# final report

Project code: B.AHW.0143

Prepared by: K McCosker[1], C Petherick[2], D Mayer[2], B Venus[2], P Letchford[3] and M McGowan[4]
[1]Department of Regional Development, Primary Industry, Fisheries and Resources; [2]Queenland Department of Primary Industries and Fisheries; [3] Cosmos-West; [4]The University of Queensland

Date published: February 2009

ISBN: 9781740362726

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

# Evaluation of the impact on animal welfare of various manipulative and surgical procedures performed on the reproductive tract of female cattle in the northern beef industry

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

# Abstract

The spaying of cattle is considered an essential management tool in parts of northern Australia. In recent years traditional flank spaying has been increasingly replaced by the Willis Dropped Ovary Technique (WDOT), which is perceived as having better welfare outcomes than flank spaying, however this perception has never been scientifically validated. Studies conducted under commercial conditions demonstrated that the acute (up to 8 hours post-procedure) physiological and behavioural responses of commercial Brahman heifers and cows to flank and WDOT spaying were virtually indistinguishable, and were significantly ($P<0.05$) greater than the responses of similar cattle to physical restraint alone. However, the longer-term inflammatory and stress responses (up to 96 hours post-procedure) were significantly ($P<0.05$) greater for flank than WDOT spaying. Physiologically, cows and heifers responded differently to WDOT spaying; heifers responded similarly to WDOT and artificial insemination (AI), but cows showed a significantly ($P< 0.05$) greater cortisol response to WDOT than AI. The mortality rates (within 42 days of procedures being performed) for yearling heifers which were only restrained (n=200), or were spayed by either flank (ovariectomy technique, n=200) or WDOT (n=200) were 0%, 2.5% and 1.5%, respectively. In a further study of WDOT spayed yearling heifers (n=574), the mortality rate was 0.5%. Overall the findings of this project demonstrate that compared to physical restraint alone surgical spaying by either method examined induced a significant ($P<0.05$) acute and early chronic increase in recognised physiological and behavioural measures of pain/stress. Research should be immediately initiated to develop practical, cost effective and safe methods to reduce these effects of spaying.

# Executive Summary

The surgical spaying of beef cattle is considered an essential management tool in northern Australia where bull control is often unreliable, and the majority of herds are continuously mated. The spaying of surplus or cull females has been shown to significantly reduce breeder mortalities by preventing 'out of season' calvings, and overstocking, and enables producers to fatten and turn-off these females. The live export trade with S.E Asia has become a major market for spayed heifers due to the strict requirement of non-pregnant females. Since the late 1990s, traditional flank spaying has been increasingly replaced by the Willis Dropped Ovary Technique (WDOT) of spaying, which is perceived by many stakeholders (including representatives of the veterinary profession and animal welfare organisations) as having better welfare outcomes than flank spaying. However, this perception has never been scientifically validated. Further, although in heifers WDOT spaying has largely replaced flank spaying, in cows flank spaying using the 'webbing' technique (excision of the oviduct) continues to be commonly performed because of the perceived lower risk of surgical haemorrhage with this technique compared to WDOT. Also it is common practice for animals to be electroimmobilised for flank spaying to reduce the risk of traumatic injuries to the animal and operators. This work was conducted to determine the welfare outcomes for cows and heifers of spaying by both flank and WDOT, and to compare these outcomes with those from other routine management practices; physical restraint alone (Control), physical restraint and electroimmobilisation, and mock artificial insemination (AI). Video footage of each these procedures being performed according to best practice, and the range of responses of cattle to them, were collected.

A series of studies were conducted in the Northern Territory during the 'first round muster' (May to August), to determine the impacts on accepted measures of animal welfare (physiological, behavioural, production and health responses were monitored) of flank and WDOT spaying of commercial Brahman heifers and cows. Study I (pilot study of physiological responses of cattle to spaying) was conducted using twenty-four, 2-year-old heifers under experimental conditions, and Studies II (main study of physiological and behavioural responses of cattle to spaying) and III (study of health and production responses of cattle to spaying) were conducted under commercial conditions, using 100 yearling heifers and 50 cull cows (II), and 600 (IIIa) and 574 (IIIb) yearling heifers respectively. Study IIIb was an additional study conducted because of a higher than expected and unexplained mortality rate in WDOT spayed cattle in study IIIa.

Studies I and II demonstrated that the acute (up to 8 hours post-procedure) physiological and behavioural responses, indicative of pain and stress, of cattle which had been either flank or WDOT spayed were virtually indistinguishable, and significantly ($P< 0.05$) greater than the responses observed in cattle which were only physically restrained. The longer-term physiological and behavioural responses indicative of pain and stress (up to 96 hours post-procedure) were significantly ($P<0.05$) greater in flank compared to WDOT spayed cattle. Physiologically, cows and heifers responded differently to WDOT spaying; heifers responded similarly to WDOT and AI, but cows showed a significantly ($P< 0.05$) greater cortisol response to WDOT than AI. For heifers there were few physiological and behavioural differences between AI and physical restraint alone. However, cows showed a greater ($P< 0.05$), acute cortisol response to AI compared to the controls. Study II also demonstrated that there were significant ($P< 0.05$) adverse, physiological and behavioural effects on the welfare of electroimmobilised cattle, particularly in cows.

In Study I significant differences ($P=0.07$) in average liveweight gain (-11 to 42 days after procedures) were not detected between the control (physical restraint alone) and spayed heifers. Similarly, in Study II no significant differences ($P<0.05$) were detected between control and spayed heifers and cows in liveweight gain during the 42 days after the procedures were performed.

However, in Study III the spayed heifers achieved a significantly ($P< 0.05$) lower liveweight gain (mean difference of 0.27 kg/day) than the controls during the 42 days after the procedures were performed.

In Study I there were no deaths in the spayed cattle, and in Study II there was a single death due to diffuse peritonitis in the flank spayed heifer group ($n = 20$) detected five days after the procedure was performed. In addition, 16% of the flank spayed animals showed visual evidence of significantly delayed or abnormal wound healing (partial to total wound dehiscence and purulent discharge from wounds) at day 42 after the procedures were performed. In Study IIIa mortality rates (deaths within 42 days of procedures) of 1.5% for WDOT, 2.5% for flank spaying and 0.0% for the control (physical restraint alone) treatments were recorded, and only 5% of the flank spayed heifers had delayed or abnormal wound healing at day 42. In Study IIIb an overall mortality rate of 0.5% was found within 42 days of WDOT spaying.

Overall the findings of this project demonstrate that compared to physical restraint alone surgical spaying by either method examined induced a significant ($P<0.05$) acute and early chronic increase in recognised physiological and behavioural measures of pain/stress. Research should be immediately initiated to develop effective, practical, and safe methods to reduce these effects of spaying. The model for defining the impact on animal welfare developed in the present project should be used to define the responses to selected methods.

In the immediate short-term it is recommended that if spaying is to be conducted then it should be done by WDOT on yearling heifers, with in the longer term it being done with appropriate analgesia. Further, due to the demonstrated marked impact of electroimmobilisation on cattle welfare, appropriately designed and constructed cattle crushes should be installed at the sites where cattle are routinely spayed so that cattle can be adequately restrained without the use of electroimmobilisation. Use of the ‘webbing technique’ rather than ovariectomy to flank spay cattle is recommended as this technique is likely to result in few if any deaths due to surgical haemorrhage.

# Contents

# 1 Background

## 1.1 Role of surgical spaying in the management of northern Australian beef herds

Surgical excision of the ovaries of heifers and cows is a procedure practised in all the major cattle producing countries of the world to permanently prevent surplus or cull females from reproducing. It has particular application in extensive rangelands where bull control is often unreliable, and many herds are continuously mated. The spaying of surplus or cull females (both heifers and cows) significantly reduces breeder mortalities, enables producers to fatten and turn-off these females, and is a critical means of managing stocking rates to control land degradation (Jubb and Letchford, 1997). Further, as the females are rendered sterile, they can continue to graze with the breeding herd until turn-off, which reduces the need for any additional mustering and handling. The live export trade with S.E Asia has become a major market for spayed heifers due to the strict requirement of non-pregnant females.

