EVALUATION OF DEHORNING DISTRESS AND ITS ALLEVIATION IN CALVES.

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ABSTRACT

In this thesis, the pain-induced distress caused by the husbandry practice of dehorning cattle is assessed and methods to alleviate it are evaluated. At the time this work was conducted there were no comprehensive studies on the effects of amputation dehorning upon the welfare of the cattle. The aims of the study were to assess the distress response after dehorning and to explore the possibilities of alleviating that distress through the use of different dehorning tools, local anaesthetic and/or cautery of the scoop wound. Changes in plasma cortisol concentrations and behaviour were used as indices of distress. It was anticipated that this research would provide scientific data to aid in the writing of welfare codes and advisory material concerning the dehorning of cattle.

The cortisol and behavioural responses of six-month-old male Friesian calves after treatment were studied. In the cortisol studies, blood samples were taken by venipuncture from the jugular vein of each calf prior to, for the first 9 hours and at 36 h after treatment. Behavioural responses were scored by point scan behaviour sampling for the first 10 h after and on day two between 26 and 29 h after treatment.

Amputation dehorning elicited a marked, biphasic cortisol response that lasted six hours. Dehorning elicited similar cortisol responses irrespective of the tool employed. ACTH bolus (iv. 0.28μg/kg) elicited a maximal cortisol response. The similarity of the magnitude of the dehorning and ACTH responses suggests that dehorning was extremely distressing. The plateauing of the plasma cortisol values between 1.5 and 3 hours after dehorning suggests the appearance of a second phase of pain, presumably from inflammation. Local anaesthesia virtually abolished the first three hours of the cortisol response after dehorning, after which cortisol concentrations rose transiently. Overall, this equated to a 50% reduction in the integrated cortisol response. Cauterizing the scoop wounds effected a marginal reduction in the cortisol response. The combination of local anaesthesia plus cauterizing the scoop wound virtually abolished the cortisol response to amputation dehorning. This striking result is reminiscent of pre-emptive analgesia. The destruction of, and the prevention of sensitization of, nociceptors in the wound is thought to contribute to this effect. The four behaviours of tail shaking, head shaking, ear flicking and rumination, met the criteria required to use behaviour as evidence of distress. The interpretation of the behaviour data corresponded with that of the cortisol data.

Taken together, the cortisol and behaviour data from this study, along with the subsequent work it generated, indicate that scoop dehorning is extremely noxious. If the cattle are older and amputation dehorning is necessary, it is recommended that local anaesthetic be given and if practicable combined with either ketoprofen (McMeekan *et al.*, 1998b) or wound cautery. However, it is preferable to dehorn calves when they are younger by cautery disbudding (Petrie *et al.*, 1996b).

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1 GENERAL INTRODUCTION

1.0 HISTORY OF THIS WORK

The research described here began in 1991, all experiments were completed by 1992 and most of the thesis had been written by 1993. The candidate then became ill and recovery was protracted. The candidate returned in 2001 to update the written material and submit the thesis for examination. At the time this work was conducted there were no comprehensive studies on the effects of amputation dehorning upon the welfare of the cattle. Subsequently a significant amount was done (Petrie *et al.*, 1996b; McMeekan *et al.*, 1997, 1998a,b, 1999; Sutherland, 1999; Stafford *et al.*, 2000). The work embodied in Chapters three and four made a significant contribution to knowledge in this area and constituted the basis of two publications (Sylvester *et al.*, 1998a,b). Chapters three and four are presented here in a form reflecting the state of knowledge in 1993. By contrast chapter five, which describes work yet to be published, is written from a contemporary standpoint. Updated information, taking account of developments in the field since 1993 has been provided in the General Discussion.

1.1 INTRODUCTION

The elimination of the problems associated with horned cattle, and the alleviation of dehorning distress may confer the most benefit to cattle welfare (Gee, 1986). Dehorning of cattle is a routine husbandry practice performed to reduce injury to stock handlers and stock, to reduce economic losses through hide and carcass damage and to improve husbandry. However, dehorning *per se* is a welfare issue because it involves tissue damage and causes significant distress. At the time this work was undertaken some research had been conducted into the effects of cautery disbudding on calf welfare (Allyn, 1991; Laden *et al.*, 1985), but there were no comprehensive studies into the effects of amputation dehorning on the welfare of cattle. In this thesis, the pain-induced distress caused by amputation dehorning is examined. The aims are firstly to characterize the distress response using changes in plasma cortisol concentrations and changes in behaviour, and second to explore the possibilities of alleviating that distress through the application of different dehorning tools, local anaesthetic and/or cauterization of the dehorning wound. It is intended that this research will provide scientific data to aid in the writing of welfare codes and advisory material concerning the dehorning of cattle.

The assessment of the emotional state of animals including pain and stress is problematic. In addition, there is considerable debate concerning the concept of stress. The debate focuses on terminology, techniques of measurement, interpretation and calibration of the response, the relative merits of psychological versus physiological indices (Barnett and Hemsworth, 1990), whether animals are sentient beings, whether we can measure subjective states and the relationship between stress and welfare. The debate has been driven by advances in our knowledge of biology and changing societal values which have influenced legislation concerning the care of animals.

In this thesis, changes in plasma cortisol concentrations and behaviour have been used to assess the pain-induced distress of dehorning. The merits and limitations of using these indices are examined in light of apparently paradoxical responses and the large inter-animal variation of response.

1.2 ANIMAL WELFARE

1.2.1 Ethics

The debate concerning the care of animals has centred on whether they are worthy of our moral concern, by examining similarities or differences according to the presence or absence of a soul, reason, language or sentience. The major contributing western philosophies as described by Rollin (1981) include the Judeo-Christian ethic that "Man has dominion over the rest of nature" (Genesis 1:28) either by God, or put "non theologically when it is asserted that man stands at the apex of the evolutionary pyramid" (Rollin, 1981). This has been used as an argument to both include animals in and exclude animals from our moral concern. The absence of a soul in animals, equated with the inability to reason, has been used as a justification for excluding animals from our moral concern (Plato [427-347 BC], Aristotle [384-322 BC], Catholic doctrine). Saint Thomas Aquinas [1225-1274] and Kant [1724-1804] believed that animals were not worthy of our moral concern but, that humans had a duty towards them as their stewards, because to be cruel to them would be intrinsically reprehensible for *humans*. Descartes [1596-1650] assumed language to be the only evidence of reason, cognition and sentience. This Cartesian view has provided justification for ignoring the suffering of animals under the auspices of humans.

Jeremy Bentham [1748-1832] disagreed with this logic and proposed that moral concern be equated with the ability to feel (sentience). He argued that "The question is not, Can they reason? nor, Can they talk? but, Can they suffer?" (in Craig and Swanson, 1994). Peter Singer in his book *Animal Liberation* (1975), popularized Bentham's argument and legitimized the concept of animal rights. Tom Regan (1983) went further, proposing

that animals have *inherent* value, interests and needs and this became the main thesis for animal rights groups.

In Western society the 1960s and 1970s was a period of increased esoterica and humanitarianism presumably as a result of basic needs being met and increased leisure time, leisure being "the mother of philosophy" (Rollin, 1981). The rights of disenfranchised humans (blacks, women, foetuses etc) were petitioned. Concern about the welfare of domestic animals increased dramatically with the publication of the book *Animal Machines* (Harrison, 1964). There followed investigations of the needs of intensively farmed animals (e.g. the *Brambell Report*, 1965) and subsequently amendments to animal care legislation in Britain.

The changing tide of social values concerning the use of animals, has been paralleled by advances in our knowledge of human and animal biology, in particular observed similarities of anatomy, physiology, and presumed sentience and consciousness. The appreciation of such similarities has been expressed by the recent creation of the field of *animal welfare science*. Unfortunately there has been a dissociation of morality *versus* the evaluative concepts of animal welfare (Van Rooijen, 1991, 1992). Whilst this may be a valuable division for the purposes of the scientific assessment of animal welfare (Broom, 1986) and the continued utilitarian treatment of animals, the *raison d'etre* of animal welfare, and the final decision as to what level of compromise constitutes poor welfare, are ultimately ethical concerns.

1.2.2 Legislation

Ethics and law are logically inseparable and causally influenced (Rollin, 1981; Battye, 1995). The emergence of the self-regulation of scientific experiments by "ethical committees", whereby the utilitarian premise of the "three R's" reduction, refinement and replacement (Russell and Burch, 1959) is employed, illustrates the current ethical obligation to minimise the costs to animals. Likewise consumers have demonstrated their changing ethical position by a shift in demand for products. Demands which were previously price and then quality are now increasingly conscience driven (O'Hara, 1992).

The British Cruelty to Animals Act (1876) served as the basis for New Zealand's Animals Protection Act (1960). With regard to animal welfare New Zealand law went further and was recognized internationally as leading the world (Bayvel, 1992). The Animals Protection Act (1960) was primarily concerned with the possible abuse of animals. Subsequent amendments focused on neglect and deprivation (O'Hara, 1992). In accordance with the increasing concern for animal welfare the Animals Protection Act (1960) was repealed by the Animal Welfare Act (1999) where the primary emphasis

was directed away from cruelty and towards an explicit emphasis of a duty of care towards animals. Transitional Provisions (sections 201, 202) have continued the effect of the Animals Protection Act (1960) as it applies to dehorning for a further three years until January 2003. Thereafter Codes of Animal Welfare (section 65 part 5) for husbandry practices will take effect. These statutory instruments will allow revision as our knowledge of biology and technology improves (Animal Welfare Act 1999). Currently it is an offence to dehorn cattle over the age of 20 months unless the operation is conducted under anaesthesia of sufficient power to prevent the animal from feeling pain (section 3y, Animals Protection Act 1960).

1.2.3 Animal welfare

There are various definitions of the terms "well-being" or "welfare". The Concise Oxford Dictionary (1986) defines well-being as the "state of being happy, healthy or prosperous", and welfare is defined similarly but includes the maintenance of this condition (well-being) by the community. The American Veterinary Medical Association (Anon, 1991) describes animal welfare as the "human responsibility that encompasses all aspects of animal well-being, including proper housing, management, nutrition, disease prevention and treatment, responsible care, humane handling, and, when necessary, humane euthanasia". Other definitions of animal welfare include an animal's "state in coping with the environment" (Broom, 1986), "mental and physical well-being" (Stamp-Dawkins, 1980); and an absence of 'suffering' a term which embraces its constituents of anxiety, fear, pain and distress (Mellor and Reid, 1994). Most definitions or assessments of animal welfare involve the satisfaction of "needs" where a lack of needs met is positively correlated with poor welfare (for example see Hurnik, 1988; Curtis, 1985).

The concept of physiological and behavioural needs for farm livestock originated with the *Brambell Report* (1965), a United Kingdom technical inquiry into the welfare of animals. The *Brambell Report* was adopted into the Council of Europe's convention for the Protection of Animals (1976). In 1979, the Farm Animal Welfare Council (UK), a quasi autonomous non-governmental organization, was formed to make recommendations to the Minister concerning the welfare of intensively housed farm animals. It used the concept of needs, derived from the *Brambell Report*, to produce a list of "five freedoms" that animals were entitled to. Webster (1986) promulgated these principles. The revised wording (Anon, 1992) of the Five Freedoms reads:

"Farm animals are entitled to:

1) Freedom from hunger and thirst - by ready access to fresh water and a diet to maintain full health and vigour;

2) Freedom from discomfort - by providing an appropriate environment, including shelter and a comfortable resting area;

3) Freedom from pain, injury or disease - by prevention or rapid diagnosis and treatment;

4) Freedom to express normal behaviour - by providing sufficient space, proper facilities and company of the animal's own kind; and

5) Freedom from fear and distress - by ensuring conditions and treatment which avoid mental suffering".

Good animal welfare has been equated with the Five Freedoms being met (Mellor, 1992; Matthews, 1992). The use of the term "freedom" is interesting in that it has moral overtones concerning the obligations of humanity towards their wards in this case animals, and would elicit a strong reaction being presumably derived from the famous "Four Human Freedoms" speech of F.D. Roosevelt (1941). It is generally accepted that the identification of physiological needs (or freedoms) is relatively easy, and can be characterized by physiology (Mellor, 1992). But, the identification and assessment of behavioural needs is more difficult. Sometimes the needs are arranged hierarchically to indicate priority, a concept similar to Maslov's (1940) hierarchy of human needs (Curtis, 1985). There is a comprehensive literature detailing the various needs of animals (see Curtis, 1985; Hurnik, 1988) all of which may be encompassed by the Five Freedoms. The Five Freedoms is a satisfactory operational definition of good animal welfare.

Good animal welfare has been defined as the absence of suffering, where suffering is an unpleasant or undesired state resulting from noxious stimuli (Stamp-Dawkins, 1980; Mellor and Reid, 1994). However, good animal welfare is more than the absence of suffering. It embraces positive attributes and is better described as satisfied when the Five freedoms are met (Mellor, 1992; Matthews, 1992). Mellor and Reid (1994) transformed the Five Freedoms into "domains of potential welfare compromise" as a means of rating scientific experiments. These domains are nutritional, environmental, health, behavioural and mental (state), and correlate to freedoms one to five respectively. This simple model highlights the fact that compromise in the first four domains will be manifest in welfare terms in the fifth domain. The fifth domain represents the components of suffering (distress, pain, fear and anxiety) and relates to the intensity of the state where it retains its original character (Mellor and Reid, 1994). This model can be applied to all situations where animals are under the auspices of humans, and is implicit within the Animal Welfare Act 1999.

1.3 STRESS

There is considerable debate concerning the concept of stress (e.g. Rushen, 1986, 1991). The debate focuses on terminology, semantics, techniques of measurement, interpretation and calibration of the response, the relative merits of psychological versus physiological factors (Barnett and Hemsworth, 1990), whether animals are sentient beings, whether we can measure subjective states and the relationship between stress and welfare.

1.3.1 Background

The physiological concept of stress originated with the recognition, by Claude Bernard [1813-1878], of the constancy of the *milieu interieur* despite a wide range of environmental conditions and metabolic needs (Reite, 1985). Cannon [1871-1945] later described this as "homeostasis". Cannon (1935) examined the "special physiological agencies" employed to maintain homeostasis in the face of environmental challenge, and described the autonomic nervous system's responses to a variety of stimuli. He termed the autonomic nervous system's response the "fight or flight syndrome" and the stimuli that caused that response he termed "stress".

In the 1930s and 1940s Hans Seyle (1936, 1946) described a non-specific response of laboratory animals to a variety of diverse noxious agents (e.g. painful stimuli, toxins, extreme temperatures). He termed the response the "General adaptation syndrome". The response primarily involved activation of the hypothalamic-pituitary-adrenal axis (HPA-axis), and was similar regardless of the agent that caused it. The syndrome had three phases, an initial alarm or "emergency phase", a "resistance phase" characterized by high levels of circulating corticosteroids, and, he theorized, a final "exhaustion phase" characterized by pathology, morbidity and possible death. This non-specific response of the adrenal cortex to noxious stimuli became the operational definition of stress in physiological research. The concept of the non-specificity of the stress response was markedly divergent from the then prevalent view that disease resulted in specific etiology.

Subsequently Mason (1968) attempted to elucidate the common factors behind the diverse agents that activated the neuroendocrine system. He proposed that *psychological* factors were the key, wherein the adrenal response resulted from emotional distress caused by the experimental paradigm rather than a non-specific stress reaction. He demonstrated that if care were taken to eliminate the distress associated with the experimental procedures, then certain stimuli which had previously elicited an adrenal response (e.g. cold, heat, hunger), would not elicit an adrenal response (Mason, 1975a,b). Hence, the non-specificity lay in the *afferent* not efferent part of the stress

response. To ascribe non-specificity to the psychological component of environmental stimuli was very different from that proposed by Seyle, who ascribed the non-specificity to the *consequences* (pituitary adrenal activation) (for review see Levine, 1985). The non-specificity of the afferent part was further emphasized by Dantzer and Mormede (1983) who proposed that the major determining factor of the response is the *emotional* state of the subject. Wiepkema and Koolhaas (1993) demonstrated that control and predictability were key elements. Dantzer and Mormede (1983) noted that animals exhibit integrated reactions to external stimuli, these being primarily hormonal and behavioural responses which occur dependently, are intimately linked and mitigate each other. Thus the perception and the ensuing behaviour of subjects are critical to the nature and intensity of the hormonal response to a stressor. Sapolsky (1990) surmised for humans and animals as clever as humans, that attitude counts and that the psychological filters through which external events are perceived can alter the physiological response to stress as much as the external events themselves.

The philosophical doctrine of solipsism (the belief that the only consciousness one can know is one's own) has presumably influenced "behaviourism" such that as expressed by Watson (1918) "states of consciousness ... are not objectively verifiable, and for that reason can never be data for science" (in Stamp-Dawkins, 1980), and therefore behavioural science measures what animals do and not what they *feel*. Whether one can measure subjective states is a separate argument from whether or not animals are sentient beings. Bradshaw (1990) suggests that "we err ... in assuming that animals have feelings on a 'just in case they do' basis". Separate from evidence of similarity in brain anatomy and physiology between humans and other animals, Stamp-Dawkins in *Animal Stress* (1980) reviewed the literature examining possible cognitive states and self awareness in animals and concludes that they are indeed conscious, have subjective feelings, and that these subjective feelings can be studied and measured by reference to the stress response.

1.3.2 Terminology

The term "stress" has been defined variously throughout history (see Reite, 1985). In the 1600s, "stress" meant hardship, adversity, affliction. By 1700 it meant the resistance of an individual to a force applied externally. This meaning was adopted into physics and engineering where stress refers to the internal forces generated within an object by the action of an external force. By 1800, in medicine, stress was believed to cause ill-health and disease. More recently, in common parlance stress has come to mean the psychological response of being unable to cope with daily problems, a definition concerned with the subjective feelings accompanying the physiological changes. In

scientific circles it has been defined as the biological response, or the stimuli which causes the stress response (tautology intended) or both.

One difficulty in the study of stress is that the term "stress" has been associated with various parts of the general adaptation syndrome. This has necessitated the use of stress qualifiers according to the "levels" of magnitude and the adaptive outcome of the animal's responses to stressors (Ewbank, 1992). The term stress has been used; a) to describe the whole of the general adaptation syndrome (Seyle, 1956); b) to denote a low level of response (Ewbank, 1973), either being the adaptive processes only (Seyle, 1974) or some damage to an animal whether adaptive or not (Fraser *et al.,* 1975); c) to denote the greater responses termed overstress (Ewbank, 1973) or the damaging and unpleasant termed "distress" (Seyle, 1974); or d) both where overstress and distress denote increasing levels of response respectively (Ewbank, 1985). Furthermore, very low levels of stress have been defined as physiological stress (Ewbank, 1985), or homeostasis, or when adaptive are not referred to as stress at all (Fraser *et al.,* 1975). However, there is a consensus to define "stress" as the biological response to challenges, and to define the stimulus that elicits a stress response as the "stressor" (Ewbank, 1992).

For the purposes of clarity, in this thesis the term "stress" will be used to refer to the biological responses of an animal to challenge. This embraces the whole response-continuum from deviations just beyond activation of minor homoeostatic mechanisms through to maximal responses. The term "distress" will be used to denote the "conscious awareness of an unpleasant state" (Ewbank, 1992; Mellor *et al.,* 2000) in order to identify the contributions of the cognitive and emotional elements to that response. Suffering is an unpleasant emotional state which occurs when its constituents, anxiety, fear, pain or distress, increase in intensity. Whilst a stress response may not involve an emotional component, suffering and its constituents (distress, pain, fear and anxiety) always do. For example, surgery conducted under general anaesthesia will elicit a stress response, but the individual is not considered to be distressed, in pain or suffering as there is no conscious awareness. To be able to experience these subjective states an individual must be both sentient and conscious.

1.3.3 Pain

The assessment of pain in animals is problematic. Pain is a perception with no physical dimensions, it is a sensory experience evoked by stimuli that cause or threaten to cause injury to tissue. Pain in humans has been described as having sensory-discriminative and motivational-affective dimensions (Melzack and Casey, 1968). Solipsism states that only our own experiences are knowable. Modern theory however, states that experience need not be direct to establish that a state or substance exists (Kitchell and Johnson,

1985). Thus by comparative anatomical, physiological and behavioural analogies that indicate a similarity of pain perception in animals and humans, we can infer that pain is experienced in animals which are unable to verbally communicate with us. Both humans and animals share similar nociceptive and pain pathways, structures and neurotransmitters (Dart, 1994a; see 3.4.4), and exhibit similar nocifensive responses to acute pain and depression responses to chronic pain. The pain threshold, that is the minimal level at which pain is detected, appears to be similar across species (Dart, 1994a). However, the pain tolerance threshold, that is the maximum level of pain tolerable, varies markedly within a species and may be influenced by physiological and motivational factors. Consciousness and sentience are requisite to pain perception. Thus whilst an unconscious animal can exhibit nocifensive reflexes and neural activity there is no awareness, and hence no perception of pain. The International Society for the Study of Pain has provided a systematic description of pain (see Table 1.1).

Table 1.1 Major attributes of pain (from Mellor *et al.*, 2000 modified from Merskey, 1979).

Attribute	Description
Purpose	Pain is understood to have evolutionary survival value
Detection	Pain sensations depend on activation of a discrete set of receptors (nociceptors) by noxious stimuli
Perception	Further processing via nerve pathways enables the noxious stimuli to be perceived as pain
Character	Pain perception varies according to site, duration and intensity of stimulus and can be modified by previous experience, emotional state and perhaps innate individual differences
Definition	Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or is describable in terms of such damage
Variation	The pain-detection threshold is apparently uniform across species, whereas the pain-tolerance threshold may be more species specific and subject to modification

In addition to having a pain warning system, mammals have an endogenous pain control system (endogenous analgesic system) which is important in the modulation of pain perception and adaptation to painful stimuli. Evidence of this system is based on two sets of observations (see Rose and Adams, 1989 for review). First there is evidence of

descending pain-control circuits involving serotonergic neuropeptides, endogenous opiates, vasopressin, neurotensisn, substance P and perhaps noradrenergic involvement. The second set of observations involve the opiate system. There are three families of endogenous opioid peptides and receptors in the body which are involved in nociceptive reflexes, analgesic activity and show naloxone antagonism. A variety of painful or stressful events induce analgesia, and it is presumed that this stress-induced analgesia is an adaptive response which permits motor activity in order to evade and thus mitigate the noxious stimulus. The animal reacts as if the pain tolerance threshold were reduced. Depending on the type, intensity and duration of stimulus, two types of stress-induced analgesia are observed, one is opioid dependent and the other is non-opioid dependent. Pain is a potent stimulus which may elicit stress responses involving the sympathetic adrenomedullary system (SNS) and the hypothalamic-pituitary-adrenal system (HPA-axis).

1.3.4 The Stress Response

The maintenance of the relative constancy of the *milieu interieur* despite a wide range of external stimuli is called homeostasis. There are primarily two homeostatic systems which respond to significant threat and injury (Mellor *et al.,* 2000). They are the SNS and the HPA-axis which collectively are responsible for the stress response. The SNS provides the physiological basis for the fight/flight behaviours and the HPA-axis provides the physiological conditions which combat injury and promote healing. Activation of the SNS increases mental alertness, energy mobilization, basal metabolism, respiratory function, cardiovascular tone and changes in perfusion from the gastro-intestinal tract to the skeletal muscles. Activation of the HPA-axis affects carbohydrate, protein and fat metabolism. This provides increased circulating concentrations of energy sources for fuelling the fight/flight responses and the synthetic processes involved in inflammation and the healing of tissues. In addition, the end product of activation of the HPA-axis, cortisol, serves to control or inhibit the process of inflammation (Goldstein *et al.,* 1992; Matteri *et al.*, 2000).

The HPA-axis responds to a range of both physical and psychogenic stimuli. With the onset of stress, transmitted by the relevant neural pathways, corticotropin-releasing factor (CRF) is secreted from the hypothalamus. CRF is secreted into the hypophysial-pituitary portal circulation, and is carried to the anterior pituitary where it stimulates the secretion of adrenocorticotropic hormone (ACTH). CRF is among the most important of the stress-related secretagogues which include vasopressin, oxytocin, and the catecholamines (adrenalin and nor-adrenalin). ACTH is released into the general circulation where it is carried to the adrenal cortex. At the adrenal cortex ACTH stimulates the secretion of corticosteroid hormones, primarily glucocorticoids, into the

circulation from whence they reach and exert their effects on target tissues throughout the body. In both humans and cattle the primary glucocorticoid is cortisol (hydrocortisone). Glucocorticoids exert their effect by interacting with cytostolic receptors which, upon binding the hormone, translocate to the nucleus and regulate genomic events (Sapolsky, 1992). Glucocorticoids mobilize energy through gluconeogenesis and inhibiting energy storage, affect the cardiovascular system, and inhibit anabolic processes including growth, reproductive, immune and inflammation systems (Saplosky, 1990). Activity of the HPA-axis is modulated by the end product cortisol (Fig 1.1).

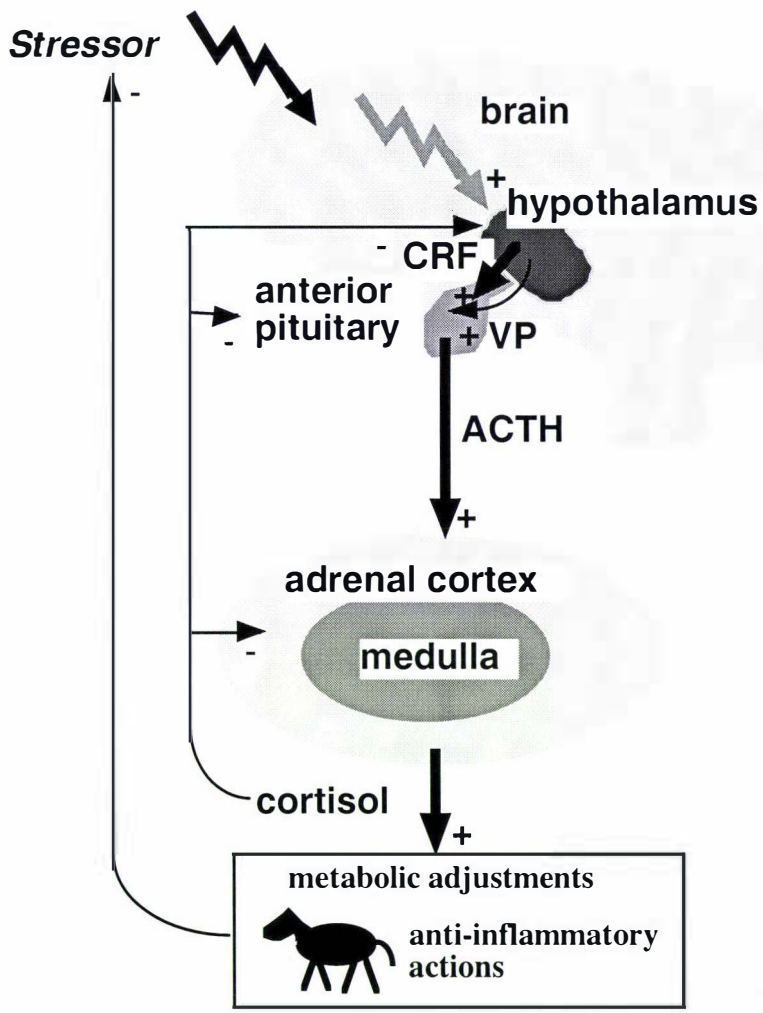


Fig 1.1 Schematic diagram of the major components of the hypothalamic-pituitary-adrenal axis. A stimulus perceived as a stressor initiates a cascade of events including release of corticotropin-releasing factor (CRH) and vasopressin (VP) from the hypothalamus. These stimulate the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn stimulates the adrenal cortex to release cortisol. Cortisol acts on a variety of target tissues to maintain homeostasis.

The activity of the HPA-axis is more complex than that described in the above review (see Sapolsky, 1992). Other pertinent features include: 1) There appears to be a dose-response relationship between the severity of the stressor and the magnitude of the adrenocortical response. Once the maximal glucocorticoid secretion is attained, further increases in ACTH concentrations do not lead to a larger secretory response but rather to a longer one (Keller-Wood *et al.,* 1983). 2) The adrenocortical axis exhibits circadian rhythmicity, with peak glucocorticoid concentrations shortly after waking and troughs around the onset of sleep (MacAdam and Eberhart, 1972; Guyton, 1992). 3) There is some debate as to whether glucocorticoids mediate the stress response (Sapolsky, 1992) or cause recovery from the stress response (Munck *et al.,* 1984).

1.4 MEASUREMENT OF STRESS

1.4.1 Cortisol and behaviour

Changes in plasma cortisol concentrations and behaviour have been used extensively to assess distress elicited by physically or emotionally noxious experiences (Mellor *et al.,* 2000). Changes in plasma cortisol concentrations and/or behaviour evoked by various husbandry procedures, including castration and/or tailing, branding, transport, unusual handling or environment and veterinary procedures, have been measured in many species including sheep, goats and cattle (e.g. Adeyemo and Heath, 1982; Alam and Dobson, 1986; Mellor and Murray, 1989a; Mellor *et al.*, 1991; Lay *et al.*, 1992a,b; Lyons *et al.*, 1993).

Whilst increased activity of the HPA-axis is the hallmark of stress (Seyle, 1936), it is recommended that a series of indices be measured to assess stress (Stamp-Dawkins, 1980). Changes in behaviour are considered a valid indicator of stress and may have advantages over measuring changes in plasma cortisol concentrations.

1.4.2 Behaviour

Changes in behaviour are often the first indication that a stress has occurred (Morton and Griffiths, 1985), and may indicate the nature of the stressor and the way in which the animal is attempting to cope with that stressor. An animal's freedom to move may be one of its most important adaptations to environmental conditions (Martin and Bateson, 1986).

Tinbergen (1963) identified four approaches for use when studying behaviour. These are: causation, ontogeny, function and phylogeny. Traditionally psychologists have focused on the causation of behaviour. This includes proximate causation questions such as "How do internal and external causal factors elicit and control the behaviour?",

and ontogenic questions such as "What internal and external factors influence the way in which the behaviour develops during the lifetime of the individual?". Ethologists have focused on the biological function of the behaviour and how it evolved, including functional questions like "What is the current survival value of the behaviour"?, and phylogenetic questions like "How did the behaviour evolve during the history of the species?". The study of behaviour from these four logically distinct approaches may help one elucidate and understand the effect of stressors and the stress responses upon the animal.

Historically there have been three approaches to identifying the behavioural expression of suffering in animals. These are (Stamp-Dawkins, 1980): 1) correlating the change in behaviour with evidence of physiological stress; 2) correlating the change in behaviour with known behavioural changes under situations which are presumed to cause stress, such as, fear, frustration and conflict; and 3) examining "abnormal" behaviours. Abnormal behaviour may be defined as a significant change from normal or usual behaviour, and maladaptive or damaging behaviours.

Ewbank (1985) suggested that in order to show a clear relationship between the behavioural change and stress four criteria must be fulfilled: 1) the stressor must be identified; 2) the physiological responses to stress must be quantified and ideally correlated with the change in behaviour; 3) the behaviour must be described and measured; and 4) damage to the physical or psychological well-being must be demonstrated.

A number of classical abnormal behaviours have been identified which include: stereotypies; anomalous reactivity including apathetic, tonic immobility or hyperactivity; self-directed abnormal behaviour (i.e. abnormal in its extent or location); inappropriately directed behaviour (towards other animals or objects); failure of function; and vacuum activities (behaviours performed in the absence of suitable stimuli) (Matthews, 1992). In addition to these behaviours, which are often associated with chronic stressors, are those particularly associated with ill-health and pain including: changes in temerament (e.g. restlessness); postural adjustments or protective responses; changes in appearance (especially body care and grooming); and avoidance of handlers or yards where an aversive situation was encountered.

There are two reasons why there may be some difficulty with the assessment of pain in cattle using behavioural indices. First, cattle are prey species and as a means of improving survival, may have evolved to not show behavioural responses to many aversive stimuli (Hinch, 1994). The apparent absence of abnormal behaviour does not prove that cattle are not stressed, but rather that they are "stoic" (KJ Stafford, personal

communication). Second, cattle are allomimetic, that is, they perform the same activity with "some degree of mutual stimulation and consequent coordination" (Fraser and Broom, 1990). This mimicry may confound the identification of the relationship between the stressor and the stress index being measured.

The use of changes in behaviour as evidence of distress requires a robust experimental design, and a well described ethogram for the species being studied. At the time this study began there had been no rigourous scientific study of the behavioural responses of calves after amputation dehorning. For this reason the behaviour study in this thesis (Chapter five) was weighted by comparison with parallel cortisol studies (Chapters three and four). Subsequent work, which built on the observations presented here, has been published (Petrie *et al.,* 1995; McMeekan *et al.,* 2000; Stafford *et al.,* 2000).

1.4.3 Interpretative problems

Stress is a part of life and is not inherently bad. Indeed Seyle (1936) stated that an absence of stress is death. The study of stress is problematic. Unlike most diseases stress has no defined etiology or prognosis. The response to stress is not as simple as Seyle (1946) proposed (Matteri *et al.,* 2000). A certain level of stress is stimulating and sought after, and yet chronic or high levels are pathological. Stress responses can be elicited by innocuous stimuli, such as eating (Mellor and Murray, 1989b) or sex (Colborn *et al.,* 1991), or by noxious stimuli either anticipated or actual. In addition, there exist some apparently paradoxical responses where interpretation of the cortisol and behavioural responses differ. A classical example being the case in broiler chickens, where behaviourally there appeared to be a flighty and calm group, but where heartrate measurements revealed that the flighty group had normal, and the calm group had elevated, physiological responses (Duncan, 1979). Another classical example is the case of sows exposed to chronic stress. Those sows that exhibited the abnormal stereotypie of crib biting had lower physiological stress responses than those sows which did not exhibit that behaviour (Fraser and Broom, 1990). Furthermore, there can be probelms with the interpretation of behavioural data. For example, the striking occurrence of agitation behaviours, has led others to overestimate its usefulness for comparing the intensity of distress (discussed in section 5.1.1). Confounding factors include the large inter-animal variation in the stress response which may be affected by previous experience, genetics, age, and physiological state; and that the act of monitoring the animals *per se* can elicit a stress response which may confound interpretation of the results (Cook *et al.,* 2000). The lack of a marked cortisol surge during surgery with local anaesthetic suggests that teleologically, the stress response evolved primarily for defence purposes (fight/flight) and secondarily as a coping mechanism when immobility and starvation occurred (Kehlet, 1988). Cortisol appears to be a phylogenetically old

tool for survival and may have little use in the modern surgical operation, in fact the suppression of the cortisol response may be beneficial (Kehlet, 1988; Dart, 1994b).

Given such paradoxical responses and problems it would appear that the measurement of stress is too daunting and condemned to the "too difficult" basket. However, Mellor *et al.,* (2000) advanced some principles and caveats to guide the quantitative and qualitative evaluation of physiological (cortisol) and behavioural responses to painful stimuli. When evaluating cortisol responses the following need to be considered: 1) what do the cortisol responses represent? 2) how can those responses be characterized quantitatively (see Chapter two)? 3) is there a correlation of the response with the presumed noxiousness of the stimuli? and 4) what are the implications of inter-animal variability? When evaluating behavioural responses the following need to be considered: 1) is the observed behaviour correlated with the noxious sensory input? 2) is it injury specific? 3) can it be used to identify different features of the distress responses? 4) is it a sensitive index of pain-induced distress? 5) is it clearly defined? and 6) can it be scaled according to different levels of pain?

1.4.4 Critique of analyses

Individual differences are relevant (Wiepkema *et al.*, 1987) and indeed central to the area of animal welfare science (Manteca and Deag, 1993), because animal welfare is concerned with the state of the individual (Broom, 1988). Manteca and Deag (1993) proposed that individual differences in temperament may be important when designing experiments and when interpreting the results from animal welfare behaviour studies. Such individual differences are *not* those well recognized to be accounted for by age, sex, genetics or previous experience. Laedwig (1991) suggested that if experimental animals are subdivided into subpopulations according to temperament then individual variation in results would be decreased. The identification of subgroups according to temperament needs to be validated against independent measures (Manteca and Deag, 1993). Whilst Manteca and Deag (1993) were addressing the idea of individual variation in behavioural responses, presumably the same variation occurs in the physiological responses and in the relative contribution of the behavioural *versus* the physiological manifestation of the stress response.

During the course of analysing the cortisol responses after dehorning, it was noted that in 28% of the cases, the cortisol concentrations resolved and remained at a new baseline value that was two standard deviations lower than the mean starting value. It was thought that these could either be a subpopulation or an artifact of data transformation. In Chapter two, the assumptions and limitations underlying transforming the cortisol data are examined. Possible explanations for these responses are advanced, and an innovative method for transforming the cortisol data is suggested. Possible differences

in the interpretation of the cortisol responses using raw or transformed data are discussed.

1.5 ALLEVIATION OF PAIN-INDUCED DISTRESS

1.5.1 Local anaesthetic

The application of local anaesthetic reduces the physiological and psychological responses to painful stimuli (Bonica, 1990b). Local anaesthetics may be considered therapeutic and prophylactic, as some of the pain-induced distress responses may counter healing, exacerbate the trauma and if severe enough promote pathology (Bonica and Buckley, 1990). When local anaesthetic is applied to the sensory nerves serving the area of trauma, it blocks all sensation from that area including nociception, rendering that peripheral section of the nociceptive pathways temporarily nonfunctional, whilst the cognitive and emotional aspects of pain perception are left functioning. It is possible that even with the efficacious application of local anaesthetic, there may yet be a distress response arising from cues other than pain. In the context of dehorning these may include: manipulation or restraint, unusual sounds or sensations, the smell of blood, changes in the weight or sensations of the head. In addition, some anaesthetics and analgesics appear to have a direct action on the function of the HPA-axis (Fox, 1994; Bonica, 1990a).

1.5.2 Cautery

Cautery has been used throughout medical history to promote hemostasis which was considered more important than the attendant retardation of wound healing. The use of cautery to decrease pain-induced distress *per se* as opposed to aiding hemostasis is a relatively novel idea. At first sight this may appear to be paradoxical because it involves the *additional* insult of a burn injury. The possibility of a therapeutic effect from cautery in surgical amputations is primarily derived from observations in this laboratory. Lester *et al.* (1991a) reported that the cortisol responses of lambs that were tail docked using a heated docking iron were lower than those when a knife was used. They proposed that the lower cortisol concentrations after cautery rather than surgical tail docking may be due to the thermal destruction of nociceptors at the site of injury. The impact of wound cautery on the cortisol response to amputation dehorning was therefore examined here (Chapter four) and has since been published (Sylvester *et al.*, 1998a) and confirmed (Sutherland, 1999).

1.6 AIMS AND FORMAT OF THE THESIS

This study was conducted in order to assess the pain-induced distress of amputation dehorning in six-month-old calves. The aims of the study were, firstly, to characterize

the pain-induced distress response, and second, to determine whether that distress could be alleviated by the application of different tools, local anaesthetic and/or cauterizing the dehorning wound. Changes in plasma cortisol concentrations and changes in behaviour were used as indices of distress. Such work has the potential to improve the welfare of cattle, contribute to our understanding of the assessment and physiology of pain-induced distress, and therefore have application to other management practices.

The work described in this thesis was preceded by a pilot study (Sylvester *et al.,* 1993) (see Appendix A) which was carried out in order to characterize the magnitude and duration of the cortisol response to dehorning through an adequate sampling frequency. This had been a problem in previous studies, resulting in the data being equivocal (Carter *et al.,* 1983; Johnston and Buckland, 1976; Boandle *et al.,* 1989).

The thesis is structured as following:

Chapter 1 *General Introduction.*
This chapter introduces some problems which arise when trying to assess the emotional state of animals, in particular, pain-induced distress, and discusses the welfare, ethical and legislative issues related to this work.

Chapter 2 *Critique of the analyses of the cortisol responses.*
Chapter two is a critique of the assessment of the cortisol responses. This is an examination of how cortisol responses are analyzed and of the importance of experimental design in animal welfare science. The assumptions and limitations of using different frames of reference for assessing cortisol data are examined using a sample data set.

Chapter 3 *The acute cortisol responses of calves following four methods of dehorning.*
Chapter three describes the effects of dehorning, using four commonly employed tools, on the plasma cortisol concentrations in order to characterize the stress response in six-month-old calves, and to assess if there is a method which causes the least distress. In addition, Chapter three establishes a frame of reference for studying cortisol responses in six-month-old cattle by identifying "baseline" and "maximal" cortisol values, the latter through conducting an ACTH challenge (Sapolsky, 1992; Verkerk *et al.,* 1993; Petrie *et al.,* 1996b). The ACTH challenge also tests the functioning of the adrenal gland component of the HPA-axis (Mellor *et al.,* 2000; Mellor and Murray, 1989b). This work has now been published (Sylvester *et al.,* 1998b; Appendix A).

Chapter 4 *Cortisol responses of calves to scoop dehorning with and without local anaesthetic and/or subsequent cautery of the wound.*

Chapter four describes the effect of prior application of local anaesthetic and/or subsequent cauterizing of the dehorning wound on plasma cortisol responses to dehorning by scoop amputation. This work has now been published (Sylvester *et al.*, 1998a; Appendix A).

Chapter 5 *The acute behavioural responses of calves to scoop dehorning with and without local anaesthetic.*

Chapter five describes the behavioural responses of calves to dehorning with and without local anaesthetic. The study was conducted in parallel, but not simultaneously with, the cortisol studies of Chapters three and four. By comparison with the cortisol studies, this study supports the credibility of using changes in behaviour as an index of distress, and thus subsequently supports the use of behaviour alone.

Chapter 6 *General Discussion.*

Chapter six is a synopsis of the original findings from this work integrated with developments in the field since 1993 (Petrie *et al.*, 1996b; McMeekan *et al.*, 1997, 1998a,b, 1999; Sutherland, 1999; Stafford *et al.*, 2000; Sutherland *et al.*, 2002a,b).

2 CRITIQUE OF THE ANALYSES OF THE CORTISOL RESPONSES

2.0 ABSTRACT

Activation of the HPA-axis, as measured by an increase in circulating cortisol, is used as evidence that stress has occurred. This response may be analysed as changes in the timecourse of plasma cortisol concentrations and/or the integrated cortisol response. The raw data may be used, or the data may be transformed to examine changes from a pretreatment value (adjusted$_{pre.tmt}$). The usual approach is to use adjusted$_{pre.tmt}$ values for these analyses. The validity of analysing data transformed in this manner was questioned when it was found that 28% of the cortisol responses resolved to values about two standard deviations below their pretreatment value, as this would underestimate the cortisol response. In order to account for these responses the data was adjusted relative to a value at a time when the response to the treatment was over. The mean value during the period 7-9 h post-treatment was used in place of the pretreatment value, and the response recalculated relative to this value (termed adjusted$_{post.tmt}$). The purposes of this chapter were a) to examine the assumptions and limitations inherent in using raw, adjusted$_{pre.tmt}$ or adjusted$_{post.tmt}$ cortisol data; and b) to determine whether resulting differences are statistically or biologically significant in the context of real data sets. The use of the post-treatment value, as opposed to the pretreatment value, as the reference point from which to compare responses, decreased the data variance but resulted in no change in the statistical differences between treatments when comparing the time integral of the response and few differences when comparing the timecourse of the response. Irrespective of which baseline adjustment was made or whether unadjusted data was used, there were no differences in the biological interpretation of the data. These results suggest that our experimental design was sufficiently robust to account for systematic or random variation, and this confirms that our methodology was sound. Whilst there appeared to be no advantage in using this technique, it is possible that under some circumstances, the post-treatment sample may more accurately represent baseline values than the pretreatment sample.

2.1 INTRODUCTION

Animals have a range of biological mechanisms to cope with challenges which threaten their survival. Activation of the HPA-axis, as measured by an increase in circulating concentrations of corticosteroids is often used as evidence that stress has occurred (Seyle, 1936; Moberg, 1985). In cattle the major glucocorticoid secreted in response to

a stressor is cortisol. The response may be analysed by examining changes in the timecourse and the integral of the cortisol response. The raw data may be used, or the data may be transformed to examine changes from a pretreatment value ($adjusted_{pre.tmt}$). The usual approach is to use $adjusted_{pre.tmt}$ values for these analyses. This is done primarily to account for any pretreatment effects and possible individual variation in baseline cortisol values. However, this transformation causes weighting of the data around the pretreatment reference point. Therefore one must be confident that the pretreatment state is representative of an unstressed state. If it is not, then the adjusted analyses may underestimate the cortisol response to the stressor.

The validity of analysing data transformed in this manner was questioned when it was found that 28% of the cortisol responses resolved to values about two standard deviations below their pretreatment value (Chapter four). Possible explanations for these responses are advanced, and an innovative method for transforming the cortisol data to account for these responses is suggested. The assumptions and limitations underlying transforming the cortisol data are examined. Possible differences in the interpretation of the cortisol responses, using either raw data or data transformed by either reference value, are discussed.

A goal of animal welfare science is to assess the effects of stressors, which can be done by examining changes in plasma cortisol concentrations. When examining the cortisol response to a stressor we are interested in answering certain questions including: What is the duration and extent of the response?; and are there significant differences between-treatments? These biological questions are answered by analysing information derived from the cortisol timecourse, and the differences are assessed statistically. An adrenal response may be elicited by the emotional distress associated with the experimental paradigm (Mason, 1968). This may complicate interpretation of the results. Because an animal's stress response is exquisitely sensitive to the vagaries of experimental design, animal welfare science requires a robust experimental design to either account for or mitigate these effects. In addition, a series of blood samples of sufficient frequency are required to characterize (pattern, magnitude and duration) the cortisol response. In the present chapter, before discussing the analysis of cortisol responses along with the underlying assumptions, consideration will be given to some aspects of scientific method pertinent to animal welfare science, namely: experimental design, data transformations and statistical theory.

2.2 EXPERIMENTAL OVERVIEW

2.2.1 Scientific method

Experimental design

The purpose of good experimental design is to eliminate confounding effects. Ideally four precautions should be taken to ensure sound design; a) control groups, b) placebos or sham procedures c) double or single blind procedures and d) random assignment. A control group is a group of subjects which are left untreated and a treatment group is a group of subjects which are given the treatment being studied. The use of control groups ensures that there is something with which to compare a treatment group so that one can determine whether or not the response is caused by the treatment or the experimental situation *per se*. In addition, pretreatment samples are taken. This act as another form of control comparison, in this case for the individual response. Placebos are inert substances which are administered in the same way as a treatment medication. Sham procedures closely resemble treatment procedures but are not intended to have the effect of the treatment. A study is double blind if neither the researcher nor the subjects know which subjects are in the control and which are in the treatment groups. Randomization is where the assignment of subjects to treatment or control groups is done by some chance-governed mechanism. If confounding effects are to be decreased, the control and treatment groups must be similar with respect to any characteristic that could affect the results (e.g. age, sex, health or size).

Data transformations

Prior to analysis, the data may be transformed mathematically including: geometric (multiplicative, logarithmic) or arithmetic (additive) manipulations. Data transformations are often used to correct for violations of normality and equal-variance assumptions, and to deal with non-linear relationships, so that correlation or regression analyses can be applied to the data. Skewed data can be normalized by taking square roots, logarithms, or reciprocals (1/x) or by squaring the data (Shott, 1990; Fowler and Cohen, 1990). It is a mathematical fact that a transformation which normalizes the data also removes the dependency of the variance upon the mean, and so ensures that a particular statistical method can be validly applied (Fowler and Cohen, 1990).

Transformations conducted to correct for baseline (or pretreatment) effects are essentially arithmetic. This is done when the focus is on isolating the response to a stimulus, that is, examining changes in concentration from a pretreatment situation. The values are adjusted by subtracting the pretreatment concentration from every cortisol concentration which renders the pretreatment sample a zero relative concentration and thus equates all individual baselines. However, the effect upon the interpretation of the

cortisol response using data transformed in this manner has apparently not been qualified.

