6 Future research

Further research needs to be done using different methodologies. Yarding and administering a different tranquilliser, with a longer half-life, or a LAN the day before any manipulations are to take place may possibly eliminate any background noise and acclimatise the deer to their surroundings.

Use of remote blood sampling and physiological monitors may be a preferred option as the deer could be moved into the paddock with the remainder of the mob and have minimal human contact.

CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

Velvet antler removal from farmed deer has the potential to become a highly controversial, moral and ethical issue in many Western countries. In New Zealand, velvet antler removal is a legal standard farming practice, provided it is undertaken in accordance with the National Animal Welfare Advisory Committee Code of Recommendations for that purpose. What began as a procedure to minimise the risks of injury to animals and people now supports a multi-million dollar industry (English, 1991).

The basic premise of most cultures and societies is that it is ethically acceptable for mankind to farm animals as long as their welfare is not compromised. The aim of the studies reported in this thesis has been to research methods that can be applied to promote and improve the welfare of stags undergoing velvet antler removal. The research extended previous studies of local anaesthetic agents for the abolition of pain during removal of velvet antler from stags, and investigated the potential of local anaesthetics, either singly, or in combination, for control of post-operative pain. A pilot study of a novel method for investigating post-operative pain was also undertaken.

7.2 Local anaesthetics

The aim of the work described in Chapter 3 was to evaluate and compare the onset and duration of analgesia produced by lignocaine, lignocaine with sodium bicarbonate and bupivacaine to determine which best fulfils the preferred criteria for a local anaesthetic: reliability, repeatability, ease of application, rapid onset, long duration and affordability.

The search for the ideal anaesthetic drug has led veterinarians to devise drug combinations that induce good-quality anaesthesia with minimal risk to the patient (Hubbell, 1994). Different local anaesthetic mixtures and concentrations were assessed for pain control during velvet antler removal and, potentially, for post-operative pain relief (Chapter 5). The first study investigated two concentrations of a lignocaine/bupivacaine mixture. The second evaluated various mixtures of

mepivacaine, lignocaine and bupivacaine. The basis for the practice of combining local anaesthetics is to compensate for short duration of action of lignocaine, and the long latency of other agents, such as bupivacaine (Raffa, 2001; Strichartz and Covino, 1990).

7.2.1 Local anaesthetic onset

Testing sensibility by an electrical stimulus every 15 seconds, rather than at 1-minute intervals as reported in previous studies, revealed that onset of analgesia occurred more rapidly in many deer than had been previously believed. The velvet removal programme manual, used as the standard in New Zealand, recommended a 4-minute wait from the time of local anaesthetic application until removal of the velvet antler to ensure antler analgesia. This, however, was a "best guess" in the absence of data when the code was developed (Wilson, pers comm.2001).

In Chapter 3, onset times of 15 seconds were recorded for the local anaesthetics used in some animals. In others, prolonged onset times of 60 seconds for LBC, 195 seconds for lignocaine and 255 seconds for bupivacaine in spikers were observed. In mature stags, the longest onset times were 120 seconds for both lignocaine and bicarbonate (LBC), and lignocaine (L). Bupivacaine had a slower mean onset than L and LBC.

While the mean onset time for LBC was not statistically significantly different from lignocaine, the observations in spikers that all antlers were desensitised by 60 seconds suggested that this mixture was more repeatable (Section 3.2.2.2). In a practical context, this would render LBC preferable to lignocaine alone if the data in spikers were repeated in adult stags. However, not all mature stags were desensitised until 120 seconds post-injection (Section 3.3.3). It is physically easier to apply local anaesthetic to spikers than to adults, possibly because of thicker subcutaneous tissue and other anatomical differences in older stags. There was no significant difference between mean onset time for lignocaine and LBC in adults, which is consistent with spikers. Thus, repeatability, in terms of analgesia onset times, is variable between age groups in this study. In order to ensure stag welfare, however, guidelines must be set at the longest onset time for a given drug and technique. For practical purposes, a zero wait time is preferred by the deer farmer. Thus, the full time/efficiency potential for local anaesthetics is yet to be achieved.