## 1.2 Surgical procedures currently used

Until 1996 the most common method of spaying cattle in northern Australia was via a flank incision through the abdominal wall. This procedure was performed by veterinarians and lay spayers without the use of local anaesthetic or prophylactic antibiotics. Further, in many cases, to ensure the procedure can be carried out with minimal risk of injury to animal or operator, the females are electroimmobilised. Generally the ovaries are excised but, increasingly with pregnant or fat females, operators excise a portion of the oviduct from each side, the so-called ‘webbing’ technique.

In the 1980’s North American veterinarians reported the use of a new per-vagina method of spaying females, the Willis dropped ovary technique (WDOT). The technique was introduced into northern Australia in 1996 and the subsequent uptake by the cattle industry and spaying technicians was rapid. The advantages over flank spaying provided by this method are: no hide damage or carcass trim required; the restraint required is similar to that required for pregnancy diagnosis; it is aesthetically more acceptable; perceived to be less invasive, causing less pain and stress; and in the hands of experienced operators it offers high processing rates (500 to 600 head per day) and apparently low mortality rate (0.5%). The major disadvantages are that the technique requires the operator to have very good per rectal palpation skills and it is not suitable for spaying recently calved females or animals greater than 4 months pregnant because of the increased risk of severe haemorrhage and/or difficulties in locating and manipulating the ovaries.

## 1.3 Reasons for conducting this study

Improvements in husbandry, nutritional management and control of diseases, particularly botulism, on increasing numbers of northern Australian properties has resulted in significant increases in the number of surplus females, and thus a sustained need for surgical spaying to be carried out. Experienced operators are spaying around 25,000 females annually, with approximately 20,000 being spayed by the WDOT and the remainder being mostly ‘webbed’ (excision of the oviduct) via a flank incision. The costs per head for WDOT spaying is approximately $5 and for flank spaying $6.50 (2007 costings).

Alternatives to surgical spaying, such as sustained release deslorelin implants have been shown to be effective in inhibiting ovulation in cattle for periods of 200 to 300 days depending on the dose rate used (D’Occhio et. al. 2002). However, these implants are currently significantly more expensive than the surgical techniques, partly because they are only registered for use in companion animals.

The northern beef cattle industry is continuing to fund investigations of non-surgical approaches for controlling reproduction in male and female cattle.

Field studies investigating the effects of spaying on animal health and productivity have been conducted in North America (Habermehl et. al.1993), and in northern Australia (Jubb et al. 2003), however, only limited observations were made of the impact of the procedures on animal welfare. There have been no published studies defining the physiological and behavioural responses to spaying in cattle. Recognising this, Meat and Livestock Australia commissioned the following project to be conducted on behalf of the northern Australian beef industry.

### 1.4 Project hypotheses

1. The Willis dropped ovary technique (WDOT) of spaying cattle provides significant benefits over flank spaying for all accepted measures of animal welfare.
2. There is no significant difference in responses of cattle to WDOT spaying and accepted non-surgical procedures, such as artificial insemination or per rectum pregnancy diagnosis, or general yard and crush handling for objective measures of animal welfare.

# 2 Project Objectives

1. Determine animal mortality and live-weight change following treatment procedures.
2. Provide accurate and objective assessments of behavioural and physiological pain and stress responses experienced during and immediately following the treatment procedures using accepted welfare measures.
3. Obtain digital, still or video footage of all techniques as performed to best practice.

# 3 Methodology

### 3.1 Study sites and research teams

With prior approval of the Northern Territory's Charles Darwin University Animal Ethics Committee (Project Reference: A06007), a series of studies were conducted at three sites within the northern half of the Northern Territory (Figure 3.1-1). A pilot study (Study I) was conducted at the Northern Territory Department of Primary Industries, Fisheries and Mines (NTDPIFM) Berrimah Research Farm, Darwin, and Studies II and III were conducted in the Victoria River District at Heytsbury Beef properties Mt Sanford Station and Pigeon Hole Station (Figure 3.1-1).

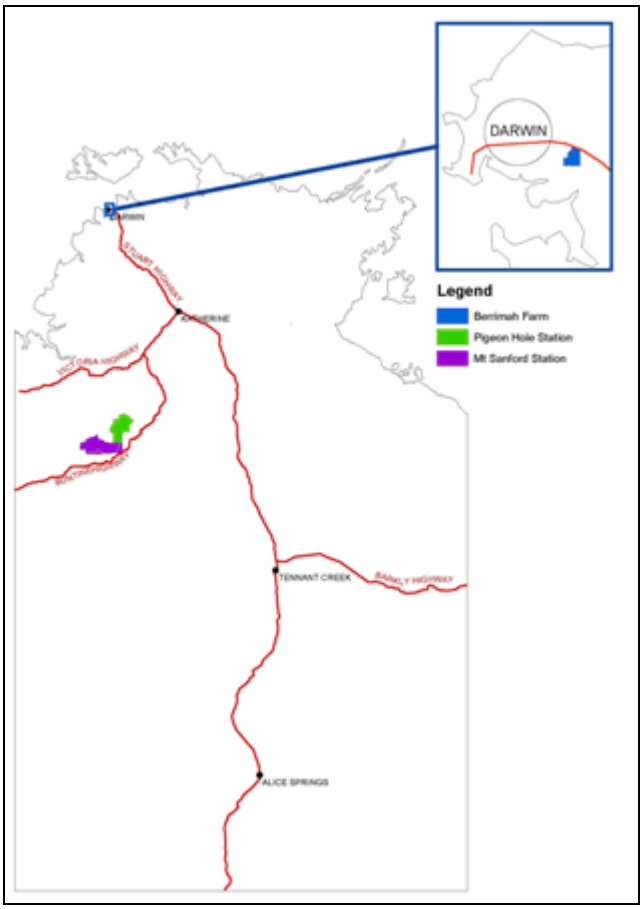



**Figure 3.1-1 Map of the Northern Territory showing the location of the study sites.**

### 3.1.1 Berrimah Research Farm

Berrimah Research Farm (12°26'48"S, 130°55'58"E), is situated near Darwin on the Stuart Highway and is approximately 215 ha in area (Figure 3.1-1). Study I was conducted between 1st May and 12th June, 2006. A Silo data drill for the duration of the study estimated that 12.2 mm rainfall fell during this period and the average relative humidity ranged between 35% and 77.8%. The minimum and maximum temperature ranges were 15.0 to 23.0°C and 27 to 32.5°C respectively (Appendix 9.3).

The main grazing resource at Berrimah Research Farm is fertilised improved pastures, predominantly Pangola grass (*Digitaria decumbens*). Yield was estimated at 3,000-3,500 kg DM/ha across the duration of the study (Plate 3.1-1). Faeces were collected at the completion of the study and pasture quality estimated using near infrared reflectance spectroscopy (NIRS). The dry matter digestibility was estimated at 57% and the dietary crude protein content at 8.1%.

a)  b) 

**Plate 3.1-1 Pasture at Berrimah Research Farm: a) Trial paddock at commencement of trial (b) at completion of trial.**

**Table 3.1-1 Study I research team**

| Organisation | Team member | Responsibilities |
|---|---|---|
| NTDPIFM | Annemarie Huey | - Pushing cattle up to race and ratcheting forward in crush<br>- Ensuring cattle were supplied to crush in an appropriate order and on time |
| QDPIF | Carol Petherick | - Data collection and record keeping<br>- Labelling of tubes |
| UQ | Michael McGowan | - Bleeding<br>- Ratcheting Up |
| NTDPIFM | Kieren McCosker | - Transportation of bloods to Berrimah Veterinary Lab<br>- Mustering of cattle<br>- Ensuring the cattle were supplied to crush in correct order and on time.<br>- Backgrounding of cattle<br>- Bleeding, wound assessment, weighing @ 21 and 42 d<br>- Head bail operation and head restraint for bleeding |
| Private | Peter Letchford | - Performing procedures |
| NTDPIFM | Berrimah Veterinary Lab | - Blood sample preparation and storage |

### 3.1.2 Mt Sanford Station

Heytesbury Beef's Mt. Sanford Station is located approximately 500 km south-west of Katherine in the southern portion of the Victoria River District. Study II was conducted at the Blackgin yards (17°12'S, 130°38'E) between 24th May and 5th July 2006 (Figure 3.1-1). An automated weather station located at Blackgin recorded that 0 mm rainfall fell during this period and the average relative humidity ranged between 32% and 59%. The minimum and maximum temperature ranges were 6 to 15°C and 20 to 30°C respectively (Appendix 9.3).