Statistical theory

When measuring any biological parameter there will be a range of values obtained as a result of biological variation. While variables such as age, sex and time of day can be controlled through good experimental design, there will be others that can not. If the measurement has been made using a random sample representative of the normal population, then an estimate of the normal range for a given physiological measurement can be calculated using standard statistical techniques. Most biological characteristics conform to a normal distribution which expresses a certain relationship between the mean value and the variance. A measurement of the variation (or error) is the standard deviation (SD), which is a measure of the average of deviations from the mean. In a normal distribution the range covered by plus or minus (±) 1SD includes 68%, ± 2SD includes 95%, and ± 3SD includes 99.73% of the observations. The standard deviation is a measure of variation of an individual measurement within the sample. A measure of the variation in the mean is the standard error of the mean (SEM, where $SEM=SD/\sqrt{n}$). This is an estimate of the SD that *would* be obtained from the mean of a given number of samples. It is possible to predict from the mean and SEM of the sample, the probability that any particular value in the general population is normal. The probability (P) of a sample being greater or less than 2SD from the mean is 5% or less, that is, the probability that the difference is due to chance is less than 5% and is therefore considered to be due to the treatment. This criterion is often used to determine if a difference is significant and is usually denoted $P<0.05$. When comparing the difference between the means of two groups of samples, the question often being asked is are they from the same population? That is, do they lie within a range represented by the SEM of each other, or not? As for a single distribution above, if the difference between the means is greater than two multiples of their SEM then the difference is considered significant. Various statistical tests are used to calculate the significance of the difference between means. In this thesis analysis of variance (ANOVA), Mann-Whitney, correlation and regression have been used.

2.2.2 Cortisol data analyses

There are various analyses and statistical tests which may be employed to assess the cortisol responses to stressors. The data are commonly analysed by examining changes in plasma cortisol concentrations with time (timecourse) and related characteristics such as maximum increment, time to maximum increment, increment at particular times after treatment, duration or perimeter of the timecourse and the integral of the timecourse (integrated response) either for the whole response or of different phases.

Timecourse analyses

The duration of the response is defined biologically and determined statistically. By definition a stressor causes increased levels of corticosteroids (Seyle, 1935), which at a later time return to pretreatment levels. The response is deemed "over" when the mean values return and stay at levels which are not significantly different from the mean pretreatment value. To assess if there are any differences between-treatments during the timecourse, the difference between the means is examined at the 5% probability level. Examination of the characteristics of the cortisol timecourse, such as duration, time to and magnitude of peak concentrations and the shape of the response, can indicate underlying physiological processes which affect that response (see 3.4.4).

Integrated response analyses

Numerical integration is used to calculate the area representative of the response (integrated cortisol response). This is the area contained under the response curve and above a pre-determined value on the y-axis. Calculation of the integrated cortisol response using adjusted data includes the area encompassed above the line y = pretreatment value (i.e. area A in Fig 2.1). By contrast, calculation of the integrated cortisol response using raw data includes the area encompassed above the line y=0 (i.e. area A + X in Fig 2.1). The integrated response may be calculated for the whole response or parts thereof.

The integrated response incorporates the features of both magnitude and duration, and renders a single value. This may be considered as a strength or limitation. The strengths include that in one number the features of both intensity and duration may be accounted for. The limitations include that the same number may represent a cortisol response that is either brief but of high magnitude, or longer lasting and of low magnitude, but it is not possible to distinguish between the responses from the one number.

2.2.3 Assumptions and limitations of baseline transformations

Raw data

Analyses may be conducted on the raw data. This is performed when conditions dictate, for example, where the pretreatment value is not representative of an unstressed state, or where the actual cortisol concentrations may be more meaningful than expressing the response as change from a pretreatment state. The use of raw data ignores differences in baseline and in fact assumes that they are similar.

The choice of a pretreatment baseline has obvious significance when considering the integrated cortisol responses (as discussed in 2.2.2). To include that area below pretreatment concentrations (i.e. area X in Fig 2.1) will mute the difference between treatments especially if that area is large compared to the area above the pretreatment

value (i.e. the response). This point is demonstrated in the theoretical situation shown in Fig 2.1. The cortisol response curve A has an area of 5 units, the cortisol response curve B has an area of 1 unit. The area below (x) both pretreatment values has an area of 1 unit. The ratio of response of B to A using raw data is 6:2, whereas, the ratio using the adjusted data is 5:1. These are quite different ratios and this demonstrates how the area below the pretreatment level may modify or mask the differences between-treatments, and may even alter the statistical significances between-treatments. Therefore when comparing integrated responses it is recommended that the adjusted data be used.

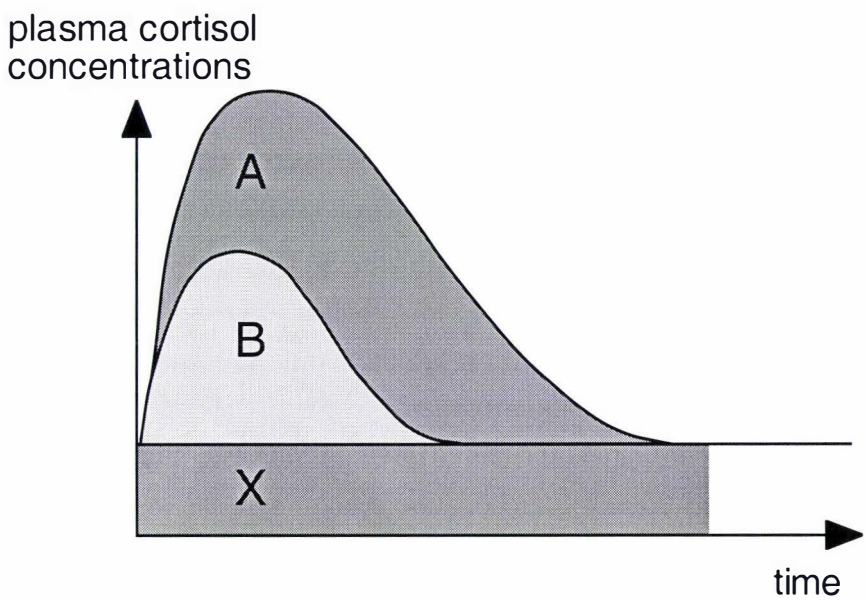


Fig 2.1 Schematic diagram showing the calculation of the integrated cortisol responses using raw and adjusted$_{pre.tmt}$ data. Using adjusted$_{pre.tmt}$ data, the integral is calculated as the area under the curve and above the pretreatment value, i.e. area A. Using the raw data, the integral is calculated as the area under the curve and down to y=0 concentration, i.e. area A + X. Where the integral of the response is small (eg. area B), then area X dominates.

Adjusted data

The most commonly employed approach for analysing the cortisol response after a stressor is to use data that have been transformed to represent changes from a pretreatment state. The pretreatment value is subtracted from all values which renders the pretreatment zero relative concentration. The pretreatment cortisol concentration is presumed to be representative of an unstressed state. This is because it is taken as quickly as possible before any increase in cortisol associated with the handling required to take that sample occurs. This pretreatment concentration, called such because it is

taken prior to any stressor effect, is assumed to be indicative of baseline cortisol concentrations, that is the basal values required for normal function which are at dynamic equilibrium, and presumably will not change with treatment. The response to the treatment is defined as the change from the pretreatment (or baseline) concentration. The pretreatment value may differ from animal to animal. Referencing all responses to the corresponding individual's pretreatment value, and denoting this as zero units, effectively equates all previous or existing stressors and stresses. In this manner the pretreatment reference point acts as a 'before experiment control' which assumes that all animals are at the same state. However, this may not be the case. It may be that for some animals the pretreatment value reflects a pre-experiment state, in particular the recent experiences of the animals. For this reason the term 'pretreatment' level is preferred to the term 'baseline' level although the latter is implied mathematically.

In summary, when analysing cortisol responses, data which have been adjusted relative to the pretreatment value are generally used because they describe changes from a pretreatment state and this accounts for individual variation in baseline values and any pretreatment effects. A major limitation underlying adjusting the data to examine changes from a pretreatment value is that the data are weighted around the pretreatment reference point, and this has most meaning when this reference is representative of an unstressed state. If however, the pretreatment value is not representative of an unstressed state, then the subsequent response may be underestimated or distorted.

2.2.4 The problem - 28% of the responses were unusual

During the course of analysing the cortisol responses elicited by the dehorning experiment (Chapter four), it was found that in 28% of the animals the cortisol concentrations resolved and remained at a new baseline value (z') (7-9 h) that was about two standard deviations lower than the mean starting value (z) (Fig 2.2). This pattern of cortisol values plateauing to levels substantially below pretreatment concentrations has not been commonly reported, nor is it our experience from similar studies (Petrie, 1994; Sylvester *et al.*, 1993). It was thought that these animals could be either outliers or a subpopulation arising either because of unique responses or their experiencing a pretreatment stress. That 28% had this feature however, merited further examination.

The calves that exhibited these responses were evenly distributed between treatments, days, time of day and order of blood sampling. For these responses, the pretreatment cortisol concentrations were *nearly* statistical outliers, that is, they were all about two standard deviations greater than the mean (m) for the unadjusted pretreatment sample (n=52). In a normal distribution only half of 5% of the observations are expected to be greater than two standard deviations (SD) from the mean, so that this disparity (in

distribution of 2.5% versus 28%) suggests that these samples belonged to another population.

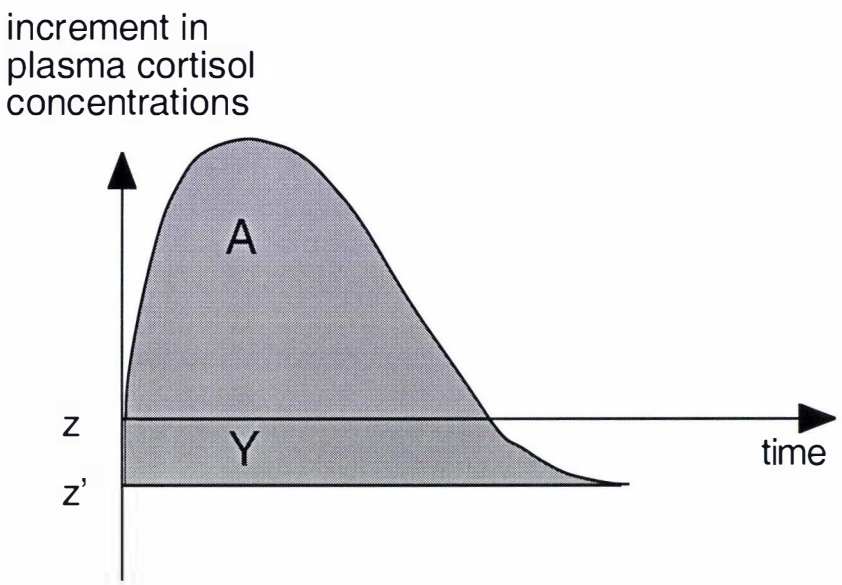


Fig 2.2 Schematic diagram of the cortisol timecourse of the subpopulation. Where the pretreatment cortisol concentration is z but the values resolve to z', thereby underestimating the integral of the response by area Y.

In addition to underestimating the integrated responses for these animals (Fig 2.2), the coefficients of variation (CV, where CV=SD/m) of the mean timecourse and the integrated responses were greater using adjusted data than those for the respective unadjusted data. This suggests that the pretreatment value was not the best choice for the reference point for analyses, and further, that the data transformation *per se* had introduced artifactual variance. This raised the question of whether the data represented a statistical abnormality or biological normality i.e. were these animals a genuine subpopulation rather than outliers *per se*.

It is possible that this subgroup was either:

a) a group with pretreatment cortisol concentrations that reflected a genuine basal state, but where the subsequent stress somehow *adjusted* their cortisol baseline to a new lower levels; or

b) a group with pretreatment cortisol concentrations that reflected a genuine basal state, but where the subsequent stress caused a *temporary refractory* period for cortisol secretion, which will later return to the pretreatment level; or

c) a group where both the pre-treatment and the 7-9 h cortisol concentrations reflected a basal state but where the difference between the two reflected a *circadian rhythm* to cortisol secretion; or

d) a group which is more sensitive to the *'pretreatment experience'* whereas by 7 to 9 hours their cortisol concentrations had returned to values more representative of their true baseline levels.

These possibilities will be considered in turn:

a) Changing baseline

It is possible that for this group of animals the pretreatment cortisol concentrations represented their genuine baseline, but that somehow the subsequent stressor adjusted their cortisol baseline to a lower level. This implies that exposure to noxious and/or novel stressors changes cortisol baselines. However, there is no evidence for stressors changing baseline cortisol concentrations once the stressor is absent. Most studies report the response to stressors, and not data on baselines *per se.* There is large variation between individuals in that response. There is evidence that the response to stressors may be affected by many factors, including previous experience. In the case of repeated stressors the response is usually the same regardless of whether it is the "first or the umpteenth stressor in a series" (Sapolsky, 1992). However in some instances facilitation or inhibition of the adrenocortical response occurs, the balance of which reflects the timing and type of the repeated stressors (for review see Sapolsky, 1992).

There were only two calves (3%) where the cortisol levels at 7-9 h were significantly greater than their respective pretreatment value. Both these calves had aberrant responses and both were excluded from the study as outliers (see Fig B.1). One of the calves subsequently died. The continued high cortisol concentrations for these two calves suggests that they were experiencing a stressor throughout the period of observation. This effect could have resulted from a combination of the treatment and a pre-existing stressor which did not manifest as a change in cortisol concentrations at the pretreatment period, but with treatment constituted a major stressor on the body.

b) Refractory period to cortisol secretion

It is possible that for those 28% of calves, their pretreatment value is their true baseline, and the decrease in concentrations after six hours reflects a temporary refractory period

to cortisol secretion. However, there appears to be no evidence of this and the activity of the HPA-axis is under rapid negative feedback regulation. There may however, be differences in the magnitude of the stress response over the circadian cycle (see Sapolsky, 1992).

c) Circadian rhythm

It is possible that for those 28% of calves, both the pretreatment and the post-treatment cortisol values were at baseline levels, and that the difference between the two reflected a circadian rhythm. There appears to be a circadian rhythmicity to glucocorticoid release, with glucocorticoid concentrations peaking after waking and being at a nadir around the onset of sleep (in Sapolsky, 1992). In cattle, cortisol concentrations are usually highest in the early morning (MacDiarmid and Cooper, 1982) and have been reported to vary by as much as 30ng/ml in the day (Fulkerson *et al.,* 1980). However, one should be cautious about comparing magnitudes between studies as differences in the absolute values may be due to differences in the cortisol assay or the experimental design. There was no apparent circadian rhythm in the cortisol concentrations of the Control calves, but this may be due to limitations of the experimental design or data variation which might obscure subtle oscillations in cortisol concentrations. The circadian rhythm may be better detected with remote sampling and a different experimental design.

d) Sensitive to pretreatment stress

It is possible that this group of animals was more sensitive to the 'pretreatment experience' and that by 7 to 9 hours their cortisol concentrations had returned to values more representative of their true baseline levels. At the time of pretreatment sampling, animals had been recently subjected to novel experiences. These novel stressors included being mustered and brought into the yards, restrained by strangers as numbers were painted onto their backs, held overnight in cattle yards and fasted. In addition, the random allocation of calves into mobs of 12 will have resulted in novel social groupings and the disruption of the normal peer associations and hierarchies, to which cattle are particularly sensitive (Hinch, 1994). It is possible that these pretreatment experiences may have affected some calves so severely that they were still stressed at the time of the pretreatment sample.

Cortisol concentrations appeared to plateau at low levels 7-9 h after treatment. By the time these last samples (7-9h) were taken there had been a uniform exposure to defined procedures over the 9 hours. Therefore there may be less variation within treatments at 7-9 hours than at the pretreatment time. This suggests that values at 7-9h may better represent baseline than those at the pretreatment time. The variation at 7-9h may largely reflect random variation in baselines whereas the variation at the pretreatment time may

largely reflect a systematic error of variation in response to recent stressors. This suggests that the mean post-treatment value between 7 and 9h could be used as the reference point instead of the pretreatment value.

In the majority of cases the cortisol concentrations before and after a stressor is over are similar. In this study, there was a greater number of post-treatment samples than pretreatment samples which gives greater confidence in the post-treatment sample than in the pre-treatment sample. It seems likely that for these 28% of calves the pretreatment value indicated a stressed state and that the post-treatment value is a better reflection of the baseline. There appears to be no evidence that cortisol baselines are either temporarily or permanently affected by stress. Therefore, it will be assumed that the cortisol concentrations have returned to basal levels.

It would be preferable to clarify if the post-treatment value was the same as the pretreatment value. This could be ascertained by taking a series of samples before and after the experiment, for example for a 24h period before and a 24h period after. In addition, this would also determine the presence and magnitude of any diurnal rhythm. However, in the current study, this was not possible for practical reasons.

2.2.5 Can post-treatment values be used as reference values?

The merit of the pretreatment, versus the post-treatment, reference point has been discussed. In the current study it seems likely that the post-treatment value is a more true representation of the baseline. Can the post-treatment value be used as a reference point? This begs the question: Is it valid to use a post-treatment sample as the pretreatment sample? The pretreatment value is understood to be representative of the state of the animal before treatment, whereas the post-treatment animal will always be post treatment, and is presumably modified in some way by the experimental experience. Therefore, because we can never be sure of what effect that experience will be, it is not possible to exclude or account for such changes. Thus to use post-treatment samples to approximate more accurately to an unstressed pretreatment value appears incorrect. However, when we reference data to the pretreatment sample, and evaluate the changes from that pretreatment state, we presume, and it is likely, that there will be a time at which the cortisol concentrations will or do return to the pretreatment value (Mellor *et al.*, 2000). This implies that the post-treatment sample and the pretreatment sample could have the same value.

The concern is that by using the pretreatment sample as that which allows for individual variation, it may not be as independent of stress as assumed, and is therefore introducing variability or limits to the analysis. By using the mean of the last three samples (7-9h), that is, after all animals have been exposed to nine hours of handling,

and cortisol concentrations are back in the region of baseline, it can argued that this cortisol value is probably closer to baseline and is thus more representative of the unstressed cortisol concentration. Neither position is certain, but, it seems likely that a post-treatment value would be more representative of the unstressed state.

There were no differences between the cortisol concentrations at pretreatment, 7-9h or at 36 h after treatment (Chapter three and four), or, in the pilot study, between cortisol concentrations at those times and at 24 h after dehorning (Sylvester *et al.,* 1993). These findings suggest that the cortisol values had returned and presumably remained at baselines by 7 h after treatment. However, cortisol samples were not taken between 9 and 36 hours. Subsequent work (Sutherland *et al.,* 2002a,b) confirmed that the cortisol concentrations remained at pretreatment levels throughout this period. This suggests that 7-9 h post-treatment sample represents baseline and is therefore a valid reference point. This approach will be used because at least that will not underestimate the duration or the magnitude of the response. This is a more conservative approach to assessing the stress, and thus errs on behalf of the animals.

In summary, when comparing the cortisol timecourse between treatments the raw data should probably be used as there should be no significant difference between the pretreatment and post-treatment values, and there may be no benefit gained from referencing the data to the pre-treatment state. Indeed more information can often be gathered from examining raw data. However, when comparing the integrated responses, data referenced to the pretreatment should be used, otherwise the area underneath the pretreatment will mute the differences between treatments. The problem arose in this case, when a large number (28%) of responses had pretreatment values which were two SD higher than their post-treatment values resulting in the integrated responses being underestimated and the timecourse being misrepresented. The question then is, what value should be subtracted as the baseline value?

There are three options. 1) This 28% of the data could be omitted. However, this is incautious as is reduces the statistical strength of the experimental design and could introduce a systemic error. 2) The standard procedure could be followed. That is, include the 28% of the data and use the pretreatment value as the reference point for comparisons. This is what has been done in Chapters three and four. 3) Include the 28% of the data and use the mean post-treatment value between 7 and 9h as the reference point from which to compare responses. As discussed above, there seems to be a better basis for this latter procedure.

2.2.6 Aims

The purposes of this chapter are to a) to examine the assumptions and limitations inherent in using raw, adjusted$_{pre.tmt}$ or adjusted$_{post.tmt}$ cortisol data; and b) to determine whether resulting differences are statistically and biologically significant in the context of real data sets. The aim here is to use some sample data to examine these issues, *not* to discuss the response to the treatment *per se*. Such a discussion is provided in Chapter four.

2.3 MATERIALS AND METHODS

The sample data (Table 2.1), to which these transformations and analyses were applied were taken from the experiment described in Chapter four. The data were from calves in response to Scoop+Cautery (n=10), Scoop (n=10), LA-Scoop (n=8) and LA-Control (n=8) treatment. However, as the aim is to examine whether transformation of the data changes the interpretation of the results, and not to discuss the response to the treatment *per se*, the data are labelled simply as data series "5", "3", "4" and "2" respectively. The experimental design is not described here, save that blood samples were taken at 0.66 (pretreatment) and 0.25 h before treatment, and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 36 h after treatment. The comparison between data series 5 and 3, and data series 4 and 2 will be examined here. To ascertain if there were any differences between these data series, statistical analyses were conducted on the timecourse and the integral of the cortisol responses after treatment. In addition to ascertain if there are any differences according to whether the data is transformed, these analyses were conducted using either 1) raw, 2) $\text{adjusted}_{\text{pre.tmt}}$ or 3) $\text{adjusted}_{\text{post.tmt}}$ data.

2.3.1 Data transformations

The data used in the analyses were transformed in the following three ways:

1) Raw data: No transformation.

2) $\text{Adjusted}_{\text{pre.tmt}}$: The pretreatment value was subtracted from all values, rendering the pretreatment as zero relative concentration.

3) $\text{Adjusted}_{\text{post.tmt}}$: The mean value of the three sample values taken at 7, 8 and 9 hours after treatment was used as if it were the pretreatment value, and this value was subtracted from all other values, making both the pretreatment and the mean of the post-treatment values zero relative concentration.

2.3.2 Statistical analyses

Statistical analyses were conducted on the timecourse and the integral of the cortisol responses after treatment using either 1) raw, 2) $\text{adjusted}_{\text{pre.tmt}}$ and 3) $\text{adjusted}_{\text{post.tmt}}$ data. Cortisol data are expressed as the mean ± the standard error of the mean (SEM). The significance of the difference between the means was determined using a two-way analysis of variance (ANOVA) with Fisher's probability of least significant differences (PLSD). All statistical calculations were conducted using a standard statistical computer package (Statsview + Graphics, Version 2, Abacus Concepts Inc., USA).

Timecourse of the cortisol response

To calculate when the cortisol response to a treatment was complete, that is when the cortisol concentrations returned to and remained at pretreatment levels, a factorial ANOVA design was used. To calculate if there were any differences in the mean timecourse of the cortisol response between treatments a repeated measures ANOVA design was used.

Integrated cortisol response

Numerical integration was used to calculate the total cortisol response, hereafter called the integrated cortisol response. The integrated cortisol response was defined as the area under the cortisol curve and above a line drawn horizontally through either, y=0, y=pretreatment value or y=post-treatment value. This produced the integral of the raw, $adjusted_{pre.tmt}$ and $adjusted_{post.tmt}$ data sets respectively. A factorial ANOVA was used to determine the difference between the mean integrated cortisol responses of different treatments.

2.3.3 Sample data

Table 2.1 Plasma cortisol concentrations (ng/ml) of calves in response to Scoop+Cautery (n=10), Scoop (n=10), LA-Scoop (n=7) and LA-Control (n=8) treatment (see Chapter four). For the purposes of this study the treatment groups were labelled simply data series 5, 3, 4 and 2 respectively.

Time (h)	Animal 1	2	3	4	5	6	7	8	9	10	mean	SEM
	Data Series #5											
-0.5	7.5	10.5	3.0	18.0	14.0	13.0	4.5	21.0	13.5	18.5	12.4	2.0
-0.3	15.0	14.5	12.5	29.5	18.5	15.5	28.5	21.0	34.0	25.5	21.5	2.5
0.3	17.0	28.0	18.5	34.0	25.0	33.0	39.5	27.5	47.0	47.0	31.7	3.5
0.5	16.5	39.0	21.5	34.5	34.0	39.0	36.5	30.0	42.5	44.5	33.8	3.0
1.0	25.0	22.5	19.5	31.5	25.5	34.0	44.0	27.0	45.5	33.5	30.8	2.9
1.5	16.5	24.0	16.0	22.5	35.5	32.0	45.5	20.5	35.0	26.5	27.4	3.2
2.0	15.5	14.0	15.5	14.0	29.5	30.0	49.0	14.5	25.0	29.0	23.6	3.8
2.5	9.0	20.5	22.5	17.5	28.5	20.0	39.5	12.5	28.0	34.5	23.3	3.2
3.0	7.0	13.5	18.0	19.5	24.0	18.0	48.0	18.0	34.0	33.0	23.3	4.0
4.0	7.0	23.0	11.5	14.0	11.0	7.5	40.5	9.5	27.5	35.0	18.7	4.0
5.0	7.0	15.0	14.0	6.5	13.0	20.0	26.5	8.0	20.0	32.0	16.2	2.8
6.0	3.0	10.0	4.5	13.0	10.0	20.5	20.0	4.5	21.5	22.0	12.9	2.5
7.0	4.0	18.5	2.5	6.0	7.0	12.5	7.5	2.5	21.5	12.0	9.4	2.2
8.0	4.5	12.5	12.5	12.0	5.5	24.5	6.0	8.5	13.5	11.0	11.1	1.9
9.0	3.5	15.5	10.0	9.5	5.0	11.5	3.5	7.5	7.5	12.0	8.6	1.3
36.0	6.5	2.5	10.5	5.5	3.0	10.5	12.5	6.0	13.0	25.5	9.6	2.2
	Data Series #3											
-0.5	4.0	8.0	17.0	7.5	5.0	12.5	3.5	5.0	10.5	7.0	8.0	1.4
-0.3	19.5	9.0	42.0	12.0	12.0	17.5	19.5	34.5	17.5	22.5	20.6	3.4
0.3	31.5	19.5	34.5	21.5	31.0	21.5	34.0	69.0	31.0	39.0	33.3	4.7
0.5	44.0	24.5	35.5	30.0	37.5	28.5	44.5	63.5	35.0	38.0	38.1	3.7
1.0	27.5	28.5	34.5	21.5	34.5	26.5	24.5	58.5	22.5	32.5	31.1	3.6
1.5	22.5	19.0	25.0	15.5	33.5	20.0	24.3	36.5	15.0	21.0	23.2	2.3
2.0	21.5	25.5	28.5	10.5	33.5	18.5	29.0	36.5	22.5	23.5	25.0	2.5
2.5	31.5	20.0	23.5	14.5	34.5	18.0	33.5	30.0	25.5	33.5	26.5	2.4
3.0	36.5	22.5	19.5	19.0	30.5	22.5	32.0	46.5	24.5	34.0	28.8	2.9
4.0	13.5	9.0	21.0	12.0	30.0	18.5	30.5	14.0	23.0	24.5	19.6	2.5
5.0	10.0	5.0	19.0	19.5	17.0	17.0	14.5	13.0	21.0	19.5	15.6	1.7
6.0	7.5	4.0	23.5	9.5	17.5	7.5	18.5	14.5	17.0	9.5	12.9	2.1
7.0	5.5	2.5	18.5	5.0	10.0	5.5	7.5	3.0	11.5	6.0	7.5	1.6
8.0	6.5	3.5	12.0	4.0	7.5	4.5	4.5	5.0	13.0	3.5	6.4	1.2
9.0	4.5	2.0	14.3	4.0	6.0	4.0	15.0	5.0	11.5	3.5	7.0	1.6
36.0	15.5	7.0	16.5	6.0	2.5	7.0	25.0	5.0	11.0	10.0	10.6	2.3

Table 2.1 cont.,

Time (h)	Animal 1	2	3	4	5	6	7	8	9	10	mean	SEM
	Data Series #4											
-0.5	8.0	5.5	9.0	*	10.5	16.0	8.5	*	15.5	n/a	10.4	1.6
-0.3	11.0	31.5	15.5		16.0	34.0	18.0		15.5		20.2	3.6
0.3	15.0	27.5	21.0		11.0	51.5	26.5		23.5		25.1	5.3
0.5	12.0	26.5	17.5		12.5	25.5	19.0		18.5		18.8	2.3
1.0	6.5	12.5	14.0		10.5	10.0	11.0		30.0		13.5	3.1
1.5	8.0	9.5	10.0		12.0	12.5	24.3		24.3		14.4	2.8
2.0	10.5	8.5	5.5		9.5	22.5	10.0		18.5		12.1	2.5
2.5	8.0	8.0	5.5		10.5	29.0	13.5		14.5		12.7	3.2
3.0	16.5	7.0	3.5		10.0	23.0	22.0		17.5		14.2	3.1
4.0	24.5	15.0	14.0		20.5	11.0	27.0		14.5		18.1	2.4
5.0	32.5	21.0	19.0		17.0	10.0	30.0		16.0		20.8	3.2
6.0	25.0	12.0	12.0		16.5	9.0	39.5		7.0		17.3	4.7
7.0	13.0	6.0	8.0		7.5	12.0	17.5		8.5		10.4	1.6
8.0	10.5	4.0	3.5		10.0	12.5	10.5		5.5		8.1	1.5
9.0	7.5	4.0	5.0		4.0	6.5	8.5		3.0		5.5	0.8
36.0	6.0	16.5	2.0		7.0	22.5	18.5		3.0		10.8	3.4
	Data Series #2											
-0.5	10.5	*	7.0	4.5	2.5	9.0	6.5	*	3.5	8.5	6.5	1.1
-0.3	17.0		13.0	8.5	14.5	25.0	22.0		18.0	22.0	17.5	2.1
0.3	15.5		7.0	9.5	17.0	22.5	19.0		11.5	15.5	14.7	1.9
0.5	19.0		3.5	9.5	14.0	15.5	22.0		9.5	14.0	13.4	2.2
1.0	11.5		6.0	4.5	4.5	7.5	12.5		8.0	13.5	8.5	1.3
1.5	7.0		6.0	4.5	3.5	6.0	8.5		8.0	24.3	8.5	2.5
2.0	9.5		4.5	5.5	5.5	4.5	12.5		6.5	6.5	6.9	1.0
2.5	14.0		7.0	7.5	7.5	4.0	11.5		6.5	8.0	8.3	1.2
3.0	13.0		13.0	8.5	4.0	8.5	8.5		11.5	8.0	9.4	1.1
4.0	5.0		3.0	7.0	2.0	3.0	9.1		10.5	3.5	5.4	1.2
5.0	7.5		10.0	7.5	2.5	2.0	4.0		4.5	5.5	5.4	1.0
6.0	3.0		7.8	4.0	2.5	2.0	12.5		15.5	17.0	8.0	2.3
7.0	2.5		5.5	4.0	1.5	6.0	6.5		4.0	7.0	4.6	0.7
8.0	4.0		8.5	3.0	3.5	5.5	11.5		9.0	8.0	6.6	1.2
9.0	4.5		5.0	5.0	2.5	3.0	5.0		4.5	7.0	4.6	0.5
36.0	2.0		4.0	3.0	1.5	4.0	8.5		2.0	2.0	3.4	0.9

* calves which were excluded from the study as outliers (see appendix B).

2.4 RESULTS

2.4.1 Cortisol timecourse analyses

The mean cortisol timecourse using adjusted$_{post.tmt}$ data compared to the adjusted$_{pre.tmt}$ data was similar, but, the mean responses of the adjusted$_{post.tmt}$ data were increased throughout by the arithmetic difference between the pretreatment and post-treatment values. This arithmetic difference ranged from 2.7 ng/ml for data series "5", to 1 ng/ml for data series "3" (see Table 2.2). For all data series the SEMs of the adjusted$_{post.tmt}$ data were smaller than those for the adjusted$_{pre.tmt}$ data (Table 2.2), especially when the response was over, that is when values returned to baseline (Fig 2.3, Fig 2.4).

There were some minor discrepancies in the comparisons between treatments of the timecourse according to how the data were transformed ($P<0.05$). Comparisons between data series "5" and "3" using raw data indicated that there were no differences, whereas using the adjusted$_{pre.tmt}$ data indicated that cortisol levels were significantly different at 0.5, 2.5 and 3h after treatment. Comparisons using the adjusted$_{post.tmt}$ data indicated that levels were significantly different at 0.5 and 3h after treatment (Fig 2.3).

Comparisons between data series "4" and "2" using raw data indicated that cortisol levels were significantly different at 0.5, 4 to 7 and at 36 h after treatment, whereas using the adjusted$_{pre.tmt}$ data indicated that cortisol levels were significantly different at 5 h after treatment only. Comparisons using the adjusted$_{post.tmt}$ data indicated that levels were significantly different at 4 to 6 h after treatment ($P<0.05$) (Fig 2.4).

Table 2.2 Statistics of the pretreatment and post-treatment cortisol reference values.

	pre.tmt			post.tmt			differences between pre.tmt and post.tmt data		
Data series	mean (ng/ml) a	SEM (ng/ml) b	CV (%) c	mean (ng/ml) d	SEM (ng/ml) e	CV (%) f	Δ mean (ng/ml) g=a-d	Δ SEM (ng/ml) h=e/b-1	Δ CV (%) i=c-f
series "5"	12.4	2.0	58	9.7	1.5	45	-2.7	-25%	-13
series "3"	8.0	1.4	54	7.0	1.3	56	-1.0	-7%	+2
series "4"	10.4	1.6	38	8.0	0.9	36	-2.4	-44%	-2
series "2"	6.5	1.1	43	5.3	0.6	33	-1.2	-46%	-10

letters indicate how columns are calculated.
CV = SD/m. Where: CV=coefficient of variation; SD=standard deviation; m=mean; Δ=difference.

2.4.2 Integrated response analyses

Regardless of the transformation employed, the mean integrated cortisol response (0-9h) of data series "5" was not significantly different from that of data series "3" (Table 2.3;). However, the coefficients of variation were different. The coefficients of variation were greatest with $adjusted_{pre.tmt}$ data, intermediate with $adjusted_{post.tmt}$ data and smallest using raw data. It appears that the using $adjusted_{post.tmt}$ data "improved" the coefficients of variation of $adjusted_{pre.tmt}$ data by about 15%. A similar trend was apparent between data sets "4" and "2" (Table 2.3;), although the order of the "improvement" in the coefficients of variation was about 40%.

Table 2.3 Integrated responses (ng.hr/ml) of data series "5", "3", "4" and "2" using raw, $adjusted_{pre.tmt}$ and $adjusted_{post.tmt}$ data (mean ± SEM).

Data series	Integral Raw		Integral $adj_{pre.tmt}$		Integral $adj_{post.tmt}$		Intgl under pre.tmt** (ng.h/ml)	ratio of Intgl* under pre.tmt to area above
	m ± SEM (ng.h/ml)	CV (%)	m ± SEM (ng.h/ml)	CV (%)	m ± SEM (ng.h/ml)	CV (%)		
	a		b		c		d	e=d/b
data "5"	179^{y} ± 21	35	81^{y} ± 20	79	87^{y} ± 19	65	98	1.2
data "3"	175^{y} ± 14	23	105^{y} ± 17	50	110^{y} ± 13	34	70	0.6
data "4"	139^{xy} ± 14	24	52^{xy} ± 14	70	65^{xy} ± 6	23	87	1.7
data "2"	73^{x} ± 7	27	22^{x} ± 5	64	25^{x} ± 3	32	51	2.3

** the integral (Intgl) of the cortisol response below the pretreatment value and above y=0 ng/ml for the period 0-9h.

* the ratio of the integral of the cortisol response under the pretreatment value to that above the pretreatment value for the period 0-9h (see Fig 2.1).

The different superscripts, x and y, within columns indicate statistically significant differences between treatments ($P<0.05$).

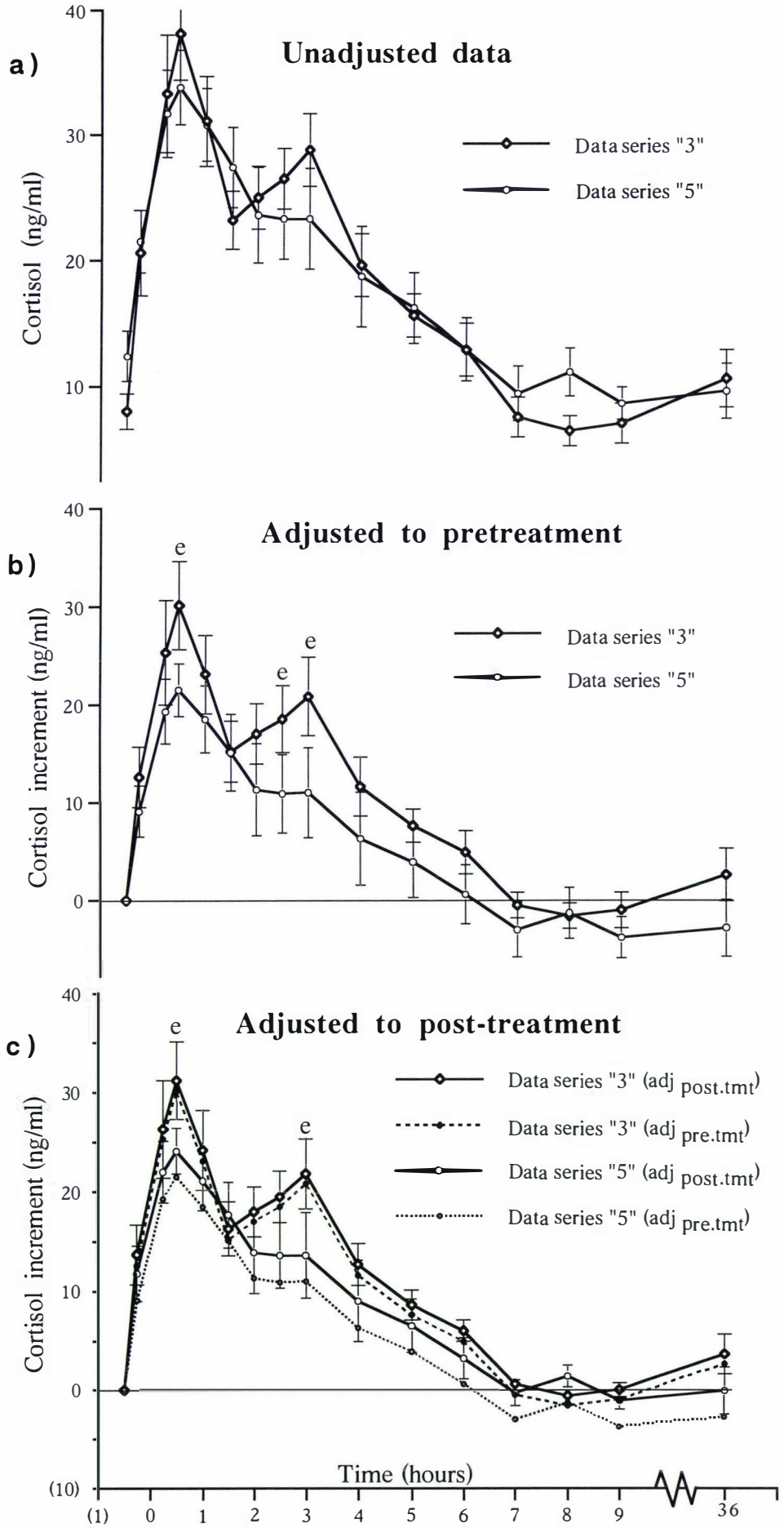


Fig 2.3 Comparison of the cortisol timecourse (mean ± SEM) between data series "3" (n=10) and "5" (n=10), using a) raw, b) adjusted$_{pre.tmt}$ and c) adjusted-$_{post.tmt}$ data. e = data series "3" is significantly different from data series "5".

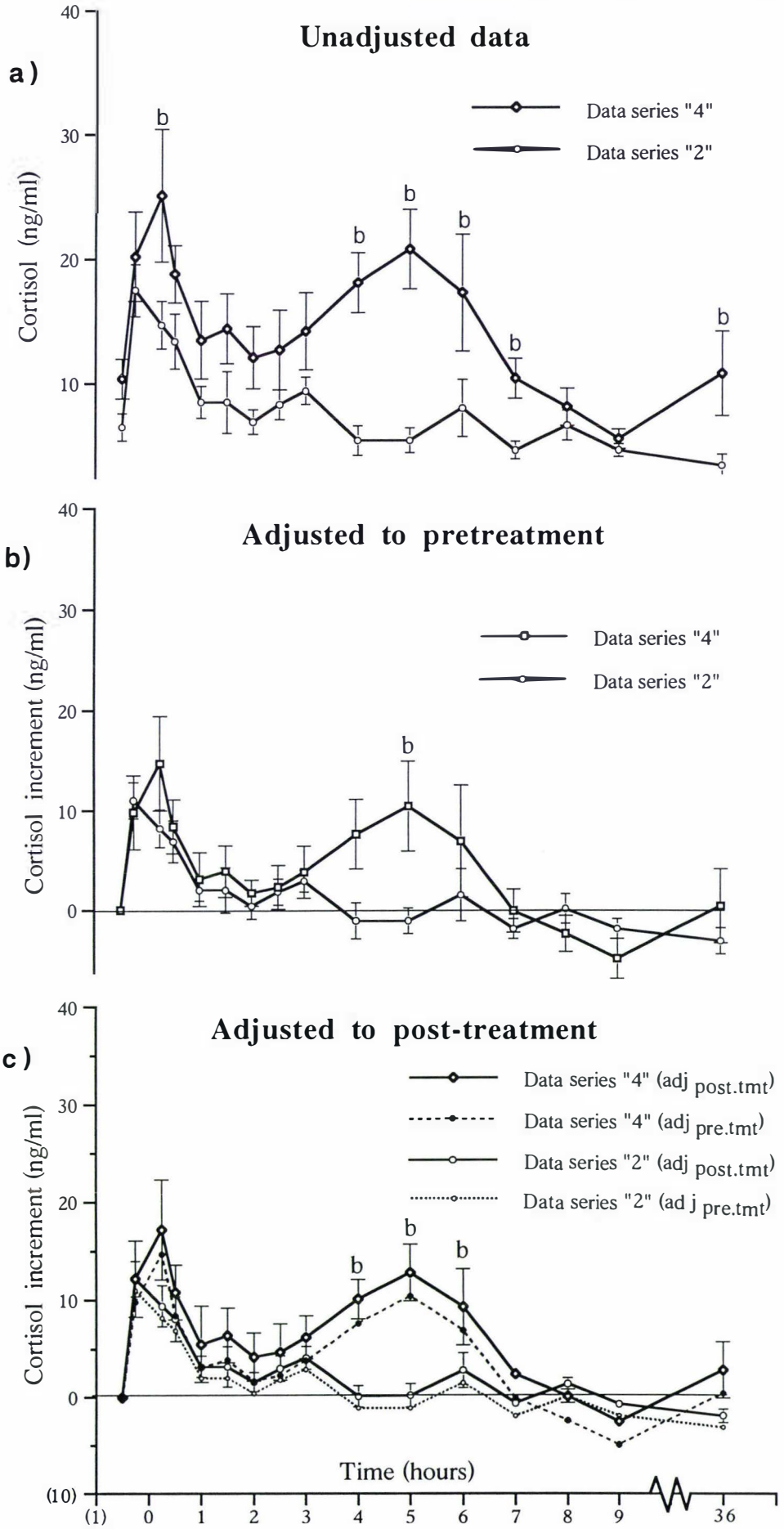


Fig 2.4 Comparison of the cortisol timecourse (mean ± SEM) between data series "4" (n=7) and data series "2" (n=8) using a) raw, b) adjusted$_{pre.tmt}$ and c) adjusted-$_{post.tmt}$ data. b = data series "4" is significantly different from data series "2".

2.5 DISCUSSION

In this chapter three methods of comparing cortisol responses have been examined, namely using raw data, adjusted$_{pre.tmt}$ and adjusted$_{pre.tmt}$ data. The use of the post-treatment value, as an alternative to the commonly used pretreatment reference, was proposed. The post-treatment value was used as the reference point for comparisons because there can be unknown factors acting which cause a higher pretreatment value and therefore using adjusted$_{pre.tmt}$ data might artifactually reduce the magnitude of the response compared to using raw data. The raw data may be used but it is assumed that differences in individual baselines, by increasing the variance of the mean adjusted$_{pre.tmt}$ data, will mute the difference between groups. This did not appear to be the case for comparisons of the timecourse, but is a problem when comparing the integral of the responses (see 2.2.3). If there are large differences in baselines then comparisons using raw data are not recommended. If there is a large variance in the mean pretreatment value then the post-treatment value may be used as the reference point. Ideally, the post-treatment should be the same as the pretreatment, and the relationship between groups should be similar whether using raw or adjusted data.

The use of the post-treatment mean, as opposed to the pretreatment mean, as the reference point from which to compare cortisol responses resulted in no change in the statistical differences between-treatments when comparing the integral of the responses and few differences when comparing the timecourse of the responses. In some cases the number of time periods for which significant differences between treatments were observed increased. In addition, using adjusted$_{post.tmt}$ data tended to decrease the variance in the mean integrated cortisol responses and to a lesser extent the variance of the timecourses. Furthermore, the reduction in the SEMs using the post-treatment adjustment increased the duration of significant differences when there existed a separation between comparative groups on the timecourse. However, in some cases, despite the improvement in the SEMs, the convergence of the mean values (as a result of differences in the pretreatment mean) reduced the significance of the differences. It is important to note that the post-treatment adjustment made the relationships between groups more similar to that observed with the raw data. However, no matter which baseline adjustment was made, or whether the unadjusted (raw) data was used, there were no differences in the biological interpretation of the data.

In every case using adjusted$_{post.tmt}$ data, the mean cortisol timecourse increased compared to that using adjusted$_{pre.tmt}$ data, although the increase was not biologically significant (range: 1-2.7ng/ml). The parsimonious explanations would be that most of the animals were already stressed to some degree by the time the first sample was taken, presumably from the novelty of the experimental situation, or that this small increase

could reflect a circadian rhythm. Cortisol levels peak in the morning just after waking and fall to a nadir during sleep in humans (Sapolsky, 1992), whereas in cattle the same relationship is observed (MacDiarmid and Cooper, 1982) but the patterns of sleep are different (Kilgour and Dalton, 1984). Alternatively, this decrease in the post-treatment period could reflect a refractory period to cortisol secretion due to the previous large cortisol surge. However, this interpretation is unlikely as previous studies have shown that cortisol surges can be elicited in succession with little effect on the subsequent responses (Mellor and Murray, 1989b; Sapolsky, 1992).

Interestingly, the variance of the pretreatment and post-treatment samples of the raw data were similar. It would appear that the pretreatment variance was primarily due to systematic variation in baseline values (i.e. 28% starting two SD greater than the mean value) whereas the post-treatment variance was primarily due to random variation. Relatively speaking the variation at the beginning and the end were similar and the responses were uniform. In general, it appeared that there was greater variation in the response to the treatment (stressor) than in the baselines. It is a common observation that there is large inter-animal variation in the response to stressors (Moberg and Mench, 2000).

The fact that there were no differences in interpretation whether one used the $\text{adjusted}_{\text{pre.tmt}}$ or $\text{adjusted}_{\text{post.tmt}}$ data suggests that the pretreatment value was not sufficiently different from the post treatment to be detected unambiguously given the data variance, or that the difference between the pretreatment and post-treatment values was compensated across the groups. In addition, the similarity in the analyses gives confidence that the data can be analysed using either the pretreatment or post-treatment reference point.

The experimental design, including the use of control groups, even distribution of groups across days, mobs and time of day and the method of blood sampling and handling was appropriate. Presumably the sample size of 10 protected the mean data from being unduly influenced by random events. This analysis has demonstrated that our methodology was sufficiently robust to account for systematic or random variation.

In this study, there appeared to be no advantage in using the post-treatment value as a reference point for comparing cortisol responses, therefore, for the remainder of the thesis the pretreatment value has been used. It is possible that under some circumstances, the post-treatment values may more accurately represent baseline than do the pretreatment values. This conundrum can only be resolved by additional sampling before and after treatment, ideally for 24 hours in each period.