In Chapter 5 (Study 1), mean time to onset of analgesia for the high concentration lignocaine/bupivacaine mixture (HLBN) was 37 seconds (range 15-90) and 55 seconds (range 15-210) for the low concentration lignocaine/bupivacaine mixture (LLBN), compared to the onset of lignocaine at 31 seconds and commercial bupivacaine at 48 seconds (Chapter 3). Study 2 showed that mepivacaine had a mean onset time of 30 seconds (range 15-75), mepivacaine/bupivacaine also had a mean onset time of 30 seconds (range 15-60), while mepivacaine/lignocaine had a mean onset time of 34 seconds (range 15-75). The mean onset time for novel bupivacaine was 86 seconds (range 15-300) which when compared to commercial bupivacaine at 48 seconds (range 15-255), suggested a difference in formulation. The longer latency for the novel formulation was also associated with longer duration.

7.2.2 Local anaesthetic duration

The novel technique used to assess duration was initially cumbersome and time consuming until adaptations were made. Bandaging the electrode to the antler allowed only three stags to be monitored at one time. Additionally, using this method with the longer acting drugs (Chapter 5) produced ischaemic desensitisation, which confounded the results. The adaptation to the "hand-held" method, therefore, allowed large numbers of animals to be assessed without ischaemia occurring.

The long duration of bupivacaine makes it a candidate for future studies addressing the management of post-operative pain. The mean duration of mepivacaine (271 minutes, range 180-375) and bupivacaine (both formulations) was significantly longer than that of lignocaine (88 minutes, range 60-120).

There appeared to be a difference in the onset and duration times of different formulations of bupivacaine. The mean duration of analgesia following the novel bupivacaine (Chapter 5) was 461 minutes (range 335-570) and was significantly longer than that for commercial bupivacaine (273 minutes, range 195-360) (Chapter 3).

BOMAC attempted to analyse the novel bupivacaineBOMAC, but there was an insufficient amount of the product remaining in the flexipaks. An investigation into the bioavailability, which is the quantification of the rate and the extent of drug absorption, is suggested. Slight changes in formulation such as product particle size,

pharmaceutical or solubility interactions may have substantial impact on bioavailability.

7.2.3 Analysis of onset and duration results

While there was no statistical difference between lignocaine and LBC in Chapter 3, the addition of sodium bicarbonate to lignocaine suggests an increase in repeatability in spikers. However, while other studies have reported a decrease in the analgesia onset time of LBC, they have also reported a decrease in the degree and duration of analgesia (Sinnott et al., 2000). Duration studies of LBC were not done because onset time data was collected first, and that showed it had little advantage over lignocaine when the data from the adult stags was analysed. Also, at this stage the logistics for duration testing were time consuming as the "hand-held" method of stimulation had not yet been adopted.

Since there was variability amongst stags, there needs to be investigation of the causes of individual variation. This would involve study of how much of the observed variation is due to differences in application of the local anaesthetic (i.e. operator variation) and how much has to do with variability of individual stags over consecutive years, differences between age groups and other animal related factors. To test for reliability there must only be one operator as there is variation between operators in administration of the local anaesthetics.

The ring block is a more repeatable method after a 1-minute wait time than the regional nerve block. With the conventional nerve block, lack of operator skill can result in failure of the anaesthetic to reach the nerve (Woodbury and Haigh, 1996). Anatomy of nerve location has been studied in red deer (Adams, 1979; Kirk and Adams, 1980) and in fallow deer and wapiti (Woodbury and Haigh, 1996). A local anaesthetic must diffuse through several physical barriers before it reaches individual neurons within a peripheral nerve. Obstacles such as fat, fibrous or scar tissue or haemorrhage in the area of injection tend to impede the diffusion of the drug toward and into the nerve (Matteson, 2000). It was found that some animals have an auxiliary nerve supply; therefore, achieving consistently effective results is made easier by placing the anaesthetic closer to the area to be blocked such as with the ring block (Woodbury and Haigh, 1996). Because of the smaller peripheral nerve branches the local anaesthetic penetrates easier into the nerve causing a rapid loss of sensation to the antler (Matthews et al., 1992).

Individual variation of animals to the local anaesthetic seems likely. Other factors such as excitability, possibly due to genetic make-up or learned responses will cause animals to struggle while restrained. Struggling increases the difficulty applying local anaesthetics. Monitoring the responses of individual adult stags over subsequent velveting seasons with the same operator could determine how significant individual variation is amongst animals. Individual responses by spikers could be measured every few weeks over one velveting season once their antlers reached the appropriate length required to test with an electrical stimulus.