Three holding paddocks (Figure 3.1-2), comprised of 'Wavehill' soil type, were utilised during the study: 'Little Blackgin Holding Paddock' (84.8ha), 'Laneway' (16.3ha) and 'Blackgin Horse Paddock' (21.5ha). Native grass pastures were the main grazing resource, with the predominant species being *Aristida latifolia*, *Dicanthium fecundum, Chrysopogan fallax, Sesbania cannabina, Astrebla* spp., *Indigophera* spp. and *Sorghum intrans* (Plate 3.1-2). Some paddocks had previously been grazed and therefore pastures had been utilised to varying degrees prior to commencement of the study: 'Little Blackgin Holding Paddock' 50% utilised, estimated 1,500-2,000 kg DM/ha yield; 'Laneway' 75-

100% utilised, 500-800 kg DM /ha yield; ‘Blackgin Horse Paddock’ <10% utilised, 2,500-3,000 kg DM /ha yield.

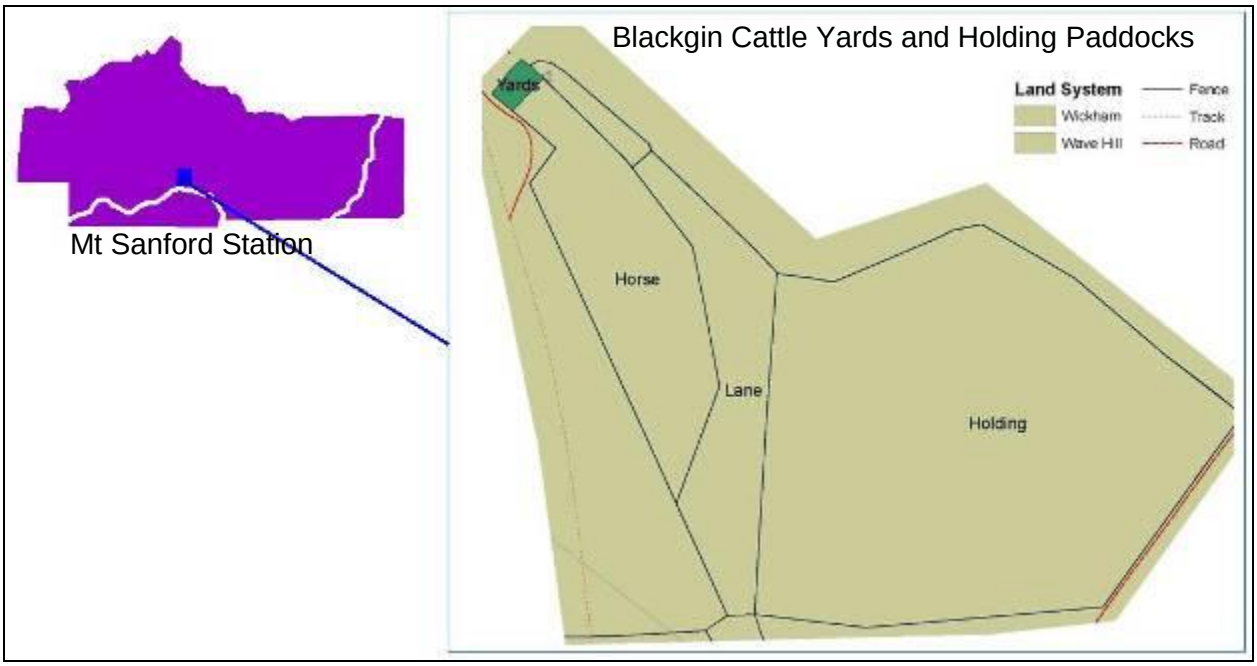



**Figure 3.1-2 Map of Mt Sanford Station showing paddocks grazed by trial animals during Study II.**

a)  b) 

c) 

**Plate 3.1-2 Paddocks grazed by trial animals during Study II at Mt Sanford Station, Northern Territory: a) Blackgin Horse Paddock, b) Laneway and c) Little Blackgin Holding Paddock.**

**Table 3.1-2 Study II research team**

| Organisation | Team member | Crush | Responsibilities |
|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| NTDPIFM | Andrew Murray | B | - | Head-bail operation and head restraint for bleeding |
| NTDPIFM | Annemarie Huey | A | - | Pushing cattle up to race and ratcheting forward in crush |
| | | | - | Ensuring cattle were supplied to pound in an appropriate order |
| QDPIF | Carol Petherick | | - | Observations of animal behaviour in the yards and paddocks |
| NTDPIFM | Caroline Smith | B | - | Data collection and record keeping |
| NTDPIFM | Gehan Jayawardahana | B | - | Bleeding |
| | | | - | Behavioural observations on approach to and in crush |
| Heytesbury | Gus Payne | A | - | Mustering |
| | | | - | Head bail operation and head restraint for bleeding |
| | | | - | Feeding of stock |
| NTDPIFM | Harmony James | A | - | Data collection and record keeping |
| NTDPIFM | Kieren McCosker | | - | Backgrounding of cattle |
| | | | - | Ensuring the cattle were supplied to crushes in correct order and on time. |
| | | | - | Bleeding, wound assessment, weighing @ 21, 42d |
| UQ | Michael McGowan | A | - | Bleeding |
| | | | - | Behavioural observations on approach to and in crush |
| UQ | Nancy Phillips | | - | Blood sample preparation and storage |
| Private | Peter Letchford | A | - | Performed all procedures |
| NTDPIFM | Sarah Streeter | | - | Ensuring cattle were supplied to pound in an appropriate order |
| QDPIF | Tracey Longhurst | | - | Observations of animal behaviour in paddocks. Assisted with blood preparation and storage |
| NTDPIFM | Trisha Cowley | B | - | Pushing cattle up to race and ratcheting forward in crush |
| | | | - | Observations of animal behaviour in paddocks |
| Private | Shirley Ross | | - | Cooking and maintaining camp area |

### 3.1.3 Pigeonhole Station

Heytesbury Beef's Pigeon Hole Station is situated approximately 390 km south-west of Katherine in the southern portion of the Victoria River District. Studies IIIa and b were conducted at 'Coles' yards (16°38'28''S, 131°12'27''E) and No.12 yards ( 30km SSW of Cole's yards) between 4$^{th}$ July and 15$^{th}$ August 2006 (Figure 3.1-1 and Figure 3.1-3). A Silo data drill for the duration of the study estimated that 1.7 mm rainfall fell during this period and the average relative humidity ranged between 29% and 74%. The minimum and maximum temperature ranges were 4.5 to 18°C and 22.5 to 33.5°C respectively (Appendix 9.3).