3 THE ACUTE CORTISOL RESPONSES OF CALVES FOLLOWING FOUR METHODS OF DEHORNING

3.0 ABSTRACT

Dehorning of cattle is a routine husbandry practice, but, there has been no comprehensive research to assess the distress associated with this procedure. Changes in plasma cortisol concentrations were used to assess the pain-induced distress in Friesian calves (six-months old, about 130 kg, n=57) during the first 9 hours and at 36 h after amputation dehorning using different tools (scoop, saw, guillotine shears or embryotomy wire), after control handling and bloodsampling and after an adrenocorticotropic hormone (ACTH) bolus (iv. 0.28 μg/kg). Calves were separated from herds at pasture and kept overnight in cattle yards with access to water but not food. On the day of the study calves were studied in mobs of 12 with equal representation of treatments in the mob. Blood was taken by venipuncture from either jugular vein while calves were held against the walls of the pen. Treatment was conducted in a cattle race. Amputation dehorning elicited a marked biphasic cortisol response that lasted for about six hours. Dehorning elicited similar ($P>0.05$) cortisol responses irrespective of the tool employed, although there is a suggestion that the depth of the wound may affect the level of distress. The cortisol response to Control handling and bloodsampling lasted 1.5 h and was significantly less in magnitude, duration and integral than that after dehorning ($P<0.05$). ACTH injection elicited a plateauing of high cortisol concentrations which decayed exponentially to pretreatment levels after 2.5 h. The plateauing of high cortisol concentrations indicates maximal cortisol secretion of the adrenal gland. The similarity in the first hour, of the responses after dehorning and ACTH, suggests that dehorning is very distressing. These results suggest that amputation dehorning is extremely noxious. Further work should be conducted to explore avenues to alleviate this pain-induced distress.

3.1 INTRODUCTION

The dehorning of cattle is a routine husbandry practice performed to reduce injury to stockhandlers and stock, to reduce economic losses through carcass bruising and hide damage (Meischke *et al.,* 1974) and to improve cattle husbandry (Anon, 1974; Ernst, 1973). Whilst dehorning is performed for the welfare of cattle it is *itself* a welfare issue, because it involves tissue damage and is presumably a painful and noxious experience. However, at the time this work was done little research had been conducted on the distress caused by dehorning and how this may affect the welfare of cattle.

Cattle may be dehorned at any age according to husbandry systems. At the time this study was conducted it was an offence to dehorn cattle over the age of 20 months unless the operation was conducted under anaesthesia of sufficient power to prevent the animal from feeling pain (section 3y Animals Protection Act, 1960). In New Zealand, dairy cattle are usually dehorned within a few weeks of birth or at weaning, and beef cattle are usually dehorned at about six months when a series of procedures such as weaning, castration and ear tagging are performed (Anon, 1974). All methods of dehorning involve either destruction or removal of the horn-forming cells (germinal tissue) and their blood vasculature (Oehme, 1974). Caustic chemicals or cautery are used to disbud young calves with minimal horn development, and surgical amputation is used to dehorn cattle older than three months (Owen, 1984). The caustic chemicals used to disbud young calves include caustic paste (potassium hydroxide) and calcium chloride injection. The chemical is applied to the emerging horn bud within one week of birth, and acts by corroding and thereby destroying the horn bud germinal tissue and its blood supply (Owen, 1984). In cautery disbudding a hot circular-shaped iron is placed over the horn and cauterizes the tissue at the base of the horn bud. This method may be used on calves aged one to eight weeks (Owen, 1984), after which time the horn bud is usually too big to allow the effective use of the cautery iron. With surgical amputation, the whole of the horn including the adjacent germinal tissue is removed. The germinal tissue has a vascular supply and is innervated. The tools used to amputate the horn include the scoop dehorner (also known as scallop-, cup-, gouge- or Barnes-dehorner), guillotine shears, saw and embryotomy wire (wire saw). In some instances only the distal part of the horn is removed which is termed tipping, but this is only a temporary solution because the horn germinal tissue remains intact and the horn re-grows.

3.1.1 Dehorning and production studies

Traditional management practices were developed and assessed for their practicality on the basis of ease of use, compatibility with existing management practices, economics, labour-costs, effects on production parameters and health and safety factors (Stafford and Mellor, 1993).

Horned cattle are more difficult to handle and transport, require more space in feedlot troughs and during transport (in Winks *et al.*, 1977), and have an increased incidence of carcass bruising (Meischke *et al.*, 1974) which occurs mainly during transport (Shaw *et al.*, 1976). For this reason it has been recommended that horned cattle be banned from, or penned separately for, transport (Animal Welfare Advisory Committee, 1994). The major incentive for complying with this recommendation might be economic rather than concern for the welfare of the cattle.

Dehorning improves the handling of cattle (Anon, 1974), reduces flight distances (K.J. Stafford, personal communication), decreases dominance at food troughs (Ernst, 1973) and reduces the feeding space and housing requirements for indoor-housed cattle (in Allyn, 1991; in Winks *et al.*, 1977). Conversely, dehorning contributes to the transmission of blood borne diseases (DiGiacomo *et al.*, 1987), decreases milk production (Eckles and Anthony, 1956) and decreases (Winks *et al.*, 1977; Loxton *et al.*, 1982; Goonewardene and Hand, 1991) or has no effect on (Laden *et al.*, 1985) subsequent bodyweight gains.

Winks *et al.* (1977) reported decreased bodyweight gains in mature Brahman crossbred steers during the six months after dehorning which were not subsequently compensated for by a similar decrease in carcass-bruise trim. They concluded that adult cattle should not be dehorned. In a follow-up study, Loxton *et al.* (1982) reported that dehorning Brahman crossbred steers of various ages (4, 7, 19, and 30 months old) led to decreased bodyweight gains for six weeks after dehorning, after which there were no differences between dehorned and horned cattle. They concluded that the age of dehorning was not critical yet they recommended that cattle be dehorned at an early age for 'welfare' reasons, but this was not explained. They also noted that the period of depressed bodyweight gains coincided with the time taken for wounds to heal properly (4-6 weeks), and like Winks *et al.* (1977) they attributed this production loss to a decreased desire to graze. Goonewardene and Hand (1991) reported significant decreases in the bodyweight gains of adult steers for the three months after dehorning, which equated to a loss of 5 kg per steer at slaughter. They also noted that the changes in bodyweights were related to feeding management systems where the difference between dehorned and horned cattle was reduced by making more feed available. It is possible that the differences in the duration of depression of bodyweight gains after dehorning of six months (Winks *et al.*, 1977), three months (Goonewardene and Hand, 1991) and six weeks (Loxton *et al.*, 1982), were due to differences in experimental design features such as age, breed, sex, or indeed feed availability. However, it is not possible to say which, and this illustrates the difficulty of drawing conclusions from the results of different studies.

Laden *et al.* (1985) reported that cautery disbudding had no effect on the bodyweight gains of 8-week-old calves four weeks after treatment. It is possible that cautery disbudding, by contrast with amputation dehorning, had minimal or no effect on bodyweight gains because the nature of the tissue insult, the resultant wound, and the inflammatory responses are different. On the other hand it is possible that had bodyweights been measured more frequently than once after treatment, differences in

bodyweight gains between the untreated and disbudded cattle may have become apparent.

3.1.2 Dehorning and welfare studies

The effects of dehorning on performance does not necessarily equate to welfare. Whilst poor production may indicate poor welfare, the reverse, good performance indicates good welfare, is not necessarily true (Stamp-Dawkins, 1980). Whilst dehorning is performed for the welfare of cattle it also raises welfare issues, because it involves tissue damage and is therefore presumably a painful and noxious experience. However, the level of that distress and the avenues for its alleviation have not been thoroughly examined. A variety of parameters have been used to assess the effects of husbandry procedures on animals. Most commonly measured are those associated with activation of the hypothalamic-pituitary-adrenal axis, usually assessed as changes in plasma cortisol concentrations (e.g. Mellor and Murray, 1989a,b; Lester *et al.*, 1991a,b; Wood *et al.*, 1991).

At the time that this study began only the effect of cautery disbudding on plasma cortisol concentrations had been clearly defined. In four- (Allyn, 1991) and eight-week-old calves (Laden *et al.*, 1985) cautery disbudding elicited a cortisol response that lasted about two hours. This was significantly greater and one hour longer than that elicited by control handling and blood sampling. In addition, there had been three other studies in which the cortisol responses of cattle to amputation dehorning (Johnston and Buckland, 1976; Carter *et al.*, 1983) or cautery disbudding (Boandle *et al.*, 1989) were reported. However, the infrequent sampling schedule in these studies means that interpretation of the partial cortisol responses is equivocal.

Changes in behaviour are considered a valid indicator of stress (Stamp-Dawkins, 1980), and have been reported following various noxious husbandry procedures including: cautery disbudding (Taschke and Folsche, 1993; Morisse *et al.*, 1995), tail docking (Petrie *et al.*, 1995), castration and/or tail docking (Robertson *et al.*, 1994; Mellor and Murray, 1989a) and branding (Lay *et al.*, 1992a,b). However, there have been no rigourous studies in which behavioural indices have been used to examine the effect of amputation dehorning on calf welfare. For this reason a study was conducted, as part of this thesis, using changes in behaviour to assess the effects of dehorning (Chapter five).

3.1.3 Aims of study

The present study was designed to address the following; first, to use plasma cortisol concentrations to characterize the stress response to dehorning in six-month-old calves; second, to investigate whether there are differences in the cortisol response when the different amputation tools of scoop, guillotine shears, saw and embryotomy wire are

employed; third to establish a frame of reference for studying cortisol responses in six-month-old cattle by identifying "baseline" and "maximal" cortisol values, the latter through conducting an ACTH challenge of sufficient dose to elicit a maximal cortisol response (Verkerk *et al.,* 1993). The ACTH challenge will also test the functioning of the adrenal component of the hypothalamic-pituitary-adrenal axis (Mellor and Murray, 1989b); and fourth, by extrapolation from the cortisol responses, to comment on the welfare implications of dehorning and the application of this to the animal husbandry practice of dehorning.

3.2 MATERIALS AND METHODS

3.2.1 Experimental design

Fifty seven male Friesian calves aged five to six months, weighing 99-159 kg (mean 130 kg) and scheduled for dehorning according to usual husbandry practice, were used in this study. They were separated into five mobs (n=12) and the study was carried out on a Massey University farm over four consecutive days in February 1992. Prior approval to conduct this study was obtained from the Massey University Animal Ethics Committee.

The evening before each experimental day at 2000 h two mobs each of 12 calves were randomly selected from the herd, numbers were painted on their backs for identification purposes, and they were penned overnight outdoors in cattle yards (10 x 10 m) with access to water but not food.

On each experimental day 24 calves were studied in two mobs of 12, commencing at 0700 h, and 1100 h respectively. Each mob was moved quietly into a small pen (3 x 4 m) where a pretreatment blood sample was taken. All blood samples were taken in that pen because its small size restricted movement of the calves and thus facilitated catching, restraint and sampling. Fifteen minutes later the calves were moved into the adjacent cattle race where they were restrained manually and the treatment was performed. Immediately after treatment they were returned to the small pen where blood samples were taken with decreasing frequency throughout the following 9 hours. Between 3 and 9 hours after treatment, when sampling was at hourly intervals, the calves were moved into an adjacent larger yard (10 x 10 m) where water was available *ad libitum*. When required for blood sampling they were quietly moved back to the smaller pen. The duration of blood sampling was about 12 minutes for the mob.

At the end of the 9-hour-sampling period the two mobs of calves were reunited and returned to pasture overnight. The following afternoon at 1600 h they were mustered into the yards and left to settle for 3 hours. This was done because mustering and bloodsampling caused elevations in plasma cortisol concentration for about three hours (Sylvester *et al.*, 1993. See Appendix A). Thereafter a blood sample was taken, the time of that sample being about 33 or 36 hours after treatment.

This whole procedure was repeated over four consecutive days in different calves. During the course of this experiment, the mustering of other stock nearby, at 1600 h and 1900 h on each day, elicited some interest from the experimental animals.

3.2.2 Blood sampling

Blood samples (10 ml) were taken by venipuncture from either jugular vein. Samples were taken at 0.25 hr before treatment (pretreatment), and at 0.25, 0.5, 1, 1.5 , 2 , 2.5, 3, 4, 5, 6, 7, 8, 9 and 36h after treatment. Intensive blood sampling was carried out during the first 9 hours because a previous study had shown that the acute plasma cortisol concentrations return to pretreatment values by about 7 hours after dehorning (Sylvester *et al.,* 1993. Appendix A), and a 36h sample was taken because it was not known whether dehorning caused a long-lasting increase of plasma cortisol concentrations. Blood samples were taken by one person whilst the calf was restrained by being held firmly against the yard wall by three other people. This procedure usually took less than 45 seconds for each calf. Calves were bled in the same order each time to test for possible effects of sampling order on plasma cortisol concentrations.

3.2.3 Treatments

The calves were randomly allocated to one of six treatments which were equally represented in each mob of 12 calves. Each calf was restrained manually for treatment while the whole mob was packed closely together in a cattle race. One person held each calf's head firmly whilst another person performed the treatment. The two operators moved down the race dealing with each calf in succession. Dehorning implements were disinfected (Hibitane concentrate 5%. Hospital Products, ICI, Australia) between animals. The amount of horn and skin tissue removed during dehorning varied depending on the horn size and head movements. The order of treatment was the same for each mob to test for possible effects of the treatment order on plasma cortisol concentrations. The time of treatment was defined as time zero for each calf.

Control calves n=10

Calves were handled to simulate the restraint required for dehorning without being dehorned.

Scoop calves n=10

Calves were dehorned using an industry standard scoop dehorner (Barnes Dehorners, Stones, USA). The scoop dehorner (Fig 3.1) has two interlocking semi-circular blades (radius of 20mm) which when engaged remove the horn and the adjacent cutaneous tissue, including the germinal tissue of the horn. This was the only dehorning method where the frontal sinus of the skull was occasionally perforated.

Saw calves n=10

Calves were dehorned using a standard butcher's saw (Fig 3.1).

Guillotine Shears calves n=10

Calves were dehorned using a small pair of industry standard guillotine shears (James Sculley, Pomeroy, PA., USA) (Fig 3.1) with a cutting hole of 60x80mm.

Embryotomy wire calves n=10

Calves were dehorned using industry standard embryotomy wire (Arnold's wire saw). The embryotomy wire is a length (0.5 m) of jagged wire with handles at both ends (Fig 3.1). To operate, both ends are held and the wire is pulled repeatedly across the base of the horn, which, in a manner similar to a saw, cuts the horn. The embryotomy wire is usually used in situations where a saw would not fit, for example, fetotomy and the cutting of boars' teeth. This method is purported to cauterise tissues (Owen, 1984; Oehme, 1974).

Adrenocorticotropic Hormone (ACTH) calves n=10

40μg (approximately 0.28 μg/kg calf) of ACTH (Synacthen, Ciba Pharmaceutical, Auckland, NZ) were injected into a jugular vein of each calf in order to elicit a maximal cortisol response and test adrenal reactivity (Mellor and Murray, 1989b; Verkerk *et al.*, 1993).

3.2.4 Plasma Cortisol Analysis

Blood samples were collected in vacutainer tubes containing lithium heparin, chilled immediately, centrifuged and the plasma was stored at -20°C until analysed. Plasma cortisol concentrations were determined by radioimmunoassay (see Appendix C). The cortisol radioimmunoassay technique was that of Ruder *et al.* (1972) as modified by Evans (1979) (see Mac Diarmid, 1982). The intra-assay and inter-assay coefficients of variation were calculated using the three sets of plasma pools and were 11% and 15% respectively. The lowest detectable concentration of cortisol was 0.3 ng/ml.

3.2.5 Statistical Analyses

Statistical analyses were conducted on 1) the non-transformed pretreatment cortisol concentrations, 2) the time-course of the cortisol responses after treatment, and 3) the integrated cortisol responses after treatment.

Cortisol data are expressed as the mean ± the standard error of the mean (SEM). The significance of the difference between the means was determined using a two-way analysis of variance (ANOVA) with Fisher's probability of least significant differences (PLSD). Except for checking whether the pretreatment data from different experimental groups (mobs) could be pooled, the cortisol concentrations were transformed as the arithmetic difference from the pretreatment value. The data were transformed in this

manner to correct for individual variation or pre-study effects in baseline cortisol values. All statistical calculations were conducted using a standard statistical computer package (Statsview + Graphics, Version 2, Abacus Concepts Inc., USA).

1) Pooling the data

To ascertain whether the results from all experimental groups (mobs) could be pooled, an ANOVA was conducted on the non-transformed pretreatment cortisol concentrations according to day, mob, and time. In addition, the possibility of a relationship between the pretreatment concentrations and the order in which the calves were sampled was examined using Pearson's correlation coefficient (r) or regression (r^2).

2) Timecourse of the cortisol response

To calculate when the cortisol response to a treatment was complete, i.e. when the cortisol concentrations returned to and remained at pretreatment levels, a factorial ANOVA design was used. To calculate if there were any differences between treatments in the mean timecourse of the cortisol response a repeated measures ANOVA design was used.

3) Integrated cortisol response

Numerical integration was used to calculate the total cortisol response, hereafter called the integrated cortisol response. The integrated cortisol response was defined as the area under the cortisol curve and above a line drawn horizontally through the pretreatment value (Mellor and Murray, 1989b). A factorial ANOVA was used to determine if there were any differences between treatments in the mean integrated cortisol responses.

The strength of using the integrated cortisol response lies in the fact that it combines in one number the features of both magnitude and duration, and so provides a means for comparing the *total* cortisol response between treatments. The limitation of this measurement is that the same number may represent both a short-lived large response or a protracted low or moderate response.

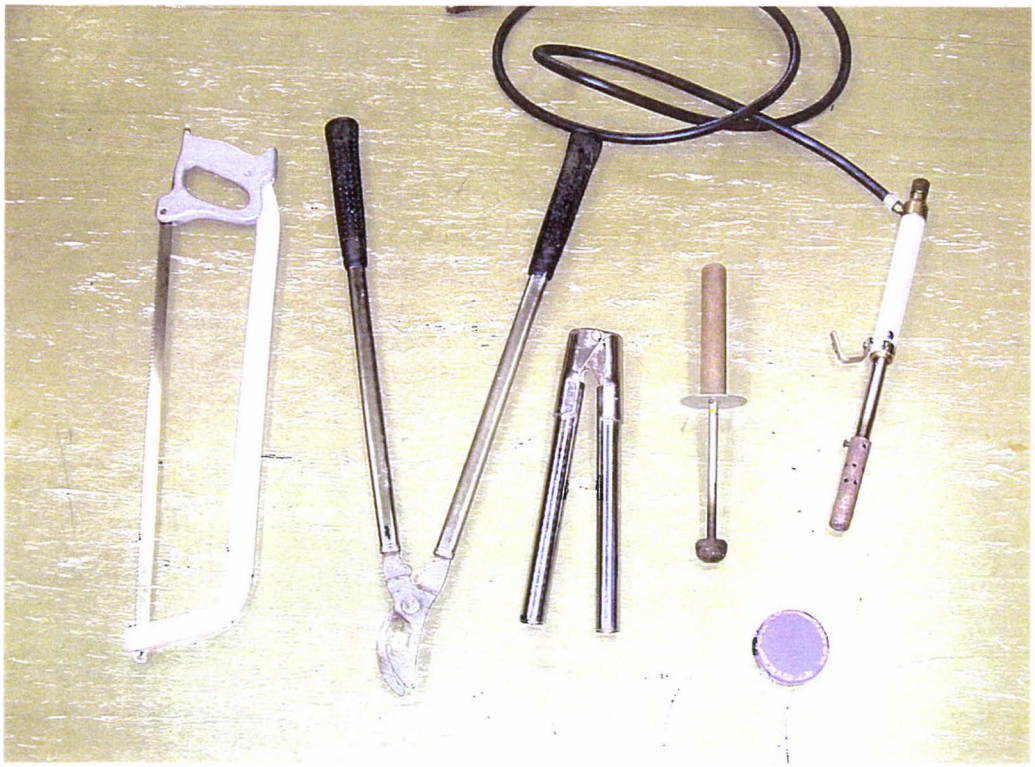


Fig 3.1 Dehorning equipment. Left to right: saw; guillotine shears; scoop; cautery irons and embryotomy wire.

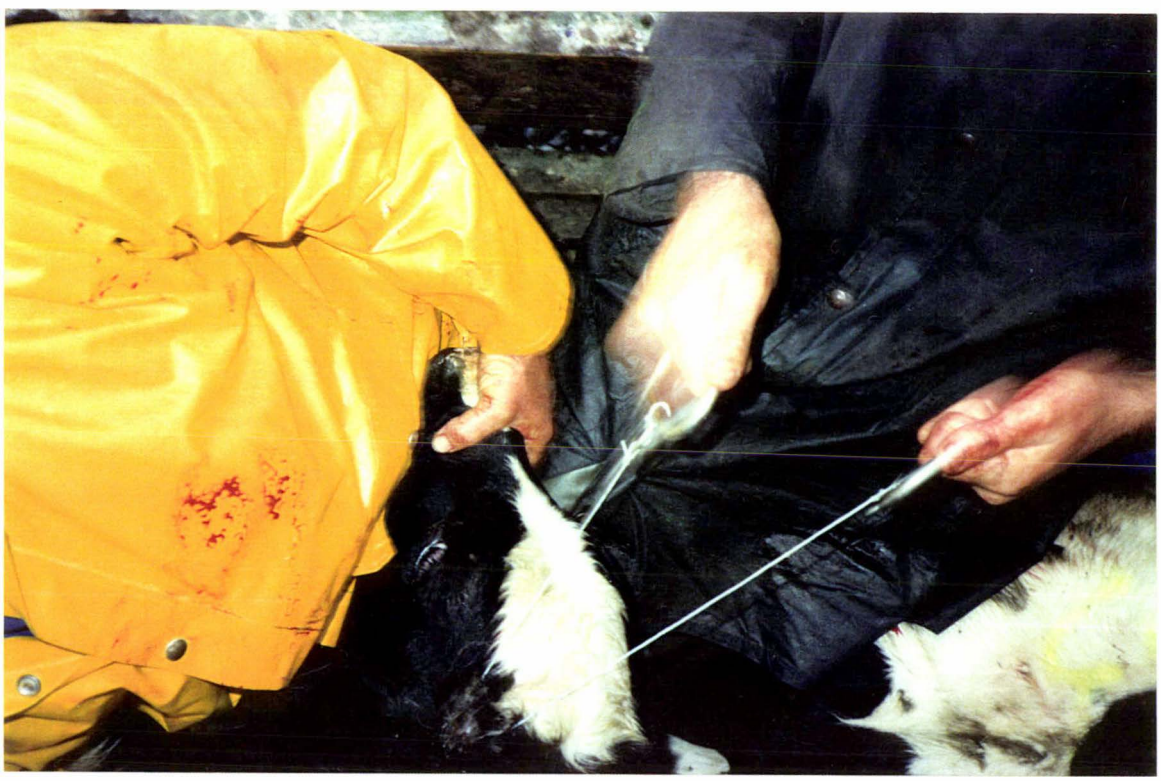


Fig 3.2 Horn removal using the embryotomy wire.

Fig 3.3 Horn removal using the scoop dehorner. Photo courtesy of Cheryl McMeekan (McMeekan, 1997).

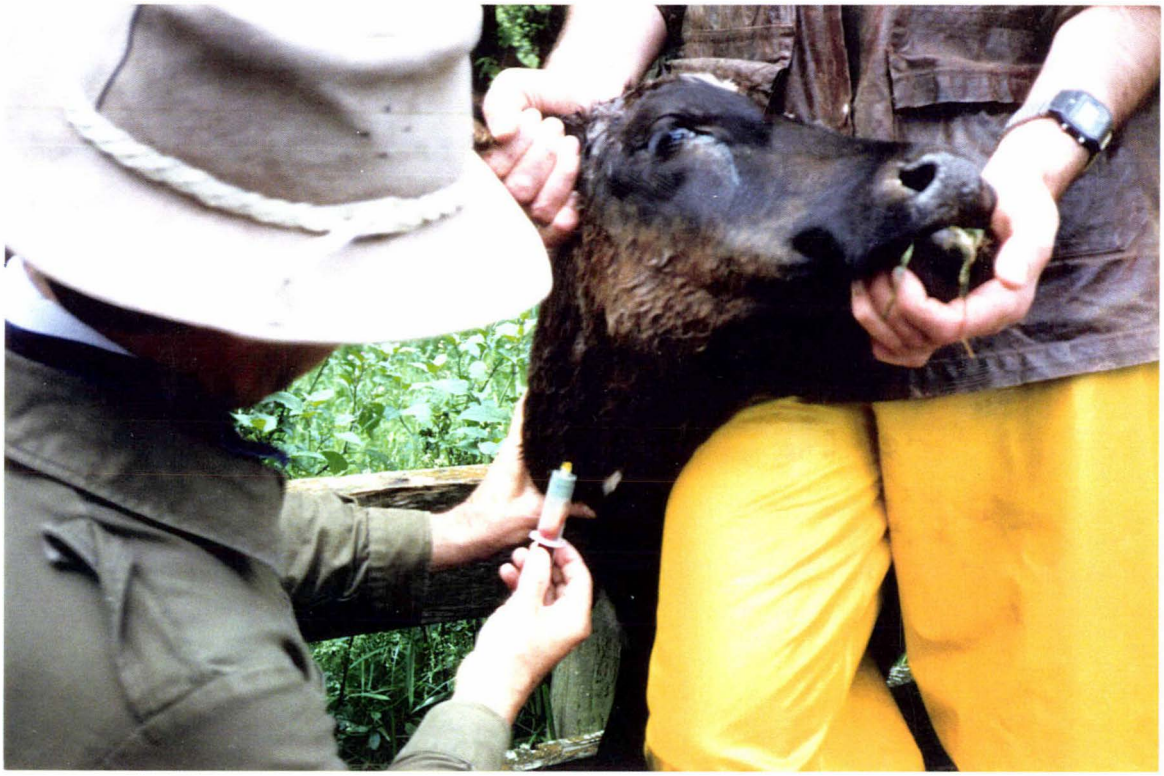


Fig 3.4 The blood sampling procedure.

3.3 RESULTS

3.3.1 Pretreatment Cortisol Concentrations

There were no significant differences ($P>0.05$) in the non-transformed pretreatment plasma cortisol concentrations between days, mobs, or treatments, nor was there a linear correlation with the order in which the animals were bled ($r^2=0.19$) (Fig 3.5). There was a significant difference ($P<0.05$) in pretreatment cortisol concentrations between the early (8.1 ± 1.2 ng/ml, n=34) and late (12.6 ± 1.4 ng/ml, n=23) mobs, but, this was considered to be of little or no biological significance in relation to the magnitude of the subsequent cortisol responses. Therefore the results from all mobs were pooled and used to calculate the mean cortisol response to each treatment. The mean pretreatment concentration was 10.1 ± 0.9 ng/ml (n=57).

After treatment, all calves showed significant transient increases in plasma cortisol concentrations, the magnitudes and durations of which varied according to the treatment. There were three distinct patterns in the timecourse of the cortisol response which were exhibited by Control, dehorned and ACTH calves respectively. The results will be discussed in these three groupings.

3.3.2 Control Calves

Restraint, blood sampling, and simulated dehorning in the race resulted in a small but significant transient rise in plasma cortisol concentrations ($P<0.05$). The maximum increment of 23 ± 4 ng/ml occurred 15 minutes after treatment, after which the cortisol concentrations decreased and returned to pretreatment values by 1.5 hours (Fig 3.6). Thereafter the mean plasma cortisol concentrations were not significantly different from pretreatment values ($P>0.05$).

3.3.3 Dehorned Calves

Amputation dehorning resulted in marked transient increases in plasma cortisol concentrations ($P<0.05$). All four amputation methods (scoop, saw, guillotine shears, and embryotomy wire) resulted in similar mean cortisol responses, with no significant differences in peak magnitude, duration or integrated cortisol responses ($P>0.05$) (Fig 3.7 and Table 3.1). The maximal increment in cortisol concentrations (33-45 ng/ml) occurred 30 minutes after treatment. During the next hour, cortisol concentrations decreased rapidly, but, between 1.5 and 3 hours values plateaued. In Guillotine shear calves the plateau values (at 2 and 2.5 hours) were less ($P<0.05$) than those of the other groups of dehorned calves. Thereafter, from 3 hours after treatment cortisol concentrations continued to decrease and returned to pretreatment values by 6 hours after treatment.

3.3.4 ACTH Calves

ACTH administration resulted in a marked transient rise in plasma cortisol concentrations, the peak increment (46 ng/ml) of which occurred 1 hour after treatment. High values were sustained for about two and a half hours ($P<0.05$), after which cortisol concentrations decreased to pretreatment values by 4 hours, and remained non-significantly below pretreatment concentrations between 4 and 9 hours (Fig 3.8). Between 6 and 9 hours the cortisol concentrations in ACTH calves were significantly less than those of Control calves, which exhibited a non-significant rise ($P>0.05$) in concentrations during this period.

3.3.5 Cortisol timecourse gradients

During the first hour after treatment the rates of rise in cortisol concentrations of ACTH and dehorned calves appeared to be similar (Fig 3.9). The initial rate of decrease of cortisol concentrations from maximal values in ACTH calves, which occurred between 2 and 4 hours, was similar to that in dehorned calves (between 0.5 and 1.5h), and in Control calves (between 0.25 and 1h) and all three were of the order of 0.40 ng/ml/min. This rate of decrease of cortisol concentrations was greater than that from the plateau to pretreatment concentrations in dehorned calves, which occurred between 3 and 6 hours, and was of the order of 0.13 ng/ml/min.

3.3.6 36 hour cortisol sample

The cortisol concentrations in Guillotine shears and Saw calves at 36 h were greater than those in the Control and ACTH calves ($P<0.05$). There were no significant differences between the pretreatment and the 36h plasma cortisol concentration within any group (Table 3.1).

3.3.7 Integrated cortisol responses

There were no significant differences ($P>0.05$) between the mean integrated cortisol responses of calves dehorned by any method (Scoop 142 $\pm$ 10 ng.hr/ml, Saw 136 $\pm$ 14 ng.hr/ml, Guillotine shears 112 $\pm$ 18 ng.hr/ml, Embryotomy wire 137 $\pm$ 21 ng.hr/ml), nor between the integrated cortisol responses of dehorned and ACTH (146 $\pm$ 16 ng.hr/ml) calves (Table 3.1). The mean integrated cortisol responses of dehorned and ACTH calves were between 3 and 3.9 times greater ($P<0.05$) than those for the Control calves (36 $\pm$ 5 ng.hr/ml).

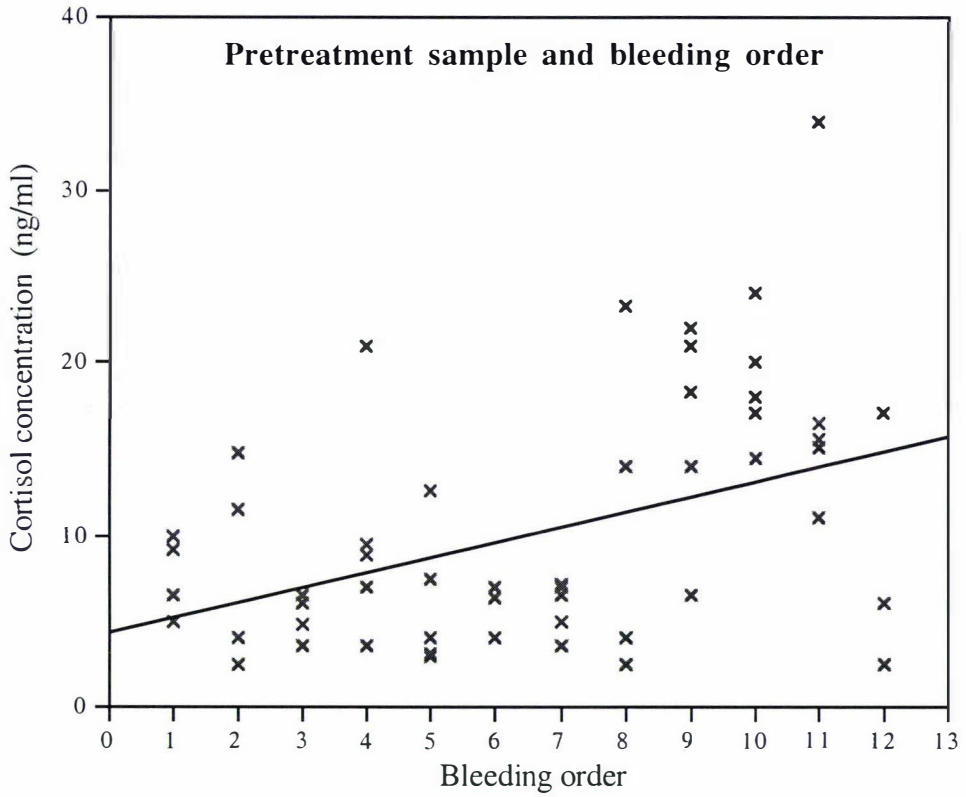


Fig 3.5 Relationship between the pretreatment plasma cortisol concentrations and the order in which the calves were sampled (bleeding order) (n=57). The straight line corresponds to a linear regression for which the correlation coefficient r^2=0.19 (i.e.weak correlation).

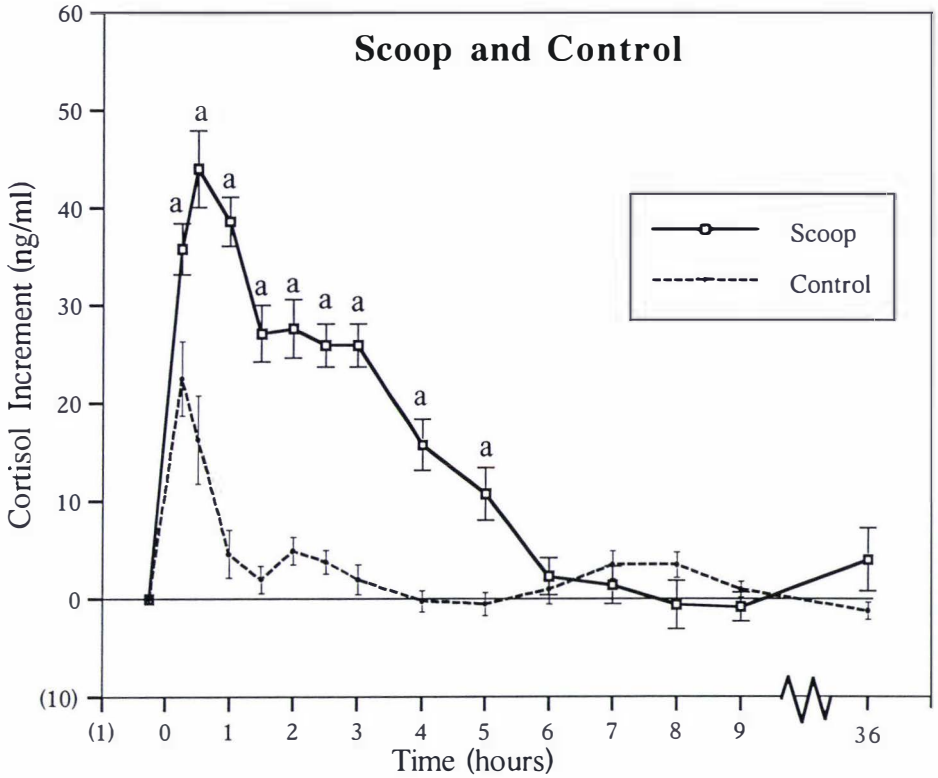


Fig 3.6 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to Scoop dehorning (n=10) and Control (n=10) treatment.
a = Scoop values are significantly different from Control values ($P<0.05$).

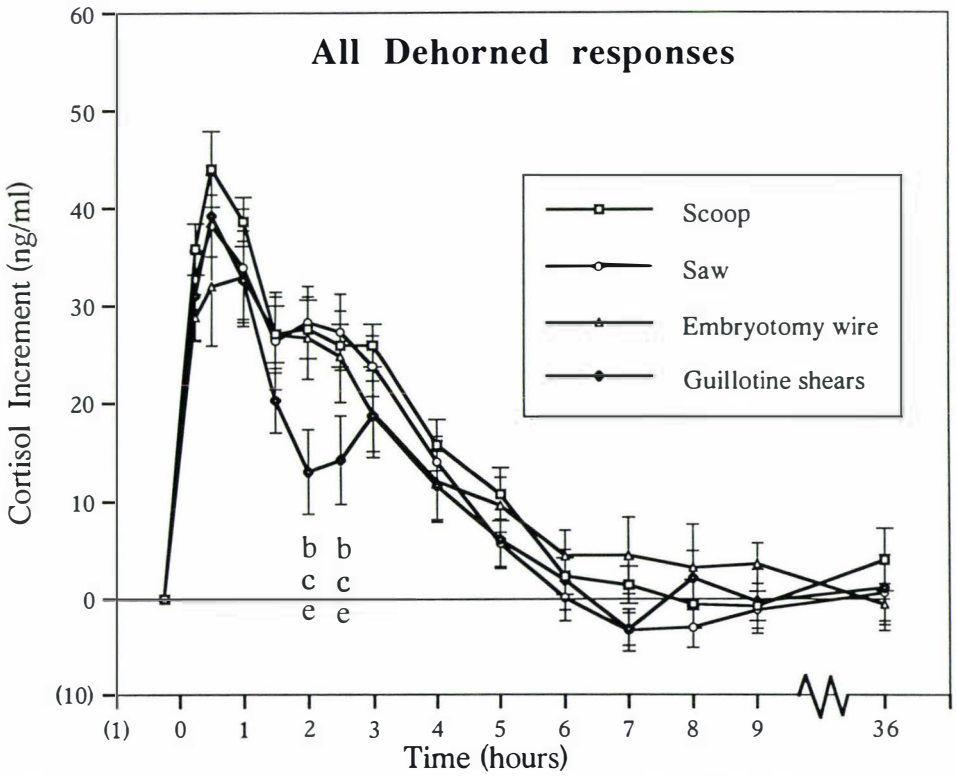


Fig 3.7 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to Scoop (n=10), Saw (n=10), Embryotomy wire (n=10) and Guillotine shears (n=10) dehorning. Guillotine shears values are significantly different from:
b Scoop; c Saw; e Embryotomy wire ($P<0.05$).

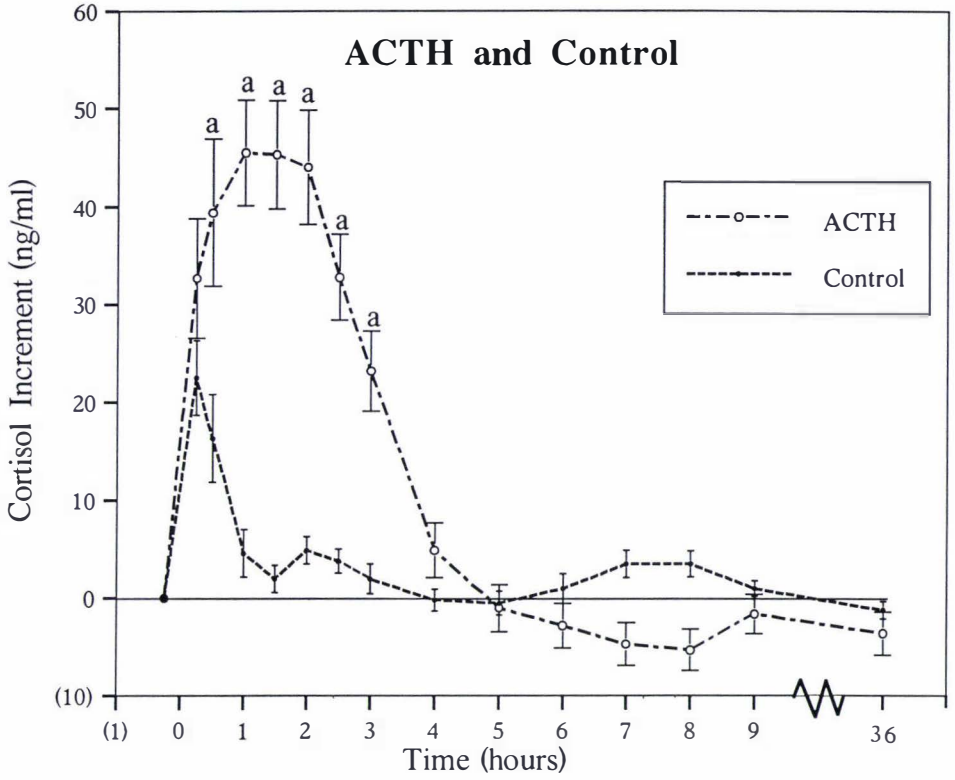


Fig 3.8 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to ACTH injection (n=7) and Control (n=10) treatment.
a =ACTH values are significantly different from Control values (P<0.05).

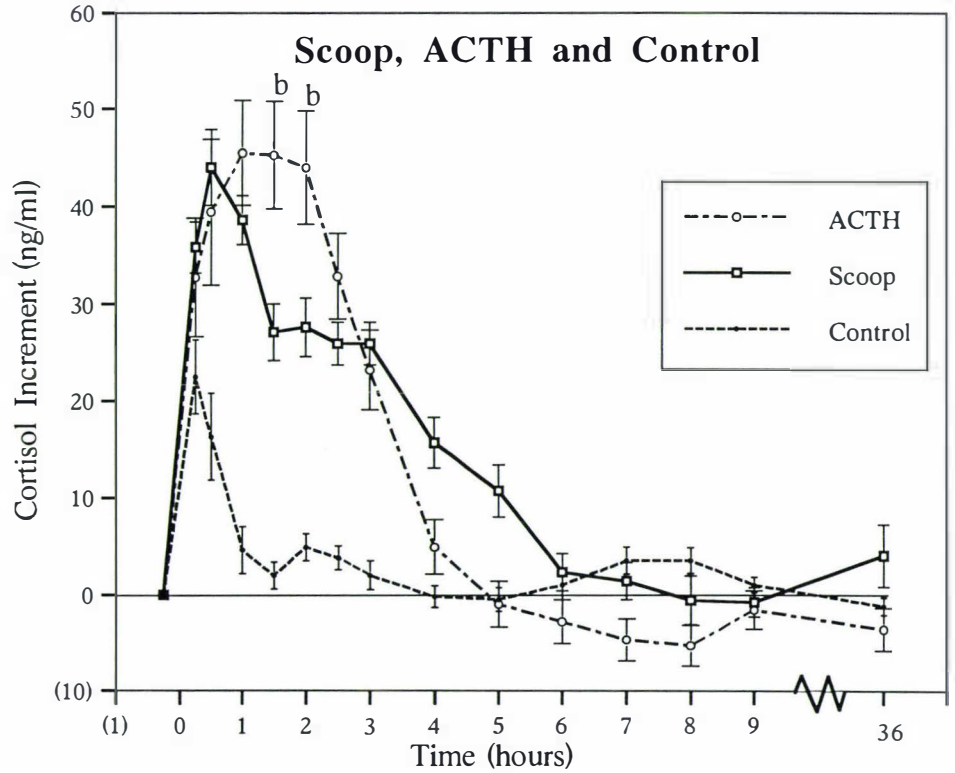


Fig 3.9 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to Scoop (n=10), ACTH (n=7) and Control (n=10) treatment.
b = ACTH values are significantly different from Scoop values (P<0.05).

Table 3.1 Characteristics of the cortisol responses for each group before and after treatment (mean±SEM). Different superscripts within columns indicate statistically significant differences between treatments.

Treatment	n	Pretreatment (ng/ml)	Peak increment (ng/ml)	Time to peak (hours)	Response duration (hours)	Integral* (ng.hr/ml)	Integral** as ratio of Control	36h (ng/ml)
Control	10	7 ± 1	23^{a} ± 4	0.25	1.5	36^{a} ± 5	1	5 ± 1
Guillotine shears	10	14 ± 2	41^{b} ± 4	0.5	6	112^{b} ± 18	3.1	16 ± 3
Embryotomy wire	10	12 ± 3	36^{b} ± 4	0.5	6	137^{b} ± 21	3.8	12 ± 4
Saw	10	11 ± 2	42^{b} ± 3	0.5	6	136^{b} ± 14	3.8	12 ± 3
Scoop	10	8 ± 2	44^{b} ± 3	0.5	6	142^{b} ± 10	3.9	11 ± 2
ACTH	7	7 ± 2	46^{b} ± 6	1	4	146^{b} ± 16	4.1	5 ± 1

* = the integral of the cortisol response. Defined as the area under the cortisol response curve and above the pretreatment level (ng.hr/ml).

** = the integral expressed as a ratio of the integral of the Control response.

3.4 DISCUSSION

Using changes in plasma cortisol concentrations as an index of pain-induced distress, the present study led to the following findings:

1) All amputation dehorning treatments resulted in similar cortisol responses regardless of the method employed. The cortisol response was marked, biphasic and lasted for about six hours.

2) The timecourse of the plasma cortisol response after ACTH administration suggests that maximum cortisol secretion had been induced, providing a reference against which to compare the other cortisol responses. The cortisol responses in the first hour after dehorning were similar to those following ACTH treatment indicating that dehorning caused maximal cortisol secretion and thus by inference was very distressing.

3) Both the duration (1.5 h) and the integral of the cortisol response of the Control calves were about a quarter of that after dehorning, so that control handling was considered to be a relatively benign experience.

4) There were three distinct patterns in the timecourse of the plasma cortisol concentrations after ACTH, Dehorning, and Control treatment respectively. Analysis of these three patterns of cortisol response suggest different underlying physiological processes and indicate avenues for alleviation of that pain-induced distress.

3.4.1 The response to dehorning

Amputation dehorning elicited a marked biphasic elevation in plasma cortisol concentrations which lasted for about six hours. There were no significant differences in the timecourse or integral of the cortisol response according to which dehorning method was used. It is not surprising that there were no significant differences in the peak magnitudes, durations, patterns or integrated cortisol responses amongst cattle dehorned by different methods (Table 3.1). This is because the methods employed involved similar surgical amputation of the horn and presumably similar trauma. Two observations suggest that the extent of the insult, or the different nature of the insult, may have affected the cortisol response. First, scoop dehorning, which caused the deepest wounds and was the only treatment where the frontal sinus of the skull was sometimes perforated, elicited cortisol responses which had the biggest (non-significantly) integrated cortisol response and peak-magnitude. Second, dehorning with the guillotine shears, which resulted in the most superficial wounds, elicited mean cortisol responses which a) at 2 and 2.5 hours after treatment were significantly less

than those for the other dehorned groups, and b) had the smallest (though non-significant) integrated response. The size and ergonomics of the guillotine shears used did not allow a deeper cut to be made. The effect of wound depth was subsequently investigated by other members of our husbandry distress research team (see below, McMeekan *et al.*, 1997).

The possibility of a synergistic effect upon the cortisol response of sampling animals in pain, either as a result of increased pain from the sampling procedure *per se*, or as a result of an increase in the negative psychological component because of the association of that pain with the operators (Hemsworth *et al.*, 1994) was discounted (Lester *et al.*, 1991b). Lester *et al.* (1991b) commenced blood sampling at different periods after surgical castration and tailing in lambs, and detected no effects of the sampling procedure on the cortisol response. They suggested that this may be because either sampling was a benign procedure, or because the most noxious stimulus, in their case surgical castration and tail docking, tended to dominate lesser noxious inputs such as sampling (Lester *et al.*, 1991b).

3.4.2 ACTH (maximal frame of reference)

Our understanding of the dynamics at each level of the HPA-axis on cortisol secretion is still rudimentary. In cattle treated with a series of increased doses of ACTH, plasma cortisol concentrations increase to a maximal value, after which further increases in ACTH dose only lengthen the duration of the plateau (Keller-Wood *et al.*, 1983; Keith *et al.*, 1986; Verkerk *et al.*, 1993). This plateauing of cortisol concentrations demonstrates that the adrenal cortex cannot secrete more cortisol in response to further increases in doses of ACTH, that is, maximal stimulation of the adrenal cortex has occurred, and by inference reflects the "maximal secretory capacity" of the adrenals to exogenous ACTH. In the present study, ACTH administration resulted in a plateauing of high plasma cortisol concentrations, which indicates the maximum cortisol secretory capacity of the adrenals. Whilst this demonstrates that the adrenal gland is responding to ACTH stimulation appropriately, it reveals nothing about the functioning of the HPA-axis proximal to the adrenals. It may be more informative to stimulate the HPA-axis at the higher level of the pituitary gland through the administration of corticotropin-releasing factor. The half hour delay in the appearance of maximal cortisol concentrations after the ACTH bolus presumably reflects the time required for the synthesis of steroid hormones (Goldstein *et al.*, 1992).