Besides evaluating the drug mixtures used in these studies on spikers, they could be evaluated in adult stags to determine if there is a difference in onset and duration times as compared with the spikers. Wilson et al. (2000) got similar results in adults and spikers. More data, however, would help determine whether spikers are suitable models, and, if so, the data could then be extrapolated to adults. The two formulations should be compared in spikers and adult stags to determine whether there is still the possibility of a difference between the two formulations of bupivacaine. Studies on how formulation affects anaesthetic performance seems a promising area for further work.

As with onset times, it may prove valuable to study duration of local anaesthetics on individual stags several times over a season to determine whether duration of analgesia is consistent and whether it is uniform for each drug. This may help differentiate whether variation in onset is due to animal as opposed to operator factors.

The novel remote technique for monitoring duration of analgesia was effective and generated minimal disturbance to the stags. However, this could only be done in deer whose antlers were not removed. There is a need for an effective non-invasive methodology for determining post-operative pain in general, and, of particular relevance to this study, ways to measure analgesia after velvet antler removal. While the remote method used here was suitable for monitoring the return of sensation in spikers not undergoing velvet antler removal, a non-invasive, repeatable methodology for monitoring post-operative pain in velveted stags is needed.

Infrared thermography, as described by Schaefer and Cook (2001), is a non-invasive instrumental measurement of radiated heat in the electromagnetic spectrum and could have application for this purpose.

7.3 Timing sequence of velveting procedures

The observation of rapid onset of analgesia when using the technique described in Chapter 3 and those of previous studies (Wilson et al., 2000a; Wilson et al., 2000b; Wilson et al., 1999a) prompted an evaluation of the timing sequence of the procedures for the removal of velvet antler (Chapter 4). Wilson et al. (1999b) suggested a wait time shorter than 4 minutes would be satisfactory if the dose and techniques for the local anaesthetic application were optimum.

By measuring the time sequence of events, it was demonstrated that the need for an unoccupied wait time would generally be unnecessary. The continuous sequence of application of analgesia to both pedicles followed by a tourniquet application and then a nick test of the velvet antler, means that the 1-minute interval is over without having to wait. The interval between injection and antler removal for the second antler is even longer than for the first. These observations were of P.R. Wilson, who is experienced and works fast and systematically. It is likely that most operators would not be as quick in removing the velvet antler, although this needs to be verified in practice.

Thus, antlers can be removed without unoccupied wait time to the deer farmer, after administration of the local anaesthetic lignocaine in a "high" dose ring block. Removal time of both antlers for lignocaine treated deer had a mean of 104 seconds (range 87-150), and a mean 167 seconds (range 117-390) for the commercial bupivacaine treatment. The results show that it is unlikely that the time interval between injection of a high dose ring block and antler removal can be much less than 60 seconds, even for experienced operators. It was proposed that the welfare of the few stags, which were not analgesic when nick-tested after a continuous sequence, was not compromised, provided a further 30-second wait time was allowed for those few stags responding to the nick test (Wilson et al., 2000a; Wilson et al., 2000b; Wilson et al., 1999a). Even with the 30-second additional wait time; the removal of the velvet antler was still accomplished under the 4 minutes required by the velveting manual standard.

The reason for this conclusion is that the approved, but sub-optimum range of techniques used in practice sometimes fail to achieve analgesia within the 4-minute wait time. Therefore, the contingency of a further wait or more local anaesthetic is stipulated by the velvet removal programme manual. While the outcome for the stag in both cases is analgesia, if the "high" dose ring block is used, the reliability of analgesia, even at 1-minute after application, is greater than achieved by the commonly used low site regional block. Thus, the net welfare cost is likely to be reduced by using optimum methods even with no unoccupied wait time.

As a result of this study combined with those of Chapter 3 and Wilson et al. (1999a, 1999b, 2000), the NVSB prior to the 2001 velvet removal season altered compliance standards in the Velvet Removal Programme to permit a 1-minute wait time provided the "high" dose ring block is applied. Thus, veterinarians and farmers using this technique do not have unoccupied wait time during velvet antler removal. This is particularly important for those systems using single animal physical restraint alone, where through put of animals is a limiting factor. Furthermore, this is a step toward encouraging more farmers to use physical restraint, thus avoiding unwanted residues of chemical immobilising agents. This is a significant issue for the deer industry (Loza, 2001). In future, if non-chemical methods for analgesia are devised, physical restraint will become the norm.