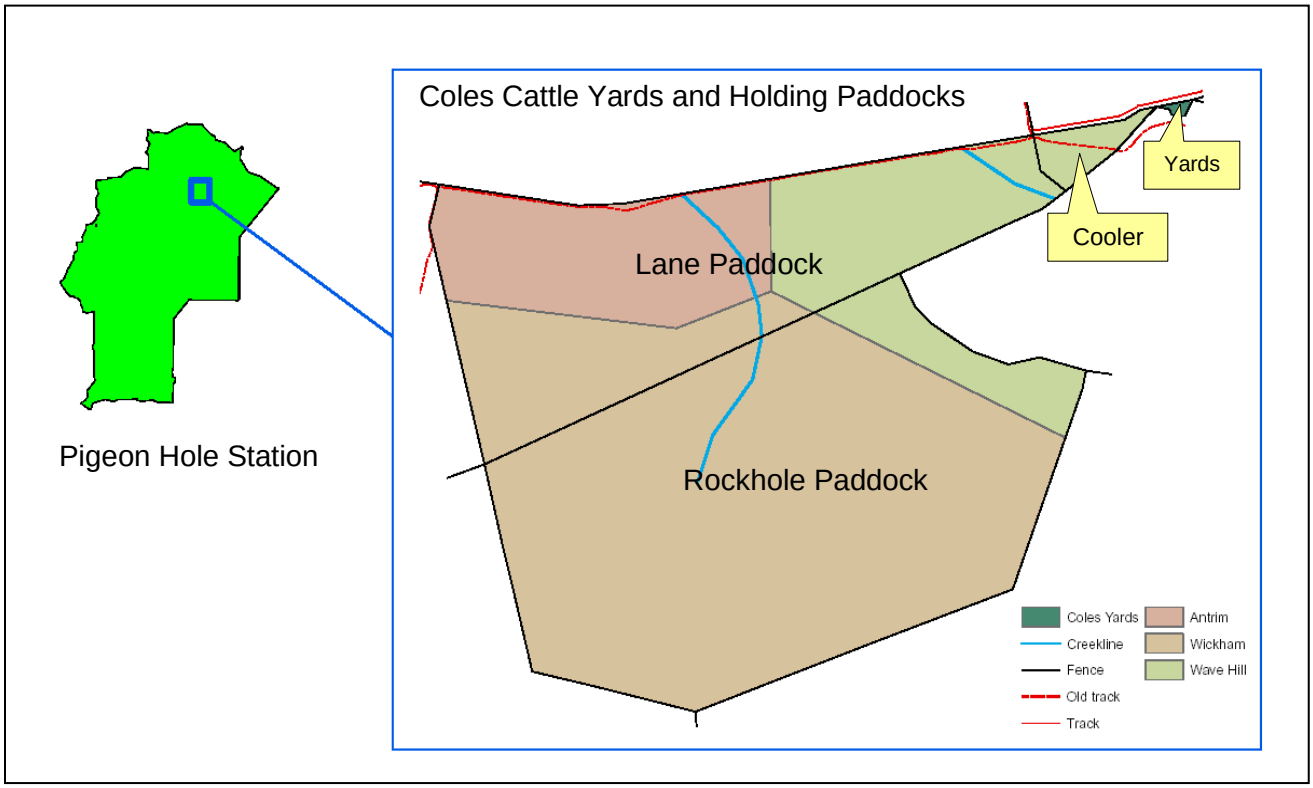



**Figure 3.1-3 Map of Pigeon Hole Station, showing paddocks utilised during study.**

Study IIIa and Study IIIb cattle (one mob only) grazed three paddocks 'Lane' (160ha), 'Rockhole' (268ha) and 'Cooler' (7ha) comprising of three main land systems; Wickham, Wavehill and Antrim (Figure 3.1-3). Native pastures were the main grazing resource consisting of *Aristida latifolia*, *Dicanthium fecundum*, *Chrysopogan fallax*, *Sesbania cannabina*, *Astrebla* spp. and *Indigophera* spp. Prior to the study these paddocks had not been grazed and yield was estimated at 2,500-3,000 kg/ha. Faecal samples were collected at the time of procedures, 21 days and 42 days post-procedures and analysed to estimate pasture quality using NIRS. The Dry Matter Digestibility was estimated to range between 49 and 53% and the dietary crude protein content between 4.1 and 6.6% (Appendix 9.2). The second mob of heifers in Study IIIb grazed a laneway (75.5ha) extending from the No.12 yards. It contained *Dicanthium* dominant pasture similar to that grazed by the heifers in Study IIIa.

**Table 3.1-3 Study III research team**

| Organisation | Team Member | Responsibilities |
|---|---|---|

| | | |
|---|---|---|
| Private | Peter Letchford | - Performing procedures |
| UQ | Michael McGowan | - Observations of animal behaviour in the yards and in paddocks<br>- Videoing procedures<br>- Paddock surveillance to assess cattle health<br>- Post mortem investigation of mortalities |
| Heytesbury | Stockcamp | - Pushing cattle up to race and ratcheting forward in crush<br>- Head-bail operation, ear punching, ear tagging<br>- Mustering |
| NTDPIFM | Annemarie Huey | - Data collection and record keeping<br>- Keeping tally of procedures and informing veterinarian of treatments to be applied |
| NTDPIFM | Trisha Cowley | - 21 and 42 d data collection and record keeping |
| NTDPIFM | Kieren McCosker | - P8 fat measurements<br>- Paddock surveillance to assess cattle health<br>- Post mortem investigation of mortalities |

## 3.2 Cattle selection and allocation to treatments

### 3.2.1 Study I

Eleven days prior to allocation 36, 2-year-old, high-grade Brahman heifers, all with electronic identification (EID) were mustered and pregnancy and lactation status, liveweight, flight speed and P8 fat depth recorded. Flight speeds were determined using a single flight time measured using a Ruddweigh flight time recorder across a distance of 1.8 m as animals exited the crush. Fat depth, at the P8 site, was recorded using an Ultramac B-10 cattle fat depth meter.

These data were subjected to a principal components analysis and animals then blocked according to weight and flight speed, so that group averages of liveweight and flight speed were the same for each treatment group. Twenty-four heifers which were either not detectably pregnant or were palpably less than 2 months pregnant were selected. Within blocks, animals were randomly allocated to one of four treatment groups, and then grouped into five replicates. Each heifer was ear-tagged with a coloured tag according to its treatment and the number of the replicate was spray painted on the offside rump.

### 3.2.2 Study II

Three days prior to allocation, 123 Brahman cows (aged 2 to 15years but mainly 8 to 13years) and 115 yearling Brahman heifers were mustered; pregnancy and lactation status, liveweight, flight speed and P8 fat depth were recorded as described for Study I. Significant outliers for body weight and P8 fat depth were rejected. Two flight speeds were recorded for cows and one for heifers. Some of the heifers had been dehorned 2 to 3 weeks prior to allocation to the study.

These data were subjected to a principal components analysis and animals then blocked according to weight and flight speed. Fifty Brahman cows less than 3 months pregnant and 100, 2-year-old, non-detectably pregnant Brahman heifers were selected. Within blocks, animals were randomly allocated to one of five treatment groups, and then grouped into 10 replicates (heifers) or five replicates (cows). Each female was ear-tagged (near-side) with a coloured tag (pink, green, white, orange and yellow) according to its treatment and the number of the replicate was spray painted on the offside rump.

### 3.2.3 Study III

For Study IIIa, 600 Brahman, 2-year-old heifers were selected for spaying as per standard station practice (liveweight <250 kg). Heifers were assigned to one of eight replicates in groups of 75 animals by order of presentation in the race. Within replicates, groups of 25 animals were randomly allocated to one of three treatment groups. At the time of treatment each heifer was tagged with a coloured tag according to the treatment applied (green, orange and pink), with the replicate number and sequential animal number recorded on it.

As a result of unexpected and unexplained mortalities in the spayed heifers in Study IIIa, an additional 574 2-year-old Brahman heifers which were being WDOT spayed, as per station practice and by the same operator on two consecutive days; 199 (the controls from Study IIIa) spayed at Coles Yards and 200 spayed at No.12 yards on the first day and 175 spayed at No.12 yards the next day, were monitored (Study IIIb).

## 3.3 Study design

### 3.3.1 Study I

The major objective of conducting this study was to evaluate and refine the planned methodology for Study II. In particular, there was a complete lack of information on the likely timing of the acute peak in bound cortisol after WDOT spaying. Therefore, an increased number of WDOT heifers were used to ensure there would be sufficient data to define the initial increase in cortisol following spaying.

Twenty-four, 2-year-old Brahman heifers were assigned as follows:

- five x physical restraint only (controls)
- five x physical restraint + ear notch
- nine x physical restraint + WDOT spay
- five x physical restraint + electroimmobilisation + flank spay (webbing technique was used as it was considered by the veterinarian to be consistent with industry practice for this age and weight of animal).