Comparison of dehorning and ACTH responses

The observation that the integrated cortisol responses for all four dehorning treatments were not significantly different from those after ACTH administration is fortuitous. This is because increasing the dose of ACTH only lengthens the duration of the plateau of

maximal cortisol concentrations (Keller-Wood *et al.*, 1983; Keith *et al.*, 1986; Verkerk *et al.*, 1993) and concomitantly increases the integrated response. However, comparing the timecourses of the cortisol responses is informative.

The rate of rise of plasma cortisol to maximal values and those maximal values attained after dehorning were similar to those after ACTH injection. Evidently dehorning caused maximal stimulation of both endogenous CRF and endogenous ACTH secretion during the first 30 minutes. It follows that dehorning was extremely distressing. In addition, this similarity in the initial cortisol response suggests that, in this case, the dynamics of the cortisol response was similar regardless of whether from nociceptive or exogenous ACTH stimulation.

The ACTH curve has an exponential decay indicative of a first order reaction process removing cortisol from the blood (Endrenyi, 1989). The decrease in cortisol concentrations in ACTH calves reflects the biological removal of both exogenous ACTH and endogenous cortisol as the levels of that ACTH returned to basal values. Whereas the ACTH calves exhibited a single phase decrease, the decrease in cortisol concentrations after dehorning had three phases. The initial rate of decrease from maximal cortisol concentrations in dehorned calves, which occurred between 0.5 and 1.5 hours, had a similar timeconstant as that of the ACTH calves and presumably likewise represented the biological removal of cortisol. In the case of dehorned calves the cortisol surge was elicited by the nociceptive barrage from tissue damage. The fall in the rate of decrease of cortisol concentrations in dehorned calves after 1.5 hours indicates the appearance of a secondary stimulus to cortisol secretion which was presumably elicited by continuing nociceptive impulses from inflamed tissues. After three hours the rate of decrease in cortisol concentrations increased again and levels returned to pretreatment values by six hours after treatment.

3.4.3 Control (minimal frame of reference)

The duration of the cortisol response after control handling and bleeding was 1.5 hours, a period which is similar to that reported in other studies on calves (Laden *et al.*, 1985; Allyn, 1991). That the integrated cortisol response was 67 to 75% less than that after dehorning, and was resolved 4.5 hours earlier, and that the rate of decrease from maximal values in the Control calves was similar to that in ACTH calves (suggesting the elimination of an initial surge of cortisol, as opposed to any on-going stimulus to cortisol secretion), all combine to indicate that the control experience, and by inference the experimental situation, was relatively benign. It is likely that any stress associated with handling and bleeding was transient and was primarily due to the novelty (Mason, 1968) of the situation.

3.4.4 Biphasic pattern of response to dehorning

There were three distinct patterns in the timecourse of the plasma cortisol concentrations after ACTH, dehorning, and control treatment respectively. Comparison of these three patterns of cortisol response provides insight regarding the underlying physiological processes and indicates avenues for alleviation of that pain-induced distress. Before analysing this, a discussion is presented of the events following tissue injury, including the anatomical and physiological substratum of pain, inflammation, healing and the 'stress' response. This following section is sourced from *The Management of Pain* edited by Bonica (1990a) which uses information from animal and human studies.

Physiological and anatomical substrates of pain

Acute pain is a complex constellation of unpleasant sensory, emotional, and mental experiences and autonomic, psychological and behavioural responses provoked by tissue damage. Injury to tissues, whether induced by disease, accident or surgical operations, constitutes a noxious stimulus and causes the release of intracellular biochemical (algogenic) substances. Noxious stimuli and these endogenous algogenic substances activate specialized high-threshold sensory receptors, called nociceptors, which transduce the stimuli into nociceptive impulses that are transmitted via various relays in the neuraxis to the central nervous system. At each relay station various autonomic, neuroendocrine, psychological or behavioural responses are initiated aimed at maintaining the body's integrity. In addition, at these relay stations the ascending nociceptive impulses are subjected to modulation from higher centres in the brain. The final perception of pain, including its sensory, evaluative and emotional aspects, and the responses to it are a complex synthesis of these inputs and modulation from the ascending and descending pathways.

Nociceptive impulses are transmitted to the central nervous system in peripheral nerves, the nerve fibres of which are classified according to conduction characteristics. With minor exceptions all nociceptive afferents conduct impulses in high threshold A delta (Aδ) and C fibres. The sensory terminal endings, the nociceptor, have no specialized structures, rather they are free nerve endings, which respond to either mechanical, thermal or chemical stimuli or a mixture of these. Nociceptors are supplied to the skin, subcutaneous tissue, fascia, muscle, periosteum, joints, bones and muscle. In human skin 95% of cutaneous nociceptors are located in the sub-epidermis, and 5% in the epidermis (Bonica, 1990c). The periosteum of bone is richly supplied with Aδ and C fibres, which run along with the blood vessels in the Haversian canals. The cancellous portion of bone has few nociceptors, and the cortex and marrow is not normally pain sensitive (Bonica, 1990c).

Injury induced nociceptive afferent input enters the neuraxis by way of the spinal or medullary dorsal horn, where second order neurones conduct the information either to higher centres or laterally to stimulate spinal reflexes. The dorsal horn is not just a simple relay station, but is a site for complex local processing of the ascending nociceptive information. This is achieved by the phenomena of central convergence and central summation of excitatory and inhibitory influences coming from the periphery, local interneurones, and from higher centres.

After modulation in the dorsal horn, some nociceptive impulses pass via second order neurones to the anterior ipsilateral horn where they stimulate somatomotor and preganglionic sympathetic neurones to provoke autonomic segmental nocifensive reflex responses. Somatomotor stimulation produces reflex withdrawal and skeletal muscle tension which becomes a new source of nociceptive impulses and reinforces the existing nociception. The stimulation of sympathetic fibres results in peripheral vasoconstriction especially in the skin and splanchnic regions, increased cardiac output and blood pressure, and decreased gastrointestinal tone. Locally the decreased microcirculation in the injured and adjacent tissues may become ischemic and generate additional nociceptive impulses.

Other nociceptive impulses are transmitted to second order neurones which pass to the contralateral ventral horn and join the ascending tracts to the brain. Nociceptive impulses originating from below the head enter the spinal dorsal horn and are transmitted to the central nervous system in three ascending pathways - the spinothalamic tract, spinoreticular tract, and spinomesencephalic tract - collectively termed the anterolateral fasciculus. In contrast, nociceptive impulses from the head originate primarily in the peripheral sensory distribution of the four cranial nerves (5, 7, 9, 10) and the first three cervical nerves. These nociceptive impulses activate second order neurones in the trigeminal brain stem nuclei and the spinal dorsal horn, which relay the information via the ventral trigeminothalamic tract to other sites in the central nervous system including the reticular activating system, thalamus, hypothalamus, periacqueductal gray, limbic system and cerebral cortex. The anatomy, physiology and biochemistry of the ventral trigeminothalamic tract is similar to the anterolateral fasciculus and they appear to terminate in similar regions of, though sometimes different nuclei in, the brain.

Suprasegmental (i.e. supraspinal) reflex responses result from nociceptive-induced stimulation of the medullary centres of ventilation and circulation, of hypothalamic autonomic and neuroendocrine centres, and limbic structures. The resultant increased sympathetic tone and accompanied catecholamine secretion enhances the effects of the spinal reflexes and further increases total peripheral resistance, cardiac output and blood

pressure. There is also an increased secretion of catabolic hormones including ACTH, cortisol and glucagon, as well as plasma cyclic adenosine mono phosphate, adenosine diphosphate, and a reciprocal decrease of anabolic hormones such as insulin. These neuroendocrine responses cause an increase in metabolism through increased concentrations of blood glucose and free fatty acids, effectively mobilizing substrates from storage to central organs and damaged tissues. Such responses are characteristic of the 'stress' response.

The degree and duration of these metabolic and endocrine changes are related to the degree and duration of tissue damage. Intense anxiety and fear can be an integral part of the pain experience and response, and can greatly enhance the hypothalamic responses through stimulation from higher cortical centres. Finally, acute pain following tissue damage is usually a self-limiting experience due to progress of the healing processes.

Components of the response to dehorning

The cortisol response to dehorning is a neuroendocrine reflex elicited by nociceptive stimulation of the HPA-axis integrated with negative emotional or evaluative enhancement from higher centres. The cortisol timecourse after dehorning was biphasic whereas that after Control and ACTH treatment was monophasic. The following logical speculation on the constituents of the cortisol response to dehorning derived from comparison of the dehorned, Control and ACTH cortisol responses (Fig 3.9), suggests underlying physiological processes and indicates possible avenues for alleviating that distress.

Rise to maximal

The plateauing of high cortisol concentrations after ACTH injection indicates maximal cortisol secretion of the adrenal gland (see section 3.4.2). The rate of rise and the maximal concentrations attained in dehorned and ACTH calves were similar and indicate that amputation dehorning elicited maximal stimulation of the adrenal cortex and was, by inference, extremely distressing. The rapid rise in plasma cortisol concentrations after dehorning was presumably elicited by the afferent barrage of nociceptive input from cut and damaged nerves, the ensuing nociceptive impulses generated by the release of algogenic substances from damaged tissues plus any psychological effect of that nociceptive input.

Decrease from maximal

The ACTH curve has an exponential decay indicative of a first order reaction process removing cortisol from the blood (Endrenyi, 1989). The decrease in cortisol concentrations in ACTH calves reflects the biological removal of both exogenous ACTH and endogenous cortisol as the levels of that ACTH returned to basal values. The

initial rate of decrease from peak cortisol concentrations in the Scoop and the Control responses has a similar timeconstant as that of the ACTH response, indicating the same underlying physiological cortisol reaction process.

Sub-maximal plateau

The deviation from exponential decay which occurs at around 1.5 hours after dehorning indicates the generation of new cortisol (see Fig 3.10). This is taken as evidence of on-going stimulation to cortisol secretion presumably from nociceptive impulses from inflamed tissues, which is represented by area B in Fig 3.10.

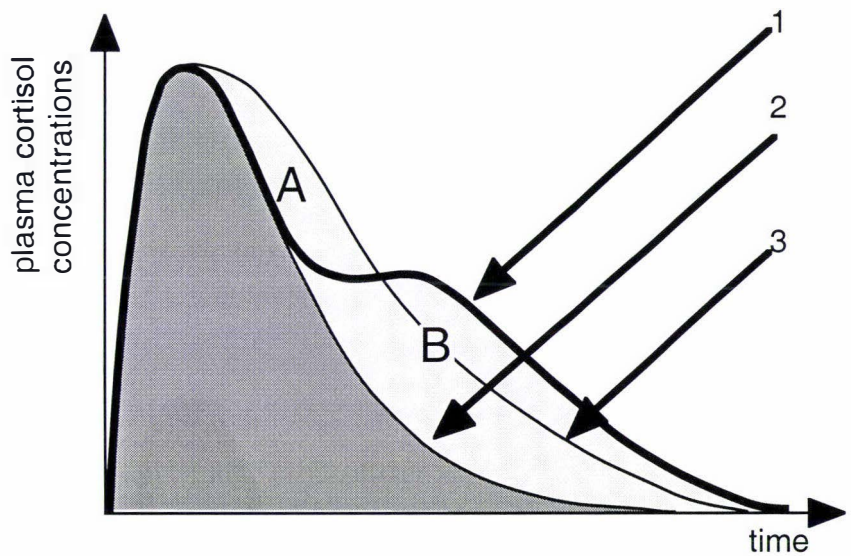


Fig 3.10 Schematic diagram of the cortisol timecourse after dehorning (curve 1). The biphasic shape suggests either the response would have followed the curve 2, but for the addition of area B (from inflammatory pain), or less likely the response would have followed curve 3, but for the loss of area A (as a result of stress-induced analgesia).

The biphasic cortisol response corresponds with the observation that tissue damage produces two phases of sensory input. The first is associated with the tissue damaging stimulus, i.e. during surgery, and the second is from the inflammation reaction to the damaged tissue. In addition, the biphasic pattern of the cortisol response corresponds to Woolf and Chong's (1993) theoretical graphs of nociceptor input depicting these two phases. It may be the case that 1.5 h after dehorning is an important time in the inflammatory process when there is an increase or change in the nature of the pain of inflammation, or both. The constituent of the response elicited by inflammation could be derived by examining the effect of an anti-inflammatory agent *versus* local anaesthetic on the cortisol response.

Alternatively, the deviation from exponential decay might be an artifact of a loss of area A (Fig 3.10). That is, the response would have followed curve 3 but for the loss of area A as a result of some effect that has waned by about three hours after treatment. The loss of area A may result from a) some limitation of cortisol secretion under physiological conditions; or a decrease in nociceptive input either b) at the level of the primary afferent input; or c) from higher centres at any nociceptive relay in the neuraxis as a result of descending inhibition. The latter is termed stress-induced analgesia (Carmody, 1992). There appear to be two forms of this. One is opiate based and the other is non-opiate based (see section 1.3.3). The presence of stress-induced analgesia may be inferred by: 1) a decreased response to a noxious stimulus at a site remote from the tissue damage; or 2) the enhancement of a "stress" response after the administration of antagonists to nociception inhibition. Such drugs include opioid antagonists, such as Naloxone, and non-opioid antagonists, such as an alpha-2 adrenergic and serotonergic antagonists. It is more likely and parsimonious that, rather than nociceptive inhibition resulting in the loss of area A, it is the pain of inflammation resulting in the appearance of area B that is the *dominant* force shaping the cortisol response curve (Fig 3.10).

Decrease to pretreatment levels

The degree and duration of the metabolic and endocrine changes subsequent to tissue injury are related to the degree and duration of tissue damage. Acute pain following tissue damage is usually a self-limiting experience due to progress of the healing processes. By six hours after dehorning cortisol concentrations have returned to pretreatment levels. This indicates that either the pain, or stress, or both, have subsided to levels that do not stimulate, or attain the threshold of, the stress response. At this stage the wound is not healed, and we would expect the continued presence of pain, though of a less intense magnitude. Presumably the quality and magnitude of pain at this time serves to promote healing behaviours (e.g. rest) and wound healing physiological processes (e.g. anabolism).

3.4.5 Choice of dehorning method

The decision as to which method of dehorning will be employed will involve considerations of practicality including ease of use, labour-costs, health and safety issues and cattle welfare. In the current study, only the acute distress response to dehorning was examined. Dehorning complications such as wound healing and infection, and the long term efficacy of the different methods were not examined.

It is noteworthy that 36h after treatment the plasma cortisol concentrations were at pretreatment values. This suggests that any pain experienced on the second day was of a low level. However, amputation dehorning wounds have been reported to take 4-6 weeks to heal (no definition given) (Loxton *et al.*, 1982), or 14 weeks to heal (by

epithelial migration) (Kihurani *et al.,* 1989). Personal experience tells us that wounds still hurt after 36h. It may be the case that the pain at 36h is much less than, or of a different nature to, that experienced in the first six hours. Alternatively the calf may still be in pain but may have habituated to the pain.

Amputation dehorning is carried out when cautery disbudding is not possible, that is on calves older than 8-12 weeks of age. The wounds inflicted are large, there may be considerable blood loss and often the frontal sinus of the skull is perforated. The presence of an open wound may increase the chance of horizontal transmission of blood borne pathogens (DiGiacomo *et al.,* 1987) and casual infections (Allyn, 1991) which impair wound healing especially in the wet season.

It is our experience that the various dehorning tools differ in their ease of use. The scoop is designed with ergonomics in mind, such that the cutting force is achieved with a push rather than a pull and thereby allows the use of body weight rather than the need for muscular force. The guillotine shears, saw and embryotomy wire require more effort to cut off the horn. The embryotomy wire results in less blood loss (Oehme, 1974; Owen, 1984; Weaver, 1986) but is exhausting to use. The latter three methods may be used on larger horns. By contrast, disbudding by cautery, which can only be done on calves less than 6-8 weeks old, is a simpler, cleaner and more sterile procedure (Allyn, 1991) than other modes of dehorning. There is no blood loss, and because there is no open wound it may be performed at any time of the year. Subsequent research showed that cautery disbudding was less distressing, as assessed by cortisol responses, than amputation dehorning in six-week-old calves (Petrie *et al.,* 1996b).

3.4.6 Critique of the experimental design

The experimental conditions used here including repeated handling and blood sampling and confinement, may have caused additional distress. Of these, repeated handling and blood sampling was the least likely to cause distress. This conclusion is based upon the following: regression analyses showed that there was no effect of repeated handling upon cortisol concentrations (Fig 3.5); pretreatment cortisol concentrations were similar to those reported previously (Herd, 1989) and for the Control calves these concentrations had returned to pretreatment levels by 1.5 hours. Furthermore, Lester *et al.* (1991b) demonstrated that repeated blood sampling and handling had no effect upon the cortisol response of lambs to surgical castration and tailing. Confinement in the yards presumably inhibited the activities of the calves, which normally spend about 33% of their time grazing and walking, 33% ruminating and 33% being idle (Fraser and Broom, 1990), and this may have caused more distress than might have occurred had the cattle been in their usual habitat. For these reasons it is possible that the cortisol responses in the present study were conservative and overestimated the distress caused

by the treatments. The use of remote portable monitors with indwelling catheters, which obviate repeated handling and allow the cattle to be studied in their normal habitat, may reveal attenuated distress responses (Ingram *et al.*, 1994).

3.4.7 Conclusions

Amputation dehorning of six-month-old calves elicited a marked, biphasic distress response that lasted for about six hours. Amputation dehorning resulted in similar cortisol responses regardless of the amputation method employed, although there is a suggestion that the depth of the wound may affect the level of distress. The plateauing of maximal cortisol concentrations after the ACTH bolus indicated that maximal secretory capacity of the adrenal gland was reached. The rate of increase and the maximal cortisol levels attained after dehorning and ACTH injection were similar indicating that dehorning caused maximal cortisol secretion by the adrenal gland and was, by inference, very distressing. The cortisol response after dehorning appears to be biphasic. The appearance of a sub-maximal plateauing of cortisol concentrations between 1.5 and 3 hours suggests the occurrence of either stress-induced analgesia, or more likely the appearance of a second phase of pain presumably from inflammation.

3.4.8 Epilogue

Subsequent studies confirmed that the cortisol response to scoop dehorning lasted about six to seven hours (Petrie *et al.*, 1996b; McMeekan *et al.*, 1997, 1998a,b). Further, as a result of the present finding regarding the similarity of cortisol responses for different dehorning methods, subsequent amputation dehorning work used the scoop only because of its ease of operation. The findings in this thesis regarding the depth of wound led to a specific study of shallow versus deep scoop dehorning, which subsequently demonstrated no significant effect of scoop wound depth (McMeekan *et al.*, 1997). Finally, the cortisol timecourse after dehorning was subsequently investigated to see if it was possible to distinguish the presumed phases of amputation pain versus inflammation-related pain in that response (McMeekan *et al.*, 1998b). These subsequent works are discussed in more detail in the General Discussion.

4 CORTISOL RESPONSES OF CALVES TO SCOOP DEHORNING WITH LOCAL ANAESTHESIA AND/OR CAUTERY OF THE WOUND

4.0 ABSTRACT

Dehorning of cattle is a routine husbandry practice which causes significant and marked distress that lasts for about six hours (Chapter three). There has been no comprehensive research into alleviation of that distress. Changes in plasma cortisol concentrations were used to assess the pain-induced distress in Friesian calves (six-months old, about 130 kg, n=59) during the first 9 hours and at 36 h after amputation dehorning with local anaesthesia (LA) and/or wound cautery. There were six treatment groups: Control, LA-Control, Scoop, LA-scoop, Scoop+cautery and LA-scoop+cautery. Calves were held overnight in cattle yards with access to water but not food. On the day of the study, calves were studied in mobs of 12 with equal representation of treatments in the mob. Blood was taken by venipuncture from either jugular vein while calves were held against the walls of the pen. Treatment was conducted in a cattle race. Local anaesthesia significantly reduced the cortisol response to dehorning by 50% ($P<0.05$). Cauterizing the scoop wound effected a marginal reduction in the cortisol response compared to dehorning alone ($P>0.05$). The combination of local anaesthetic plus cauterizing the scoop wound virtually abolished the cortisol response to amputation dehorning. These results suggest that local anaesthetic should be administered before dehorning and that if possible the scoop wound should be cauterized. However, further research is needed to determine the duration of the apparent cautery-induced analgesia, its effects upon wound healing and the practicalities of the additional handling and care required to carry out this procedure.

4.1 INTRODUCTION

Dehorning of cattle is a routine husbandry practice performed to reduce injury to stock handlers and stock, to reduce economic losses through hide and carcass damage and to improve husbandry. The dehorning of cattle is a welfare issue because it causes significant and marked distress that lasts for about six hours, as judged from plasma cortisol concentrations (Chapter three). It is likely that the cortisol response elicited by dehorning is due to pain and may therefore be alleviated by applying strategies which alter the perception of pain such as systemic analgesics, general anaesthetics, sedatives and tranquillisers, or strategies which decrease the transmission of nociception from the wound such as local or regional anaesthetics and possibly cauterising of the wound, or by a combination of these techniques. Alternatively, there may be a method of dehorning which evokes less distress than other methods (Chapter three). At the time this work was

done little research had been conducted on the assessment or alleviation of the pain-induced distress caused by amputation dehorning.

In accordance with the increasing concern for animal welfare the Animals Protection Act (1960) was repealed by the Animal Welfare Act (1999). Transitional Provisions (sections 201, 202) have continued the effect of the Animals Protection Act (1960) as it applies to dehorning for up to a further three years. Currently it is an offence to dehorn cattle over the age of 20 months unless the operation is conducted under anaesthesia of sufficient power to prevent the animal from feeling pain (section 3y, Animals Protection Act 1960). Cattle younger than 20 months have no such legislative protection. However, it is likely that this arbitrary age limit will be decreased to a no less arbitrary age limit of three months (K.J Stafford, and D.J Mellor, personal communication).

It is possible that the different methods of dehorning may evoke dissimilar distress responses because of variations in the nature or magnitude of the insult. The cortisol responses of six-month-old calves dehorned by scoop, saw, guillotine shears, or embryotomy wire were similar, however, there was an indication that the guillotine shears may cause slightly less distress than the other methods, possibly because the wound is more superficial (Chapter three).

Local anaesthetics are drugs that block all types of nerve conduction when applied locally to nerves in appropriate concentrations. Thus they cause both sensory and motor paralysis in the area with no loss of consciousness or impairment of central control of autonomic functions. Effective loss of sensory input to the brain from the bovine horn and the surrounding tissue is accomplished by a regional nerve block of the cornual nerve (Weaver, 1986) (see Fig 4.1). Local anaesthetic has been shown to virtually eliminate the cortisol and behavioural responses after ring castration and tailing in lambs (Wood *et al.*, 1991).

The rationale for cauterizing wounds as a means of alleviating pain comes from historical anecdotes (Lord Nelson, 1810), medical literature (Freund and Marvin, 1990), and previous work done in this laboratory (Lester *et al.*, 1991a). Prior to the advent of modern medicine it was common practice to cauterize wounds for the purposes of haemostasis and anti-sepsis. Patients suffering from third degree burns report less pain than those suffering from second or first degree burns (Freund and Marvin, 1990). This is because the nociceptors have been destroyed (Freund and Marvin, 1990). Lester *et al.* (1991a) reported a lower acute cortisol response after docking lambs tails with the heated docking-iron than when a knife was used. They attributed this to a beneficial by-product of the docking iron which through cauterizing the wound, may destroy nociceptors at the wound, and thus decrease pain transmission and accordingly distress.

Cauterizing the wound may therefore alleviate the distress associated with some surgical husbandry practices. Husbandry tools which incorporate this cauterizing effect include docking irons, hot iron brands, disbudding irons and, possibly, the embryotomy wire - which heats up when it is used (Oehme, 1974; Owen, 1984).

4.1.1 Aims of study

The aims of the current study were to investigate the alleviation of amputation dehorning distress. In particular the objectives were; 1) to characterize the cortisol response elicited by amputation dehorning in six-month-old cattle; 2) to investigate the effects of local anaesthesia on the cortisol response to dehorning; 3) to investigate whether cauterizing the dehorning wound confers any benefit on the cortisol response to dehorning; 4) to ascertain whether the effect on the cortisol response to dehorning with local anaesthesia combined with wound cautery is additive, multiplicative or whether one feature of the treatment dominates. It is intended that this research will proved scientific data to aid in the writing of welfare codes and advisory material concerning the dehorning of cattle. In addition, this study may provide further insight into the physiology of pain-induced distress which could have application to other husbandry procedures.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design

The major features of the experimental design were the same as that described in Chapter three, except that 10 minutes after the pretreatment blood sample, local anaesthetic was administered to those calves scheduled to receive it, and a second pretreatment blood sample was taken fifteen minutes later from all calves. Thereafter there were no differences except for the treatments. The main features were as follows:

Fifty-nine male Friesian calves aged five to six months, weighing 101-149 kg (mean 128 kg) and scheduled for dehorning according to usual farm practice, were used in this study. Prior approval to conduct this study was obtained from the Massey University Animal Ethics Committee. The cattle were divided into five mobs of 12 calves, with an even distribution of calves receiving the different treatments represented in each mob. The study was carried out over four days in February 1992.

The evening before each experimental day at 2000 h, two mobs each of 12 calves were randomly selected from the herd, numbers were painted on their backs for identification purposes, and they were penned overnight outdoors in cattle yards (10 x 10 m) with access to water but not to food.

On each experimental day investigation began in the first mob at 0700 h and the second mob at 1100 h. Each mob of 12 calves was quietly moved into a small pen (3 x 4 m) where a pretreatment blood sample was taken. Ten minutes later 6 ml of a local anaesthetic were administered to each cornual nerve for those animals scheduled to receive it. Fifteen minutes later the efficacy of anaesthesia was tested, and if required another dose of local anaesthetic was administered to that cornual nerve. At this juncture another blood sample was taken from all calves. This extra blood sample was taken in order to check for the effect of the administration of the local anaesthetic on plasma cortisol concentrations. Fifteen minutes later the calves were moved into an adjacent cattle race where the treatment was performed. Immediately after treatment they were returned to the small pen where blood samples were taken with decreasing frequency throughout the subsequent 9 hours (see below). Between 3 and 9 hours after treatment, when sampling was at hourly intervals, the calves were moved into an adjacent larger yard (10 x 10 m) where water was available *ad libitum*. When required for sampling they were quietly moved back to the smaller pen. The duration of blood sampling on each occasion was about 12 minutes for the mob.

At the end of the 9 hour sampling period the two mobs of calves were reunited and returned to pasture overnight. The following afternoon (1600 h) they were mustered into

the yards and left to settle for three hours (Sylvester *et al.*, 1993. Appendix A), after which a blood sample was taken, the time of that sample being about 33 or 36 hours after treatment.

This whole procedure was repeated over four consecutive days in different calves. During the course of this experiment, the mustering of other stock nearby, at 1600 h and 1900 h on each day, elicited some interest from the experimental animals.

4.2.2 Blood sampling

Blood samples (10 ml) were taken by venipuncture from either jugular vein. Blood samples were taken at 0.66 (pretreatment) and 0.25 h before treatment, and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 36 h after treatment. Intensive blood sampling was carried out during the first 9 hours because a previous study had shown that plasma cortisol concentrations returned to pretreatment values by about 6 hours after dehorning (Sylvester *et al.*, 1993. Appendix A), and a 36 h sample was taken because it was not known whether dehorning caused a long-lasting increase of plasma cortisol concentrations. Blood samples were taken by one person whilst the calf was restrained against the yard wall by three other people. This procedure usually took less than 45 seconds for each calf. Calves were bled in the same order each time to test for possible effects of the blood sampling order on plasma cortisol concentrations.

4.2.3 Treatments

The calves were randomly allocated to one of six treatments which were equally represented in each mob of 12 calves. Each calf was restrained manually for treatment while the mob was packed closely together in a race. One person held each calf's head firmly whilst another performed the treatment. The two operators moved down the race dealing with each calf in succession. Between animals, dehorning implements were disinfected (Hibitane concentrate 5%. Hospital Products, ICI Australia). The order of treatment was the same for each mob in order to test for possible effects of the treatment order on plasma cortisol concentrations. The time of treatment was defined as time zero for each calf.

Control n=10

Calves were handled in the race to simulate the restraint required for dehorning but without being dehorned.

Local Anaesthetic Control (LA-Control) n=10

Local anaesthetic was administered to calves 0.5 h before Control treatment as above. Local anaesthetic (6 ml of 2% lignocaine hydrochloride. Ethical Agents ltd, Auckland, New Zealand) was injected near each cornual nerve where it crosses between the

postorbital bar and the horn (Weaver, 1986) (see Fig 4.1). The cornual nerve receives sensory information from the horn and the immediately surrounding tissues.

Anaesthesia, which occurs 10-15 minutes after lignocaine administration (Ritchie and Greene, 1990), was tested by pricking the skin around the horn with a hypodermic needle and observing if there was a response (for instance ear, eye or head flinches). If a response to this stimulus did occur, another 6 ml of local anaesthetic were injected near the corresponding cornual nerve.

Scoop n=10

Calves were dehorned using an industry standard dehorning scoop (Barnes Dehorners, Stones, USA). The scoop (Fig 3.1) consists of two interlocking semi-circular blades (diameter about 45 mm) which when engaged removed the horn and the adjacent cutaneous tissue, including the germinal tissue of the horn. This procedure often resulted in the frontal sinus of the skull being perforated. The amount of horn and skin tissue removed during dehorning depended on the horn size and head movements.

Local Anaesthetic followed by Scoop (LA-Scoop) n=9

Local anaesthetic was administered and it efficacy was tested as described for LA-Control calves, after which the calves were dehorned as described for Scoop calves.

Scoop and Cautery (Scoop+Cautery) n=10

Calves were dehorned as described for Scoop calves, and immediately afterwards each wound was cauterized with a custom built iron which was heated over a gas flame. The cauterizing iron was shaped as a half sphere with a diameter of 30 mm (Fig 4.2). When sufficiently hot to burn wood it was applied firmly over the wound to cauterize the tissues. This usually took about 6 seconds per wound.

Local Anaesthetic followed by Scoop and Cautery (LA-Scoop+Cautery) n=10

Local anaesthetic was administered and its efficacy was tested as described for LA-Control calves, thereafter the calves were dehorned and the wound was cauterized as described for Scoop+Cautery calves.

4.2.4 Plasma Cortisol Analysis

Blood samples were collected in lithium heparinised vacutainers, chilled immediately, centrifuged and the plasma stored frozen at -20°C until analysed. Plasma cortisol concentrations were determined by radioimmunoassay using the technique Ruder *et al.* (1972) as modified by Evans (1979) [see Mac Diarmid (1982) and Appendix C]. The intra-assay and inter-assay coefficients of variation were 9% and 14% respectively. The smallest detectable concentration of cortisol was 0.3 ng/ml.

4.2.5 Statistical Analyses

Statistical analyses were conducted on 1) the non-transformed pretreatment cortisol concentrations, 2) the time-course of the cortisol responses after treatment, and 3) the integrated cortisol responses after treatment.

Cortisol data are expressed as the mean ± the standard error of the mean (SEM). The significance of the difference between the means was determined using a two-way analysis of variance (ANOVA) with Fisher's probability of least significant differences (PLSD). A probability of 5% or less ($P<0.05$) was considered to indicate a significant difference. Except for checking whether the data from different experimental mobs could be pooled, the cortisol concentrations were transformed as the arithmetic difference from the pretreatment value. The data were transformed in this manner to correct for individual variation or pre-study effects in baseline cortisol values. All statistical calculations were conducted using a standard statistical computer package (Statsview + Graphics, Version 2, Abacus Concepts Inc., USA).

1) Pooling the data

To ascertain whether the results from all experimental groups (mobs) could be pooled an ANOVA was conducted on the non-transformed pretreatment cortisol concentrations according to day, mob, and time. In addition, the possibility of a relationship between the pretreatment concentrations and the order in which the calves were sampled was examined using Pearson's correlation coefficient (r) or regression (r^2).

2) Timecourse of the cortisol response

To calculate when the cortisol response to a treatment was complete, i.e. when the cortisol concentrations returned to and remained at pretreatment levels, a factorial ANOVA design was used. To calculate if there were any differences between treatments in the mean cortisol timecourse, a repeated measures ANOVA design was used.

3) Integrated cortisol response

Numerical integration was used to calculate the total cortisol response, hereafter called the integrated cortisol response. The integrated cortisol response was defined as the area under the cortisol curve and above a line drawn horizontally through the pretreatment value (Mellor and Murray, 1989b). A factorial ANOVA was used to determine if there were any differences in the mean integrated cortisol responses, between treatments.

The strength of using the integrated cortisol response lies in the fact that it combines in one number the features of both magnitude and duration, and so provides a means for comparing the *total* cortisol response between treatments. The limitation of this

measurement is that the same number may represent both a short-lived large response or a protracted low or moderate response.

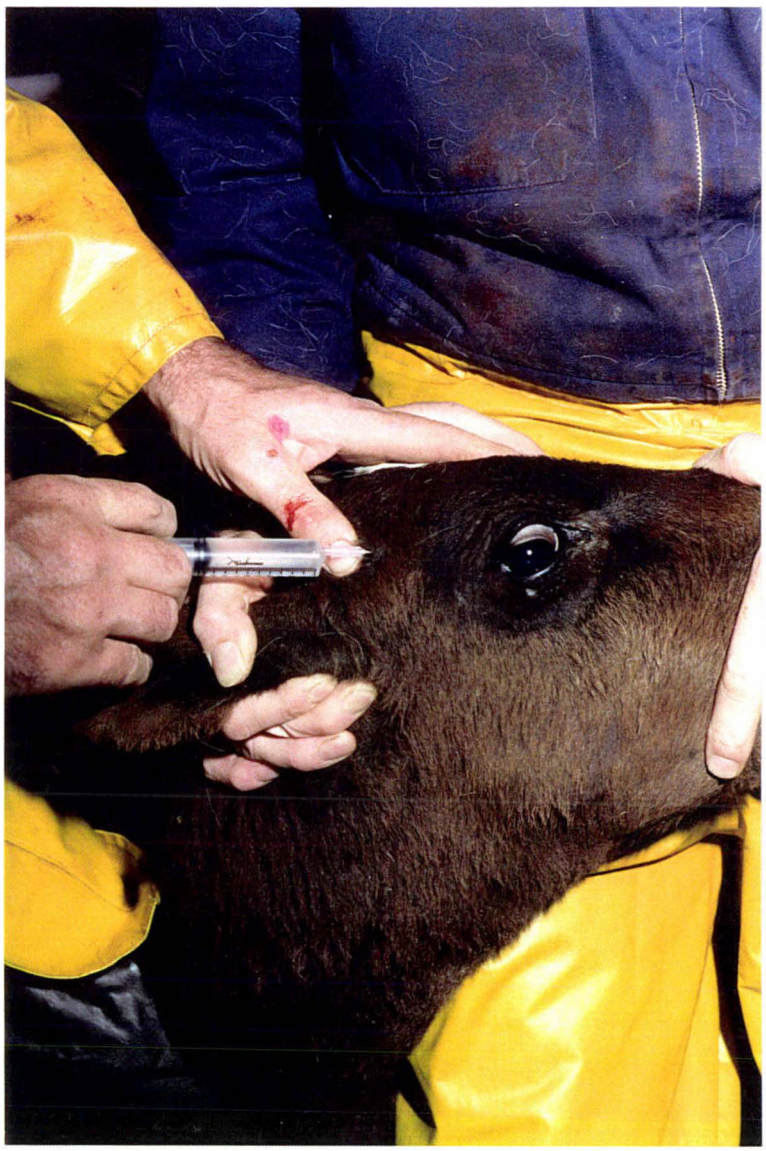


Fig 4.1 Injection of local anaesthetic. Photo courtesy of Cheryl McMeekan (McMeekan, 1997).

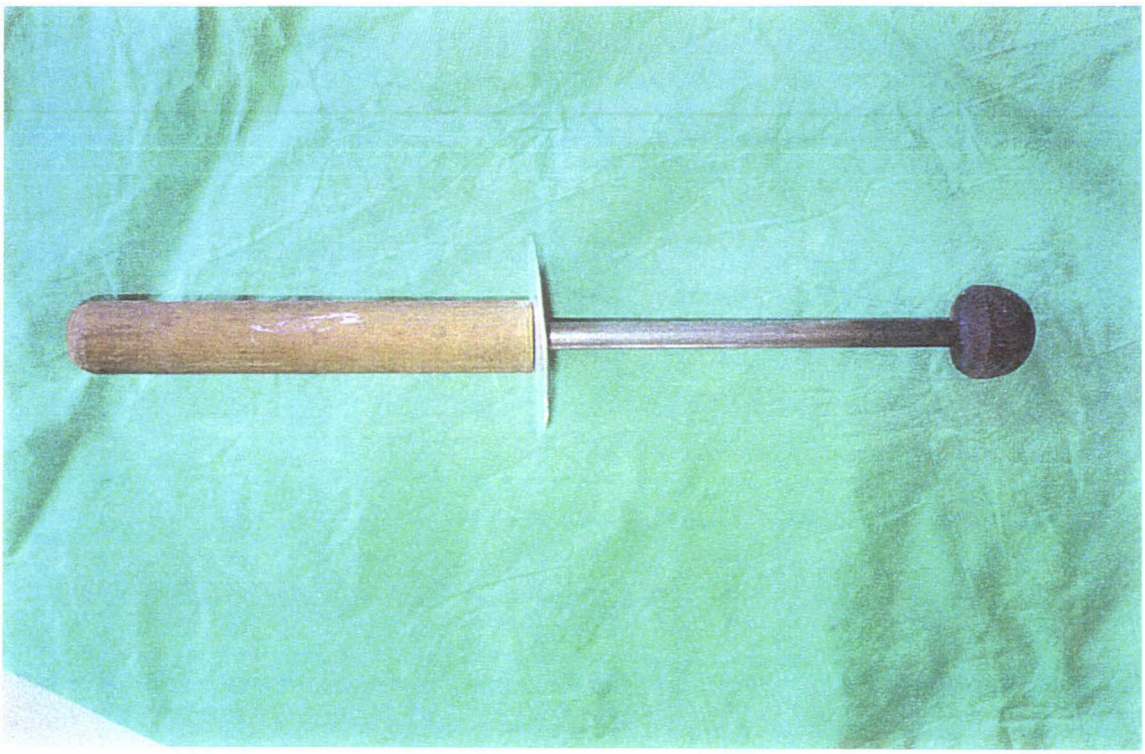


Fig 4.2 The cautery iron. (Photo from Sutherland, 1999).

4.3 RESULTS

4.3.1 Pretreatment Cortisol Concentrations

The non-transformed pretreatment plasma cortisol concentrations were examined for the effects of extraneous factors. There were no significant effects upon either the first or second pretreatment cortisol sample of days, treatments, mobs, or time of day (i.e. early versus late mobs) (Table 4.1) ($P>0.05$ in all cases). Nor was there a difference in the plasma cortisol concentrations of the second pretreatment sample between those calves which did (n=23) and those that did not (n=29) receive local anaesthetic ($P>0.05$). There was no linear correlation between the pretreatment concentrations and the order in which the animals were bled (first sample: $r^2=0.23$, second sample: $r^2=0.06$) (Fig 4.3). Therefore the data from all experimental mobs were pooled and used to calculate the mean response for each treatment. The overall mean pretreatment cortisol concentrations (n=52) for the first and second samples were 9.8 ± 0.7 and 19.5 ± 1.1 ng/ml respectively.

4.3.2 Data excluded from the analyses as outliers

The data from seven calves were excluded from the study as outliers. The determination of an outlier was made by satisfying two criteria which indicated that the calves were abnormally or excessively distressed prior to and/or throughout the study: 1) if examination of the cortisol timecourse showed a unique and aberrant pattern, and 2) where statistical analysis of the cortisol response found the pretreatment and/or the integrated cortisol response were greater than those encompassed by two standard deviations from the mean for that treatment. For calves #105 (LA-Scoop), #81 (LA-Scoop+Cautery), #70 (Control), #90 (LA-Control), and #66 (LA-Control) it was concluded that they were suffering from a previous distress prior to and during the study, which masked the effect of the treatment. For calves #79 (LA-Scoop) and #69 (LA-Scoop+Cautery) it was concluded that the local anaesthetic had *not* worked in decreasing the initial distress of dehorning. As individual differences are central to animal welfare science (Wiepkema *et al.*, 1987; Manteca and Deag, 1993; Broom, 1988) details of these responses are provided in Appendix B.

4.3.3 Cortisol responses to treatment

All calves showed significant transient increases in plasma cortisol concentrations after treatment, the magnitudes and durations of which varied according to the treatment.

Control and LA-Control

Control and LA-Control calves exhibited similar small but significant transient increases in plasma cortisol concentrations which returned to pretreatment levels by 1 hour after treatment. There were no significant differences between Control and LA-control calves in the peak increment (Control 8 ng/ml, LA-Control 11 ng/ml), duration, or integrated

response (0-9h: Control 19 ± 4 ng.hr/ml, LA-Control 22 ± 5 ng.hr/ml) (see Fig 4.4 and Table 4.1). For both, cortisol concentrations decreased below pretreatment values at 9 and 36 hours but this was significant only in Control calves.

Scoop

Scoop dehorning (Fig 4.5) elicited a marked significant transient increase in plasma cortisol concentrations, with the maximal increment (30 ng/ml) occurring 0.5 h after treatment. During the next hour, the mean cortisol concentrations decreased rapidly to values which plateaued between 1.5 and 3 hours. Thereafter cortisol concentrations continued to decrease returning to pretreatment levels by 6 hours after treatment. The integrated cortisol response (0-9h) was 105 ± 17 ng.hr/ml.

LA-Scoop

Calves which received local anaesthetic prior to scoop dehorning exhibited a *biphasic* response (Fig 4.6). There was an initial small but significant transient increase in mean plasma cortisol concentrations, the peak increment (15 ng/ml) of which occurred 15 minutes after treatment. Thereafter cortisol concentrations decreased and returned to pretreatment levels by 1 h and remained there until 3 hours after treatment. After 3 h cortisol concentrations rose again, the peak increment (10 ng/ml) of this second rise, which was of similar magnitude to the first rise, occurred at 5 h. However, the timecourse of this second rise was more protracted than that of the first rise. Cortisol concentrations then decreased and returned to pretreatment values by 6 h where they remained. The integrated cortisol response (0-9h) was 52 ± 14 ng.hr/ml.

Scoop compared to LA-Scoop and LA-Control

Prior application of local anaesthetic abolished the initial cortisol response exhibited by Scoop calves for the first three hours after treatment, so that the cortisol concentrations of LA-Scoop calves were similar to those of LA-Control calves during this period (Fig 4.6). After 3 hours the responses diverged, and whilst LA-Control calves remained at pretreatment values, the cortisol concentrations of LA-Scoop calves increased to levels similar to those in Scoop calves, and thereafter in both groups cortisol concentrations decreased in a similar fashion (Fig 4.7).

The integrated cortisol response (0-9h) of LA-Scoop calves (52 ± 14 ng.hr/ml) was 50% less ($P<0.05$) than for Scoop calves (105 ± 17 ng.hr/ml) and 2.4 times greater than for LA-Control calves (22 ± 5 ng.hr/ml ($P<0.05$) (Table 4.1).

Scoop+Cautery

Cauterizing the wound after scoop dehorning resulted in a marked significant transient increase in mean plasma cortisol concentrations, the peak increment (22 ng/ml) of which

occurred 0.5 h after treatment (Fig 4.8). Thereafter, mean cortisol concentrations decreased and returned to pretreatment values by 5 hours. Between 2 and 3 hours the rate of fall of cortisol concentrations decreased resulting in a plateauing of cortisol concentrations. The integrated cortisol response (0-9h) was 78 ± 20 ng.hr/ml.

Scoop+Cautery compared to Scoop

The *pattern* of the cortisol timecourse in Scoop+Cautery calves was essentially similar to that in Scoop calves. However, the cortisol concentrations in Scoop+Cautery calves were lower than those in Scoop calves at every sampling time, but only significantly so at 0.5, 2.5, and 3 hours after treatment (Fig 4.9). The plateauing of cortisol concentrations between 1.5 and 3 hours was less marked in Scoop+Cautery calves than in Scoop calves.

The integrated cortisol response (0-9h) of Scoop+Cautery calves (78 ± 20 ng.hr/ml) was 25% less than that for Scoop calves (105 ± 17 ng.hr/ml), but, this difference was not significant, presumably because of the high coefficients of variation (79% and 50%, respectively) (Table 4.1).

LA-Scoop+Cautery

LA-Scoop+Cautery calves (Fig 4.10) exhibited a small but significant transient increase in mean plasma cortisol concentrations, the peak increment (10 ng/ml) of which occurred at 0.25 h after treatment. The mean cortisol concentrations then decreased to pretreatment values by 1 hour after treatment where they remained, except for a small but significant increase ($P<0.05$) at 3 hours. The integrated cortisol response (0-9h) was 26 ± 7 ng.hr/ml.

LA-Scoop+Cautery compared to LA-Scoop and LA-Control

Local anaesthetic combined with cauterization of the scoop wound virtually abolished the second rise in cortisol concentrations exhibited by LA-Scoop calves between 4 and 6 h. The cortisol concentrations in LA-Scoop+Cautery calves were similar to those in the LA-control calves throughout the period of observation, and there were no significant differences in peak-magnitudes, durations, or integrated responses (see Fig 4.11 and Table 4.1).

The integrated cortisol response (0-9h) of the LA-Scoop+Cautery calves (26 ± 7 ng.hr/ml) was 50% less than that for the LA-Scoop (52 ± 14 ng.hr/ml) calves, however, these differences were not significant ($P>0.05$) (Table 4.1).

Comparison of the 36 h cortisol samples

For all groups the plasma cortisol concentrations 36 h after treatment were not significantly different from their respective pretreatment values. But, between treatments

those in the Control and LA Control calves were marginally lower from those in LA-Scoop+cautery, LA-Scoop, Scoop+cautery and Scoop calves (P>0.05) (Table 4.1) .

4.3.4 Test of duration of analgesia (skin prick test)

The needle-prick testing of the skin adjacent to the horn buds in the LA-Control calves indicated that sensation began to return to the area 2.5 to 3 h after treatment.

Of the 23 calves which were given local anaesthetic, eight required a second application on one side as anaesthesia was incomplete. After analysis of their cortisol responses, two calves were excluded as outliers as it was obvious that the local anaesthetic had not worked in decreasing the response to dehorning. Interestingly six of the seven calves which were excluded as outliers had received local anaesthetic.