Furthermore, physical restraint has been shown to be aversive in deer (Carragher et al., 1997; Grigor et al., 1998c; Matthews et al., 1990; Pollard, 1993; Pollard et al., 1994). Reduction of restraint time would, therefore, benefit the stags' welfare. Unlike tail docking or polling of horns, which are performed once in the animal's life, removing velvet antler is an annual procedure. With the alteration of the compliance standards stags may now be restrained and subjected to the actual velveting procedure for only a few minutes per year, therefore, minimising any compromise to their welfare. Thus, this research has identified a way to improve the welfare of stags during velvet antler removal.

7.4 Pilot trial of methods to evaluate post-operative pain

The research presented in Chapter 7 was a pilot trial to study whether the use of the tranquilliser, azaperone, could be used to reduce the effects of confounding environmental factors such as handling and restraint, thereby, allowing for the measurement of post-operative pain over time. The longer-term welfare aim was to

assess the significance of pain after velvet antler removal and, if significant, to determine whether it could be alleviated by the administration of non-steroidal anti-inflammatory drugs.

Plasma cortisol was chosen as the sole parameter in this study as cortisol has been used previously in deer to assess stress levels. There was no detectable difference in mean plasma cortisol concentrations between the two treatments. Despite the use of the tranquilliser, azaperone, both groups exhibited elevated concentrations of cortisol in the plasma samples compared to other cortisol data on deer. As a result, it was concluded that azaperone was not a suitable agent for decreasing or eliminating background noise in testing deer.

Measurements of cortisol are the most common method of evaluating handling stresses, but it is a time dependent measure. Evaluations of handling and velveting stress will be more accurate if behavioural reactions, heart rate, and other blood biochemical parameters are also measured (Grandin, 2000a).

Further research needs to be done to evaluate post-operative pain. It would seem to be good management practice to habituate deer to yarding and restraint from an early age without having them undergo any other aversive procedure, as this should keep their stress levels controlled. Yarding and administering a different tranquilliser or a long-acting neuroleptic (LAN) the day before any manipulations are to take place may then eliminate any background noise and acclimatise the deer to their surroundings. It may also prove valuable to assess four treatments instead of two. The other two treatment groups would not receive the tranquilliser or LAN, but one of the two groups would receive a NSAID after velveting. Use of remote blood sampling and physiological monitors may be a preferred option as the deer could be moved into the paddock with the remainder of the herd and have minimal human contact.

To evaluate the duration of post-operative pain on mature velveted stags, a nerve conduction experiment could be applied. While it is invasive, it is administered under general anaesthesia. When a general anaesthetic is applied, the brain's memory of surgical pain is dulled, but the nerves still react with electrical and chemical signals in response to a painful stimulus (Roberge and McEwen, 1998).

This procedure would reduce the confounding effects of stress and cognitive awareness.

Another method would involve minimum alveolar concentration of a volatile anaesthetic, which would maintain deer in a light plane of anaesthesia. This would allow the cardiovascular and neurological responses to be measured while undergoing velvet antler removal. Comparisons could be made of the responses among analgesia treatments such as lignocaine and compression along with control groups

7.5 Conclusion

Research in this thesis has shown:

- Onset of analgesia occurred in some stags within 15-seconds of local anaesthetic application.
- The novel technique for monitoring duration of analgesia was effective.
- In spikers, all antlers were desensitised within 60 seconds when using LBC.
- Combinations of local analgesics have an onset generally equivalent to that of the most rapid acting components, and duration generally equivalent to that of the longer acting component, but this is dependent on the concentration of the active ingredient.
- Mepivacaine and bupivacaine (both bupivacaine formulations) produce significantly longer duration of analgesia than lignocaine.
- There appears to be a difference in the onset and duration of analgesia caused by different formulations of bupivacaine.
- Antlers can be removed without unoccupied wait time after administration of the local anaesthetic lignocaine using the high dose ring block, without increasing the welfare cost to the stag.
- The tranquilliser, azaperone, did not reduce the confounding stress factors of yarding, handling and restraint. Efficacy of NSAIDs in reducing post-operative pain could not be assessed. The raised cortisol levels in both groups suggested that this was a result of the azaperone administration, but whether it was due to route of administration or dose rate is unknown.