Five replicates each consisting of one animal from each treatment, except for the WDOT group where in four of the replicates there were two heifers spayed using this technique, were processed on a single day. A sampling schedule was developed based on the following estimates of how long it would take to complete each procedure including blood sampling: 3 minutes for flank spaying, 2 minutes for WDOT, and 1 minute for physical restraint alone or ear notch. The procedures were commenced at 7 am, with replicates 1 to 3 being processed sequentially. Three and half hours afterwards replicates 4 and 5 were commenced. This schedule was designed to enable the intensive blood sampling schedule to be executed. Each heifer was bled immediately prior to conduct of each procedure (time 0), and then the aim was to sample at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 24, 48, 72, 96 hours afterwards.

The physiological responses to the procedures were defined by measuring:

- Bound and unbound cortisol concentrations for all sampling times
- Haptoglobin concentrations determined at 0, 24, 48, 72 and 96 hours
- Creatinine phosphokinase (CPK) and Aspartate aminotransferase (AST) concentrations determined at 0, 8, 24, 48, 72 and 96 hours
- Non-esterified fatty acids (NEFA) concentrations determined at 0, 8, 24, 48, 72 and 96 hours

The very frequent blood sampling during the first 4 hours after the procedures were performed was employed to ensure that the acute bound cortisol response was adequately defined for each procedure especially WDOT. The time each blood sample was taken and each procedure completed was recorded.

No systematic recording of animal behaviours was done in this study, rather an assessment was made of what behaviours might be observed and what schedule of behaviour observations could be integrated with the blood sampling schedule.

The impact of the procedures on animal health and production were assessed as follows:

- at 24, 48, 72, 96 hours and 21 and 42 days after the procedure the general health of each heifer was assessed and scored (see section 3.10 for details)
- for the flank spayed heifers a wound healing score was recorded at 21 and 42 days (see section 3.10 for details)
- body weights were recorded at 21 and 42 days and average daily gains for each period and overall determined

### 3.3.2 Study II

The major objective of this study was to measure, under typical commercial conditions, in *Bos indicus* female cattle, the physiological and behavioural responses to spaying. As well as a standard control group, which had no procedures performed on them except physical restraint in a crush, an additional 'control' group for each spay procedure was added; for the WDOT spayed cattle this was a similar group of cattle which were mock artificially inseminated (AI) enabling assessment of the impact of rectal palpation and manipulation of the reproductive tract versus palpation, manipulation and ovariectomy (WDOT). Cattle which are to be spayed using the flank technique are commonly electroimmobilsed first, and thus the additional 'control' for this procedure was a group of similar cattle which were only electroimmobilsed (see 3.4.3), enabling assessment of the impact of electroimmobilisation versus electroimmobilisation and flank laparotomy and ovariectomy. Thus there were 5 treatment groups.

This study was carried out during the first round of mustering at Mt Sanford station when typically surplus heifers and cull cows are spayed for subsequent turn-off at the first-round muster the following year. Twenty heifers and 10 cows were assigned to each of the control, AI, WDOT, electroimmobilisation (EI) and flank spay (ovariectomy technique, Flank) treatment groups. The cattle were grouped according to blocking (see above) into replicates of 10 animals each, with two cattle from each treatment group. Based on the findings on how long it took to carry out procedures in Study I, it was decided to process five replicates per day according to the following schedule; Day 1, five replicates of heifers, Day 2, five replicates of cows, Day 3, five replicates of heifers. This study design was selected to ensure that all blood samples could be collected during daylight hours and that there would be sufficient time after the last blood sample was collected on the day procedures were done to enable a period of uninterrupted observation of the behaviours of the processed cattle. Following analysis and review of the findings of Study I the following sampling schedule was used to define the physiological responses to the procedures:

- Bound cortisol concentrations determined at 0, 1, 2, 3, 4, 6, 8, 24 and 96 hours
- Unbound cortisol concentrations determined at 0, 8, 24 and 96 hours
- Haptoglobin concentrations determined at 0, 24 and 96 hours
- CPK and AST concentrations determined at 0, 8, 24 and 96 hours
- NEFA's concentrations determined at 0, 8, 24 and 96 hours

The time each blood sample was taken and each procedure completed, was recorded.

Assessments of the behaviour of the cattle were carried out as cattle were:

- moved up the race
- restrained in the crush
- held in holding yards
- held in holding paddocks.

These assessments are described in detail in 3.8 below.

The impact of the procedures on animal health and production were assessed as follows:

- at 24, 48, 72, 96 hours and 21 and 42 days after the procedure, the general health of each heifer was assessed and scored (see section 3.10 for details)
- for the flank spayed heifers a wound healing score was recorded at 21 and 42 days (see section 3.10 for details)
- body weights were recorded at 96 hours and at 21 and 42 days, and average daily gains for each period and overall determined.

### 3.3.3 Study IIIa

The major objective of Study III was to define, under typical commercial conditions, the impacts on animal health and production of WDOT and flank spaying. This study was carried out during the first round of mustering at Pigeon Hole station when, typically, surplus heifers and cull cows are spayed for subsequent turn-off at the first-round muster the following year. Wherever possible station managers prefer to spay cattle during the first-round muster, as the environmental temperatures are low and the risk of mortalities is considered lower at this time than during the hotter months of the year. Although the vast majority of surplus heifers are typically spayed on stations using WDOT (P. Letchford, pers. comm.), an equal number of heifers in this study were flank spayed to enable the impact of both techniques to be determined. Further, although the veterinarian would normally use the webbing technique for flank spaying to reduce the risk of mortalities (P Letchford pers.comm. 2006), to enable an unbiased comparison to be made between the two procedures, the flank spayed cattle were ovariectomised.

Six-hundred yearling Brahman heifers from a larger mob of cull heifers were drafted off and assigned as follows:

- 200 x physical restraint only (controls)
- 200 x WDOT spay
- 200 x flank spay.

The procedures were completed in replicates of 25 over 2 days (15 replicates on Day 1 and 9 replicates on Day 2). The procedures were commenced at first light and completed just after sundown, typical of normal station practice. The time each procedure was completed was recorded.

Based on the behavioural observation conducted in Study II, a number of behaviours, for which the incidence could be readily estimated in large groups of cattle, were recorded. From 0 to 6 hours after spaying, groups of heifers (25 – 150) were observed approximately three times an hour for a period of 10 minutes on each occasion. Subsequently, the health of cattle was assessed by regular patrolling of holding paddocks between 36 and 84 hours after the procedures were performed then again on Days 7, 10, 21 and 42. Wherever possible all mortalities detected were subjected to a systematic post mortem examination.

### 3.3.4 Study IIIb

As a result of higher than expected mortalities in the WDOT group in Study IIIa and deaths occurring outside the scheduled monitoring period (which precluded post-mortem examination), a further study to define the mortality rate and timing and cause of mortalities in WDOT spayed cattle was conducted at Pigeon Hole station. Two mobs of yearling heifers (the control group from Study IIIa [n = 199] and another very similar mob of 375 yearlings) were WDOT spayed. After the procedures were performed they were monitored by regular patrolling of their holding paddocks twice daily until Day 14. Again, wherever possible, all mortalities detected were subjected to a systematic post mortem examination.

## 3.4 Procedures

All of the following manipulative and surgical procedures were carried out on appropriately restrained cattle without the use of local anaesthetic, consistent with industry practice. There is no regulation requiring use of local anaesthetic for spaying of cattle in the Northern Territory. All procedures were carried out by a veterinarian (P Letchford) highly experienced in performing these procedures, typically carrying out WDOT spaying on 20,000 head and flank spaying (mainly webbing technique) on 5,000 head per annum.

### 3.4.1 Control (physical restraint only)

Each animal was physically restrained in a commercial cattle crush with the head caught in a parallel closing head bail. They were restrained for a period of 1 minute after blood sampling (Studies I and II), or for the time required to ear tag, weigh and record P8 fat depth, approximately 30 s. (Study III).

### 3.4.2 Physical restraint +AI

The cattle were physically restrained as described above, with a kick gate swung behind the back legs. After wiping the vulva clean, an artificial insemination gun (0.25 mL Cassou) was inserted into the vagina and manipulated by transrectal palpation of the cervix to enable passage of the tip of the gun through the cervix to the body of the uterus. The procedure took approximately 1 minute to perform.