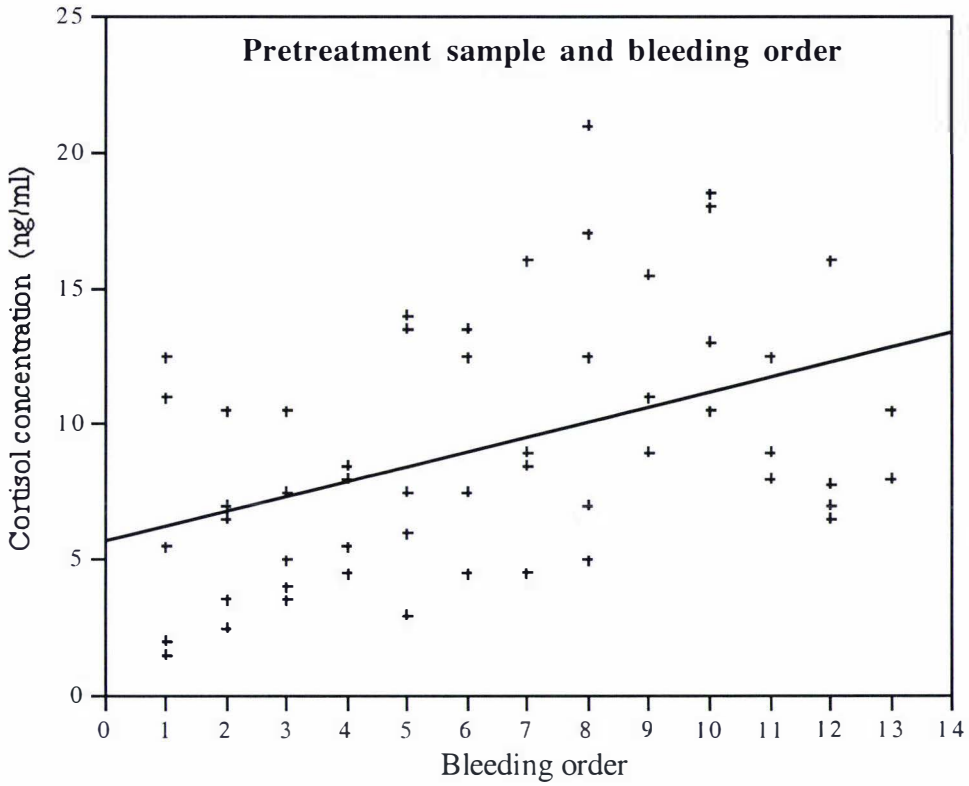


Fig 4.3 Relationship between the pretreatment plasma cortisol concentrations and the order in which the calves were sampled (bleeding order) (n=52). The straight line corresponds to a linear regression for which the correlation coefficient r^2=0.23 (i.e. weak correlation).

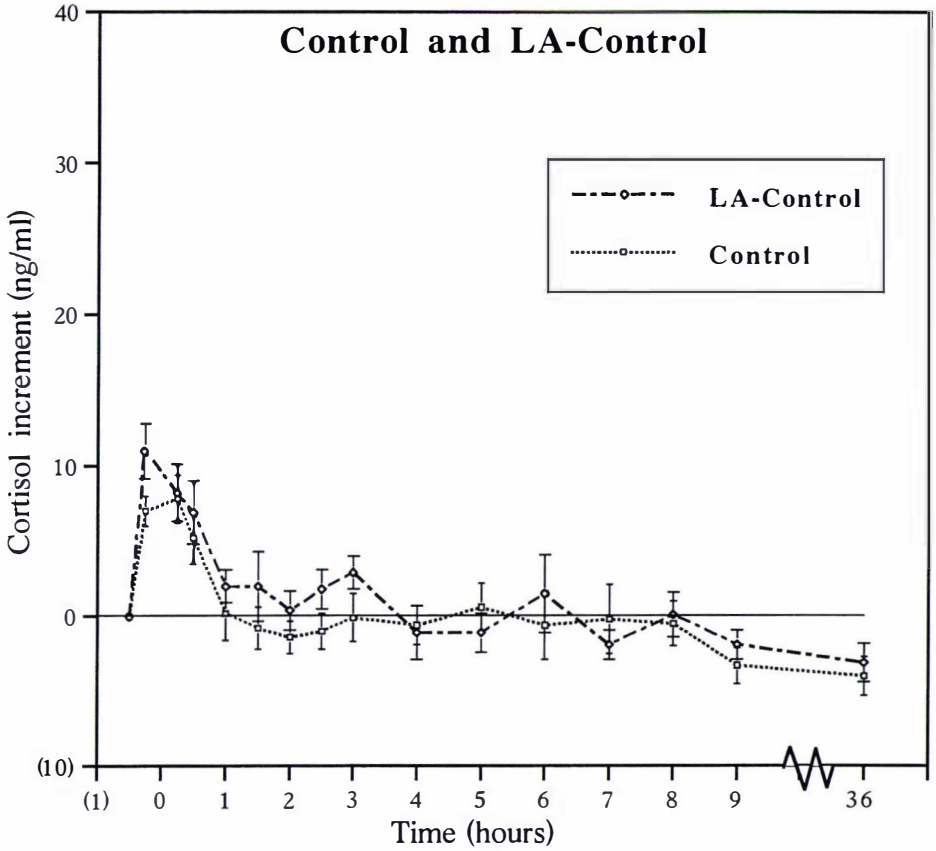


Fig 4.4 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to Control (n=9) and LA-Control (n=8) treatment.
There were no significant differences between treatments (P>0.05).

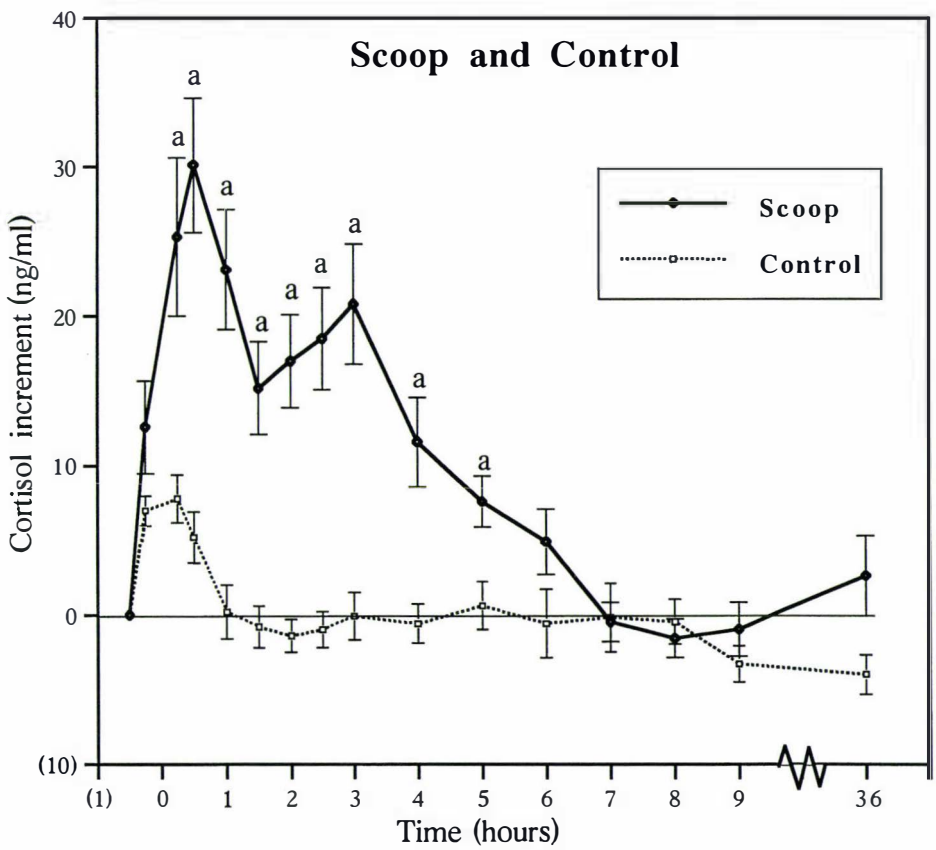


Fig 4.5 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to Scoop (n=10) and Control (n=9) treatment.
a = Scoop values are significantly different from Control values (P<0.05).

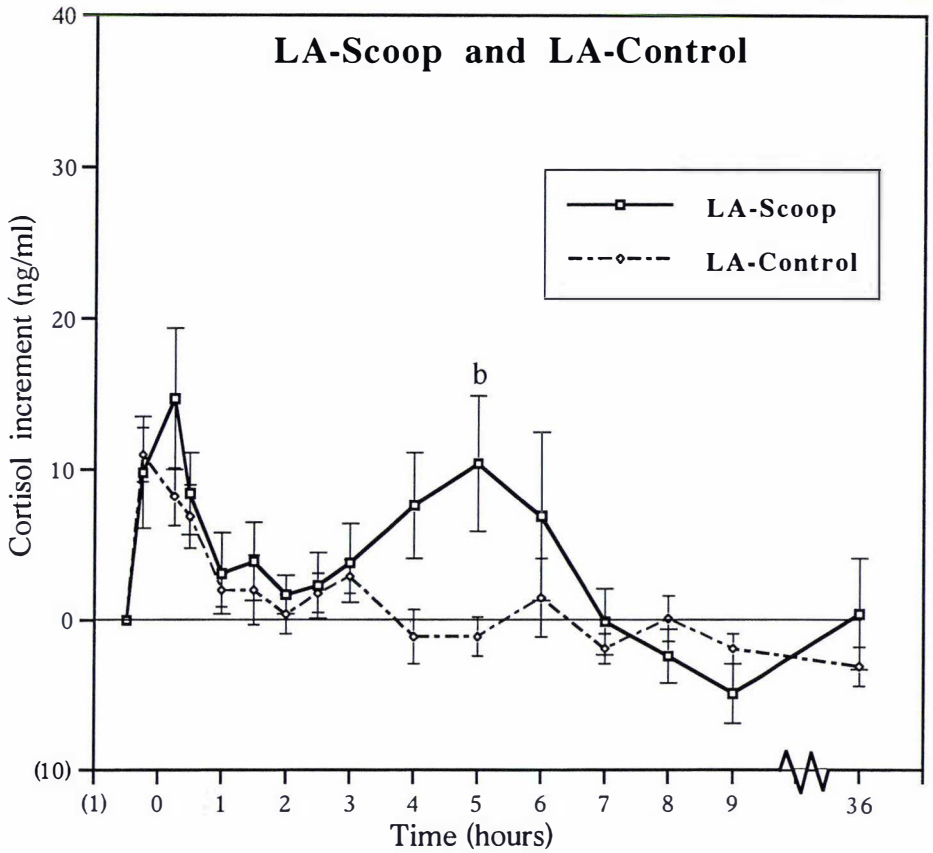


Fig 4.6 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to LA-Scoop (n=7) and LA-Control (n=8) treatment.
b=LA-Scoop values are significantly different from LA-Control values (P<0.05).

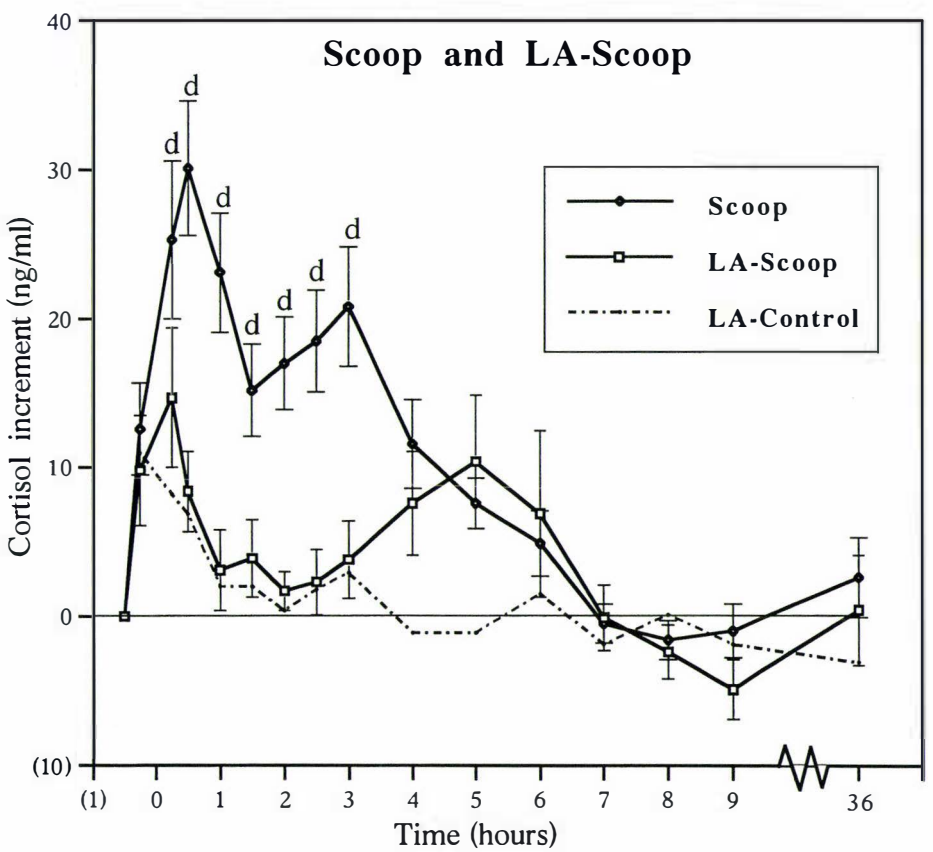


Fig 4.7 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to Scoop (n=10) and LA-Scoop (n=7) treatment.
d = Scoop values are significantly different from LA-Scoop values (P<0.05).

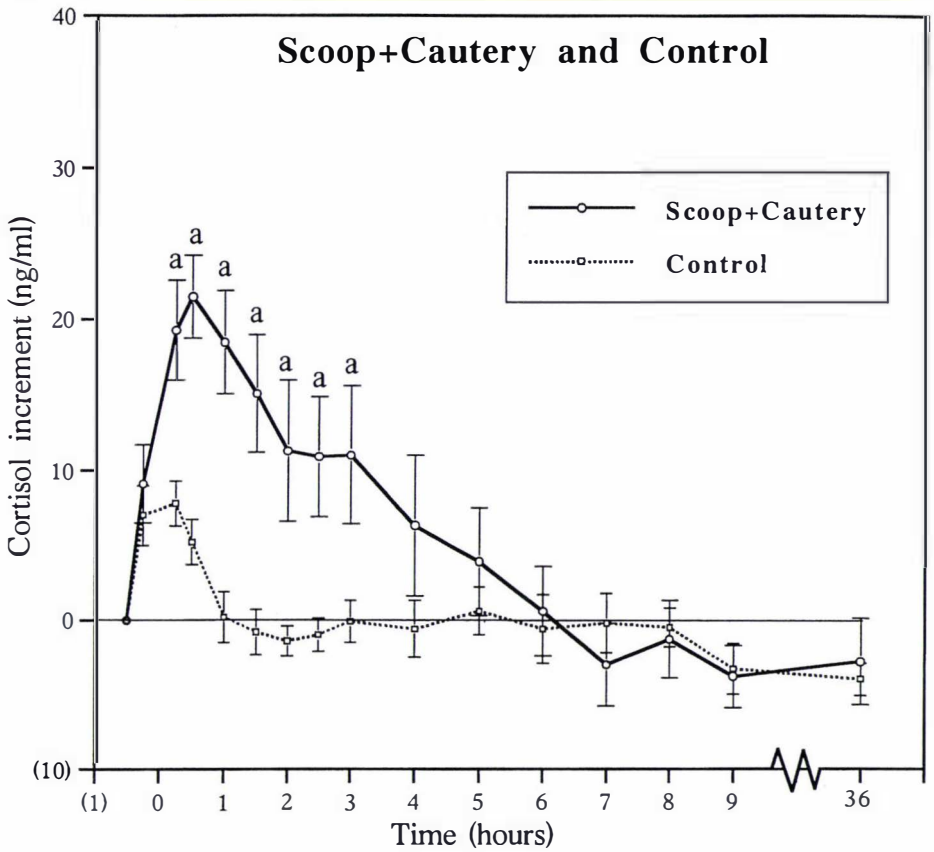


Fig 4.8 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to Scoop+Cautery (n=10) and Control (n=9) treatment.
a = Scoop+Cautery values are significantly different from Control (P<0.05).

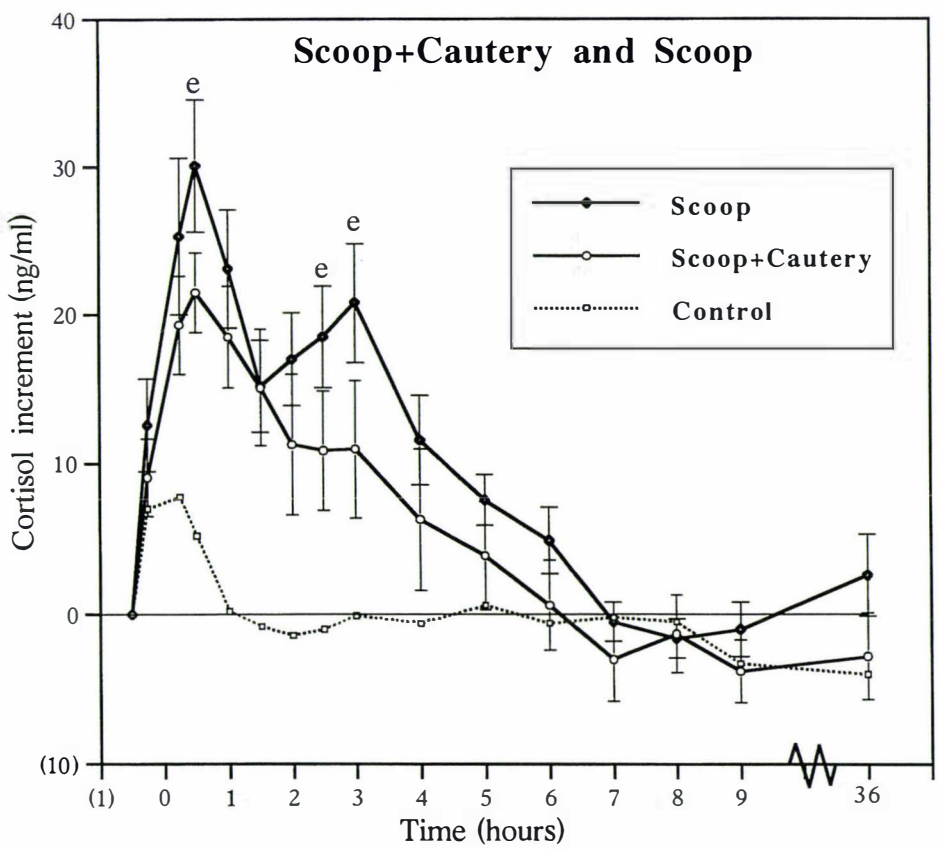


Fig 4.9 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to Scoop+Cautery (n=10) and Scoop (n=10) treatment.
e=Scoop values are significantly different from Scoop+Cautery values (P<0.05).

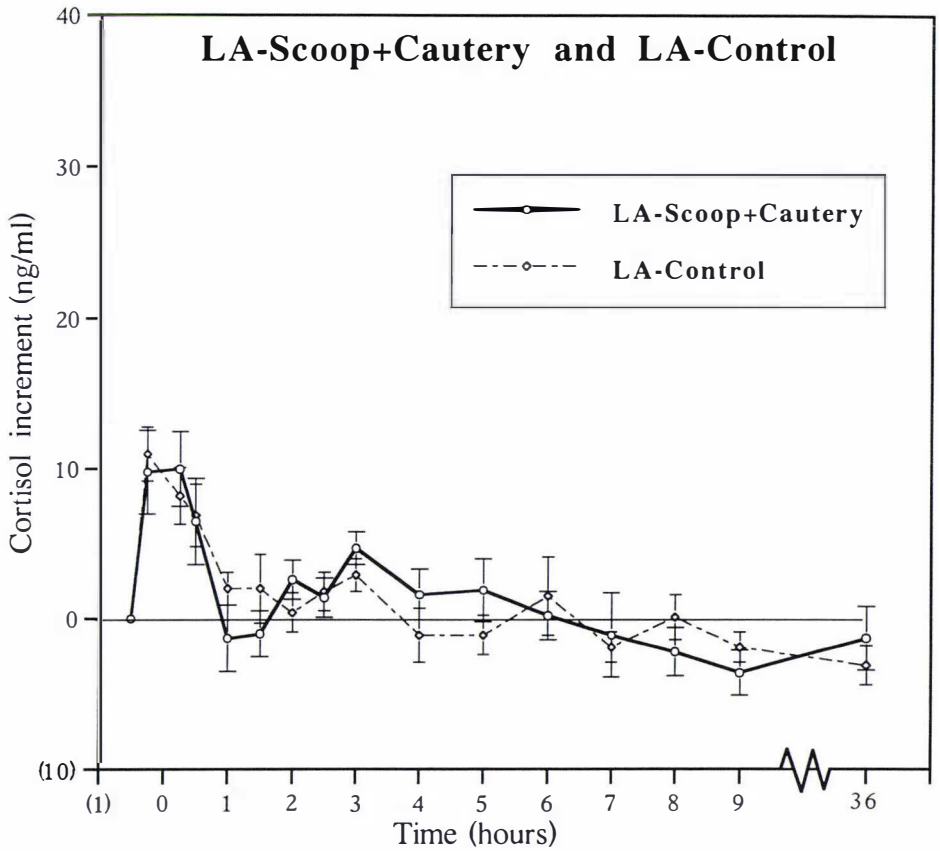


Fig 4.10 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to LA-Scoop+Cautery (n=8) and LA-Control (n=8) treatment.
There were no significant differences between treatments (P>0.05).

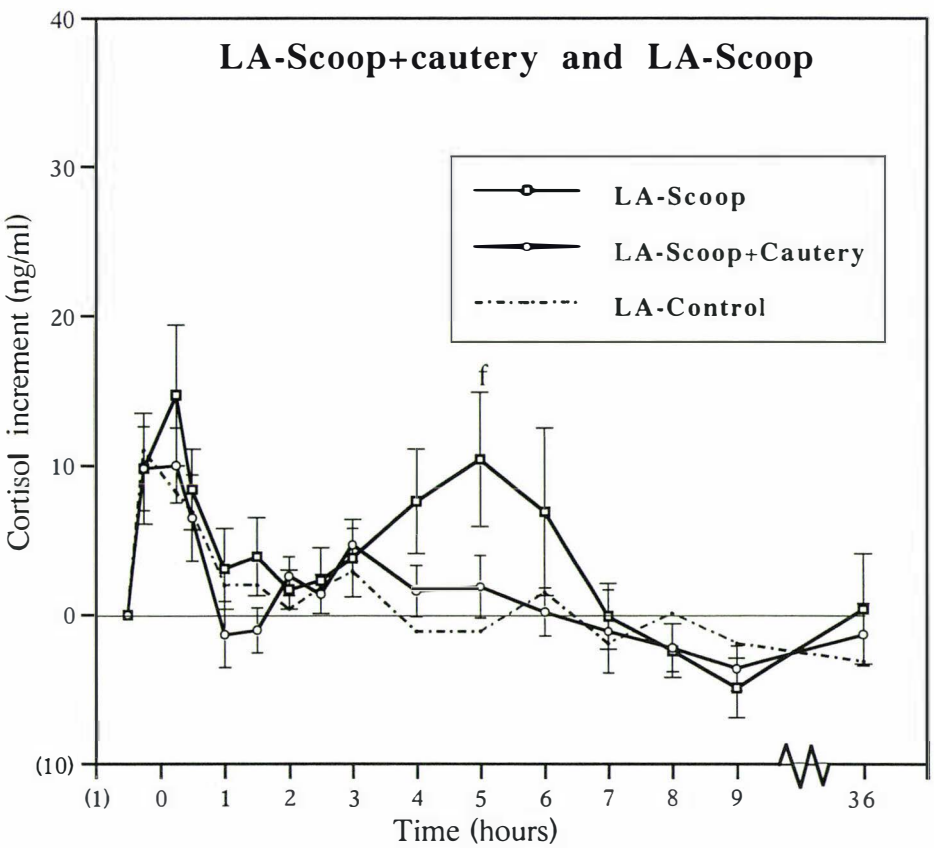


Fig 4.11 Changes in plasma cortisol concentrations (mean ± SEM) of calves in response to LA-Scoop+Cautery (n=8) and LA-Scoop (n=7) treatment.
f = LA-Scoop is significantly different from LA-Scoop+Cautery (P<0.05).

Table 4.1 Characteristics of the cortisol responses (mean ± SEM) for each group before and after treatment. Different superscripts within columns indicate statistically significant differences between treatments.

Treatment	n	Pretreatment(ng/ml)	Peak increment (ng/ml)	Time to peak (hours)	Response duration (hours)	Integral* (ng.hr/ml)	Integral** as ratio of Control	36h (ng/ml)
Control	9	8 ± 2	8 ± 2	0.25	1	19^{a} ± 4	1	4 ± 1
LA-Control	8	7 ± 1	11 ± 2	0.25	1	22^{a} ± 5	1.2	3 ± 1
LA-Scoop+Ct	8	9 ± 1	10 ± 3	0.25	1	26^{a} ± 7	1.4	8 ± 2
LA-Scoop	7	10 ± 2	15 ± 5	0.25	6	52^{ab} ± 14	2.7	11 ± 3
Scoop+Ct	10	12 ± 2	22 ± 3	0.5	6	78^{b} ± 20	4.1	10 ± 2
Scoop	10	8 ± 1	30 ± 5	0.5	6	105^{b} ± 17	5.5	11 ± 2

* = the integral of the cortisol response. Defined as the area under the cortisol response curve and above the pretreatment level (ng.hr/ml).

** = the integral expressed as a ratio of the integral of the Control response.

4.4 DISCUSSION

Using changes in plasma cortisol concentrations as an index of distress, the present study led to the following findings:

1) The time course of the changes in plasma cortisol concentrations in calves after Scoop dehorning in this study was similar to that described in Chapter three. The response was marked, biphasic and lasted for about six hours, indicating that dehorning is an extremely noxious experience.

2) Injection of local anaesthetic prior to Scoop dehorning virtually abolished the first three hours of the cortisol response elicited after dehorning. This equated to a 50% reduction in the integrated cortisol response (0-9h) and indicates that local anaesthetic should be given to alleviate the pain-induced distress of dehorning.

3) Cauterizing the wounds after scoop dehorning appeared to marginally reduce the cortisol response compared to dehorning alone. This suggests that there may be some benefit in cauterizing the dehorning wound. However, the behavioural response to the cautery indicated that it was very aversive.

4) Surprisingly, the combination of local anaesthetic before and cauterizing the wound after dehorning virtually eliminated the cortisol response. The cortisol response was similar to the control response which suggests that pain-induced distress was either absent or minimal. The apparent benefit of the combined regime has parallels with recent discoveries in pain physiology and merits further investigation.

Scoop dehorning elicited a marked biphasic elevation in plasma cortisol concentrations which lasted for about six hours. The similarity of the Scoop response with the ACTH response suggests that scoop dehorning was an extremely distressing experience, as described in the previous study (Chapter three). The predominant stimuli to cortisol secretion were presumably noxious input of nociception from damaged tissues and psychological interpretation of that from higher centres. By contrast, the cortisol response elicited by control handling and blood sampling lasted for 1.5 hours and was of low magnitude, and similar to that described for Control calves in Chapter three. This was taken as evidence that control handling and bleeding was a relatively benign experience and any distress associated with it was minimal and caused by the novelty of the experimental condition.

4.4.1 Local anaesthesia

Prior injection of local anaesthetic virtually abolished the cortisol response to scoop dehorning for the first three hours. During this period the plasma cortisol concentrations

in LA-Scoop calves were similar to those in LA-Control and Control calves. This similarity indicates that the local anaesthetic eliminated or substantially decreased the initial nociceptive barrage due to damage to peripheral nerves, along with any subsequent nociceptive input from inflamed damaged tissues for the first three hours. Presumably the small cortisol response exhibited by these three groups during this period, was elicited by psychological response to the novelty of the experimental situation, which stimulates the HPA-axis from the higher centres of the brain. This similarity of the LA-Scoop response to the Control response is surprising as one might expect the other non-noxious and novel stimuli, such as changes in sensation, weight of the head or dripping blood, to elicit a greater cortisol response than that exhibited by the Control calves. In addition, the similarity of the cortisol responses of LA-Control and Control calves supports the findings that the administration of local anaesthetic *per se* causes minimal distress (Petrie, 1994; Petrie *et al.*, 1993).

The increase in cortisol concentrations in LA-Scoop calves after 3 hours presumably reflected the return of sensation to the area served by the cornual nerve and the first appearance of noxious stimuli associated with the tissue damage to that area. The return of sensation was evidenced by a) pricking the cutaneous area served by the cornual nerve with a hypodermic needle, at about 2-3h calves began to respond to this stimulus; b) at about 3 hours there was an increase in the general activity of LA-Scoop calves, in particular increased occurrences of tail shaking, head shaking, ear flicking and walking (see Chapter five); and c) the duration of action of lignocaine is 60 to 120 minutes, but this may be extended by increasing the amount of drug injected (Ritchie and Greene, 1990). Similar behavioural responses were exhibited by six-week-old calves after dehorning with local anaesthetic or after ring tail-docking with epidural anaesthesia (Petrie, 1994). That the response is elicited by pain-inducing stimuli and not other stimuli is deduced from the following. The similarity of the LA-Control and Control responses suggests that there is no additional distress associated with the return of sensation to the head, or the wearing-off of local anaesthetic *per se*. The similarity of the LA-Scoop and LA-Control response during the first three hours, suggests that there is no additional effect of local factors such as hemorrhage or algogenic substances eliciting a cortisol response through systemic means. Presumably this continues to be the case when the local anaesthetic wears off. These findings support the conclusion that the increase of cortisol concentrations in LA-Scoop calves after three hours was elicited by the appearance of pain-induced distress.

Interestingly the increase in cortisol concentrations at 3h in LA-Scoop calves rose to the level of that in Scoop calves at the same time, after which they both followed a similar course back to pretreatment levels, so that between 4 and 6 hours the responses were

similar. Nerve blockade eliminates sensory transmission from the area of trauma, and therefore the spinal and supraspinal reflexes which are responsible for the catabolic and altered circulation state of the stress response to surgery do not occur. Based on the cortisol responses, it would appear that the sequence of the local events of inflammation was similar to that which occurred after dehorning. At the local level, tissue damage causes the release of algogenic substances, resulting in local vasodilation and extravasation of other inflammatory mediators. These algogenic substances may persist for hours or days and become a source of nociceptive impulses when local anaesthetics wear-off. Nociceptive input from such sources elicited a stress response as indicated by the second cortisol rise, but with character different from that in calves which did not receive local anaesthetic, and yet after three hours it followed the same sequence as those calves which did not receive local anaesthetic. Overall, the effect of the local anaesthetic equated to a 50% reduction in the integrated cortisol response (0-9h) compared to scoop dehorning without local anaesthetic. This suggests that there may be merit in investigating the effect of a long-acting local anaesthetic, that is, one which is of duration longer than the response to dehorning; or in investigating the effect of a long acting anti-inflammatory agent.

4.4.2 Cautery

The marginal reduction in the cortisol response with the addition of wound cautery, compared to the scoop calves, suggests that cauterizing the wound confers some benefit. This effect was anticipated from previous studies (Lester *et al.*, 1991a) and from observations that third degree burns are analgesic (Freund and Marvin, 1990). However, there was the possibility that wound cautery could increase the cortisol response as it was an insult additional to the dehorning.

A knowledge of the anatomy of the skin, in particular the location of nociceptors therein, and histological examination of burn injuries, help us understand why some burn injuries are painful and others are analgesic. Pain receptors, pathways and transmitters are similar in humans and animals (Bonica, 1990a; in Dart, 1994a). In humans 95% of cutaneous nociceptors are located in the subepidermis, and 5% in the epidermis. With first degree burns only parts of the epidermis are destroyed resulting in mild discomfort. In second degree burns the epidermis and variable amounts of dermis are destroyed. These wounds are extremely painful due to damaged nerves and other intact nerve endings which, with the protective layer of skin gone, are sensitized and exposed to stimulation. A third degree burn causes complete destruction of the skin leaving the initial wound analgesic, although there may be a component of dull throbbing pain. Around the periphery of third degree burns are areas of second degree burn which are painful. Over time as the wound heals and the neural tissue is reorganized there appears sharp pain. Thus, third degree burns are

analgesic because the relative number of nociceptors in the damaged area have been decreased by ablation (Freund and Marvin, 1990).

The marginal reduction in the cortisol response with the addition of wound cautery, compared to the scoop calves contrasts with the markedly smaller cortisol responses of lambs tail-docked with a heated docking-iron rather than a sharp knife. The destruction of nociceptors in the wound site has been advanced as a possible reason for the decreased cortisol responses after those amputations (Lester *et al.*, 1991a; Petrie *et al.*, 1996a) or procedures (Petrie *et al.*, 1996b) which incorporate cautery as opposed to those which do not (Lester *et al.*, 1991a; Kent *et al.*, 1993). It is possible that destruction of nociceptors is more effective when tissues are severed by cautery than when wounds caused by cutting are subsequently cauterized. Indeed it is surprising that the effect of amputation followed by cauterization of wound was less than that from amputation alone - as the latter involves the additional insult of a burn. Alternatively, the nature of the insult may be different in that more damage was inflicted on bony structures rather than cutaneous tissue. However, the periosteum of bone has a rich supply of nociceptors and fascia is also innervated with nociceptors (Bonica, 1990c). Nonetheless, these results provide a partial confirmation of the benefit of cautery in surgical amputation as advanced by Lester *et al.* (1991a).

4.4.3 Combined regimes (LA-Scoop+Cautery)

The virtual elimination of the cortisol response to scoop dehorning throughout the nine hour period of observation by the prior injection of local anaesthetic and subsequent cauterizing of the wound was surprising and striking (Fig 4.10), especially in view of the marginal reduction effected by cauterising the scoop wounds without prior local anaesthesia.

The increase in cortisol concentrations after the local anaesthetic wore off in LA-Scoop calves demonstrates that there is sufficient pain during this period to elicit a delayed cortisol rise. Somehow the additional effect of cauterizing the wound prevented the delayed rise in cortisol concentrations when the local anaesthetic wore off. The synergistic effect of the combined regime of local anaesthetic plus cautery is reminiscent of pre-emptive analgesia and merits further investigation.

Woolf and Chong (1993) reviewed the literature on various pain management therapies, and showed that a knowledge of the physiological mechanisms by which pain is evoked, and a change in attitude towards providing pain relief before pain is present, results in a decrease in post-operative pain relief requested by human patients. Originally anaesthesia was used to allow surgical manipulations, as opposed to pain relief *per se*. Post-operative pain relief is often given *after* pain has manifested. Woolf and Chong (1993) observed

that when the noxious sensory barrage of surgery was blocked by the use of local or regional anaesthesia there was a reduced intensity and delayed onset in the appearance of post-operative pain. This has been termed "pre-emptive analgesia". The suggested mechanism for this phenomenon is that the surgery-induced sensory barrage decreases the pain threshold, resulting in an increased sensitivity to stimuli including pain (Wall, 1988; Katz *et al.,* 1992; Woolf and Chong, 1993). If this afferent barrage can be prevented, through the use of local anaesthetic, then, when the local anaesthetic wears off the experience of pain is reduced, because there has been no reduction in the pain threshold.

The virtual elimination of the cortisol response to dehorning by the combined regime of local anaesthesia and cautery may be analogous with the findings of Woolf and Chong (1993). The increase in plasma cortisol concentrations at 3h after dehorning with local anaesthesia indicates that the nociception at this stage is of sufficient magnitude to elicit a sizeable cortisol response and, by inference, may sensitize the pain pathways. It has been suggested that cautery destroys nociceptors in the wound and thereby decreases nociception (Lester *et al.,* 1991a). The marginal decrease in the cortisol response throughout from cauterizing the scoop wounds alone suggests that if the number of functioning nocireceptors has been decreased by the cautery, then those that remain functioning have had their threshold decreased (i.e. are sensitized) by the afferent barrage from dehorning. The mechanism of the effect from the combined regime of local anaesthetic and wound cautery may be that the local anaesthetic has prevented the nociceptor barrage caused by the amputation of tissues, and hence that there is no change to the nociceptor pain threshold. Consequently when the local anaesthetic wears off, the few functional nociceptors have not had a change in their pain threshold and the magnitude of the noxious stimulation cannot elicit a cortisol surge.

Before commenting on the overall efficacy of this procedure in reducing the pain-induced distress of dehorning the following points need to be addressed: 1) How long does this analgesic effect last? This study monitored the acute cortisol response. The similarity of the 36h and pretreatment sample suggests that there was little distress experienced on day two. 2) Does the apparent analgesia outlast the time required for the wound to properly heal? This is important because as noted above, when third degree burns heal and the neural tissue is reorganized sharp pain will appear (Freund and Marvin, 1990). 3) Would this new pain be less noxious overall than that experienced by dehorning alone? 4) Will there be a negative impact on wound healing process? Historically cautery has been applied to wounds where the risk of hemorrhage was greater than the risk from the attendant delayed healing. It has been recommended to use a firing iron after dehorning to accomplish haemostasis (Oehme, 1974). The marginal decrease in the cortisol response after cauterizing the scoop wound would not offset the risks of delayed wound healing.

The benefit derived from the combination of local anaesthetic and wound cautery after scoop dehorning, would need to be weighed against the additional handling and great care required to carry out this procedure.

4.4.4 Critique of the experimental design

As discussed in Chapter three (see 3.4.6), the experimental conditions of repeated handling and blood sampling and confinement may have caused additional distress. In a similar experimental situation repeated handling and blood sampling had no apparent effect upon the cortisol response (Lester *et al.*, 1991b). Presumably confinement in the yards was an additional source of distress for the calves. This confinement would have frustrated their pursuit of normal activities, such as grazing and walking. However, the use of appropriate control groups makes allowances for such confounding effects. These problems could be obviated by the use of remote sampling devices (Ingram *et al.*, 1994).

This study, with its intensive sampling in the first nine hours, monitored only the acute cortisol responses to dehorning. In order to fully assess the distress of dehorning, the chronic effects such as continued pain or problems associated with wound healing need to be investigated. The similarity of the 9h and 36 h cortisol values with the pretreatment values suggests that there was little distress experienced on day two.

4.4.5 Conclusions

The application of local anaesthetic reduced the cortisol response to dehorning by 50%. This suggests that there would be merit in investigating the effect of a local anaesthetic or an anti-inflammatory agent which has a duration of action longer than the response to dehorning (6h). The combination of local anaesthetic prior to, and cauterizing the wound after, scoop dehorning, virtually abolished the cortisol response to amputation dehorning.

These results suggest that local anaesthetic should be administered before dehorning and that if possible scoop wound should be cauterized. However, further research is needed to determine the duration of the apparent cautery-induced analgesia, its effects upon wound healing and the practicalities of the additional handling and care required to carry out this procedure.

4.4.6 Epilogue

Subsequent work showed that the local anaesthetic merely delayed the response to dehorning, as opposed to reducing it *overall* (Petrie *et al.*, 1996b; McMeekan *et al.*, 1998a; Sutherland, 1999; Sutherland *et al.*, 2002b). This is in contrast to the findings here and is discussed in the General Discussion (Chapter six). The virtual elimination of the cortisol response to dehorning by the combined regime of local anaesthetic plus

cauterizing the scoop wound was confirmed, and in addition, it was shown that cortisol concentrations remained at Control values for at least 24 hours (Sutherland *et al.,* 2002a).

5 BEHAVIOURAL RESPONSES OF CALVES TO SCOOP-AMPUTATION DEHORNING WITH AND WITHOUT LOCAL ANAESTHESIA

5.0 ABSTRACT

Dehorning of cattle is a routine husbandry practice which causes significant and marked distress that lasts for about six hours (Chapter three), but may be alleviated by local anaesthesia (Chapter four). The aim of the current study was to use behavioural indices to assess the distress of dehorning and its alleviation. Changes in behaviour were used to assess the pain-induced distress in Friesian calves (six months old, about 130 kg, n=60) during the first 10 h and between 26 and 29 h after amputation dehorning with or without local anaesthesia (LA). There were three treatment groups: Control, Scoop and LA-Scoop. Treatment was conducted in a cattle race. Thirty calves were studied at a time in three mobs of 10 held in pens. Calf behaviour was recorded on video and on data sheets using point scan behaviour sampling. There were distinct behavioural profiles according to the treatment received. The four behaviours of tail shaking, head shaking, ear flicking and rumination satisfied the criteria required to use behaviour as evidence of distress. The first three behaviours occurred in an inverse relation to rumination. Calves dehorned without local anaesthetic exhibited a high incidence of tail shaking, head shaking and ear flicking that lasted for six hours, whereas Control calves had significantly lower ($P<0.05$) incidences of these behaviours and a high incidence of rumination. When given prior local anaesthetic the behaviour of dehorned calves was similar ($P>0.05$) to that of the Control calves for two hours, and thereafter tail shaking, head shaking and ear flicking increased to levels similar to those in the Scoop calves. Both the physiological study (Chapter four) and the current study suggest that dehorning is a painful experience that lasts about six hours and that the application of local anaesthetic alleviates that pain for about the first two hours. For this reason it is recommended that local anaesthetic be administered when conducting amputation dehorning.

5.1 INTRODUCTION

Dehorning of cattle is a routine husbandry practice which may cause short term pain and distress, but is performed to reduce injury to stock and stockhandlers, to decrease carcass bruising and hide damage (Meischke *et al.*, 1974), and to improve the handling of cattle (Anon, 1974). At the time this work was done there had been little research conducted to evaluate the distress of dehorning cattle. Using changes in plasma cortisol concentrations as an index of distress, amputation dehorning elicited a marked and

biphasic cortisol response that lasted about six hours and was considered to be an extremely noxious experience (Chapter three). Local anaesthesia reduced the dehorning response for the first three hours, after which there was a small cortisol rise, such that, overall local anaesthetic reduced the cortisol response by 50% (Chapter four). By contrast, the combination of local anaesthetic and cautery of the scoop wound virtually eliminated the cortisol response to dehorning (Chapter four). The aim of the current study was to assess and further qualify the distress of dehorning using behavioural indices. As there had been no rigourous scientific research evaluating the behavioural responses of calves to amputation dehorning, the behaviour study in this thesis was weighted by comparison with parallel cortisol studies (Chapters three and four). Subsequent work, which built on the observations presented here, has been published (Petrie *et al.,* 1995; McMeekan *et al.,* 1999; Stafford *et al.,* 2000).

Animals have to contend with a complex dynamic environment where they are continually experiencing physical, social, psychological and environmental factors including injury and disease, which may compromise their well-being. They have a variety of biological mechanisms available to cope with challenges. These include, primarily, activation of the autonomic nervous system and the HPA-axis, both of which are often accompanied by behavioural changes (Moberg, 1985; Stamp-Dawkins, 1980). These physiological responses and their outcomes are intimately linked in a feedback and feedforward relationship (Dantzer and Mormede, 1983) and are designed to reduce the effect of the stressor. Increased activity of the HPA-axis and to a lesser extent sympathetic outflow are the hallmarks of stress. There is no single reliable or definitive indicator of stress, and it is recommended that, if possible, a variety of biological parameters be measured (Stamp-Dawkins, 1980). Changes in behaviour are considered a valid indicator of stress and may have advantages over measuring changes in plasma cortisol concentrations.

Changes in behaviour are often the first indication that a stress has occurred (Morton and Griffiths, 1985; Stamp-Dawkins, 1980), and an animal's freedom to move may be one of its most important adaptations to environmental conditions (Martin and Bateson, 1986). Changes in behaviour may also indicate the nature of the stressor and the way in which the animal is attempting to cope with it (Matthews, 1992). Behavioural measurements are non-invasive and so minimize the complicating effect of sampling upon the response of the animal, and once categorized may be used by anybody with minimal expertise if behaviour measurement rules are observed (Fraser and Broom, 1990).

In ruminant species such as cattle, behavioural responses to many stimuli are subtle and therefore a good knowledge of normal behaviour is required (Hinch, 1994). The

apparent absence of abnormal behaviour does not imply that cattle are not stressed, but rather that they may be better described as "stoic" (K.J Stafford, personal communication). Cattle are gregarious creatures with social organization based on dominance hierarchies, making them sensitive to spacing and density changes (Hinch, 1994). The initial responses of cattle to a stressor are orientation towards the stimulus, suppression of normal activities, and preparation for fight or flight. Thereafter, behavioural changes may include increased aggressiveness or decreased amicable behaviours; changes in activity level, either the increased activity of frustration at having the motivation to express a normal behaviour thwarted, or decreased activity and the adoption of the submissive posture indicative of social tension, pain or ill-health; reduction in maintenance behaviours such as grooming, feeding and stretching associated with changes in appearance and posture; and with chronic stressors the possible appearance of stereotypies (Hinch, 1994). In addition to these behaviours the expression of pain may be evinced by vocalization, increased aggression especially if flight is thwarted, favouring of the traumatized area, severely depressed activity and the adoption of an immobile and unresponsive stance, which may include a fixed stare with the head and neck extended towards the ground (Hinch, 1994).

Whilst ruminant species such as cattle may be less expressive in their behavioural responses to pain-induced distress compared to other mammals (Hinch, 1994), changes in behaviour have been assessed following noxious procedures including tail docking (Petrie *et al.*, 1996a), castration (Robertson *et al.*, 1994) and branding (Lay *et al.*, 1992a,b). In particular, cautery disbudding elicited increased escape behaviours (including rearing, head pushing, tail wagging) during, and foot stomping, head shaking, ear flicking, vocalization and restlessness after the procedure (Taschke and Folsche, 1993, 1995; Morisse *et al.*, 1995; Petrie *et al.*, 1996b).

5.1.1 Interpretative problems

In addition to the interpretative problems associated with the assessment of the subjective states of pain and stress discussed in section 1.4.3, there can be problems interpretating behavioural responses *per se* as demonstrated below.

Behaviours indicative of injury, disease or pain include a change in active (restlessness) and/or inactive (e.g. inappetance) behaviours (Fraser and Broom, 1990; e.g. Lester *et al.*, 1996). Animals may either increase or decrease their activity to avoid noxious stimuli. Whilst studies have been conducted showing changes in behaviour correlated to presumed distressful experiences, there have also been studies where there was no apparent change in behaviour following a presumed noxious experience. For example, Shutt *et al.* (1987), did not observe any changes in behaviour following the presumably painful operations of excising the skin around the anus (mulesing) or

grinding down the teeth in sheep. Such studies have been interpreted in a variety of ways. In some cases they have been used to infer an absence of pain or distress directly, or indirectly as a result of stress-induced analgesia, or they have been taken as evidence that grazing prey species have evolved the capacity to suppress the expression of such behaviours as a means of improving survival (Shutt *et al.,* 1987, 1988). The striking occurrence of agitation behaviours, has led others to overestimate its usefulness for comparing the intensity of distress. For example, on these grounds Shutt *et al.* (1988) and Molony *et al.* (1993) erroneously concluded that the knife caused less distress than the rings for castration and/or tailing in lambs. Furthermore, these conclusions were not consistent with their respective cortisol data (Shutt *et al.,* 1988; Kent *et al.*, 1993). See critiques of these studies by Mellor and Holmes (1988) and Lester *et al.* (1996). Lester *et al.* (1996) concluded that for the behavioural assessment of distress intensity to be credible, there needs to be a validating continuum between behaviours, and therefore, behaviours which are treatment specific cannot be used alone to gauge relative intensities of distress. In order to address such problems, Mellor *et al.,* (2000) advanced some principles and caveats to guide the quantitative and qualitative evaluation of physiological (cortisol) and behavioural responses to painful stimuli (see 5.4 and 1.4.3).

5.1.2 Aims of study

The objectives of the current study were: 1) to characterize the behavioural response of six-month-old calves to dehorning; 2) to determine if the administration of local anaesthetic might mitigate that response; 3) to compare the behavioural changes with the plasma cortisol concentration changes (Chapter four); 4) to determine whether it is possible to identify behaviours elicited by pain-induced distress *versus* non-noxious stimuli; and 5) to determine if it is possible to identify behaviours indicative of generic *versus* dehorning pain-induced distress by comparison with other studies.

5.2 MATERIALS AND METHODS

5.2.1 Experimental design

Sixty male Friesian calves aged five to six months, weighing about 130 kg, and scheduled for dehorning according to usual husbandry practice on a Massey University farm, were observed in this study. Prior approval to conduct this study was obtained from the Massey University Animal Ethics Committee.

The calves were randomly allocated to one of three treatments (n=20 in each case) and were observed on the two days following treatment. In order to allow for more accurate behavioural monitoring 30 animals were studied at a time. Therefore the study was spread over two successive periods of two days. On days one and two the first 30 calves were observed, and on days three and four the next 30 calves were similarly observed.