Further research is required for assessing post-operative pain in stags and the ways to alleviate it, either by long acting local analgesia or systemic analgesia. The importance of self-monitoring welfare issues is imperative for the deer industry, especially as it relates to velvet antler removal. The New Zealand deer industry can be considered to be in a potentially fragile and precarious position in relation to welfare issues. The mere hint of any unethical practices pertaining to the removal of velvet antler could result in severe economic boycotts or disruption from animal welfare and animal rights groups in New Zealand. Therefore, the deer industry needs to undertake further research to improve and enhance deer welfare and to address both real and potential concerns of the marketplace, and society as a whole.

7.5.1 Industry adoption of the research findings

As a result of the studies in Chapters 3 and 4, and previous studies (Wilson et al., 2000a; Wilson et al., 2000b; Wilson et al., 1999a; Wilson et al., 1999b), the NVSB amended the compliance standards of the Velvet Removal Programme to allow a 1-minute wait time when a "high" dose ring block is applied. It is anticipated that this change will encourage more deer farmers to cease using chemical restraint and its attendant risks, and utilise physical restraint instead for velvet antler removal. The stag's welfare should be improved, as analgesia will be achieved using "best practice" methods and restraint times will be reduced.

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APPENDIX 1 - RAW DATA

TABLE A1. RAW DATA OF TIME TO ONSET FOR BC FOR STUDY 1, CHAPTER 3. BASELINE VOLTAGE AND ONSET TIME WERE DETERMINED USING A GRASS STIMULATOR. *SEETABLES 3.1,3.3

ANIMAL ID	TREATMENT ORDER	ANTLER	CIRCUMFERENCE	DATE	VOLTAGE	TIME (SEC)
04		L	0	-NOV		5
06		L	3	0-OCT		40
08		L	2	4-OCT		5
14		R	2	0-OCT	.5	5
15		L	0.5	-NOV		5
15		R	0	4-NOV		0
16		R	2	-NOV		5
18		R	1.5	4-OCT		5
19		R	1	0-OCT		5
19		L	0	6-OCT		5
21		R	2	0-OCT	0	5
26		L	1	4-OCT	.5	0
32		R	1	4-NOV		5
32		L	0	6-NOV		0
33		R	0	-NOV		5
35		L	4	0-OCT		5
37		L	1	-NOV		5
39		L	1	-NOV		5
47		R	2	0-OCT		0
47		L	2	-NOV		5
49		R	2	6-OCT		5
49		L	2	-NOV		0
50		L	1.5	4-OCT		5
50		R	1	0-OCT		55
52		R	1.5	-NOV		5
57		R	1.5	6-OCT		5
62		L	2.5	0-OCT		95
62		R	2.5	-NOV	.5	5

TABLE A2. RAW DATA OF TIME TO ONSET FOR LBC FOR STUDY 1, CHAPTER 3. BASELINE VOLTAGE AND ONSET TIME WERE DETERMINED USING A GRASS STIMULATOR. *SEE TABLES 3.1, 3.3

Animal ID	Treatment order	Antler	Circumference	Date	Voltage	Time (sec)
904	5	R	10	2-Nov	7	15
906	3	L	12	14-Nov	4	15
906	2	R	12.5	16-Nov	3	15
908	5	R	12.5	17-Oct	4.2	15
908	3	L	11	7-Nov	6	15
909	3	L	10	7-Nov	5	15
909	5	R	10	14-Nov	4	15
915	6	R	10	2-Nov	4	60
915	3	L	11	16-Nov	3	15
916	2	L	12	30-Oct	3	15
918	2	L	11	30-Oct	3	15
919	3	R	10	2-Nov	3	15
921	2	R	11	26-Oct	2	15
926	5	R	12.5	17-Oct	5	15
932	6	L	11	9-Nov	3	30
933	4	L	10	2-Nov	4	15
935	6	L	14	17-Oct	5.2	15
935	2	R	11	7-Nov	6	15
937	5	R	12.5	17-Oct	5.4	30
937	3	R	11	7-Nov	5	15
939	6	L	12	26-Oct	4	15
939	2	R	12	2-Nov	3	15
947	6	L	12	26-Oct	2	45
949	6	L	12.5	16-Oct	2.5	15
950	3	L	11	7-Nov	6	15
952	5	L	12	20-Oct	2	60
957	2	L	12	2-Nov	7	15
962	6	L	12.5	17-Oct	3.4	30