### 3.4.3 Physical restraint + Electroimmobilisation

The cattle were physically restrained as described above, and then the two electrodes (approximately 10 gauge x 3 cm sharpened probes) of the “Stockstill” (standard model) immobiliser unit were attached on the same side of the animal to the upper lip and either the base of the tail or rump area (Plate 3.4-1). The Stockstill unit is powered by a 6 volt battery. The maximum output of the Stockstill unit is 240 mAmps and it delivers an electric pulse of one second duration every 20 seconds. The unit was turned on and the amperage adjusted to cause skeletal muscles to contract resulting in immobilisation, but still allowing the animal to breathe. The animals were electoimmobilised for approximately 1 minute, the average time cattle are fully electroimmobilised for a flank spay to be performed.


**Plate 3.4-1 Veterinarian adjusting Stockstill® to electroimmobilise animal**

### 3.4.4 Physical restraint + Earnotching

The cattle were restrained as above and then, using standard ear marking pliers, a 2 cm diameter hole was punched into the pinna of the left ear (Plate 3.4-2). All cattle which are spayed must be identified by this procedure to comply with legislation. The animal was physically restrained for at least 1 minute.

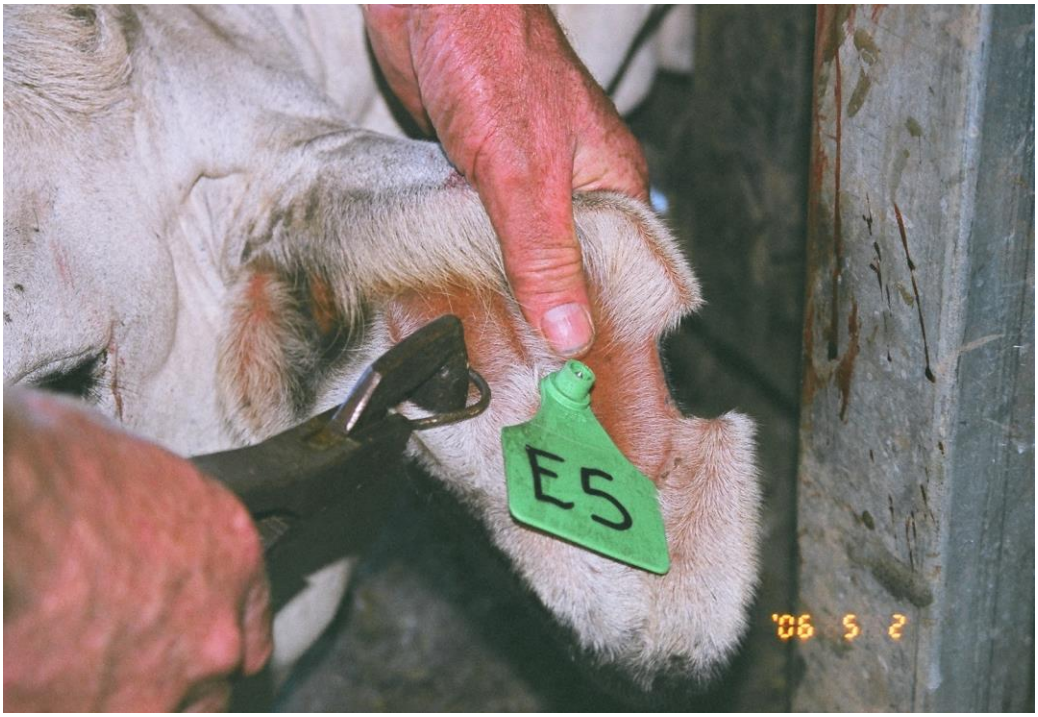



**Plate 3.4-2 Ear notching procedure.**

### 3.4.5 Physical restraint + Ovariectomy by WDOT + Earnotching

The cattle were physically restrained as described and with the kick gate swung behind the back legs. They were WDOT spayed as described by de Witte et al. (2006). After wiping the vulva clean the disinfected ovariotome was introduced into the vagina (Plate 3.4-3), inserted through the vaginal fornix into the caudal abdominal cavity, then each ovary was manipulated into the cutting slot of the ovariotome by transrectal paplaption and severed. Immediately after the spay procedure was completed the female was ear notched as described above. The whole procedure took 1 to 2 minutes to perform.

**Plate 3.4-3 Willis ovariotome being introduced into the vulva**

### 3.4.6 Physical restraint + Electroimmobilisation + Ovariectomy via Flank + Earnotch

The cattle were physically restrained and electoimmobilised as described above. The skin of the caudal left flank was then cleaned using a disinfectant solution with the excess solution being wiped off by the back of the right hand. A 12-15 cm incision was made with a Number 23 scalpel blade in a cranio-ventral direction along the dorsal border of the abdominal oblique muscle, approximately 10 cm ventral to its insertion on the *tuber coxae*. A small incision was also made in the aponeurosis of the *external abdominal oblique* muscle and the fingers of the left hand, shaped to form a cone, were pushed between the *internal abdominal oblique* and the deeper *transverse abdominal* muscle and through the peritoneum. The uterus and ovaries were then located and the right ovary selected and secured between the thumb and fingers. A plastic Spaymate 23 instrument was then introduced into the incision sliding it along the left arm as a guide to the ovary. The ovary was rotated to present the distinct cranial edge of the ovarian attachments to the opening of the Spaymate 23 with its concealed scalpel blade cutting edge. The attachments were fed into the mouth of the Spaymate 23 until the ovary was severed. This was then repeated with the left ovary while still retaining the right ovary in the hand. The hand and ovaries were then withdrawn and the skin incision was then sutured with a continuous ‘blanket” suture pattern using a No. 11 scalpel blade mounted on a 15 cm handle and cotton twine (Kinnears Fine Cotton 660 TEX P/#79177) (Figure 3.4-1). After completion of suturing the apposition of the wound edges was checked and aligned as necessary with gentle manual pressure. In Studies I and II Cetrigen (Virbac Pty Ltd) wound spray was applied to the wound because the cattle were regularly handled within the first 24 hours after the procedure was performed. However, in Study III no Cetrigen was applied to be consistent with industry practice. Immediately after the spay procedure was completed the female was ear notched as described above. On average the entire procedure was completed in approximately 3 minutes.

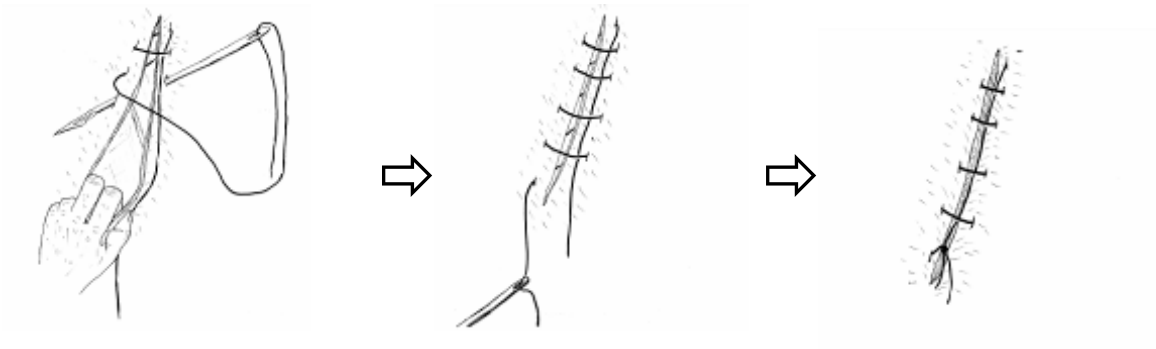



**Figure 3.4-1 Flank suturing**

### 3.4.7 Restraint + Electroimmobilisation + Webbed via Flank + Earnotch

The cattle were physically restrained and electroimmobilised as described above. Using the same type of flank incision as described above, the right ovary was grasped, and then two forefingers were slid caudo-dorsally along the lateral aspect of the ovarian attachments to locate the oviduct or "web" (Plate 3.4-4). The plastic Spaymate 23 instrument was then introduced into the incision sliding it along the left arm as a guide to the tight anterior edge of the membrane. The whole length of the membrane was fed around into the Spaymate and severed (Plate 3.4-5). This was then repeated with the left ovary while retaining the severed "web" in the palm of the left hand. The hand was then withdrawn and the incision sutured as described above. Immediately after the spay procedure was completed the female was ear notched as described above. On average the procedure was completed in approximately 3 minutes.