On the morning of the first day of the study at 0800 h, 30 calves were separated from the herd, randomly allocated to one of the three treatment groups, and had numbers spray painted on their backs for identification purposes. Calves receiving the same treatment were handled and penned as a group. Each group of 10 calves was tightly packed into a race where treatment was performed which took about six minutes for the whole group. Thereafter the group was quietly herded 20 metres into nearby covered yards (5 x 5 m) with access to water but not food. The allocation of the treatments to each group was unknown to the observer, although the absence of scoop wounds made Control calves easy to identify. The calves were observed for 10 hours. Afterwards the calves were let out to pasture and reunited with the herd. On the morning of the second day of the study, the same calves were separated from the herd, allocated to the yards according to treatment, allowed to settle for two hours and observed for three hours between 1000 h and 1300 h. Thereafter the calves were reunited with the herd.

5.2.2 Treatments

Treatment was performed in a cattle race, with two operators moving sequentially through the calves, one restraining the calf and the other performing the treatment. Between animals, the scoop dehorning tool was disinfected (Hibitane concentrate 5%. Hospital Products, ICI Australia). The amount of horn and skin tissue removed during dehorning varied depending on the horn size and head movements.

Control n=20

Calves were handled to simulate the restraint required for dehorning but without being dehorned.

Scoop n=19

The calves were dehorned using an industry standard dehorning scoop (Barnes Dehorners, Stones, USA). The scoop dehorner (Fig 3.1) has two interlocking semi-circular blades (diameter about 45 mm) which when engaged removed the horn and the adjacent cutaneous tissue, including the germinal tissue of the horn.

LA-Scoop n=20

Local anaesthetic (6 ml of 2% lignocaine hydrochloride. Ethical Agents ltd, Auckland, New Zealand) was injected near each cornual nerve at a site a little below the lateral ridge of the frontal bone (temporal ridge) about halfway between the lateral canthus (outer corner of the eye) and the horn (Weaver, 1986) (see Fig 4.1). Anaesthesia, which occurs 10-15 minutes after lignocaine administration (Ritchie and Greene, 1990), was tested by pricking the skin around the horn with a hypodermic needle and observing if there was a response (for instance ear or eye flicks). If a response to this stimulus did occur, another 6 ml of local anaesthetic were injected near the corresponding cornual nerve. Thereafter calves were dehorned as described for Scoop calves. No data were collected on how many calves required an additional dose of local anaesthetic.

5.2.3 Behaviour measurements

The calf behaviour was recorded using "instantaneous point scan *behaviour* sampling". That is, the *number of animals* exhibiting particular behaviours was scored, as opposed to the behaviours that a *particular* calf was exhibiting. Because of this, it was not possible to correlate behaviours that may have been expressed simultaneously in individual animals. No data were collected on the duration, frequency or intensity of the behaviours.

On the first day after treatment behaviours were scored every 15 minutes until five hours, and then every 30 minutes until 10 hours after treatment. On the second day after treatment behaviours were scored every 30 minutes for three hours. The behaviours scored were:

Behavioural States

Ruminating:	Calves ruminating whilst either standing or lying.
Lying:	Calves in sternal recumbency.
Head down:	Head below a horizontal plane drawn level with the withers

Behavioural Events

Ear flicking:	Vigorous shaking of one or both of the ears independently of a head shake.

Head shaking:	Vigorous shaking of the head around a rostral to caudal axis.
Tail shaking:	Rapid movement of the tail to and fro across the calf's hind quarters.
Walking:	Calves pacing or walking, defined as more than four steps.
Leg scratching:	Elevation of hind leg making contact with the head.
Head rubbing:	Rubbing of the head on any object or another calf.
Neck extending:	Extension of the jaw and neck towards the ground.
Riding:	Calves mounting, or attempting to mount another calf.
Vocalizing:	Any bawling made by the calves.

Continuous observations

In addition to the scan sampling, continuous monitoring of the calves was conducted to score any other behaviours not listed above. Because head rub, neck extension, leg scratch, riding and vocalizing occurred infrequently they were also monitored and scored when they occurred. These results are not reported here.

Restlessness index

There were three dominant and frequently occurring apparent restless behaviours and these were summed to produce a "restlessness index". The separate behaviours that were summed to produce this value were: tail shaking, head shaking and ear flicking. It is important to note that each behaviour independently exhibited a pattern of incidence that was similar to the overall pattern of the restlessness.

The term restlessness index is used to distinguish this, which is a composite score of three behaviours, from the term restlessness score which has been used to describe the occurrence of getting up and down in lambs (Mellor and Murray, 1989a).

5.2.4 Data Analyses

General

The data collected were counts of occurrences of behaviours. Such data does not necessarily meet the criteria of the normal (gaussian) distribution and therefore non-parametric statistical tests should be conducted on them (Lehner, 1992). However, if the number of observations is "large enough" such data may approximate a normal distribution and parametric tests may be used (Shott, 1990). In this case where n=20 it was considered safer to conduct non-parametric analyses on the data. The analyses were

based on instantaneous sampling of the occurrences of a behaviour during pre-determined time periods. The time periods were 0-2h, 2-6h, 6-10 h, 0-10h and 26-29h after treatment. In each of these intervals there were 8, 14, 8, 30 and 7 samplings respectively.

The decision as to which periods of observations should be used for statistical comparisons was based upon the need to distinguish the behavioural response during the action of the local anaesthetic and during and after the period of distress. The importance of distinguishing the first two hours from the remaining periods was to examine the effect of the local anaesthetic. The decision to use two hours was based on the following: a) the duration of action for lignocaine is about 120 minutes (Ritchie and Greene, 1990); b) the skin prick test indicated a return of sensation to the horn area about 2.5 hours after treatment (Chapter four); c) the study of the cortisol response to dehorning with and without local anaesthetic (Chapter four) indicated that analgesia was effective in delaying the cortisol rise until about 2.5 hours after treatment; and d) these periods for comparison have been used in similar trials (Petrie *et al.,* 1996a,b). The reason for comparing the responses before six hours with that after six hours was based upon data which showed that the cortisol response to dehorning with or without a local anaesthetic was resolved by six hours (Chapters three and four). And finally to conduct many more comparisons, for example, every hour, would have necessitated a few hundred tests which would have resulted in too many type 1 and type 2 statistical errors.

Mann-Whitney test

Mann-Whitney tests were conducted to ascertain if there were differences between treatments. Because the data from within-treatment comparisons are usually not independent it is recommended to use the Friedman or Wilcoxon test (Shott, 1990, 1991). However, in this study behaviour sampling and not calf sampling was scored, it was not known whether the calves exhibiting a particular behaviour were the same as those exhibiting that behaviour at another sampling. Thus the data were neither truly independent nor truly not-independent (as they could have been the same calves). As it was impossible to analyse the data as not-independent they were therefore analysed as independent. Consequently Mann-Whitney tests, which assume data independence, were conducted to ascertain if there were any within treatment differences.

The Mann-Whitney test is a non-parametric technique for comparing the medians of two unmatched samples, where the values of the observations are converted into their ranks. It is important to note that whilst the data cited in the text and graphs are the arithmetic mean ± SEM, statistical analyses were conducted on the *medians.* The Mann-Whitney tests were conducted using Statsview+Graphics software package (Version 2,

Abacus concepts Inc., USA). In addition, random manual Mann-Whitney tests were conducted to check the results [for calculation see Fowler and Cohen (1990)].

The Mann-Whitney test does not require that the samples be matched, nor does it require that there be the same number of sampling observations. It is worthwhile noting that for the within treatment comparisons the first period was two hours long with 8 observations, the middle period was four hours long with 14 observations and the last period was four hours long with 8 observations. Neither the different number of observations nor the different duration of the observation periods will render the mathematical analysis invalid because the number of calves exhibiting the behaviour at any instant was measured not the total amount of the activity. For example, the incidence of tail shaking not the sum total of tail flicks was measured. Rather only the reliability and variation of the measurement may be affected, i.e. the reliability of a short duration event increases with the number of observations (Martin and Bateson, 1986).

The Mann-Whitney test appeared to break down when comparisons were conducted where one test group had low occurrences of a particular behaviour and the other test group had no such occurrences. This was manifest as a significant discrepancy in the p value (probability value) between that adjusted and that not adjusted for ties (values of equal rank). In fact many statistical tests break down if the observations compare less than 5% of the total sample [e.g. Chi-square test (Fowler and Cohen, 1990)]. To get around this problem the following procedure was followed, either a) the results were compared with one-way ANOVA - accepting the problems of assuming a normal distribution, and a 95% agreement was found with the Mann-Whitney's more conservative p value; or b) the data used in the analyses were collected from the whole observation period (0-10h). This produced more data for the comparisons leading to a more reliable Mann-Whitney test. Perhaps, in these cases the statistical result should be considered less important as there may simply be an underlying biological question unanswerable through statistics. For example, where there is no difference between two treatments and one group has a low incidence and another group has no occurrence of the bchaviour, it may be of high biological significance that one group *never* exhibited the behaviour.

Data presentation

The data presented in the graphs are the number of calves exhibiting a behaviour for the group (n=20 in each case). Whereas, the data presented in the table are the percentage (mean $\pm$ SEM) of calves exhibiting a behaviour, for each period. The mean $\pm$ SEM percentage frequency is shown as this provides additional information about the data which medians alone fail to do. Statistical analyses were conducted on the data from

pre-determined periods, these being 0-2h, 2-6h, 6-10h and 0-10h, and significant differences are shown using different letters of the alphabet: a, b and c indicating differences within treatments; x, y and z indicating differences between treatments.

The data from day one are graphed, whereas the data from day two after treatment are only reported in the text and are not graphed. In addition, no statistical tests were conducted on the day two data due to the low incidence of occurrence. However, the data are described in terms of the mean (± sem) incidence.

5.3 RESULTS

The experiment went well. However, one Scoop calf was withdrawn from the study as it went berserk, becoming extremely active and unsettled, running and jumping the fences after dehorning, which disturbed the other calves in the trial.

The data were analysed and the responses to each treatment were compared. There were no significant differences between similarly treated calves in the two independent studies, and therefore both sets of data for day one after treatment were pooled. However, because of insufficient incidences of behaviours from which to check data independence, the data from day two were not pooled. The results from day one are discussed separately from those of day two after treatment (Table 5.1; Table 5.2 respectively).

All calves were restless for the first 15 to 30 min after treatment and their arrival elicited restlessness in those calves already penned. This restlessness did not necessarily correlate with the scan observation times. The behaviours that constituted this initial restlessness were primarily walking and milling around, tail shaking, bawling, head shaking and ear flicking. Riding attempts were rare and only occurred in the Control calves during this settling-down period.

5.3.1 Behavioural comparisons (Day one)

Ruminating

Control calves

The maximum number of calves ruminating at any one time was 60% and this occurred at 0.75h after treatment. During the course of the day the mean number of Control calves ruminating decreased, 28 $\pm$ 7% ruminated in the first two hours, 15 $\pm$ 4% ruminated during the following four hours and 9 $\pm$ 4% ruminated in the last four hours, and this decrease was significant ($P<0.05$ Mann-Whitney) (Fig 5.1). It is important to note the underlying trend of decreasing rumination in the Control calves when interpreting the trends in the dehorned calves.

Scoop calves

There was little or no rumination in Scoop calves (4 $\pm$ 3% 0-2h, 2 $\pm$ 1% 2-6h) until the last period (6-10h) when a small but statistically significant number of calves (9 $\pm$ 2%) ruminated ($P<0.05$ Mann-Whitney).

LA-Scoop calves

The maximum number of calves ruminating was 50% and this occurred at 0.75h after treatment. Thereafter the number of calves ruminating decreased significantly from

20 ± 7% (0-2h) to 2 ± 1% (2-6h) and then remained at low levels (4 ± 2% 6-10h) (P<0.05 Mann-Whitney).

Between treatments

Over the 10 hour observation period the mean number of Control calves ruminating (17 ± 3%) was significantly greater (P<0.05 Mann-Whitney) than that for either Scoop (4 ± 1%) or LA-Scoop (7 ± 2%) calves which exhibited similar numbers (Fig 5.1). There was virtually no rumination during the first half hour irrespective of treatment.

During the first two hours after treatment the number of LA-Scoop calves (20 ± 7%) and Control calves (28 ± 7%) ruminating was similar (P<0.05 Mann-Whitney). The number of LA-Scoop calves ruminating between 2 and 6h was similar to the number of Scoop calves ruminating between 0 and 6h and both were significantly less than that of Control calves for the corresponding periods. But, during the last period (6-10h) there were no differences between Control (9 ± 4%) LA-Scoop (4 ± 2%) or Scoop (9 ± 2%) calves (P>0.05 Mann-Whitney).

Tail shaking

Control calves

The mean number of Control calves tail shaking (12 ± 1%) over the ten hours was distributed as 9 ± 3% during the first two hours, 17 ± 1% during the next four hours and 8 ± 2% during the last four hours (Fig 5.2). The number of calves tail shaking in the middle period (2-6h) was significantly greater than that in both the earlier (0-2h) and later (6-10h) periods (P<0.05 Mann-Whitney).

Scoop calves

There were no differences in the number of Scoop calves tail shaking over time (0-2h 26 ± 3%, 2-6h 31 ± 3%, and 6-10h 23 ± 2%) (P>0.05 Mann-Whitney).

LA-Scoop calves

The number of LA-Scoop calves tail shaking in the first period (0-2h 9 ± 2%) was significantly less (P<0.05 Mann-Whitney) than that in the next two periods (2-6h 37 ± 3%, 6-10h 27 ± 5%), which were similar.

Between treatments

Over the 10 hour observation period the mean number of Control calves tail shaking (12 ± 1%) was significantly less (P<0.05 Mann-Whitney) than that tail shaking for either Scoop (28 ± 2%) or LA-Scoop (27 ± 3%) calves which were similar (Fig 5.2).

The number of Scoop and LA-Scoop calves tail shaking was significantly greater than those of the Control calves for most periods except the first period (0-2h) in LA-Scoop calves when the number of calves tail shaking was similar to that in Control calves.

Head shaking

Control calves

There were only three incidences of head shaking in Control calves and these occurred at about one hour after treatment (Fig 5.3).

Scoop calves

Scoop calves exhibited a high incidence of head shaking for the first six hours (0-2h 19 ± 4%, 2-6h 15 ± 2%) followed by a significant decrease to minimal levels (6-10h 2 ± 1%). The maximum number of calves head shaking (45%) at any one time occurred at the first observation (0.25h).

LA-Scoop calves

In LA-Scoop calves there was a low incidence of head shaking in the first period (0-2h 6 ± 2%), most of which occurred in the first hour, and this was significantly less than that in the next period (2-6h 13 ± 2%). This was followed by a small decrease in head shaking during the last period (6-10h 11 ± 3%), which was of intermediate magnitude between the former two periods and statistically similar to both ($P>0.05$ Mann-Whitney).

Between treatments

Over the 10 hour observation period the mean number of Control calves head shaking (0.5 ± 0.4%) was significantly less ($P<0.05$ Mann-Whitney) than that for either LA-Scoop (10 ± 1%) or Scoop (13 ± 2%) calves which were similar (Fig 5.3).

Calves which were dehorned with or without local anaesthetic had a significantly higher ($P<0.05$ Mann-Whitney) incidence of head shaking in most periods than Control calves except for the LA-Scoop calves in the first period (0-2h) and Scoop calves in the last period (6-10h) when values were similar to those in Control calves.

Ear flicking

Control calves

There were only three incidences of ear flicking in Control calves and these occurred at about one hour after treatment (Fig 5.4).

Scoop calves

Scoop calves exhibited a high incidence of ear flicking for the first six hours. The number of calves ear flicking in the first period (0-2h 14 ± 3%) was significantly

greater than that in the second period (2-6h 7 ± 2%) which was followed by a significant decrease to minimal levels in the last period (6-10h 4 ± 2%). The maximum number of calves ear flicking (35%) occurred at the first observation (0.25h).

LA-Scoop calves

LA-Scoop calves exhibited a low incidence of ear flicking in the first period (0-2h) (4 ± 3%), most of which occurred in the first hour, followed by a significant increase in ear flicking (2-6h 15 ± 2%; 6-10h 14 ± 4%) ($P<0.05$ Mann-Whitney).

Between treatments

Over the 10 hour observation period the mean number of Control calves ear flicking (0.5 ± 0.4%) was significantly less ($P<0.05$ Mann-Whitney) than that for either LA-Scoop (12 ± 2%) or Scoop (8 ± 1%) calves which were similar (Fig 5.4).

Calves which were dehorned with or without local anaesthetic had a significantly higher ($P<0.05$ Mann-Whitney) incidence of ear flicking in most periods than Control calves except for the LA-Scoop calves in the first period (0-2h) when values were similar to those in Control calves.

Neck extending and leg scratching to face (infrequent)

The numbers of calves exhibiting either of these behaviours were low and the incidence infrequent, rendering analyses for the three partial periods unreliable. Analyses on the data from the whole observation period (0-10h) were reliable and are reported here:

Neck extending

There were no occurrences of neck extending in Control calves. Over the 10 hour observation period there few (0.7 ± 0.4%) occurrences of neck extending in LA-Scoop calves and this was statistically similar to that in Control calves ($P>0.05$). The incidence of neck extending in both Control and LA-Scoop calves was less than that in Scoop calves (2 ± 1%. $P<0.05$). The instances of neck extending in LA-Scoop calves occurred between 3 and 4.25h whereas neck extending in Scoop calves occurred between 0 and 5h (Fig 5.5).

Leg scratching to face

Over the 10 hour observation period the number of Control calves scratching their face with their hind leg (0.2 ± 0.2%) was significantly less ($P<0.05$ Mann-Whitney) than that for either LA-Scoop (2 ± 1%) or Scoop (3 ± 1%) calves which were themselves similar. There was no apparent pattern over time (Fig 5.6).

Lying and Walking (time of day)

Other than an initial period (0-0.5h) of restlessness which affected the amount of walking and lying, both behaviours appeared to be related to the time of day rather than the treatment received. Walking behaviour appeared to occur as the compliment of lying which is expected because walking and lying are mutually exclusive behaviours. But walking isn't exactly the compliment of lying - as the missing component of standing has not been included.

Lying

Within each treatment there was an increase in the number of calves lying during the course of the day (Table 5.1), these increases were statistically significantly as follows: for Control calves the number lying in the first two periods (0-2h 3 ± 1%, 2-6h 5 ± 2%) was less than that lying in last period (6-10h 18 ± 4%), whereas for LA-Scoop calves the number lying during each successive period was greater than in the previous period (0-2h 0 ± 0%, 2-6h 8 ± 1%), 6-10h 18 ± 3%), and for Scoop calves the number lying in first period (0-2h 0 ± 0%) was less than that in the last two periods (2-6h 18 ± 3%, 6-10h 29 ± 4%).

Between treatments

Over the 10 hour observation period the number of Scoop calves lying (16 ± 3%) was significantly greater than that for either LA-Scoop (9 ± 2%) or Control (8 ± 2%) calves which were similar ($P<0.05$ Mann-Whitney). During the day this pattern of scoop calves lying the most occurred but was only significant in the middle period (2-6h).

Walking

Within each treatment there was a decrease in the number of calves walking over the course of the day (Table 5.1), these decreases were statistically significantly as follows: for both Control and Scoop calves the number walking in the first two periods (Control: 0-2h 7 ± 2%, 2-6h 4 ± 1%; Scoop: 0-2h 11 ± 3%, 2-6h 9 ± 2%) was greater than in the last period (for both 6-10h 0 ± 0%), whereas for LA-Scoop calves the number walking in the middle period (2-6h 13 ± 2%) was greater than in the first (0-2h 6 ± 4%) and in the last period (6-10h 4 ± 2%).

Between treatments

Over the 10 hour observation period the number of LA-Scoop calves walking (9 ± 2%) was significantly greater than that for Control calves (5 ± 2%) whereas the number of Scoop calves walking was of intermediate value (7 ± 2%) and not significantly different from either.

Vocalization and riding (external events)

Vocalization

Incidences of bawling appeared to be sporadic and occurred in response to external events such as being mustered into the yards, and the mustering of other stock nearby. Such external events did not necessarily occur at the predetermined scan time.

Riding

Over the course of the entire study there were only four riding attempts observed. These were initiated by Control calves during the initial settling period and three of them were by the same calf.

Head rubbing and head down (no pattern)

Head rubbing

The number of calves head rubbing was low and the incidence infrequent (Table 5.1), rendering analyses for the three periods unreliable. Analyses on the data from the whole observation period (0-10h) are reliable and are reported here. Over the 10 hour observation period there were no differences between treatments in the number of calves head rubbing (Control: 3 ± 1%, LA-Scoop: 2 ± 1%, Scoop: 2 ± 1%).

Head down

Over the 10 hour observation period there were no differences between treatments in the number of calves with their heads down (Control: 55 ± 3%, LA-Scoop: 56 ± 2%, Scoop: 52 ± 2%) (Table 5.1).

Restlessness index (day one)

The dominant and frequently occurring behaviours of tail shaking, head shaking and ear flicking are now discussed on terms of a summed index as described in section 5.2 (Fig 5.7).

Control calves

The mean incidence of restlessness in Control calves (0-10h 13 ± 2%) was low. There were minor but significant differences between periods: the restlessness in the second period (2-6h 17 ± 1%) was significantly greater than that in the last period (6-10h 8 ± 2%) and both were similar to that in the first period (0-2h 13 ± 5%) (Table 5.1) ($P<0.05$ Mann-Whitney).

Scoop calves

Scoop calves exhibited a high incidence of restlessness for the first six hours (0-2h 59 ± 9%, 2-6h 53 ± 5%) followed by a significant decrease in restlessness thereafter (6-10h 31 ± 3%).

LA-Scoop calves

There was a low incidence of restlessness in LA-Scoop calves during the first period (0-2h 19± 4%), most of which occurred in the first hour, and this was significantly less than that between 2 and 10h (2-6h 64 ± 6%; 6-10h 52 ± 10%) ($P<0.05$ Mann-Whitney).

Between treatments

Over the 10 hour observation period the mean incidence of restlessness in Control calves (13 ± 2%) was significantly less than that for either LA-Scoop (49± 5%) or Scoop (49 ± 4%) calves which were similar ($P<0.05$ Mann-Whitney) (Fig 5.7).

Calves which were dehorned with or without local anaesthetic had a significantly higher incidence of restlessness in most periods than Control calves except for the LA-Scoop calves in the first period (0-2h) when values were similar to those in Control calves ($P<0.05$ Mann-Whitney).

5.3.2 Summary of the behaviour of Control, Scoop and LA-Scoop calves (day one)

All calves were restless for the first 0.5 hour after treatment. Thereafter, the main differences in the behaviour of calves, according to treatment, were in changes of the pattern of restlessness over time and in the occurrence of rumination, which appeared to exhibit an inverse relationship to each other. The separate behaviours of tail shaking, head shaking and ear flicking were summed to produce a "restlessness index". It is important to note that each behaviour independently exhibited a *pattern* of incidence that was similar to the overall pattern of restlessness.

Behaviour of Control calves

The Control calves stood still and ruminated with decreasing incidence throughout the observation period (Fig 5.7). There were only three occurrences each of head shaking and ear flicking, and two of those coincided with the passing of a railway train nearby (about 100 m) which aroused all calves regardless of treatment. Over the 10 hour study period about 12% of the Control calves flicked their tails intermittently and the incidence of walking was low (5 ± 2%), did not occur after six hours, and its nature was a slow amble of a few paces. The incidence of head rubbing was low (3 ± 1%) and infrequent. There was only one occurrence of a leg scratch and this was to the eye region. Neck extending did not occur in this group.

Behaviour of Scoop calves

Scoop calves exhibited a high level of restlessness immediately after treatment which lasted for six hours (Fig 5.7). This activity was constituted predominantly from 28% of

the calves tail shaking, 18% head shaking and 12% ear flicking. In addition, there was some (10%) walking (pacing) and low sporadic incidences of neck extension and leg scratch to face occurred (<3% for both. Note that all numbers are the weighted means of the first and second periods). Neck extension did not occur after five hours and leg scratch occurred sporadically with no apparent time course pattern. There was virtually no rumination (2.5% 0-6h).

Six hours after treatment the incidence of those behaviours had decreased (head shaking, ear flicking and pacing <4% for each) except for tail shaking which appeared to continue at high levels (24%). The incidence of rumination increased and approached levels similar to those in the Control calves.

Behaviour of LA-Scoop calves

For the first two hours after treatment the behaviour of LA-Scoop calves was similar to that of Control calves: 20% of the calves were ruminating, 9% tail flicking and there was virtually no head shaking, ear flicking or walking (<6% for each) (Fig 5.7). After two hours restlessness increased and remained high. Like Scoop calves between 0-6h, this activity was reflected primarily by 37% of the calves tail shaking, 13% head shaking and 15% ear flicking. In addition, there was some (3%) pacing (walking) and there were low and infrequent incidences of leg scratching and neck extending (<4% for each). Leg scratching occurred without pattern for the first seven hours and neck extending only occurred between 3.25 and 4.25 hours after treatment.

Whilst the restlessness index in the last period (6-10h 52%) was statistically similar to that in the middle period (2-6h 64%) there was an obvious decrease in restlessness after 7.5h, but this decrease was rendered invisible by the grouping of the data. Between 6 and 10h the number of calves ruminating was similar to that in Control calves ($P>0.05$); this appears to be as a result of decreasing levels in the Control group.

5.3.3 Behavioural comparisons (Day two)

It was not possible to analyse the data from day two using the Mann-Whitney test because of the low incidence of all behaviours, the only exception being tail shaking and rumination. Having remarked so, there appeared to be no differences between treatments on either study day. But, there appeared to be some differences in the behaviour and bleeding in calves according to the day the study was conducted (see discussion 5.4.5). It is possible that weather played a role. For that reason the day two data from the different studies are discussed separately. The following reports the four behaviours of rumination, tail shaking, head shaking and ear flicking (see Table 5.2).

In the first study the weather was sunny and hot, and there appeared to be a lot of flies present. There were no apparent differences between treatments in the incidence of tail shaking (Control 9±2%; Scoop 15±2%; LA-Scoop 13±2%) or rumination (Control 14±4%; Scoop 11±2%; LA-Scoop 12±3%). For both tail shaking and rumination the levels observed on day two were similar to those exhibited by the Control calves on day one (2-6h). For both, the data were acquired at the same time of the day. Head shaking and ear flicking were not observed in Control calves and were infrequent in calves dehorned with or without local anaesthetic.

In the second study it rained all day, was cold and flies were scare. There appeared to be no differences in behaviour between treatments. The incidence of rumination was low (Control 6±3%; Scoop 6±3%; LA-Scoop 9±2%) and there was no tail shaking or head shaking and only one ear flick was observed.

Table 5.1 Percentage of calves (mean ± sem) exhibiting a particular behaviour during the periods 0-2h, 2-6h 6-10h, or 0-10h after treatment. Values with different letters differ significantly ($P<0.05$), where:
a,b,c indicate differences within treatments,
x,y,z indicate differences between treatments,

	Time periods							
Behaviour	**0-10h**		**0-2h**		**2-6h**		**6-10h**	
Ruminating								
Control	17 ± 3	**x**	28 ± 7	**xa**	15 ± 3	**xa/b**	9 ± 4	**xb**
LA-Scoop	7 ± 2	**y**	20 ± 7	**xa**	1 ± 1	**yb**	4 ± 2	**xb**
Scoop	4 ± 1	**y**	4 ± 3	**ya/b**	1 ± 1	**ya**	9 ± 2	**xb**
Tail shaking								
Control	12 ± 1	**x**	9 ± 3	**xa**	17 ± 1	**xb**	8 ± 2	**xa**
LA-Scoop	27 ± 3	**y**	9 ± 2	**xa**	37 ± 3	**yb**	27 ± 4	**yb**
Scoop	28 ± 2	**y**	26 ± 3	**ya**	31 ± 3	**ya**	24 ± 2	**ya**
Head shaking								
Control	1 ± 0	**x**	2 ± 1	**xa**	0 ± 0	**xa/b**	0 ± 0	**xb**
LA-Scoop	10 ± 1	**y**	6 ± 2	**xa**	13 ± 2	**yb**	11 ± 3	**ya/b**
Scoop	13 ± 2	**y**	19 ± 4	**ya**	15 ± 2	**ya**	3 ± 1	**xb**
Ear flicking								
Control	0.5 ± 0.4	**x**	2 ± 1	**xa**	0 ± 0	**xa**	0 ± 0	**xa**
LA-Scoop	12 ± 2	**y**	4 ± 3	**xa**	15 ± 2	**zb**	14 ± 4	**yb**
Scoop	8 ± 1	**y**	14 ± 3	**ya**	7 ± 2	**ya/b**	4 ± 2	**yb**
Restlessness								
Control	13 ± 2	**x**	13 ± 5	**xa/b**	17 ± 1	**xa**	8 ± 2	**xb**
LA-Scoop	49 ± 5	**y**	19 ± 4	**xa**	64 ± 6	**yb**	52 ± 10	**yb**
Scoop	49 ± 4	**y**	59 ± 9	**ya**	53 ± 5	**ya**	31 ± 3	**yb**

Table 5.1 cont.,

Behaviour	Time periods 0-10h		0-2h		2-6h		6-10h	
Neck extending*								
Control	0 ± 0	x	0 ± 0	xa	0 ± 0	xa	0 ± 0	xa
LA-Scoop	0.7 ± 0.4	x	0 ± 0	xa	1 ± 1	xa	0 ± 0	xa
Scoop	2 ± 1	y	4 ± 2	ya	2 ± 1	xa/b	0 ± 0	xb
Leg scratching to face*								
Control	0.2 ± 0.2	x	0 ± 0	xa	0 ± 0	xa	0 ± 0	xa
LA-Scoop	2 ± 1	y	2 ± 1	xa	3 ± 1	ya	1 ± 2	xa
Scoop	3 ± 1	y	3 ± 1	xa	2 ± 1	x/ya	4 ± 4	xa
Walking**								
Control	5 ± 2	x	7 ± 2	xa	4 ± 1	xa	6 ± 6	xa
LA-Scoop	9 ± 2	y	6 ± 4	xa	13 ± 2	yb	4 ± 2	xa
Scoop	7 ± 2	x/y	11 ± 3	xa	9 ± 2	x/ya	0 ± 0	xb
Lying**								
Control	8 ± 2	x	3 ± 1	xa	5 ± 2	xa	18 ± 4	xb
LA-Scoop	9 ± 2	x	0 ± 0	xa	8 ± 1	xb	18 ± 3	xc
Scoop	16 ± 3	y	0 ± 0	xa	18 ± 3	yb	29 ± 4	xb
Head rubbing#*								
Control	3 ± 1	x	3 ± 1	x/ya	3 ± 1	xa	1 ± 1	xa
LA-Scoop	2 ± 1	x	2 ± 1	ya	3 ± 1	xa	1 ± 1	xa
Scoop	2 ± 1	x	6 ± 1	xa	1 ± 1	xb	1 ± 1	xb
Head down#								
Control	55 ± 3	x	49 ± 6	xa/b	63 ± 3	xb	48 ± 4	xa
LA-Scoop	56 ± 2	x	51 ± 3	xa	55 ± 4	x/ya	60 ± 4	xa
Scoop	52 ± 2	x	48 ± 5	xa	54 ± 2	ya	52 ± 3	xa

* behaviours occurring with low incidence

** behaviours where the pattern of incidence appears related to the time of day not treatment

\# behaviours with no apparent pattern of incidence

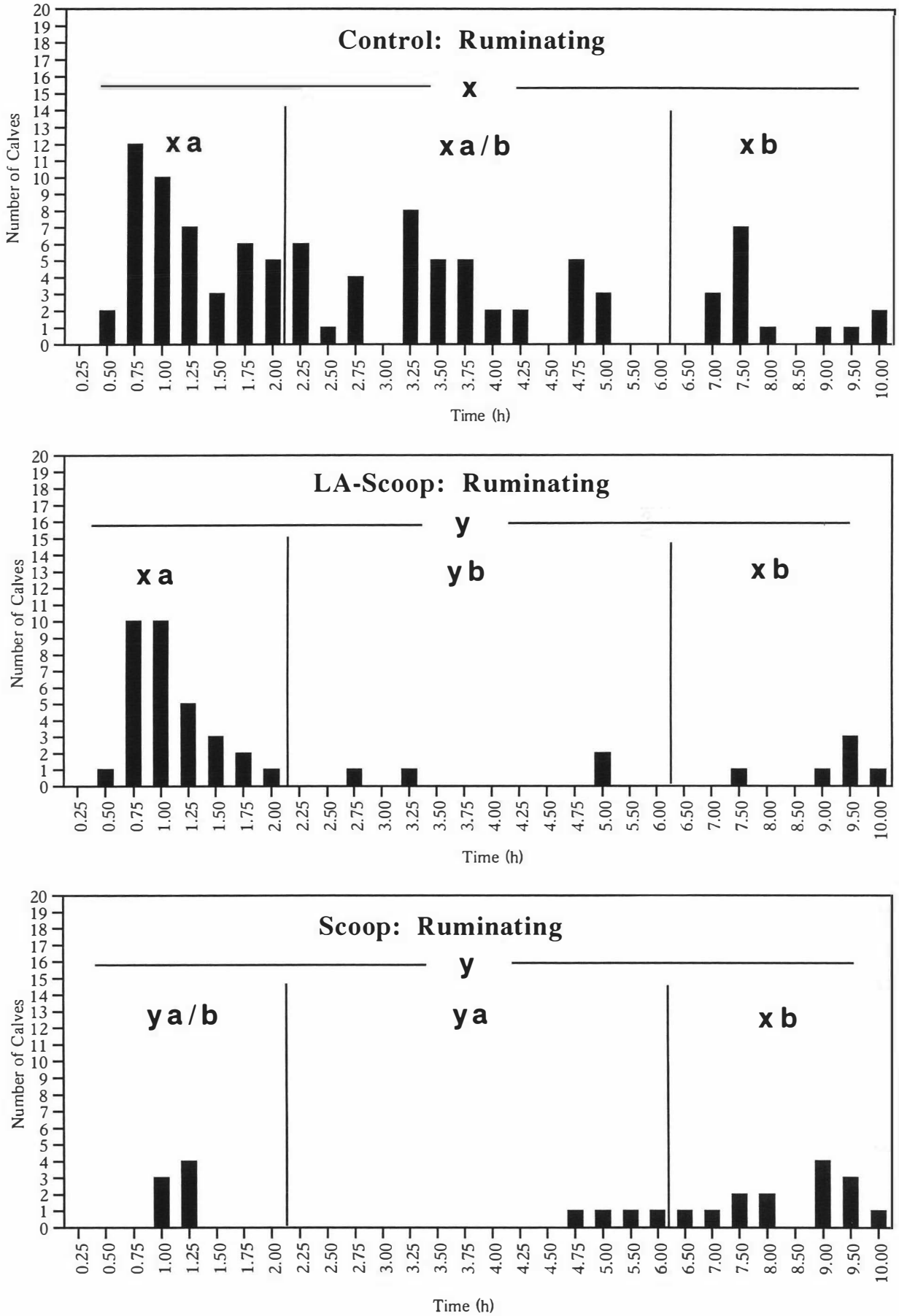


Fig 5.1 Number of calves ruminating after treatment. Analyses were conducted for the periods: 0-2h, 2-6h, 6-10h & 0-10h. Values with different letters differ significantly ($P<0.05$. Mann-Whitney), where:
a,b,c indicate differences within treatments,
x,y,z indicate differences between treatments.

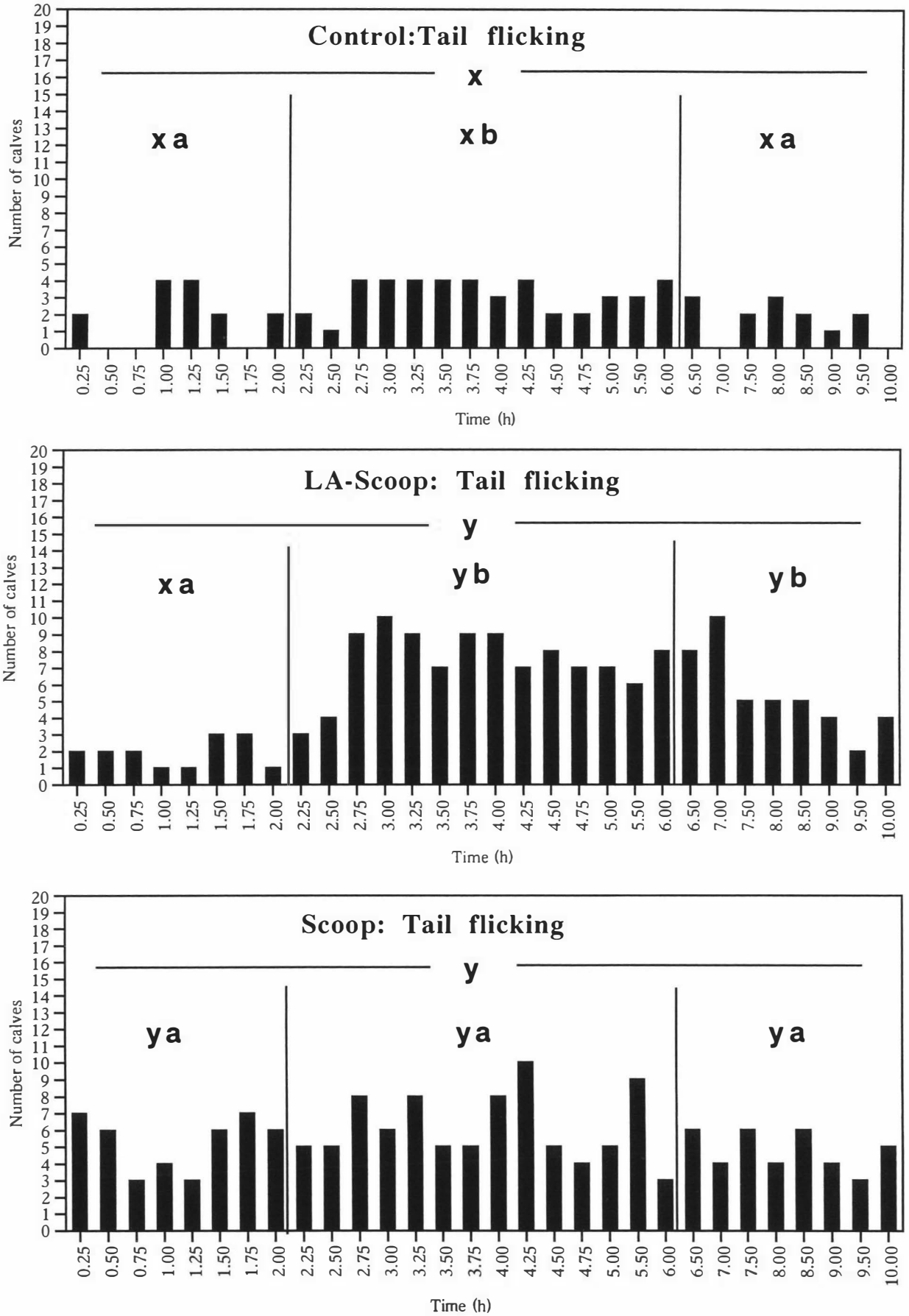


Fig 5.2 Number of calves tail flicking after treatment. Analyses were conducted for the periods: 0-2h, 2-6h, 6-10h & 0-10h. Values with different letters differ significantly ($P<0.05$. Mann-Whitney), where:
a,b,c indicate differences within treatments,
x,y,z indicate differences between treatments.

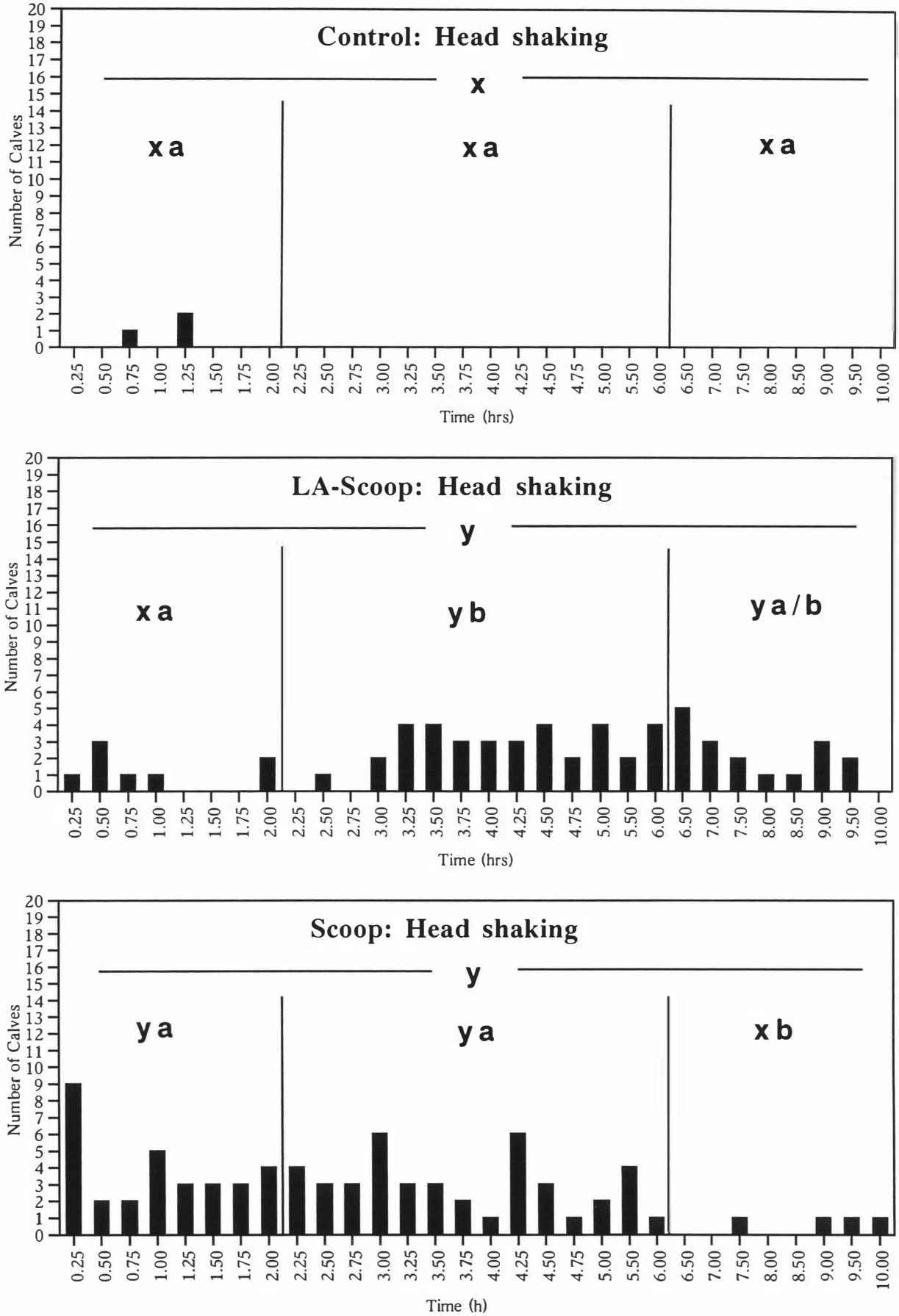
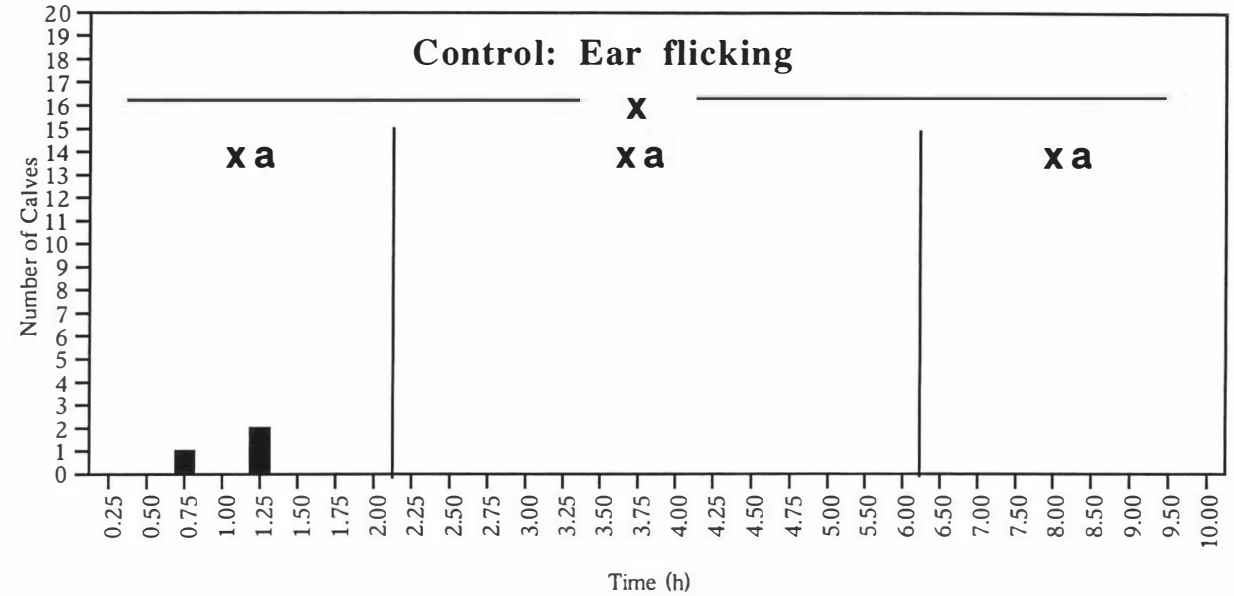
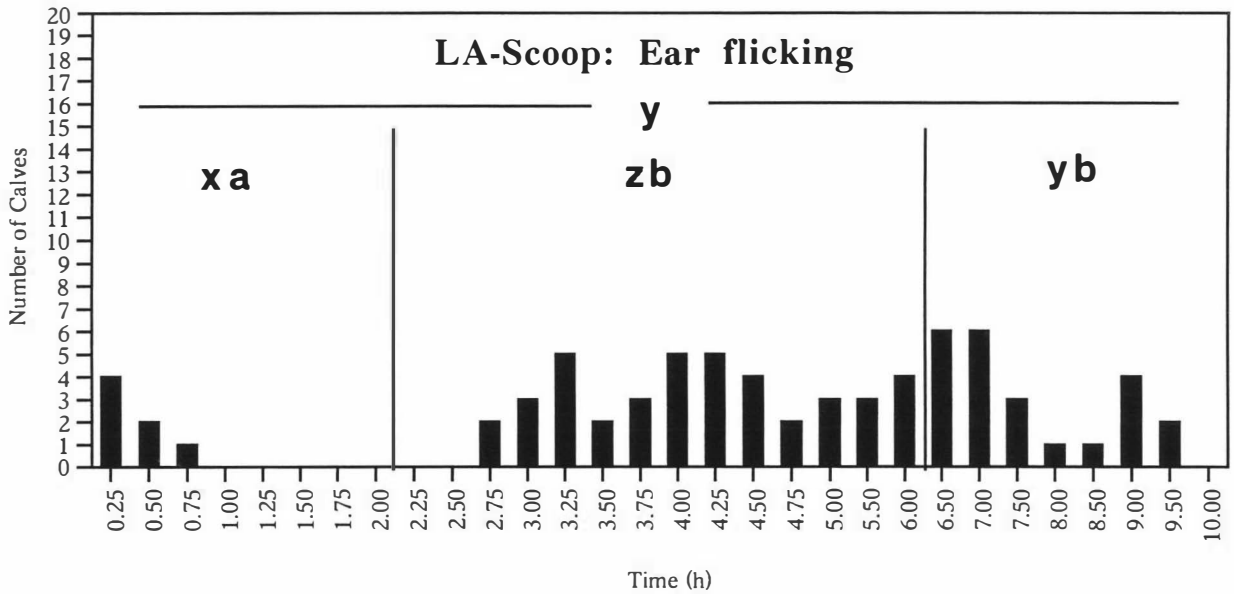


Fig 5.3 Number of calves head shaking after treatment. Analyses were conducted for the periods: 0-2h, 2-6h, 6-10h & 0-10h. Values with different letters differ significantly ($P<0.05$. Mann-Whitney),
a,b,c indicate differences within treatments,
x,y,z indicate differences between treatments.