TABLE A3. RAW DATA OF TIME TO ONSET FOR L FOR STUDY 1, CHAPTER 3. BASELINE VOLTAGE AND ONSET TIME WERE DETERMINED USING A GRASS STIMULATOR. *SEE TABLES 3.1, 3.3

Animal	Treatment order	Antler	Circumference	Date	Voltage	Time (sec)
904	5	R	11	14-Nov	5.5	15
904	4	L	11	16-Nov	5	15
906	2	R	12	24-Oct	2	15
908	5	R	11	30-Oct	2.5	15
909	5	L	10.5	16-Nov	4	15
914	1	R	12.5	17-Oct	5	60
916	2	L	13	17-Oct	2.2	15
916	1	R	13	24-Oct	4	15
918	2	L	11	17-Oct	3	30
918	4	R	11	7-Nov	5	15
919	4	L	10	9-Nov	5	15
921	2	R	13	16/10/00	2.5	15
921	4	L	12	2-Nov	5	15
926	5	R	10	30-Oct	2	60
926	4	L	10	7-Nov	7	15
932	1	R	10	2-Nov	3	30
933	5	L	10	14-Nov	7	15
933	4	R	10	16-Nov	3	15
935	2	R	11.5	24-Oct	6	30
937	5	R	12	30-Oct	4	195
939	2	R	12	20-Oct	7	15
947	4	R	12	2-Nov	3	15
949	1	R	12.5	20-Oct	6	15
950	1	R	14	17-Oct	4	90
952	1	R	11	26-Oct	5	30
952	5	L	11	2-Nov	4	15
957	2	L	13	20-Oct	4	15
962	1	R	12	24-Oct	2	45

TABLE A4. RAW DATA OF TIME TO ONSET FOR LIGNOCAINE W/SODIUM BICARBONATE AND FOR TIMES OF VELVET ANTLER REMOVAL FOR STUDY 2, CHAPTER 3. *SEE TABLES 3.6, 3.7

ANIMAL ID	ANTLER	CIRCUMFERENCE	DATE	VOLTAGE	ONSET TIME (SEC)	KICK TEST	ANTLER REMOVED	RESPONSE
Orange	L	19	17-Oct	7	15	44	44	N
No Tag	L	18	17-Oct	0	15	39	39	N
23	R	17	17-Oct	8	15	37	37	N
319	R	16	17-Oct	10	15	45	45	N
266	L	15	17-Oct	4	15	31	31	N
272	L	16	17-Oct	8	15	52	52	N
134	L	18	17-Oct	10	15	32	32	Y
23	L	20	24-Oct	2.5	15	43	65	Y
216	R	17	24-Oct	7	15	39	45	Y
613	L	17	24-Oct	5	15	55	55	N
138	R	19	28-Oct	5.5	15	33	33	Y
310	R	14	28-Oct	5.5	15	35	35	N
357	L	14	4-Nov	6	15	33	33	N
324	R	15	17-Oct	6	30	49	49	N
195	R	16	17-Oct	6	30	47	47	N
267	L	20	31-Oct	5	30	52	52	N
353	L	15	31-Oct	4	30	53	53	N
323	R	26	4-Nov	6	30	64	102	Y
615	R	21	28-Oct	6.5	45	64	64	N
364	L	15	4-Nov	3.5	45	74	96	Y
30	R	18	17-Oct	3.5	60	77	77	N
125	L	18	28-Oct	4	60	80	80	N
918	R	21	14-Oct	2.8	90	120	120	N
303	R	14	31-Oct	7	90	108	108	N
Orange	R	21	4-Nov	8	90	104	104	N
618	L	19	14-Oct	0	120	136	136	N

TABLE A5. RAW DATA OF TIME TO ONSET FOR LIGNOCAINE AND FOR TIMES OF VELVET ANTLER REMOVAL FOR STUDY 2, CHAPTER 3. *SEE TABLES 3.6, 3.7