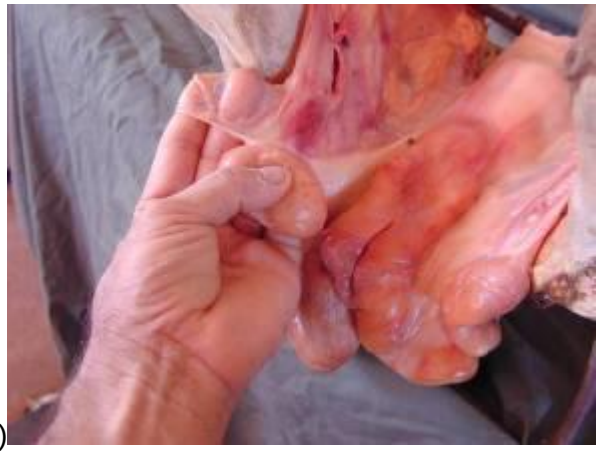
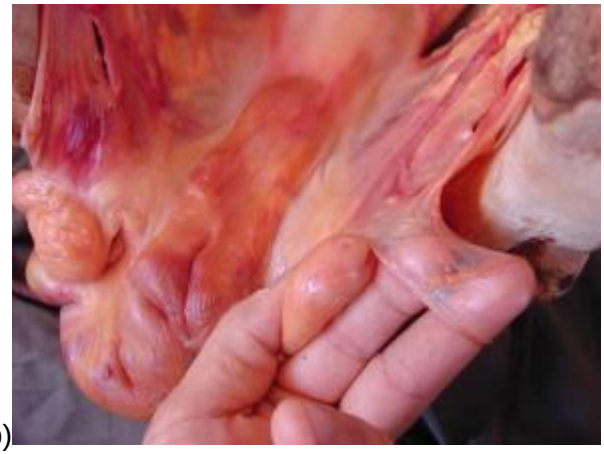
a)  b) 

**Plate 3.4-4 Locating the "web" a) right ovary and b) left ovary**

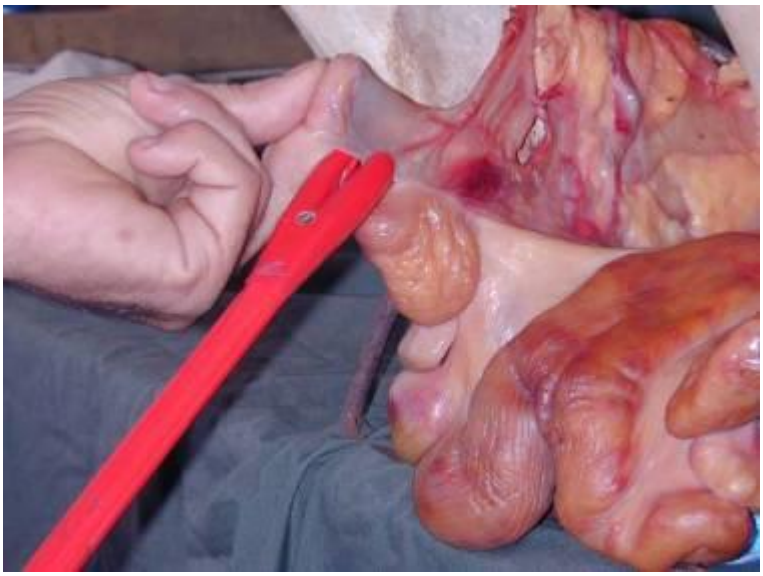


**Plate 3.4-5 Severing the right "web". Note how the thumb has slid up to clamp with the fore fingers to entrap the web.**

## 3.5 Facilities and Handling of cattle

### 3.5.1 Study I – Berrimah Research Farm

A small set of steel cattle yards, which were well watered and shaded, located at Berrimah Research Farm were used for Study I. The yards had a concreted processing area that was well covered and fitted with adequate bench space, a hydraulic assisted Country Industries Australia (CIA) *Interrogator* crush and a weigh box (Figure 3.5-1). A checker plate floor was fitted to the crush providing adequate grip for electroimmobilised animals. The weigh box was fitted with a Tru-test Ezi-weigh data logger connected to Tru-test HD1010 weigh beams situated beneath the box.

To ensure the easy movement of cattle during sampling, hessian was attached to the pound, race and laneway and the yards arranged to allow cattle to be directed on either an inner and outer loop (Figure 3.5-1). The inner loop ensured the forward movement of cattle and enabled rapid blood sampling of scheduled replicates.


**Plate 3.5-1 The processing area of the Berrimah Research Farm Cattle Yards during Study I. Note the hessian fixed to the yards to facilitate the easy movement of animals.**

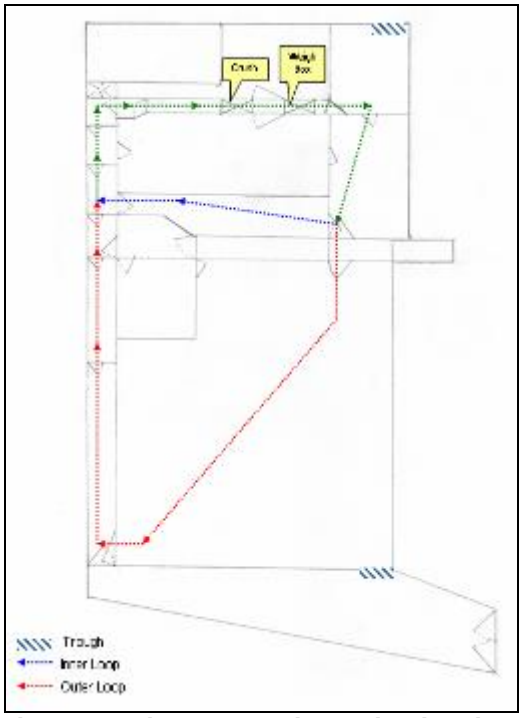



**Figure 3.5-1 Diagram of Berrimah Research Farm cattle yards showing the flow of cattle along 'inner' and 'outer' loops. Note: diagram not to scale.**

Cattle were moved though the yards using a "low stress" handling approach. In high pressure areas (pound, force and race) only small groups of two or three animals were handled at a time. Only two or three animals were in the race at one time.

Using a network of laneways, trial animals could be easily mustered into the yards from any of the three paddocks they grazed during the trial. Animals were usually mustered either on foot or on motorbike.

### 3.5.2 Study II - Mt Sanford Station

A commercial set of steel yards and surrounding holding paddocks were utilised during Study II. To ensure blood collections took place at designated times and to maximise the number of replicates able to be processed daily, the yard was modified using portable panels to allow an additional crush (Crush B) to be installed and to allow cattle to flow through the yards via two loops, similar to that used in Study I (Figure 3.5-). All animals passed through the pound where they were directed to crush A or B (both containing CIA *Immobilizers*) (Figure 3.5-). Hessian was fixed to pressure points of the yards to assist in the movement of animals. Backs of Toyota Landcruisers were used to provide bench space at both crushes ( Plate 3.5-2).