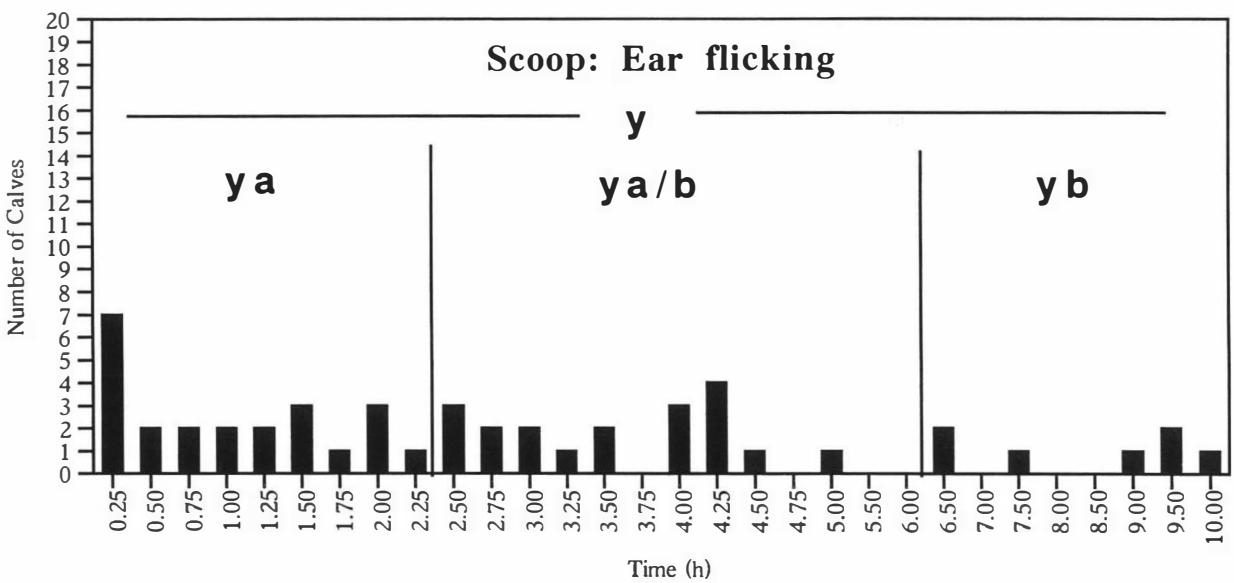


Fig 5.4 Number of calves ear flicking after treatment. Analyses were conducted for the periods: 0-2h, 2-6h, 6-10h & 0-10h. Values with different letters differ significantly ($P<0.05$. Mann-Whitney), where:
a,b,c indicate differences within treatments,
x,y,z indicate differences between treatments.

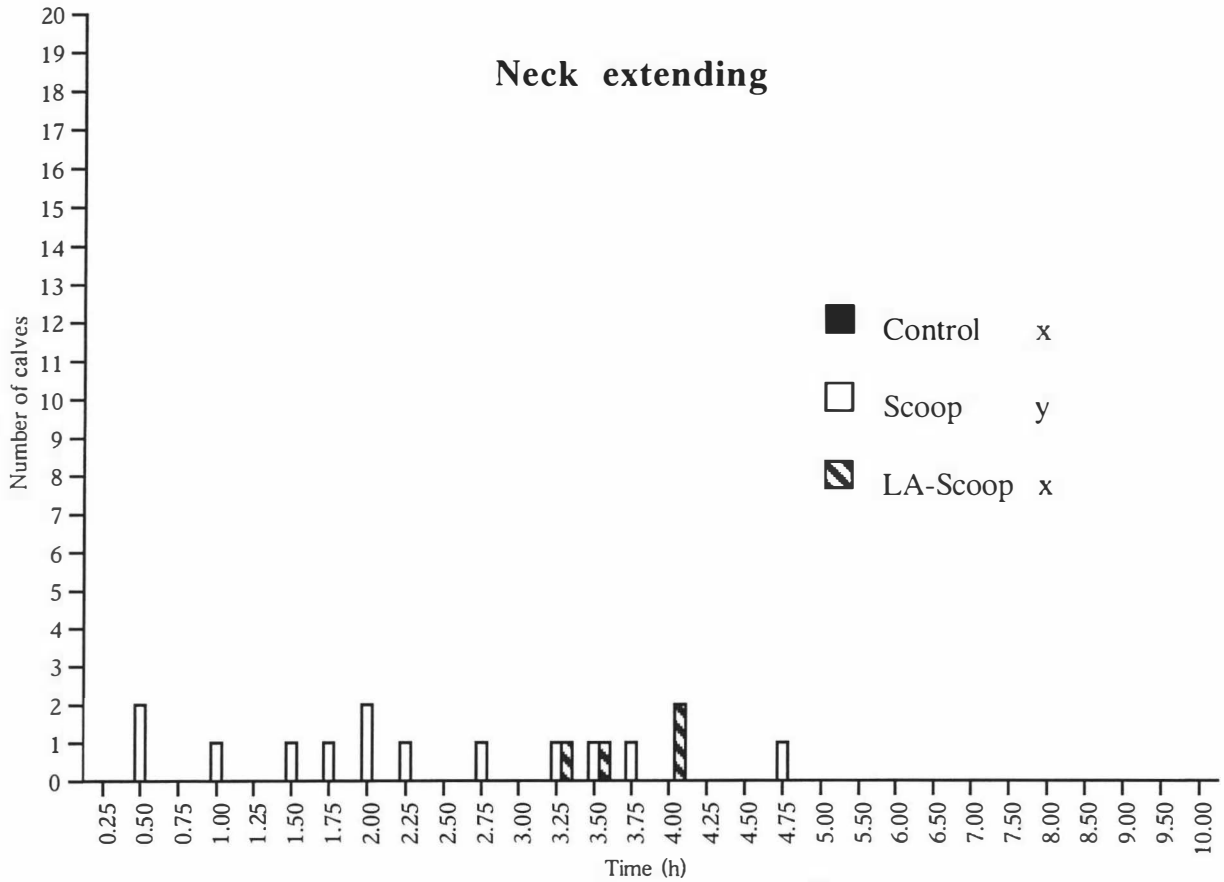


Fig 5.5 Number of calves neck extending after treatment for the 10h observation period only. Different letters (x, y, and z) indicate significant differences between treatments (P<0.05).

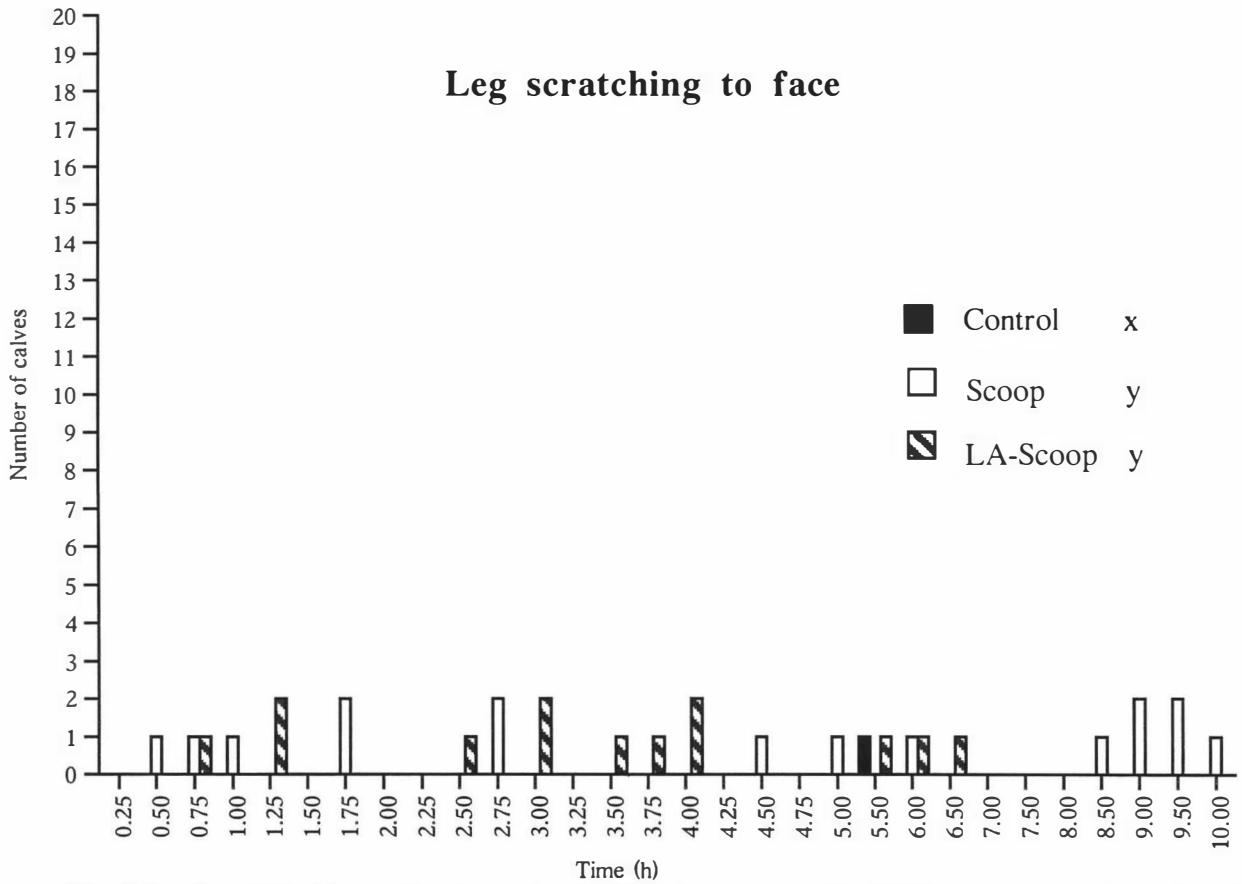


Fig 5.6 Number of calves leg scratching to face after treatment for the 10h observation period only. Different letters (x, y, and z) indicate significant differences between treatments (P<0.05).

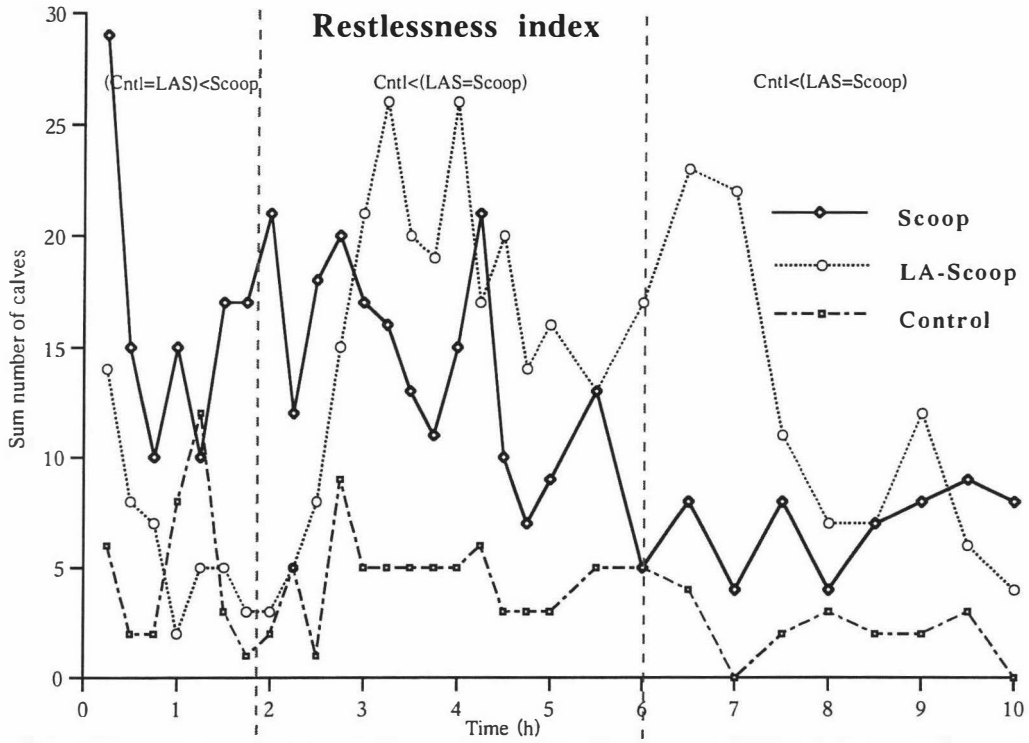


Fig 5.7 The timecourse of the restlessness index after Scoop, LA-Scoop and Control treatment (0-10h). Significant differences between treatments are indicated by mathematical notation on the graph (P<0.05). The restlessness index is the sum of the number of calves tail flicking, head shaking or ear flicking.

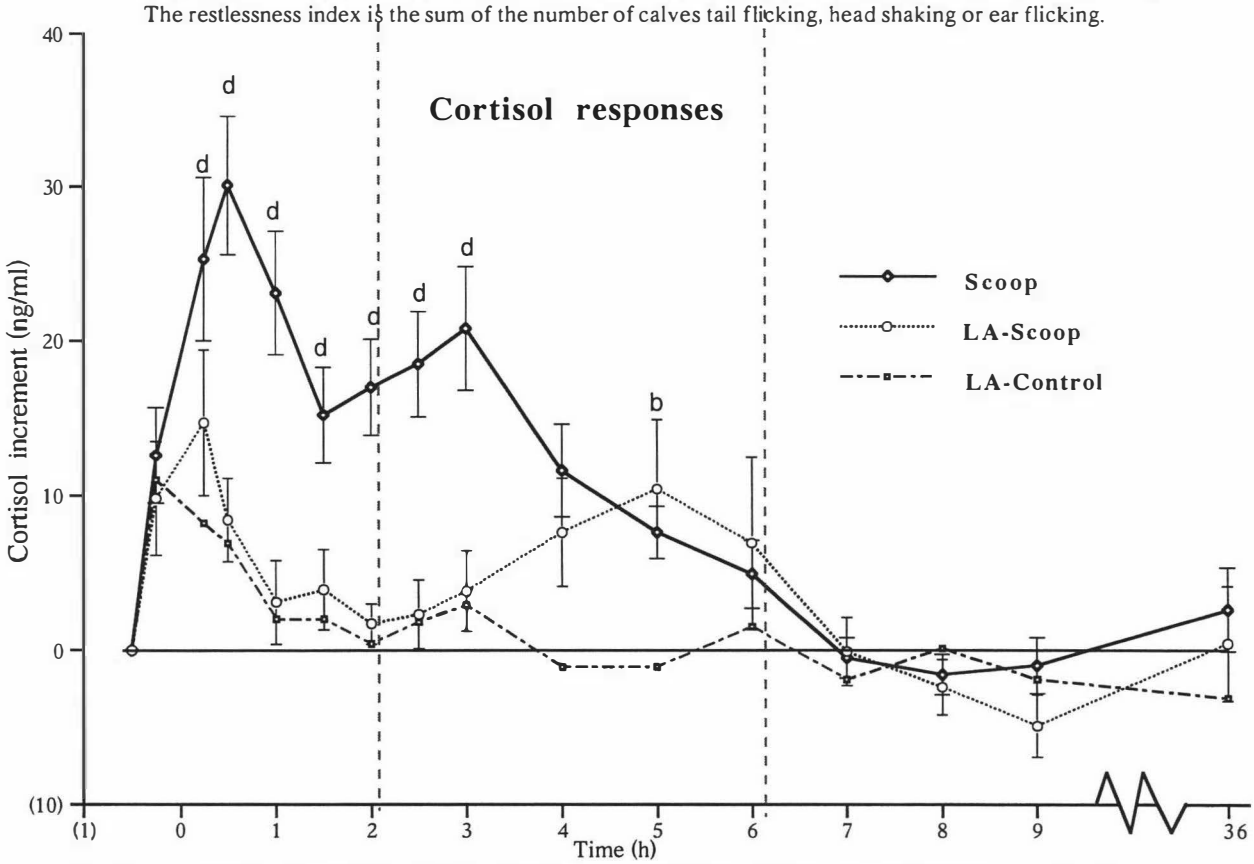


Fig 5.8 For comparison with Fig 5.11. The timecourse of the cortisol response (mean ± sem) of calves after Scoop, LA-Scoop and Control treatment (data from Chapter four). Significantly differences (P<0.05) between treatments are indicated: b: LA-Scoop ≠ LA-Control; d: Scoop ≠ LA-Scoop.

Table 5.2 The percentage of calves head shaking, ear flicking, tail flicking or ruminating on day two after treatment (mean±sem), according to the day the study was conducted.

Behaviour	Treatment		
Group 1 (hot weather)	Control	Scoop	LA-Scoop
Head shaking	0±0	1±1	1±1
Ear flicking	0±0	4±2	5±2
Tail flicking	9±2	15±2	13±2
Rumination	14±3	11±2	12±3
Group 2 (cold weather)			
Head shaking	0±0	0±0	0±0
Ear flicking	0±0	0±0	1±1
Tail flicking	0±0	0±0	0±0
Rumination	6±3	6±2	9±2

For group 1 the weather was hot and sunny, for group 2 the weather was cold and raining.

5.4 DISCUSSION

Using behavioural measurements as an index of the acute pain-induced distress apparently caused by dehorning with or without local anaesthetic, the present study led to the following findings:

1) There were distinct behavioural profiles according to the treatment received which were presumably indicative of distressed and non-distressed states.

2) Calves dehorned without local anaesthetic exhibited a high incidence of restlessness that lasted for six hours, which presumably indicated the acute pain-induced distress of dehorning.

3) Prior application of a local anaesthetic virtually abolished the behavioural response after Scoop dehorning for the first two hours. Thereafter, between 2 and 6 hours, restlessness increased to levels similar to those of Scoop calves during the first six hours. It would appear that local anaesthetic *delayed* the distress response.

4) The timecourse of the restlessness in the Control, Scoop and LA-Scoop calves corresponded to the timecourse of plasma cortisol concentrations in those groups respectively (Chapter four).

5) The incidence of rumination appeared to occur in an inverse relationship to restlessness. Both were apparently sensitive indices of pain-induced distress.

The results will be discussed and the following aspects considered. The criteria to be met for using changes in behaviour to indicate changes in emotional state will be described. Limitations upon interpretation of the data are discussed and design modifications are suggested to assist future work should any be conducted.

In the present study, twelve behaviours were measured in order to assess the pain-induced distress that calves experience when being dehorned with or without local anaesthetic. Of these the four behaviours of tail shaking, head shaking, ear flicking and rumination were found to be the most reliable and informative. These four behaviours satisfied the following criteria required to use behaviour as evidence of pain-induced distress (Stamp-Dawkins, 1980; Ewbank, 1985; Mellor *et al.,* 2000): a) they showed distinct differences between treatment; and b) interpretation was in agreement with a similar physiological study (Chapter four); plus c) these behaviours occurred in all groups being compared and assured a validating continuum (Lester *et al.,* 1996) thus

avoiding the problems associated with drawing conclusions based on behaviours which do not occur in one group (see Lester *et al's.* (1996) critique of Molony *et al.* (1993) and Shutt *et al.* (1988)). Furthermore, these behaviours occurred in sufficient frequency that the Mann-Whitney statistical analyses were reliable. The three behaviours of tail shaking, head shaking and ear flicking were summed to produce a "restlessness index" and the limitations of using these derived data are discussed below. The remaining eight behaviours measured were either related to the time of day (lying, walking), external events (vocalization), had no pattern according to treatment (head down position) and/or the incidence of occurrence was extremely low rendering statistical analysis unreliable (riding, head rubbing, neck extending and leg scratch to face). Therefore the following discussion will focus on the four behaviours of tail shaking, head shaking, ear flicking and rumination and on the restlessness index. None-the-less occasional references will be made to the incidences of other behaviours.

5.4.1 Dehorning

Scoop calves exhibited a high level of restlessness immediately after treatment which lasted about six hours. This activity consisted of tail shaking (28%), head shaking (18%), ear flicking (12%) as well as pacing (10%) and infrequent leg scratching and neck extending (<3% for each). This was in striking contrast to the lack of activity in the Control calves which mainly stood still, gently flicked their tails (12%) and ruminated (24%). The behavioural response of the Control calves presumably reflected the psychological reaction to the novelty of the experimental situation which included disruptions to social spacing and hierarchies, to which cattle are particularly sensitive (Hinch, 1994), as well as any reaction to the proximity of distressed and bleeding calves. The high incidence of rumination and the lack of restlessness in Control calves suggest that the experimental situation caused little distress.

The differences in behaviour between Scoop and Control calves are presumably because the Scoop calves experienced pain-induced distress. This finding is supported by the lack of rumination in Scoop calves (compared to Control calves) as rumination decreases when animals are stressed or in pain (Fraser and Broom, 1990). It is also supported by the fact that the timecourse of the restlessness in Scoop calves corresponded with the timecourse of the plasma cortisol concentrations after dehorning (Chapter four), the latter being taken as evidence of distress. Furthermore, local anaesthetic virtually eliminated the behavioural differences between Scoop and Control calves for the first two hours after treatment and confirmed that these behaviours indicate pain-induced distress and not a reaction to non-noxious stimuli.

The changes in behaviour of the Scoop calves, in particular tail shaking, head shaking, ear flicking and general activity, are similar to those reported during and after cautery

disbudding (Taschke and Folsch, 1993, 1995; Morisse *et al.*, 1995) and amputation dehorning (McMeekan *et al.*, 1999). In addition, the six hour duration of the behavioural response is similar to that reported by McMeekan *et al.* (1999).

5.4.2 Local anaesthetic

The application of local anaesthetic prior to dehorning virtually eliminated the behavioural response exhibited by Scoop calves during the first two hours such that the behaviour was similar to that of the Control calves. This is taken as evidence that the local anaesthetic decreased or eliminated the nociceptive barrage from damaged nerve and inflamed tissue and that the calves were experiencing minimal distress at this time. Furthermore, these results support the finding that the application of the local anaesthetic *per se* caused minimal distress (Morisse *et al.*, 1995; Petrie *et al.*, 1995, 1996a,b; Graf and Senn, 1999).

After 2 hours there was an increase in restlessness in LA-Scoop calves and this behavioural profile (37% tail shaking, 13% head shaking, 15% ear flicking, 13% pacing) was similar to that of Scoop calves for the first six hours ($P>0.05$). This similarity in behaviour suggests that during this period, the LA-Scoop calves were experiencing pain-induced distress. The marked increase in restlessness in LA-Scoop calves after two hours presumably reflected the return of sensation to the area served by the cornual nerve, and concomitantly the first appearance of noxious inputs associated with the tissue damage to that area. This is supported by an assessment of the efficacy of anaesthesia which was tested by pricking the cutaneous area served by the cornual nerve with a hypodermic needle. At about 2.5h calves began to respond to this stimulus, and in addition, plasma cortisol concentrations increased at this time (Chapter four). Similar responses were reported after dehorning plus local anaesthesia (Petrie *et al.*, 1996b) and after tail-docking plus epidural local anaesthesia (Petrie *et al.*, 1996a) in calves.

Unfortunately in the current study there was no LA-Control group and therefore it cannot be demonstrated that the delayed rise in restlessness was not due to the local anaesthetic wearing off, or any other sensation as distinct from the pain. Behavioural responses can vary according to the site and duration of the stimulus. It is possible that the behaviours directed to the region of trauma may not reflect noxiousness, but rather the annoyance or discomfort from dripping blood, flies, movement of cold air into and out of the cranial sinuses or a change in the weight distribution of the head. The similarity of the behaviour of the Control and LA-Scoop calves in the first two hours, during analgesia, suggests that these associated non-noxious stimuli have minimal effects on behaviour compared to the effect of dehorning on behaviour. This similarity is surprising given that one might have expected more differences elicited by bleeding

from the scoop wounds, associated flies or other sensations (e.g. loss of sensation), all of which were absent in the Control calves. Furthermore, the cortisol data support the inference that non-noxious stimuli have minimal effects (Chapter four). The similarity of the cortisol responses of the LA-Control and Control calves suggests that the administration and the wearing-off of the local anaesthetic have minimal effect. The similarity of the cortisol response of LA-Scoop and LA-Control calves in the first three hours suggests that the delayed rise in cortisol in LA-Scoop calves after 3h is elicited by pain-induced distress rather than other non-noxious stimuli. Both the behaviour and the cortisol data indicate that after two hours the LA-Scoop calves experienced pain.

In some physiological studies the effect of local anaesthetic on the timecourse of the cortisol response following cautery disbudding has not been apparent. This has occurred where either the cortisol response elicited by the procedure was similar to the cortisol response after control handling and bleeding, which suggests that the procedure causes minimal distress (Petrie *et al.,* 1996a,b), or where the sampling scheme was not of sufficient frequency or duration to detect the changes in plasma cortisol concentrations (Morisse *et al.*, 1995). Furthermore, the effect of local anaesthetic can be obscured by the occurrence of a high number of cases where there is incomplete anaesthesia (see Chapter four). This may be as a result of operator imprecision or variations of innervation (Weaver, 1986). Morisse *et al.* (1995) reported a 40% occurrence of incomplete anaesthesia. In the current study the number of times it was necessary to administer local anaesthetic twice was not recorded, but, in the cortisol study (Chapter four) 34% (ie. 8/23) of the calves required a second dose. It was apparent from the timecourse of the cortisol concentrations that two calves had incomplete anaesthesia and they were therefore excluded from the study as outliers. Interestingly six of the seven outliers, the data from which were excluded from the results, received local anaesthetic. It is possible that these aberrant responses were idiosyncratic responses caused by inadvertent intravenous injection (Weaver, 1986). In many studies the application of local anaesthetic decreased the occurrence of escape behaviours, such as tripping, rearing and backwards locomotion, during the noxious procedure (Morisse *et al.*, 1995). Indeed, this is a general observation (L.R Matthews; D.J Mellor; K.J Stafford, personal communication). In those studies above where no apparent therapeutic effect of local anaesthesia was measured it was none-the-less recommended. This was because of anecdotal observations of decreased escape and avoidance behaviours during a noxious procedure when local anaesthetic was given [e.g. see Mellor and Stafford's (1997) comments on Petrie *et al.* (1996b)]. Common sense and experience tell us that local anaesthetics relieve pain. Where the effect of local anaesthetic is not measured, but is observed anecdotally, perhaps it is our ethical duty to either act conservatively on behalf

of the animal and give local anaesthetic or, refine these experiments in order to measure the effect of local anaesthetic.

5.4.3 Differences between the behaviour and cortisol data

Both the behaviour and the cortisol data indicated that the response to scoop dehorning lasted about six hours and that the local anaesthetic reduced that response for the first two to three hours. Furthermore, the cortisol data indicated that local anaesthetic halved the distress response to dehorning (Chapter four). However the quantitation of the effect of local anaesthetic using the behaviour data is problematic. It is clear that the local anaesthetic decreased the behavioural response to dehorning for about the first two hours. The problem lies in assessing the magnitude of the behavioural response in the middle period (2-6h) and assessing when exactly the LA-Scoop response is back to Control levels. At a casual glance it appears that the local anaesthetic has merely displaced the response by two hours because the magnitude and duration (2-7.5h) appears similar to that of the scoop response (0-6h) (Fig 5.7). However the statistical analyses indicated that the behavioural responses of Scoop and LA-Scoop calves between 2 and 6 hours were similar (i.e. when both experienced pain) and between 6 and 10 hours were also similar (i.e. response over). This suggests that the LA-Scoop response is two hours (33%) shorter than the Scoop response. It is not possible to deduce from these data whether the local anaesthetic has merely displaced the response or whether it has reduced the response by up to a third. This may be because of the grouping of the data for statistical analyses, or problems with weighting and comparing behaviours.

If local anaesthesia delayed the behavioural response, rather than decreasing it, this would suggest one of the following physiological etiologies (discussed in Chapter four): a) Central effect: that somehow the local anaesthetic has prevented the endogenous stress-induced analgesia, which may occur after the Scoop dehorning, from being activated; or b) Local effect: that the reduced stress response in the first two hours (whether this be as a direct result of the pain, or the indirectly from algogenic substances) has somehow affected the wound healing process so that the pain experienced is greater at this later stage than would have occurred had there been no local anaesthetic; or c) Perception effect: that the wearing off of local anaesthetic and the return of sensation, including the first experience of pain sensation, somehow combines to heighten the perception or experience of pain.

Perhaps the behavioural data provide a more sensitive index of duration or magnitude of response than the cortisol data. It is not possible to deduce from these data whether the local anaesthetic has merely displaced the response or whether it has reduced the response by up to a third. Nor is it possible to state which approach (cortisol *versus*

behavioural indices) more accurately compares the scoop to LA Scoop response. Given the limitations of the experimental design of the behaviour study we are more confident in the cortisol data. However, dehorning studies since have shown that local anaesthetic merely delayed the cortisol response (Petrie *et al.*, 1996b; McMeekan *et al.*, 1998a; Sutherland, 1999).

5.4.4 Interpretation issues concerning specific behaviours

Rumination

The ruminant as an eat and hide animal retires to a safe place to ruminate (Kilgour and Dalton, 1984). The feed acquisition behaviours of grazing and ruminating are decreased when animals are stressed (Schalk and Amadon, 1928) or in pain (Fraser and Broom, 1990). This finding has been confirmed in the present study where rumination occurred in an inverse relationship to restlessness. This observation supports the view that rumination may be a sensitive index of stress.

The apparent similarity in the incidence of rumination in Control, Scoop and LA-Scoop calves after six hours should be approached with caution. This similarity may be fortuitous. The decrease in rumination throughout the day in Control calves was presumably due to decreasing rumen content. But for calves which had been dehorned, with or without the local anaesthetic, the increase in rumination after six hours may have reflected full rumen contents, rather than indicating a decrease in pain-induced distress. It is possible that had food been provided throughout the day, the difference in occurrence of rumination between dehorned and Control calves might have persisted beyond six hours. It is interesting that near the end of the study period there was no apparent increase in restlessness elicited by the additional stressor of hunger.

There was virtually no rumination during periods of high restlessness. It is interesting to note however, that in Scoop calves between 0.5 and 1.5 hours after treatment, there was a transient decrease in restlessness and a transient increase in rumination which corresponded to a decrease in plasma cortisol concentrations from peak values (Chapter four). These three events indicate that there is a decrease or change in noxious input during this period, which may be due to decreased or changed nociceptive input from either the wound site or as a result of descending inhibition (i.e. stress-induced analgesia).

Tail shaking

After six hours the behavioural profile in Scoop calves was similar to that of the Control calves, and the behavioural profile in LA-Scoop calves was approaching that of the Control calves. However, tail shaking in both Scoop and LA Scoop calves continued at high levels. McMeekan *et al.* (1999) reported similar continued tail shaking after

dehorning of calves, and this tail shaking returned to levels observed in Control calves by 24h. It is possible that a high level of tail shaking reflects an urge to escape a stimulus, or it may reflect general distress. Tail shaking presumably involves less effort and, after an insult to the head region, elicits less pain, than head shaking or ear flicking. Alternatively it may be that tail shaking is a social signal and that these cattle are broadcasting their discomfort to the rest of the herd.

Neck extending

Neck extending is associated with animals that are in pain or diseased (Hinch, 1994). In this study it was an infrequent abnormal behaviour which only occurred during periods of maximal restlessness. In Scoop calves neck extending only occurred during the first six hours and in LA-Scoop calves between 3 and 4h. The occurrence of this behaviour corresponded to high cortisol levels (Chapter four) which suggests that neck extending is a behaviour indicating extreme pain.

Leg scratching

Leg scratching occurred with low incidence and without pattern in calves dehorned with or without local anaesthetic, suggesting that this is a response to non-noxious stimuli, perhaps the dripping blood or the presence of flies.

Restlessness index

The restlessness of the LA-Scoop calves between 2 and 6 hours, appears to be similar to that in Scoop calves during the first six hours. Evaluation of these differences is complicated by the restlessness index being the sum of three behaviours. Should the values be compared arithmetically? To do so could magnify the apparent distress response, or imply that those three behaviours had equal weighting with respect to distress. Had the behaviours been scored to incorporate intensity, duration or number of bouts it may still not have been possible to arithmetically compare the data. Composite measures can be justified, when the behaviours are related (Martin and Bateson, 1986). For simplicity, and given the lack of evidence to the contrary, the behaviours were given equal weighting. However, this approach should be viewed with caution (Mellor *et al.,* 2000). Note that the restlessness index used here is not the same as the restlessness score that has been defined as the number of times an animal gets up and down (Mellor and Murray, 1989a; Lester *et al.,* 1996; Petrie *et al.,* 1996a).

Noxious stimulation, in addition to metabolic and neuroendocrine changes, elicits classical withdrawal reflexes and/or defence and escape behaviours, termed the "fight or flight" response, which are designed to minimize further injury, and later on the noxious stimulation elicits behaviours aimed at promoting healing. Pain has been associated with decreased activity (Fraser and Broom,1990), increased restlessness or both

(Lester *et al.*, 1996). Decreased activity presumably minimizes further episodes of pain and promotes healing through rest. Increased activity presumably reflects an animal's urge to escape the source of the noxious stimulus. The walking, or rather pacing or running, presumably reflected an urge to escape the noxious stimuli. Head shaking, ear flicking or leg scratching to the face may be an attempt to dislodge the source of the noxious stimulus or a response to non-noxious stimuli, for example the dripping of blood. Presumably there is a conflict of motivation between the desire to escape and the desire to decrease further episodes of pain caused by movement, combined with the desire to blend in with the herd and thus avoid predation.

5.4.5 Day two after treatment

There appeared to be no differences in the behaviour of Control, Scoop or LA-Scoop calves on day two after treatment, and this behaviour appeared to be similar to that of the Control calves on day one. However, there appeared to be subtle differences in the behaviour and bleeding of dehorned calves, according to the weather. In the first study the weather was sunny and hot and flies were abundant. In the second study it rained all day and was cold and flies were scarce.

On the hot day some calves irrespective of treatment shook their tails at a level similar to that of the Control calves on day one. Only those calves dehorned with or without local anaesthetic exhibited infrequent head shaking and ear flicking, whereas, on the cold day there was no tail shaking, no head shaking and only one occurrence of ear flick observed. On the day that it was was hot there was more bleeding: one calf exhibited arterial bleeding from its scoop wound, another calf dripped blood, and three calves oozed blood all day, one of these having blood in its nostrils. There was blood covering much of the face of these five calves and to a lesser extent over the heads of the remainder of the group. It was necessary to apply a tourniquet and later a bandage and antibiotic to the calf that with arterial hemorrhage. By contrast, in the second study, when it was raining and cold, there was less evidence of bleeding. Some calves had a lot of blood on their face, and three calves had evidence of fresh blood in their nostrils. Our data does not allow us to analyse whether these differences in behaviour or bleeding between days were significant. Furthermore, it is not possible to say whether these differences in behaviour reflect differences in the pain-induced distress experienced as the different environmental conditions may affect not only the magnitude of non-noxious stimuli but also the pain-induced distress. However, the data suggest that the weather could have a significant impact on the experience and outcome of such procedures, and should be taken into account when dehorning.

5.4.6 Critique and recommendations to experimental design

A degree of caution should be exercised when drawing firm conclusions from this study for the following reasons: Whilst marked differences in the pattern of restlessness were observed, there were few occasions where the occurrence of a particular behaviour exceeded 30%. For this reason it is likely that any external event, for example, the mustering of stock nearby, while possibly affecting only a small number of calves, could have a substantial effect on the data. The low incidence of active behaviours of calves in all treatments may have been a result of the experimental environment, a response for improving the chances of survival in the face of predation or possibly an artifact of the scoring scheme. Further complicating factors include the fact that cattle are alliomimetic and that there was no LA-Control group. In addition, there were minor differences in the experimental design of the cortisol study (Chapter four) and the behaviour study. Each of these is discussed below.

Experimental situation

The behaviour experiment was conducted in sheep yards which were small (5x5m), covered and had a stony floor. This was done in order to simulate the experimental conditions of, and allow comparison with, the parallel cortisol study (Chapter four). Confinement in these environmentally sterile yards inhibited the expression of many normal, active behaviours. Grazing cattle spend most of their time in the feed acquisition activities of grazing and walking (33%) and ruminating (33%), and about 33% of their time idling (i.e. either standing or lying) (Fraser and Broom, 1990). Grazing was not possible and therefore presumably the incidence of rumination decreased during the day as a consequence of diminishing rumen contents. In addition, the occurrence of rumination may have been low because the environment was stressful. The usual incidence of lying may have been affected by this change of grazing behaviour, and/or the inability to avoid the accumulation of faeces in a confined area. The perturbing effect of the experimental situation can be accounted for by the use of controls. In the current study the Control calves were inactive compared to cattle at pasture. They stood still and ruminated with decreasing frequency (from 28% to 9%) throughout the day. This indicates that the experimental situation affected their usual behaviour but caused minimal distress. Apparently ruminants do not show overt behavioural responses to pain or distress as a survival mechanism for protection from predation (Hinch, 1994). In the current study it is likely that the apparent lack of behaviour was a result of the experimental situation.

Behaviour sampling scheme

Calf behaviour was recorded using "instantaneous point scan behaviour sampling". That is, the number of animals exhibiting particular behaviours at a point in time was scored.

It was decided to behaviour sample in this way so as to make possible the scoring of a large number of calves in a short period of time. This resulted in the following limitations in interpretation of the data:

Because the *number* of animals exhibiting a particular behaviour was scored and not the behaviour that an individual calf was exhibiting, it was not possible to correlate behaviours which may have been expressed simultaneously in individuals, or to identify outliers or subpopulations (see Chapter two). In the dehorning study where changes in plasma cortisol concentrations were measured (Chapter four), there were clearly identifiable outliers. For example, there were two calves in which it appeared that the local anaesthetic had not worked, and these animals were excluded from the results so as not to distort the mean response.

Because the sampling scheme involved *instantaneous* point scan sampling and the behaviours were simply recorded as either present or absent, no information on the frequency or duration of a behaviour bout was gathered. This information is obtained by conducting period sampling, where the duration and number of bouts of particular behaviours are recorded during a period, and provides more information about the animal's behaviour. Instantaneous scan sampling is a recommended technique if the frequency of occurrence of behaviour is high and the time between observations is low, in that case the resulting data will be a true snapshot of behavioural activity (Martin and Bateson, 1986). If the frequency of observation is high, then some information about the frequency and duration of bouts can be inferred from the data (Fraser and Broom, 1990). However, where frequency or duration of a behaviour is low it is possible that the behaviour may not be recorded at all, resulting in a distortion of results (Mellor and Murray, 1989a).

While not measured in this study, the intensity of a behaviour can be measured in point scan sampling. The importance of such quantitation is that it is possible that a difference in the intensity of a behaviour may later be classified as a different behaviour. This was of particular relevance to the scoring of walking and tail shaking; for example, with respect to walking a slow rate is a walk, a medium rate is pacing and a fast rate may be a run. For tail shaking it appeared that Control calves slowly flicked their tails, while dehorned calves, during periods of high restlessness, lashed their tails, this behaviour being fast, sustained and with the tail traversing a greater distance than that observed in Control calves. In the current study, period sampling, with information on duration, frequency and intensity, may have been preferable for scoring behaviours, especially in the case of tail shaking and walking. However, this may have made little difference to the interpretation of the data, presumably only exaggerating the difference between Scoop and Control calves. For the scoring of behavioural states such as ruminating or

lying where the duration of a bout was relatively long, the type of sampling has little impact upon the results.

No LA-Control group

Good experimental design incorporates control groups to account for extraneous factors and this allows changes to be correlated with treatment (Shott, 1990). In the current study there was a Control group but there was no LA-Control group. It was decided to omit this latter group because of a shortage of calves and because the parallel physiological study (Chapter four) showed that there was no difference between the LA-Control and the Control calves. However, it is possible that while this similarity may be the case for plasma cortisol, it may not be the case for behaviour. Thus, it was not possible to determine the contribution if any, of the possible negative effects of the administration or the wearing-off of the local anaesthetic on the behavioural response. Subsequent work indicated that the effect of local anaesthetic *per se* is negligible (Morisse *et al.*, 1995; Graf and Senn, 1999).

Comparison of the behaviour and cortisol data

While the behaviour study was conducted in an experimental situation similar to the cortisol study, there were some design differences between the two. The main difference was that unlike the behaviour study, the cortisol study involved repeated handling and invasive blood sampling. Because of the possible confounding effects of sampling on the interpretation of data from welfare studies, it is recommended to blood sample with minimal interference. This may be done by using a remote sampler device (see Cook *et al.*, 2000). In the absence of such equipment, the calves could be behaviour and blood sampled and their behaviour compared to a group which is just behaviour sampled; or separate behaviour and cortisol sampling trials could be conducted in parallel. There are advantages and disadvantages with both of these approaches. Furthermore, cattle are alliomimetic, that is, they tend to copy each other. This may complicate interpretation of their behaviour. In the cortisol study each experimental mob had calves from all treatment groups, whereas the calves in the behaviour study were grouped according to treatment. The former approach would presumably decrease, and the latter approach would presumably increase, the differences between treatments. But which approach is more correct? Whilst there are some limitations in the comparison of the two studies, there was none-the-less good agreement in the interpretation of the results.

5.4.7 Conclusions

Amputation dehorning elicited a significant increase in restless behaviours that lasted for about six hours. Local anaesthetic virtually abolished the behavioural response to scoop dehorning for the first two hours. These results suggest that dehorning is a painful

experience that lasts about six hours and that the application of local anaesthetic alleviates that pain for about the first two hours. These finding are consistent with the physiological study (Chapter four). Better quality information may have been gathered had period sampling of behaviour in the paddock been used. However, it is unlikely that the pattern of response would have differed. On the basis of the behaviour study it is recommended that local anaesthetic be administered when conducting amputation dehorning.

5.4.8 Epilogue

Since this research was conducted more recent work has been done (McMeekan *et al.*, 1999; Stafford *et al.*, 2000). These works confirmed many of the behavioural changes observed here and are discussed in the General Discussion.

6 GENERAL DISCUSSION

In this thesis, the pain-induced distress caused by the husbandry practice of dehorning cattle is assessed and methods to alleviate it are evaluated. Cattle are dehorned to reduce injury to stock handlers and stock and to improve husbandry. At the time this work was conducted there were no comprehensive studies on the effects of amputation dehorning on the welfare of the cattle. The aims of the study were to assess the distress response after dehorning and to explore the possibilities of alleviating that distress through the use of different dehorning tools, local anaesthetic and/or cautery of the scoop wound. Plasma cortisol concentrations and changes in behaviour were used as indices of distress. Subsequent to this work a series of studies investigating the distress of amputation dehorning were conducted (see 6.2) to which this work made a significant contribution.

6.1 CONCLUSIONS

Using changes in plasma cortisol concentrations and behaviour as indices of the acute pain-induced distress apparently caused by the different treatments, the present study led to the following findings:

1) All dehorning amputation methods resulted in similar cortisol responses irrespective of the tool used. The response was marked, biphasic and lasted for about six hours.

2) The timecourse of the plasma cortisol response after ACTH administration suggests that maximum cortisol secretion had been induced. The cortisol responses in the first hour after dehorning were similar to those following ACTH treatment indicating that dehorning initially caused maximal cortisol secretion and by inference was very distressing.

3) Injection of local anaesthetic prior to Scoop dehorning virtually abolished the behaviour and cortisol responses elicited in the first two to three hours after dehorning. Overall, this equated to a 50% reduction in the cortisol response to dehorning.

4) Cauterizing the wounds after scoop dehorning appeared to marginally reduce the cortisol response compared to scoop dehorning alone. This suggests that there

may be some benefit in cauterizing the dehorning wounds. However, the behavioural response to the cautery indicated that it was aversive.

5) The combination of local anaesthetic and cauterizing the scoop wound virtually eliminated the cortisol response after scoop dehorning. This suggests that pain-induced distress was minimal. The apparent benefit of the combined regime has parallels with recent discoveries in pain physiology and merits further investigation.

6) Control handling and bloodsampling and the administration of the local anaesthetic caused minor distress which lasted for 1.5 hours.

7) The four behaviours of tail shaking, ear flicking, head shaking and rumination met the criteria for use to indicate distress. Interpretation of the behaviour data corresponded with that of the cortisol data.

8) There were three distinct patterns in the timecourse of the plasma cortisol concentrations after ACTH, Dehorning and Control treatment respectively suggesting different underlying physiological processes, the understanding of which may provide insight for the alleviation of pain-induced distress.

6.1.1 Dehorning

Amputation dehorning of six-month-old calves elicited a marked, biphasic cortisol response (Chapter three) and a high level of restless behaviours (Chapter five) both of which lasted for about six hours. All the dehorning amputation methods used resulted in similar cortisol responses, although there is a suggestion that the depth of the wound may affect the level of distress. The plateauing of high cortisol concentrations after the ACTH bolus indicated that maximal secretory capacity of the adrenal gland was reached (Sapolsky, 1992). The rate of increase and the maximal cortisol levels attained after dehorning and ACTH injection were similar, indicating that dehorning caused maximal cortisol secretion by the adrenal gland and was, by inference, very distressing. The cortisol response after dehorning appears to be biphasic. The appearance of a sub-maximal plateauing of cortisol concentrations between 1.5 and 3 hours suggests the occurrence of either stress-induced analgesia (between 1 and 3 h), or more likely the appearance of a second phase of pain presumably from inflammation (between 1.5 and 6 h).

6.1.2 Local anaesthetic

Injection of local anaesthetic near each cornual nerve before scoop dehorning almost abolished the first three hours of the cortisol and the behaviour response after dehorning. Noxious sensory input from the wounds persisted beyond the three hour

action of the local anaesthetic, leading to an increase in plasma cortisol concentrations after the local anaesthetic effects wore off. This equated to a 50% reduction in the integrated cortisol response. The extent of the benefit of local anaesthetic is not clear from the behaviour data, being somewhere between 33% and 0%, the latter representing a delay only. This possible disparity between the cortisol and behaviour data may arise from problems associated with local anaesthetic failures, assessment of behavioural indices or design limitations of the behaviour study.

There were distinct behavioural profiles according to the treatment received which presumably indicated distressed and non-distressed states. The four behaviours of tail shaking, head shaking, ear flicking and rumination satisfied the criteria required to use behaviour as evidence of pain-induced distress (Stamp-Dawkins, 1980; Ewbank, 1985; Mellor *et al.,* 2000). Whilst there was agreement in the behaviour and cortisol studies, it was not possible to determine the exact magnitude or duration of the responses from the behaviour data. This is a problem of how to compare, or give weight to, different behaviours.

It is apparent from the cortisol responses that there can be a high rate of local anaesthesia failure (40%, Morisse *et al,* 1995). This may be caused by imprecision of site due to operator failure or the struggling movements of the calf, variations of innervation, or significant innervation from the medial or caudal aspect of the horn (Weaver, 1986). In the study described in Chapter four there was an initial 34% anaesthesia failure rate, and those calves were given an additional dose of local anaesthetic. None-the-less 26% of calves which received local anaesthetic, were excluded from the study as outliers. It was apparent that in some cases there had been insufficient anaesthesia, while in other cases there were bizarre cortisol responses which may have been caused by inadvertent intra-venous application, idiosyncratic responses or by factors not related to the treatment.

A criticism of the behaviour study is that the sampling scheme did not allow for the identification of outliers. Anaesthesia failure rates of 34% would presumably mask the benefit of local anaesthetic. Whilst this may reflect the situation in the field, we are none-the-less obliged to ameliorate this. It was obvious that those calves that were dehorned with local anaesthetic exhibited less escape behaviours than those calves dehorned without local anaesthetic. Unfortunately, we did not score the behaviour during the dehorning procedure. Had we scored the responses during the dehorning procedure, the therapeutic effect of local anaesthetic would have been more apparent. It is possible that better quality information may have been gathered had period sampling of individual calf behaviour in the field been conducted. However, it is unlikely that the pattern of response would differ. Both the physiological study (Chapter four) and the

behaviour study (Chapter five) suggest that dehorning is a painful experience that lasts about six hours and that the application of local anaesthetic alleviates that pain for about the first two to three hours. It is recommended that local anaesthetic be administered when conducting amputation dehorning.