ANIMAL ID	ANTLER	CIRCUMFERENCE	DATE	VOLTAGE	ONSET TIME SEC)	NICK TEST	CUT TIME	RESPONSE
G18	R	19	14-Oct	4.5	15	45	45	N
Orange	R	19	17-Oct	5	15	33	33	N
319	L	16	17-Oct	8	15	33	33	N
30	L	18	17-Oct	4	15	43	43	N
216	L	14	17-Oct	3	15	37	45	N
134	R	18	17-Oct	10	15	31	31	N
615	R	20	24-Oct	3.5	15	48	48	N
139	R	17	24-Oct	5.5	15	43	43	N
613	R	19	24-Oct	9	15	34	34	N
267	R	21	31-Oct	4	15	36	36	N
322	R	17	31-Oct	4	15	35	35	N
303	L	14	31-Oct	8	15	38	45	Y
353	R	16	31-Oct	8	15	39	39	N
364	R	15	4-Nov	4	15	42	42	N
Orange	L	20	4-Nov	5.5	15	41	41	N
323	L	16	4-Nov	7	15	42	55	Y
23	L	18	17-Oct	4	30	48	48	N
266	R	16	17-Oct	3	30	47	47	N
125	R	18	28-Oct	5	30	65	65	N
309	R	17	31-Oct	3	30	49	49	Y
357	R	14	4-Nov	5	30	53	53	Y
No Tag	R	18	17-Oct	6	45	60	60	N
310	L	15	28-Oct	5.5	60	80	80	N
94	L	15	31-Oct	3	75	97	97	N
918	L	18	17-Oct	2	120	140	159	Y
195	L	15	17-Oct	5	120	132	132	N

APPENDIX 2 - DATA FORMS FOR RESEARCH

PROJECT NAME: ONSET OF ANALGESIA STUDIES

DATE: LOCATION: RESPONSE TO STIMULUS: RANGE OF 1-10 (TIME IN SECONDS)

ANIMAL ID	TREAT-MENT	ANTLER/ CM	BASELINE 0	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	

PROJECT NAME:		LENGTH OF DURATION STUDIES																					
DATE:		LOCATION:						REMOTE STIMULATOR (TRITRONICS "BEAGLER") TIME IS MEASURED IN MINUTES															
ANTLER ID	TREAT TIME	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270	285	300	315	330

DATA SHEET: TIME SEQUENCE TRIAL

ANIMAL NO: DATE:

ANTLER 1: L / R ANTLER 2: L / R

PEDICLE _____CM. PEDICLE _____CM.

TIME IN SECONDS

BLOCK 1: START: 0 SECONDS (TIME 0)
COMPLETE: _____

BLOCK 2: START: _____
COMPLETE:_____

TOURNIQUET ON:_____

TEST CUT ANTLER 1: (1) _____Y/N
(2)_____
(3)_____
(4)_____

REMOVAL TIME ANTLER 1:_____

TEST CUT ANTER 2: (1)_____ Y/N
(2)_____
(3)_____
(4)_____

REMOVAL TIME ANTLER 2:_____

COMMENTS/OBSERVATIONS:_____
_____
_____

DATA SHEET: ADULT STAG ANTLER ANALGESIA TRIAL 2000

Sheet No: _____ Date: _____

Stag No.: _____Age:_____ Antler L / R Pedicle Circ. _____cm

Treatment No: _____ 1 = Lignocaine alone, 1ml/cm
2 = Lignocaine + bicarbonate

Control voltage: _____

Electrical test:	Volts:	Behaviour score	Categorical: Yes/No
15 sec	_____	_____	
30 sec	_____	_____	
45 sec	_____	_____	
60 sec	_____	_____	
75 sec	_____	_____	
90 sec	_____	_____	
105 sec	_____	_____	
120 sec	_____	_____	
135 sec	_____	_____	
150 sec	_____	_____	
165 sec	_____	_____	
180 sec	_____	_____	
195sec	_____	_____	
210 sec	_____	_____	
225 sec	_____	_____	
240 sec	_____	_____	

Cut Time _____sec **Response**: Yes / No

Time _____ sec "

Time _____sec "

Other Observations: _____

_____

_____