There was little evidence to suggest that the experimental design affected the cortisol responses. The Control and LA-Control procedures elicited small cortisol responses which were over by 1.5 hours. The similarity of the Control and LA-Control responses supports the finding that the administration of local anaesthetic *per se* causes minimal distress (Petrie *et al.,* 1996a,b; McMeekan *et al.,* 1998a,b; Sutherland, 1999; Graf and Senn, 1999). In addition, the similar cortisol concentrations in LA-Control and LA-Scoop calves between 0 and 3h indicates that the increase in cortisol concentrations in LA-Scoop calves after three hours was caused by the appearance of pain-induced distress with the return of sensation, and not hemorrhage or the local anaesthetic wearing off *per se.*

6.1.3 Local anaesthesia and cautery of the wounds

The combination of local anaesthetic and cauterizing the scoop wound virtually abolished the cortisol response to amputation dehorning. This result was surprising, especially in view of the marginal reduction from cauterizing the scoop wounds without local anaesthetic. On the one hand, we anticipated some reduction in the cortisol response with cautery as indicated from previous studies (Lester *et al.,* 1991a) and from the fact that third degree burns are analgesic compared to second or first degree burns. On the other hand, it was possible that wound cautery could increase the cortisol response because it was an *additional* insult to the dehorning, and not the means by which the tissue was removed. Furthermore, the behaviour of the calves during the cauterizing without local anaesthetic indicated that this was a noxious experience.

The increase in cortisol concentrations after the local anaesthetic wore off in LA-Scoop calves demonstrates that there is sufficient pain during this period to elicit a delayed cortisol rise. Somehow the additional effect of cauterizing the wound prevented the delayed rise in cortisol concentrations when the local anaesthetic wore off. It has been suggested that cautery destroys nociceptors in the wound and thereby decreases nociception (Lester *et al.,* 1991a). The magnitude of the benefit of local anaesthetic plus wound cautery was striking. By contrast, the marginal decrease in the cortisol response from cauterizing the wounds alone suggests that if the number of functioning nocireceptors has decreased, then those that are functioning have had their threshold decreased (i.e. are sensitized) by the afferent barrage from dehorning. The synergistic effect of the combined regime of local anaesthetic plus cautery is reminiscent of pre-emptive analgesia (see section 6.3).

6.1.4 Recommendations

Both the cortisol and behavioural data indicate the benefit of giving local anaesthetic when dehorning. The combination of local anaesthetic prior to, and cauterizing the wound after scoop dehorning, is recommended provided that the additional handling and great care required for this procedure, can be managed. If a scoop dehorner which incorporated the cauterizing effect were to be developed then this would be a highly recommended technique.

6.2 SUBSEQUENT WORK

Since this study was conducted a series of studies were undertaken to examine the distress of amputation dehorning (Cooper *et al.*, 1995; Petrie *et al.,* 1996b; McMeekan *et al.,* 1997, 1998a,b, 1999; Sutherland, 1999; Stafford *et al.*, 2000; Sutherland *et al.*, 2002a,b; Mellor *et al.*, 2002) and cautery disbudding (Taschke and Folsch, 1993, 1995; Wohlt *et al.,* 1994; Morisse *et al.,* 1995; Petrie *et al.,* 1996b; Grondahl-Nielsen *et al.,* 1999; Graf and Senn, 1999; Faulkner and Weary, 2000).

6.2.1 Amputation dehorning

Subsequent studies confirmed that the cortisol response after scoop dehorning lasted six to seven hours, was marked and biphasic (Petrie *et al.,* 1996b; McMeekan *et al.,* 1997, 1998a,b, 1999; Sutherland, 1999). Further, as a result of the present finding regarding the similarity of cortisol responses for different dehorning methods, in subsequent amputation dehorning studies by our team, the scoop was used because of its ease of operation. The findings in this thesis regarding the depth of wound led to a specific study of shallow versus deep scoop dehorning, which subsequently demonstrated no effect from scoop wound depth (McMeekan *et al.,* 1997).

Subsequent work showed that local anaesthetic eliminated the cortisol response to dehorning during its period of action, but once the anaesthesia wore off cortisol concentrations rose irrespective of whether the duration of anaesthesia was 2, 4, 5, or 8 hours, such that the integrated cortisol responses with and without local anaesthetic were similar (Petrie *et al.,* 1996b; McMeekan *et al.,* 1998a; Sutherland, 1999; Sutherland *et al.*, 2002a,b). The nature and magnitude of the delayed rise in cortisol concentrations after dehorning with local anaesthesia is different from that after dehorning alone. Whereas the response to dehorning is biphasic, the delayed cortisol response to dehorning with local anaesthesia has one phase and concentrations do not attain maximal values. McMeekan *et al.* (1998b) proposed that the lack of apparent benefit of local anaesthetic on the cortisol response *overall* may be due to a delay in the resolution of inflammation because of the lack of the initial maximal cortisol surge, since cortisol has potent anti-inflammatory actions (Goldstein *et al.,* 1992; Buckwalter,

1995). The apparent lack of benefit of local anaesthesia on the cortisol response overall is in contrast to the findings of the current study and other studies where behavioural and/or physiological indices indicate that local anaesthesia reduces the responses during and/or after cautery dehorning (Morisse *et al.,* 1995; Graf and Senn, 1999; Grondahl-Nielsen *et al.*, 1999). Indeed, because of such observations it is usually recommended that local anaesthesia be given. Perhaps the reasons for the differences in the effect of local anaesthetic are age related. In the current study the calves were six months old, whereas the calves in the subsequent studies were 3-4 months (McMeekan *et al.,* 1998a; Sutherland, 1999; Sutherland *et al.*, 2002a,b) and 6-8 weeks old (Petrie *et al.,* 1996b).

Understanding the physiological basis of the distress response is important as it may provide insight for alleviation of that distress. The biphasic shape of the cortisol response after dehorning suggested that there may be two phases of response, the first caused by the nociceptor barrage from the amputation itself, and the second reflecting nociception from inflammation. The cortisol response after dehorning was subsequently investigated to see if it was possible to distinguish the presumed phases of pain elicited from the afferent barrage versus pain elicited by inflammation (McMeekan *et al.,* 1998b).

The combined regime of local anaesthetic plus the non-steroidal anti-inflammatory drug (NSAID) ketoprofen virtually abolished the cortisol response to dehorning (McMeekan *et al.,* 1998b). Ketoprofen alone effected a marginal reduction in the cortisol response in the first two hours after dehorning and abolished the response thereafter. This suggested that a major component of the dehorned response after two hours was due to inflammation. The therapeutic effect of local anaesthetic and the NSAID ketoprofen was confirmed (Sutherland, 1999; Sutherland *et al.*, 2000b), but in that study there was a decrease of small magnitude as opposed to elimination of the cortisol response. It was suggested that the difference in the magnitude of the effect could be due to the differences of duration of action of local anaesthetic *per se*, of five hours (Sutherland, 1999; Sutherland *et al.,* 2002b) compared to two hours (McMeekan *et al.*, 1998b), or indirectly in that by the time the 5 h local anaesthetic has worn-off, the ketoprofen concentration is so low that the occurrence of inflammatory pain elicits a small second cortisol rise. Local anaesthetic lasting 5 h combined with elevated cortisol concentrations (induced by ACTH administration) effected a small reduction in the delayed cortisol response once the local anaesthetic wore off. Local anaesthetic (5 h) plus the NSAID phenylbutazone was not effective in reducing that delayed cortisol response (Sutherland *et al.,* 2002b). The lack of effect of phenylbutazone was attributed to it being less potent in its anti-inflammatory (Kantor, 1986) or anti-nociceptive action. The significant decrease in the cortisol response to dehorning by the combined regime

of local anaesthetic plus cauterizing the scoop wound was confirmed (Sutherland *et al.*, 2002a), however, the magnitude of the decrease was less than that reported by Sylvester *et al.* (1998a).

6.2.2 Disbudding

A series of studies have been conducted on the effects of cautery disbudding. To date Petrie *et al.* (1996b) is the only study where the physiological responses to cautery disbudding and amputation dehorning were compared. In six-week-old calves the cortisol response to amputation dehorning is marked, biphasic and lasts about 6.5 hours, whereas the response to cautery disbudding is monophasic and lasts about 1.5 hours. Other studies have shown that the cortisol response to cautery disbudding lasts about 1-2 hours (Laden *et al.*, 1985; Taschke and Folsch, 1993, 1995; Wohlt *et al.*, 1994; Petrie *et al.*, 1996b; Graf and Senn, 1999; Faulkner and Weary, 2000) or less than 4 hours (Morisse *et al.*, 1995; Grondahl-Nielsen *et al.*, 1999). In some studies the application of local anaesthetic has decreased the cortisol and behavioural responses to disbudding (Graf and Senn, 1999) and in other studies there has been no apparent *overall* effect of local anaesthetic (Petrie *et al.*, 1996b). In the latter case it would appear that the *overall* effect of local anaesthetic was obscured by the fact that the responses to cautery dehorning and to control handling and sampling are similar. The effects of sedation (xylazine), local anaesthetic (2 h) (lidocaine or lignocaine) plus an analgesic (either butorphanol or ketoprofen) have been investigated (Grondahl-Nielsen *et al.*, 1999; Faulkner and Weary, 2000). In one study the xylazine caused such deep sedation that the behaviour and heart rate data were equivocal (Grondahl-Nielsen *et al.*, 1999), in another xylazine and local anaesthetic resulted in peak distress behaviours at six hours after disbudding which were eliminated with the addition of ketoprofen (Faulkner and Weary, 2000). Whilst these results may indicate the benefit of ketoprofen when disbudding, they also suggest that sedation *per se* might be distressing. Disbudding by caustic chemical is more distressing than by cautery, and this greater response is reduced by the application of local anaesthetic (Morisse *et al.*, 1995).

6.3 PAIN-INDUCED DISTRESS - INSIGHTS

Understanding the physiological basis of the distress response is important as it provides insight for avenues of alleviation of that distress. This has benefit for husbandry and scientific procedures. In the last ten years the pain-induced distress response elicited by various husbandry practices including dehorning, disbudding, castration and tailing, and the strategies for its alleviation, have been extensively investigated. The distress response appears to have various components including handling and acute and chronic phases. These components may be affected by age, method and pharmacological and/or physical alleviation strategies.

The mustering and restraint required to carry out husbandry or scientific procedures usually evoke a distress response, the magnitude and duration of which will depend on the temperament of the animal and whether it is familiar with the handlers and/or conditions. Taming and training animals before an experiment reduces the behavioural and physiological responses to the experimental situation (Mellor, 2000), however, such training is problematic as it may alter the animal's responses to the treatment or not be representative of their response in their natural environment, which may be what is of interest. For instance, the cortisol response in lambs subjected to handling plus venipuncture is similar to that when ring tail-docked (Lester *et al.,* 1991a), but if lambs are familiar with the experimental conditions the response to handling plus venipuncture is less than the response to ring tail-docking (Mellor and Murray, 1989a). This raises the issue of the extent to which the stress response to the experimental paradigm complicates interpretation of the results (Mason, 1968; Mellor, 2000). On the one hand, where the response to the noxious procedure is marked, as in the case of surgical castration in lambs, the response does not appear to be affected by the repeated handling and venipuncture (Lester *et al.,* 1991b). On the other hand, when blood samples are taken remotely via indwelling catheters, the 'Dracpac' or via transcutaneous biosensors the physiological responses are lower (Cook *et al.*, 2000).

There appears to be no convincing evidence that age affects distress; indeed there are interpretative problems comparing such responses because of age-related differences in the pharmacokinetics of the HPA-axis (Mellor and Murray, 1989b; Sapolsky, 1992; Mellor and Stafford, 1999a). Where there is more than one method available to achieve the same husbandry goal, there is a need for rigourous scientific evaluation in order to choose a method which causes the least distress. For instance, castration and/or tailing of lambs using rings or the docking iron is less distressing than surgical removal (for review see Mellor and Stafford, 1999a, 2000), and in calves, whilst there are no major differences in the distress responses when different methods of amputating the horn are used (Sylvester *et al.,* 1998b), cautery disbudding elicits significantly less distress than amputation dehorning (Petrie *et al.,* 1996b).

Any surgical intrusion causes tissue damage with attendant acute pain and distress. This is usually a self limiting experience due to the progress of the healing processes. Recent advances in our knowledge of the physiology of pain have driven changes in pain management strategies. When the noxious sensory barrage of surgery is blocked by the use of local or regional anaesthesia there is a reduced intensity and delayed onset in the appearance of post-operative pain. This has been termed pre-emptive analgesia (Woolf and Chong, 1993). The suggested mechanism for this phenomenon is that the surgery-induced sensory barrage decreases the pain threshold resulting in an increased

sensitivity to stimuli including pain (Wall, 1988; Katz *et al.,* 1992; Woolf and Chong, 1993). If this afferent barrage can be prevented, through the use of local anaesthetic, then, when the local anaesthetic wears off the experience of pain is reduced, because there has been no reduction in the pain threshold.

Pre-emptive analgesia may be achieved by pharmacological or physical means. For example, when the pain-producing stimuli outlast the duration of analgesia resulting in a significant distress response thereafter, as in the case of amputation dehorning, a combination of local anaesthetic plus the NSAID ketoprofen (McMeekan *et al.,* 1998b) is effective; or, the effective duration of local anaesthetic can be increased as in the case of castration and/or tailing when rings are used. The ring, by obstructing blood flow, prevents the clearance of local anaesthetic from tissues distal to the ring (Mellor and Stafford, 1999a; 2000). It is also possible to decrease the transmission of nerve impulses through physical means. This may be direct or indirect. Examples of direct methods are procedures which incorporate cautery such as tail-docking with a docking iron (Lester *et al.,* 1991b) or cauterizing dehorning wounds (Sylvester *et al.,* 1998a), or procedures which crush nerves such as the castration clamp (Mellor and Stafford, 1999a, 2000). In the former two, cautery appears to decrease the number of functional nocireceptors, and in the latter one the transmission of impulses in the nociceptors is inexorably disabled (Mellor and Stafford, 1999a, 2000). Examples of indirect methods are procedures where the rings are used. The rings decrease blood flow which causes anoxia of tissues and eventually disables pain reception (Mellor and Stafford, 1999a, 2000).

The chronic welfare aspects of such husbandry procedures have not been well researched and may include infection and morbidity, and chronic pain arising from infection, hyperalgesia, phantom pain or neuropathies, or indeed social problems for instance, the tail docking of cattle, in addition to removing their fly swats, may remove a means to communicate (Mellor and Stafford, 2000).

6.4 Future directions and Critique of the experimental design

It is now widely accepted that in order to understand the response of an animal to stress it is necessary to measure several variables. These variables may range from subtle changes at the level of the central nervous system to systemic responses, and include neurohumoral, hormonal and behavioural indices (for review see Mellor *et al.,* 2000). Recent advances in biomedical technology provide the opportunity for sophisticated assessment of the stress responses of animals. Such technology includes the use of telemetry and remote (i.e. hands-off) samplers including remote catheter systems (Ingram *et al.,* 1994), portable devices such as the 'DracPac' and free-range physiological monitors, and the use of electrodes to measure neural-evoked responses, biosensors and microdialysis techniques (for review see Cook *et al.,* 2000). This remote

sampling approach would allow the measurement of baseline levels for a full 24 hours before and after treatment, the merit of which was pointed out in Chapter two. In addition, the use of more sophisticated behavioural assessments such as preference testing, aversive tests or behavioural demand function may tell us more about what the animal is feeling than examining simple changes in behaviour (for review see Cook *et al.*, 2000).

In the present study, changes in plasma cortisol concentrations and behaviour were used to assess the pain-induced distress elicited by dehorning. It is possible that the response time of the HPA-axis makes it insensitive as a means of distinguishing between different levels of stress elicited within the first few minutes after a noxious insult, and for this reason changes in the sympathetic nervous system or central nervous system may be more useful during this period (Mellor and Stafford, 1999a). The act of studying an animal affects the response and may confound interpretation of results, and for this reason there is merit in conducting hands-off assessment of stress responses. In chapter two, however, it was shown that the experimental design used here was sufficiently robust to account for fluctuations in the pretreatment state (Chapter two). Future research should exploit advances in technology. With regard to more direct measurement of pain, perhaps measurement of nerve impulse traffic in nociceptors or samples from the region of trauma may provide extra information about pain, although pain is not nociception but rather a subjective experience. With respect to animal stress and animal welfare, incremental improvements in husbandry practices may be most practicable (Mellor and Stafford, 1999b, 2001). There are obvious benefits from continued research in the area of pain physiology as demonstrated by such surprising discoveries as pre-emptive analgesia and the striking benefits of local anaesthetic plus wound cautery.

Enfin

It has been said that a society can be judged on how civilized it is by how it treats its prisoners (Dostoevsky 1821-1881), but it may be just as meaningful to see how society cares for its other disenfranchised members including animals. It is our ethical duty to not just reduce the pain and stress that animals experience under our auspices, but in fact strive to ameliorate their condition. In addition to intrinsic reasons, we should be kind because it is good to do so (Proverbs 12:10). Furthermore, it has been demonstrated that it pays to be kind (Sigmund, 2002).

fin

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APPENDIX A: PRESENTATIONS AND PUBLICATIONS OF THIS WORK

Oral presentations

May 1993 Sylvester SP, Mellor DJ, Stafford KJ, Bruce RA, Ward RN. "*Cortisol responses to dehorning in six-month-old calves*". Proceedings of the twentieth annual New Zealand Physiological Society Conference. Vol., 13. May 1993. Abstracts published. ISSN 1171-8013

June 1993 "*Assessment and alleviation of dehorning distress in calves*".Closed animal welfare workshop at Massey University.

Dec 1993 "*Assessment and alleviation of dehorning distress in calves*". MAF Policy meeting. Paraparaumu, Wellington.

Publications

Sylvester SP, Mellor DJ, Stafford KJ, Bruce RA and Ward RN. "*Cortisol responses to dehorning in six-month-old calves*". Proceedings of the twentieth annual New Zealand Physiological Society Conference. Vol., 13. May 1993. Abstracts published. ISSN 1171-8013

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Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA,Ward RN. Acute cortisol responses of calves to four methods of dehorning by amputation. *Australian Veterinary Journal* 1998; **76** p123-126.

Sylvester SP, Mellor DJ, Stafford KJ, Bruce RA and Ward RN. "*Cortisol responses to dehorning in six-month-old calves*". Proceedings of the twentieth annual New Zealand Physiological Society Conference. Vol., 13. May 1993. Abstracts published. ISSN 1171-8013

CORTISOL RESPONSES TO DIFFERENT METHODS OF DEHORNING IN 6 MONTH OLD CALVES. Shauna.P.Sylvester[1], D.J.Mellor[1], K.J.Stafford[2], R.A.Bruce[2], & R.N.Ward[1]. Departments of [1]Physiology and Anatomy, and [2] Veterinary Clinical Sciences, Massey University, Palmerston North.

Dehorning is a routine husbandry practise designed to decrease injury to cattle and stock handlers. It is presumed to be a noxious experience, but the levels of distress caused by different methods and efficacy of alleviation have not been assessed. The present study is an early phase of such an investigation. The objectives were to compare cortisol responses to dehorning by scoop and guillotine shears in order to make recommendations about the least distressful treatment whilst taking into account practical considerations. There were three treatments (9 calves in each): Control handling, dehorning by scoop and dehorning by guillotine shears. All treatments were bled before treatment and regularly thereafter for nine hours. Plasma cortisol concentrations were determined by radio-immunoassay. Cortisol concentrations above pretreatment concentrations were calculated. Control handling, dehorning by scoop and dehorning by guillotine shears caused mean ± SEM maximum rises of 23 ± 0.7 ng/ml , 45 ± 2.6 ng/ml , 40 ± 2.2ng/ml ng/ml respectively; cortisol concentrations returned to pretreatment values at about 4 hr, 7 hr and 5.5 hr respectively. It is concluded that dehorning caused markedly greater cortisol responses than control handling. The significance of the differences between the responses using the two dehorning methods has yet to be determined.

Acute cortisol responses of calves to four methods of dehorning by amputation

SP SYLVESTER[a], KJ STAFFORD[bc], DJ MELLOR[a], RA BRUCE[b] and RN WARD[a]

Objective To measure the plasma cortisol response in calves dehorned by four different methods (scoop, guillotine shears, saw, embryotomy wire) for 9 h after dehorning.

Design A physiological study with controls.

Procedure Horn amputation was carried out on calves restrained manually in a race.

Results The four methods of dehorning provoked similarly increased cortisol responses which lasted for 6 h. During the first hour after dehorning the plasma cortisol concentrations were similar to those following ACTH injection. The overall cortisol response to control handling was about 30% of the responses to dehorning.

Conclusions The similarity of the cortisol responses produced by the four methods of dehorning suggests that the distress experienced by calves following dehorning by amputation is similar regardless of method used.

Aust Vet J 1998;76:123-126

Key words: Calf, dehorning, scoop, embryotomy wire, saw, shears, cortisol.

ACTH Adrenocorticotropic hormone

Dehorning of cattle is a routine husbandry practise which improves the safety of stockhandlers and reduces injury, bruising of carcasses and damage to hides. Caustic chemicals or cautery are used to disbud younger calves and amputation is used to dehorn older animals. All methods of dehorning are probably painful. Animals experiencing pain exhibit increased activity of the hypothalamic-pituitary-adrenal axis as indicated by an increase in plasma cortisol concentration.

The changes in plasma cortisol concentration have been used as one index for assessing the distress caused by cattle husbandry practices[1,2] including cautery disbudding[3,4] and scoop amputation dehorning.[5] However no comparison has been made of the cortisol response to different methods of amputation dehorning and by inference the distress caused by these amputation techniques. Consequently the cortisol responses to amputation dehorning by scoop, saw, guillotine shears and embryotomy wire are reported here.

Materials and methods

Animals

Fifty-seven male Friesian calves aged 5 to 6 months and weighing 99 to 159 kg (mean 130 kg) scheduled for dehorning according to usual farm practise were used in this study. They were divided into five groups and the study was carried out over 3 days (Table 1). On the evenings before each experiment day, calves were randomly selected from the herd, identification numbers were painted on their backs, and they were penned overnight in outside cattle yards (10 × 10 m) with access to water but not to food. The following morning one group was moved into a small pen (3 × 4 m) at 0700 h and a pretreatment blood sample was taken. At 1100 h when these animals had been moved out of the sampling pen, the second group was placed in it. All blood samples were taken in that pen because its small size facilitated sampling by restricting movement of the calves. Fifteen minutes later the calves were moved into an adjacent race where they were restrained and dehorned. Immediately after dehorning the calves were returned to the small pen where blood samples were taken with decreasing frequency throughout the subsequent 9 h. Between 3 and 9 h after treatment, when sampling was at hourly intervals, the calves were let into an adjacent larger yard (10 × 10 m) where water was available. When required for sampling they were moved back to the smaller pen slowly and without undue interference. The duration of blood sampling was about 12 min for each group.

At the end of each 9 h sampling period the calves were returned to pasture. The following day calves were mustered into the yards and left to settle for 3 h, after which a blood sample was taken about 36 h after treatment.

Blood sampling

Blood samples (10 mL) were taken by venipuncture from either jugular vein 0.25 h before treatment (pretreatment), and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 36 h after treatment. Blood samples were taken by one person whilst the calf was restrained by two or three other people. Calves were bled in the same order each time to test for possible effects of the blood sampling order on plasma cortisol concentrations.

Table 1. The number of animals receiving the different treatments in each group during the 3 days of the experiment.

		Day 1 group		Day 2 group		Day 3 group
Treatment	Total	1	2	3	4	5
Control	10	2	2	2	3	1
Scoop	10	2	2	3	2	1
Guillotine shears	10	2	2	2	2	2
Saw	10	2	2	2	2	2
Embryotomy wire	10	2	2	2	2	2
ACTH	7	2	2	1	1	1

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Treatments

The calves were randomly allocated to six treatments which were usually represented by 2 animals in each group of 12 calves. The calves were restrained manually for treatment in a cattle race (Table 1). One person held each calf's head firmly whilst another performed the treatment. The two operators moved down the race dealing with each calf in succession. Between animals, dehorning implements were disinfected (Hibitane Concentrate 5%,Hospital Products, ICI Australia). The amount of horn and skin tissue removed during dehorning varied depending on the horn size, being greater for the larger horns.

Control

Ten calves were handled to simulate the restraint required for dehorning without being dehorned.

Scoop

Ten calves were dehorned using a standard dehorning scoop (Barnes Dehorners, Stones, USA).[4]

Saw

Ten calves were dehorned using a butcher's saw.

Guillotine Shears

Ten calves were dehorned using a small shears (James Sculley, Pomeroy, PA, USA) with a cutting hole of 60 × 80mm.

Embryotomy wire

Ten calves were dehorned using embryotomy wire (Arnold's Addrem wire saw).

ACTH injection

Forty mg (approximately 0.28 mg/kg live weight) of ACTH (Synacthen, Ciba Pharmaceutical, Auckland, New Zealand) was injected into the jugular vein of seven calves to elicit a cortisol response representing the maximum cortisol secretion during the first 2 h after injection.[4]

Plasma cortisol analysis

Blood samples were collected in vacutainer tubes with lithium heparin, chilled immediately, centrifuged and the plasma stored frozen at -20°C until analysed. Plasma cortisol concentrations were determined by radioimmunoassay.[6] The intra-assay and inter-assay coefficients of variation were 11% and 15% respectively. The smallest detectable concentration of cortisol was 0.81 nmol/L.

Statistical analyses

Cortisol results are expressed as the mean ± SE. Statistical analysis was conducted on plasma cortisol concentrations and on the integrated cortisol responses during selected periods after treatment. The integrated cortisol response is defined as the area between a horizontal line drawn through the pretreatment concentration and the cortisol response curve.

To ascertain whether day, group, or time had a significant effect on the pretreatment cortisol concentrations a two-way analysis of variance (ANOVA) using Fisher's probability of least significant differences (PLSD) was carried out. In addition the possibility of a linear or polynomial relationship between the pretreatment concentrations and the order in which the calves were sampled was examined by calculating Pearson's correlation coefficient (r) or simple regression (r^2).

Two-way ANOVA using Fisher's PLSD was used to determine the significance of the differences between means in the timecourse of the cortisol response. Between-treatment comparisons used a factorial ANOVA design. The within-treatments comparison was a repeated measures ANOVA design. This involved the comparison of the mean pretreatment cortisol concentration to those at subsequent time periods to ascertain when the cortisol response was over, that is when the concentrations were statistically similar to the pretreatment value.

Numerical integration was used to calculate areas representative of the total response (integrated cortisol response). A two-way factorial ANOVA using Fisher's PLSD was used to determine the significance of the differences between the means of the total integrated cortisol response of the different treatments.

Unless otherwise stated, cortisol concentrations have been reported as adjusted values by subtracting the pretreatment concentration from all subsequent concentrations of the cortisol response, so that the cortisol responses are expressed as changes in cortisol concentration with time.

All statistical calculations were conducted using a standard statistical computer package (Statsview + Graphics, Version 2, Abacus Concepts Inc., USA).

Results

There were no significant differences ($P < 0.05$) in the pretreatment plasma cortisol concentrations between days, groups or treatments, nor was there a linear correlation with the order in which the animals were bled ($r^2 = 0.02$). There was a significant ($P < 0.05$) difference between pretreatment cortisol values in the early 0700 h (21.9 ± 3.2 nmol/L, n = 34) and the late 1100 h (34.0 ± 3.8 nmol/L, n = 23) groups, but this was of

Table 2. Plasma cortisol concentrations (nmol/L, mean ± SEM) in calves before and 36 h after dehorning by one of four methods or having been injected with ACTH.

	n	Pretreatment nmol/L	36 h after treatment nmol/L	MR[a] nmol/L	ICR[b] nmol/L.h^{-1}
Control	10	17.6 ± 1.9	14.0 ± 2.7	62.1 ± 10.8	94.5 ± 13.5
Scoop	10	21.9 ± 6.5	30.8 ± 5.1	118.8 ± 8.1	391.5 ± 29.7
Guillotine shears	10	38.6 ± 6.2	42.9 ± 7.8	102.6 ±10.8	286.2 ± 48.6
Saw	10	29.4 ± 6.5	32.9 ± 6.7	105.3 ± 8.1	340.2 ± 37.8
Embryotomy wire	10	32.9 ± 8.4	31.1 ± 9.5	89.1± 8.1	367.2 ± 54.0
ACTH[c]	7	17.6 ± 5.4	13.0 ±1.9	124.2 ± 13.5	353.7 ± 43.2

[a]MR = Mean maximum increase in plasma cortisol concentrations
[b]ICR = The integrated cortisol response
[c]ACTH = Calves injected with adrenocorticotrophic hormone

little consequence when compared to the plasma cortisol response to dehorning. Therefore the results from all groups were pooled and used to calculate the mean cortisol response to each treatment. The overall mean pretreatment concentration was 27.3 ± 2.4 nmol/L (n = 57). The pretreatment concentrations for each treatment are shown in Table 2.

After treatment, all calves showed transient increases in plasma cortisol concentration ($P < 0.05$), the magnitudes and durations of which varied according to the treatment.

Control

Restraint and blood sampling resulted in a small transient rise in plasma cortisol concentrations ($P < 0.05$). The maximum increment of 62 nmol/L occurred 15 minutes after treatment, after which the cortisol concentrations decreased and returned to pretreatment values by 1 h (Figure 1).

Dehorned treatment

All four amputation dehorning methods (scoop, saw, guillotine shears and embryotomy wire), resulted in similar cortisol responses (Figure 2), with no significant differences in the maximum rise in cortisol concentrations (89 to 119 nmol/L) or in the duration of increased cortisol concentrations. The maximum plasma cortisol concentrations occurred 0.5 to 1.0 h after treatment. The mean cortisol concentrations then declined rapidly to plateau values which were maintained between 1.5 and 3 h. The values at 2 and 2.5 h in the calves which were dehorned by guillotine shears were smaller ($P < 0.05$) than those of the other groups of dehorned calves (Figure 2). Thereafter, from 3 h, cortisol secretion continued to decrease and the plasma cortisol concentration returned to pretreatment values by 6 h (Figure 2).

ACTH

ACTH administration resulted in marked increases in plasma cortisol concentrations, with a mean peak rise of 124 nmol/L 1 h after treatment. Large values were sustained for about 2.5 h, after which concentrations decreased and returned to pretreatment values by 4 h. Thereafter the concentrations declined further and remained smaller than pretreatment concentrations between 4 and 9 h (Figure 1). Between 6 and 9 h the cortisol concentrations in ACTH calves were significantly smaller than those of control calves, which exhibited a nonsignificant increase in concentrations during this period.

Integrated cortisol responses

There were no significant differences ($P < 0.05$) between the mean integrated cortisol responses of calves dehorned by any method, nor between the responses of dehorned and ACTH calves (Table 2). The integrated responses (nmol/L.h^{-1}) of dehorned and ACTH calves were between 3 to 3.9 times greater ($P < 0.05$) than the control response (Table 2).

Discussion

In 5 to 6-month-old calves the four methods of dehorning provoked similar marked cortisol responses which lasted for about 6 h. The cortisol responses in the first hour after dehorning were similar to those following the ACTH injection, indicating that dehorning caused maximum cortisol secretion during this period. The cortisol responses of the control calves were significantly smaller than those of dehorned calves. The difference in the pretreatment cortisol values between the early (0700 h) and late (1100 h) groups was considered to be of no biological significance in relation to the magnitude of the subsequent cortisol responses.

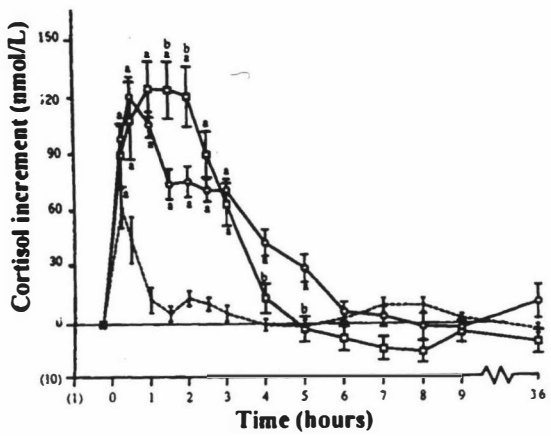

Figure 1. The mean cortisol responses of calves to scoop dehorning (circles), ACTH injection (squares) and control handling (broken line). a = significantly different ($P < 0.05$) from control values; b = ACTH calves are significantly ($P < 0.05$) different from scoop calves

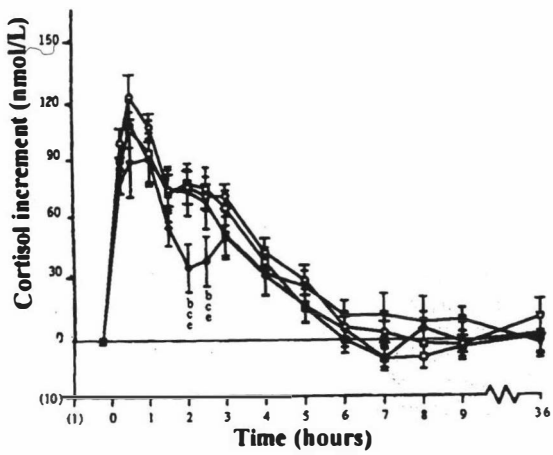

Figure 2. The mean cortisol responses of calves to four methods of dehorning; scoop (circles), guillotine shears (diamond), saw (square) and embryotomy wire (broken line). b, c and d = responses in scoop, saw and embryotomy wire groups, respectively, are significantly different ($P < 0.05$) from those in calves dehorned by the guillotine shears.

It is not surprising that there were no significant differences in the peak magnitudes, durations, patterns or integrated cortisol responses between these four dehorning treatments, because they all involved surgical amputation of the horn, which presumably would result in similar trauma. The only differences were in the tools used to achieve this. Two observations suggest that the depth of wound or the different nature of the wound may have affected the cortisol response. First, scoop dehorning

which caused the deepest wounds, often perforating the frontal sinus of the skull, elicited cortisol responses which had the biggest (nonsignificantly) integrated cortisol response and peak magnitude. Secondly, dehorning with the guillotine shears caused quite superficial wounds and elicited cortisol responses which at 2 and 2.5 h after treatment were significantly less than those for all other dehorned calves. The shears also elicited the smallest (nonsignificant) integrated cortisol response. However this difference is not likely to have been due to wound depth because similar cortisol responses occurred in calves dehorned with scoops, which caused shallow or deep wounds.[5]

The pattern of cortisol concentrations seen after amputation dehorning in this study in 5 to 6-month-old calves was similar to the patterns seen in 6-week-old[4] and 14-week-old[5] calves after scoop dehorning. The initial increase to peak concentrations after scoop dehorning apparently represents maximal cortisol secretion because a similar rate and peak concentration occurred after injection of ACTH in a quantity sufficient to maintain the peak concentration for 2 h after injection (Figure 1). The cortisol response to dehorning appears to be divisible into three components: the initial increase to peak values and subsequent rapid decrease (the first 1.5 h), the plateau stage and then a decline to pretreatment values between 3 and 6 h after dehorning. The possibility of an exaggerated effect upon the cortisol response of sampling animals in pain, either as a result of increased pain from the sampling procedure, or an increase in the negative psychological component because of the association of that pain with the operators, has been examined in lambs[7] by commencing blood sampling at different times after surgical castration and tailing of lambs. As no effects on the cortisol response were detected, Lester et al[7] suggested that this may be because either resampling itself was a benign procedure, or because the most noxious stimulus (in their case castration and docking) tended to dominate lesser noxious inputs (that is, sampling) from elsewhere. The effect of repeated sampling in the present study is unknown but the cortisol responses found here would represent at least the maximum expected under field conditions, when repeated handling and blood sampling does not occur after dehorning.

The duration of the cortisol response after control handling and sampling was 1.5 h, which is similar to that reported in other studies on calves.[3-5] That the integrated cortisol response of control calves was one third to a quarter of that after dehorning and was resolved 5 h earlier, suggests that the combination of handling and blood sampling was a benign procedure in comparison with dehorning, and that the predominant component of the response was the novelty of the situation. This is consistent with other studies in lambs[8] and calves.[4] It is possible that using an indwelling catheter rather than repeat venpuncture might have produced a smaller cortisol response. The absence of any effect on cortisol concentration at 36 h indicates that chronic distress may be minimal.

On the basis of this study there appears to be little difference in the cortisol response and by inference the distress experienced by calves with any one method of amputating horns. However, the rate of wound healing was not investigated.

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APPENDIX B: DATA EXCLUDED FROM THE ANALYSES

As judged by their cortisol responses calves that were abnormally or excessively distressed prior to and throughout the study were excluded from the results as outliers. The determination of an outlier was made by satisfying two criteria: 1) if examination of the cortisol timecourse showed a unique and aberrant pattern, and 2) where statistical analysis of the cortisol response showed that the pretreatment and/or the integrated cortisol response were greater than those encompassed by two standard deviations from the mean for that treatment. The individual cortisol responses of the calves which were excluded as outliers are shown in Fig B.1, Fig B.2, Fig B.3.

Two calves exhibited aberrant responses prior to and during the study: For both calf #105 (LA-Scoop) and calf #81 (LA-Scoop+Cautery) the pretreatment cortisol concentrations and the integrated cortisol responses (unadjusted areas) were greater than those encompassed by two standard deviations from the mean response (see Fig B.1). Both calves had a cortisol timecourse pattern which was unique and aberrant, such that, for calf #105 cortisol concentrations increased throughout the study period, and for calf #81 cortisol concentrations oscillated from high to low throughout (see Fig B.1). In addition, calf #105 had an haematocrit estimated at about half that of other calves (20% compared to the usual 40%), and it subsequently died on day two of the study. It was therefore concluded that both animals were suffering from a previous distress prior to and during the study, and thus were excluded so as not to distort the mean cortisol responses of each treatment.

In two cases the local anaesthetic did not work: The pretreatment cortisol concentrations of calf #79 (LA-Scoop) and calf #69 (LA-Scoop+Cautery) were not statistical outliers. However, their post-treatment cortisol responses were unlike that of any other individual in their respective treatments, and were in fact similar to those found in the Scoop and Scoop+Cautery treatments respectively. The integrated responses (unadjusted data) for both were greater than those encompassed by two standard deviations from the mean for their treatment group. It was concluded that the local anaesthetic had *not* worked in decreasing the initial distress of dehorning, therefore these calves' responses were excluded from the calculation of the respective mean response (see Fig B.2).

Three calves exhibited a pre-existing stress: Calves #70 (Control), #90 (LA-Control), and #66 (LA-Control) all had pretreatment cortisol concentrations and integrated cortisol responses (unadjusted data) which were greater than those encompassed by two standard deviations from the mean of their respective treatment group. In addition, at about four hours after treatment, all three calves exhibited a drop in cortisol concentrations which was substantially below pretreatment levels and levels remained there. This suggests that

there may have been an unknown stressor that in its own right or combined with the treatment maintained a stress which masked the effect of the treatment alone for the first four hours. Presumably after four hours the stressors were absent, because cortisol concentrations fell to levels substantially below pretreatment values. The low values after four hours suggest that the baseline in these calves if unstressed would have been similarly low (see Fig B.3).

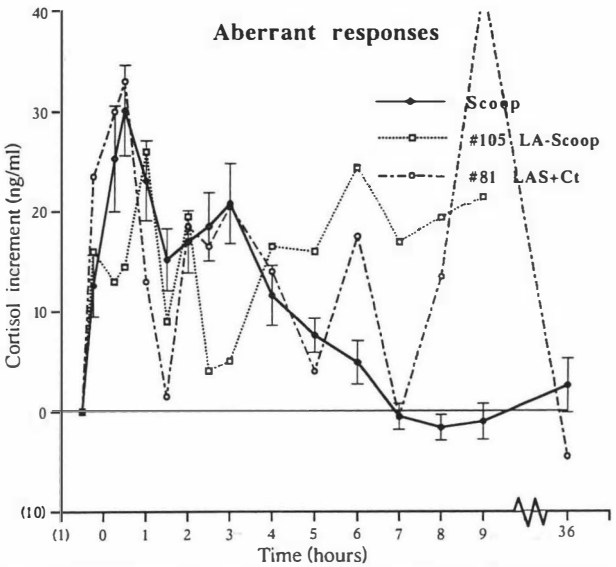

Fig B.1 Comparison of changes in plasma cortisol concentrations of calves #81 (LAS+Ct) and #105 (LA-Scoop) to the mean (± SEM) Scoop response. For both these calves their cortisol responses were aberrant.

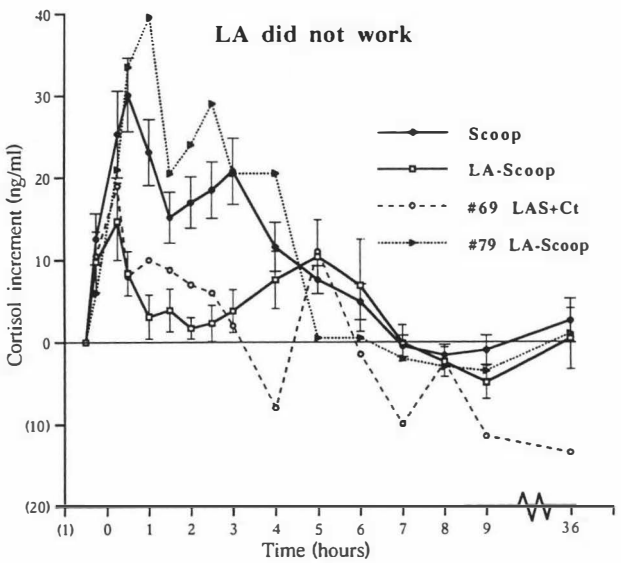

Fig B.2 Comparison of changes in plasma cortisol concentrations of calves #69 (LAS+Ct) and # 79 (LAS) to the mean (± SEM) Scoop and LA-Scoop response. For both these calves it appeared that the local anaesthetic did not work.

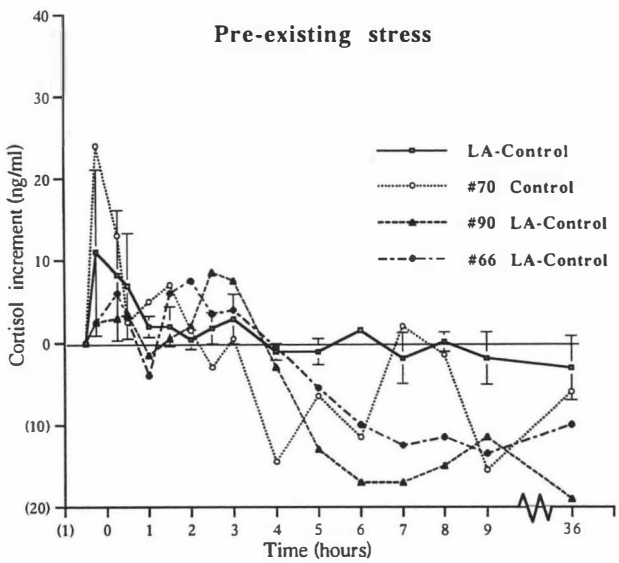

Fig B.3 Comparison of changes in plasma cortisol concentrations of calves #70 (Control) and #90 and #66 (LA-Control) to the mean (± SEM) LA-Control response. For these three calves their cortisol responses indicated that they were experiencing a stress prior to the pre-treatment sample.

APPENDIX C: THE CORTISOL RADIOIMMUNOASSAY

Plasma Cortisol Analysis

Blood samples were collected in vacutainer tubes containing lithium heparin, chilled immediately, centrifuged and the plasma was stored at -20°C until analysed. Plasma cortisol concentrations were determined by radioimmunoassay. The cortisol radioimmunoassay technique was that of Ruder *et al.* (1972) as modified by Evans (1979) [see Mac Diarmid (1982)].

Radioimmunoassay theory

The radioimmunoassay (RIA) takes advantage of the competitive binding of unlabelled antigen (C) and radioactively-labelled antigen (C*) to the antibody (Ab-C). In this RIA the antigen is cortisol (C or C*), the unlabelled-antigen is our sample (C) and the antibody is antiserum to cortisol (Ab-C). The amount of radioactively-labelled antigen and the amount of antibody is fixed and known, and to this is added an unknown amount of unlabelled antigen (i.e. our sample). The greater the amount of unlabelled cortisol the more it competes with and the smaller the amount of radioactive cortisol that can bind to the cortisol antibody. After incubation of the C, C*, and Ab-C, the cortisol that is bound as the antigen-antibody complex is separated from the unbound antigen, and the radioactivity of the bound phase is measured. The unlabelled antigen will either be a series of samples of known concentration, called standards, or the unknown sample. A standard curve of concentration versus radioactivity is constructed using the known standards, and by reference to this the amount of cortisol in the unknown sample is calculated.

Materials

The assay buffer was 0.01 M phosphate-buffered saline pH 7.3, containing 0.1 % (W/V) gelatin and 0.01 % sodium merthiolate.

Bovine gamma globulin (BgG) (Sigma Chemical Co., St Louis, USA) was dissolved in assay buffer to give a 1.5 % (W/V) working solution of non-specific protein.

Polyethylene glycol 4000 (BDH Chemicals ltd., Poole, UK) was dissolved in distilled water to give a 16.2 % (W/V) solution which was used to separate bound from unbound tritiated cortisol (^{3}H-C).

The scintillation fluid was toluene:triton x-100 (2:1) containing 3 g of 2, 5 diphenyloxazole (PPO) (sigma chemical Co., St Louis, USA) and 100 mg of 1, 4-bis (2-(5-phenyloxazolyl)) benzene phenyloxazolyphenyl-oxazolyl-phenyl) (POPOP) (Sigma Chemical Co., St Louis, USA) per litre.

The cortisol antiserum (cortisol-3-oxime-bovine serum albumin. F3-314. Endocrine Sciences Products, Tarzana, CA., USA) was stored frozen at a 1:200 dilution in assay buffer. Immediately before use it was further diluted in assay buffer to a 1:2000 dilution.

The tracer used was tritiated cortisol ([1,2,6,7-^{3}H] cortisol. Amersham, Life Sciences) with a specific activity of 2.33 TBq/nmol, and a radioactive concentration of 63 Ci/mmol.

Dichloromethane

Ethanol

The Radioimmunoassay procedure

The cortisol in the plasma samples (0.5 ml aliquots) was extracted by overnight incubation in 5 ml of dichloromethane. The solvent was transferred to test tubes and evaporated under a stream of air, and then the samples were resuspended in 0.5 ml of ethanol to make up to the original sample volume.

Aliquots (0.1 ml) of the samples, or the series of standard cortisol solutions (made-up in ethanol) or the plasma "pools" were added to assay test tubes. This was done in duplicate. The solution was evaporated under a stream of warm air. To each tube was added 0.1 ml of cortisol antiserum, 0.1 ml of bovine-gamma-globulin, and 0.1 ml of tracer (^{3}H-Cortisol). The appropriate controls for the radioimmunoassay were made up as shown in Table C.1. The tubes were vortexed and incubated overnight at 4°C to allow for equilibrium between the free and bound phase of the reagents.

Separation of the free and bound cortisol was achieved by the addition of 1 ml of polyethylene glycol to each tube, which was then vortexed and incubated for 10 minutes at 4°C. The bound phase (the precipitate) was collected by centrifuging the solution at 2,000 rpm for 20 minutes and aspirating off the supernatant. The precipitate was redissolved in 1 ml of water. This solution was decanted into scintillation vials to which 6 ml of scintillation fluid was added. The radioactivity in each vial was measured using a Beckman LS 8000 scintillation counter. A standard curve was constructed using the data from the series of standard cortisol solutions. The concentrations of the unknown samples were calculated from this by extrapolation (Industry standard computer package, Animal Science Dept., Massey University, New Zealand). Each assay run took around 3 days.

The intra-assay and inter-assay coefficients of variation were calculated using the three sets of plasma pools and were about 10% and 14% respectively. The lowest detectable concentration of cortisol was 0.3 ng/ml.

Table C.1 The setting up of the radioimmunoassay and the appropriate controls.

cocktail	Total	Blank	Samples
tracer (^{3}H-C)	0.1 ml	0.1 ml	0.1 ml
BgG	—	0.1 ml	0.1 ml
buffer	—	0.1 ml	—
antiserum (Ab-C)	—	—	0.1 ml
H_2O	0.9 ml	—	—