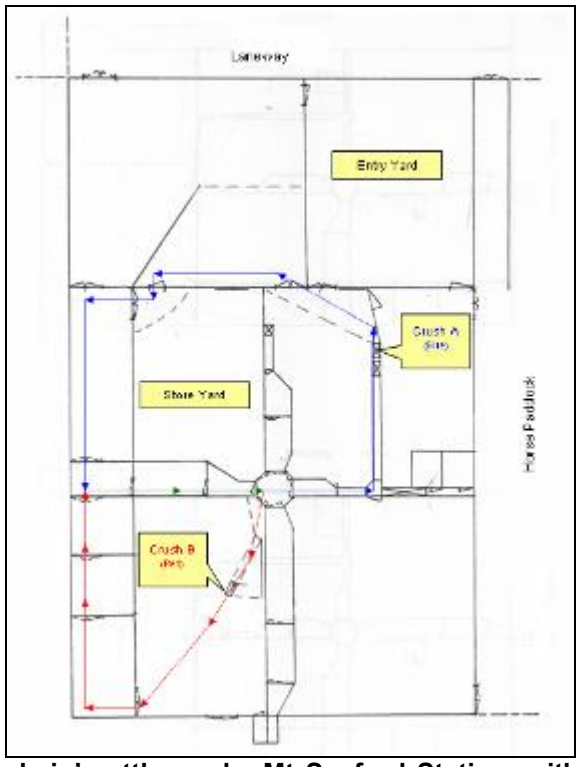



**Figure 3.5-2 Diagram of 'Blackgin' cattle yards, Mt Sanford Station, with flow of cattle shown. Note: diagram not to scale.**

a)  b) 

**Plate 3.5-2 The two crushes used during Study II a) Crush A and b) Crush B.**

Trial animals were mustered into the yards from the 'Laneway' into the 'Entry Yard'. Any drafting that was then required was done by moving the animals through the pound. Animals were released from the yards out of the 'Entry Yard' to either the 'Laneway' or 'Blackgin Horse Paddock'. Trial animals that were retained over night were held in the 'Store Yard' (Figure 3.5-2).

Motorbikes were usually used to muster paddocks. The 42 days muster (final muster) was completed by helicopter to ensure that no animals were missed.

### 3.5.3 Study III – Pigeon Hole Station

A commercial set of steel yards and surrounding holding paddocks were utilised during Study III. The yards had a concreted processing area that was well covered, had adequate bench space, and a CIA *Immobilizer* crush with Tru-test HD1010 weigh beams fixed beneath it. A checker plate floor was fitted to the crush providing adequate grip for electroimmobilised animals. The flow of cattle during procedures and processing is shown in Figure 3.5-3.

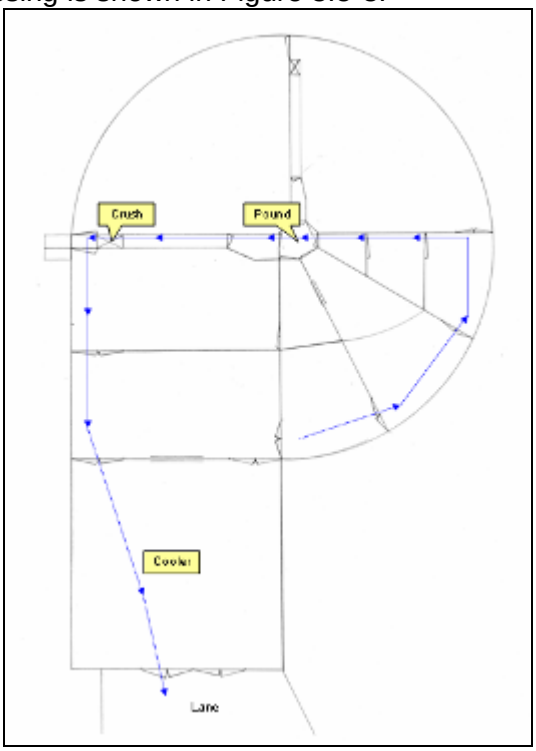



**Figure 3.5-3 Diagram of 'Coles cattle yards, Pigeon Hole Station, with flow of cattle shown. Note: diagram not to scale.**

Animals were pooled in the yards after procedures and then, as a group, released into the cooler. At the time of procedures, pooled animals were released to walk out of the yards every couple of hours. Once animals had settled in the cooler they were given access to graze the 'Lane' (Figure 3.1-).

The mustering of trial paddocks was completed by helicopter the day before the procedures were performed. Animals were mustered to the 'cooler' and then stockman yarded the animals on horseback.

## 3.6 Blood sampling, processing and storage

In Studies I and II immediately after the cattle were physically restrained a rail was placed behind the hindlegs and ratcheted forward so that the neck of the animal protruded fully through the head bail and could be easily turned to the side to enable the blood sampling to be done. Ten mL blood samples were collected by jugular venipuncture into labelled lithium heparin or plain Vacutainer tubes (Becton Dickenson, Plymouth, U.K.), and the samples were then held at <10$^{o}$C in a portable

refrigerator. Within one hour of collection the blood samples were either taken to the Berrimah Veterinary Diagnostic Laboratory (Study I) or the NTDPIFM Mt Sanford field laboratory for processing. The blood samples were centrifuged at 1,500 g for 20 minutes (refrigerated centrifuge, Berrimah; Clements GS200 centrifuge, Mt Sanford). The plasma and/or sera were then decanted into duplicate, labelled, 5 mL, screw-capped storage tubes (Sarstedt Australia Pty Ltd, Adelaide, Australia), and frozen at -20˚C until assay.

## 3.7 Assessment of the physiological responses to procedures

Pain is a subjective experience that cannot be measured directly, but it has been assessed extensively, indirectly via measurement of peripheral cortisol concentrations (Mellor et al. 2000). Corticosteroids are not stored in the adrenal glands and must be synthesised in response to secretion of corticotrophic releasing hormone from the hypothalamus followed by secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. Hence there is always a lag period after an animal experiences pain before a peak secretion of cortisol occurs. Cortisol is transported in the blood in bound and unbound forms; 80% of the total cortisol is bound to transcortin or corticosteroid binding globulin (CBG); 7% is bound to albumin. Only about 10% of total cortisol in cattle is unbound (Gayrard et. al.,1996). If an animal is repeatedly exposed to a stressor or experiences sustained stress from a single event the relative amount of unbound cortisol in the blood may be increased (Hemsworth and Barnett, 2000). Also changes in the amount of the binding protein CBG will influence the amount of bound and unbound cortisol in the blood. Roux et. al., (2003) reported that in human patients undergoing major elective surgery there was a 30% decrease in CBG. It might be expected that there would be a difference in the unbound cortisol concentrations of cattle undergoing flank spaying (significantly invasive procedure) versus WDOT spaying (likely to be a less invasive procedure). Also, although following an acute discharge of cortisol from the adrenals there maybe down-regulation of pituitary secretion of ACTH (resulting in a decrease in cortisol secretion), in some cases the hypothalamic-pituitary axis becomes sensitised such that further exposure to a stressor (e.g. repeated handling through a crush for the purposes of collection of jugular blood samples) may result in a greater cortisol response than initially observed.

Bound or total cortisol concentrations are usually used to define the acute pain/stress response in animals, whereas unbound cortisol concentrations are more commonly used to define the longer term or chronic pain/stress response (Hemsworth and Barnett, 2000). Under normal physiological conditions unbound cortisol concentrations increase almost proportionally to total cortisol concentrations. However when an animal experiences significant ongoing distress, because the total cortisol concentrations are near to or higher than the number of binding sites for CBG there may be a proportionally greater increase in unbound cortisol. Breuer et al (1998) found that twice-daily negative handling of dairy heifers over a 5 week period resulted in a significant increase in unbound cortisol concentration compared to positively handled heifers, however total cortisol concentrations did not differ between the 2 groups of animals. Finally, as it has been consistently reported that there is considerable variation between individual animals in the duration and magnitude of their cortisol response to painful procedures, the mean response derived from analysis of responses from a statistically appropriate number of animals should always be used to compare different treatments.

The physiological pain/stress response to each procedure assessed in Studies I and II were defined by measuring the plasma concentrations of bound and unbound cortisol during the acute response period (0 to 8 hours), the immediate post-acute response period (8 to 24 hours) and the early chronic response period (24 to 96 hours). The time periods selected for analysis were derived from Stafford and Mellor (2005) review of the physiological responses of calves to amputation dehorning and are consistent with the general principles of pain induced stress responses (reviewed by

Hemsworth and Barnett, 2000). Almost all the studies of the physiological response of cattle to routine surgical husbandry procedures have been conducted in *Bos taurus* breed calves <12months of age. The lack of published data on the responses of *Bos indicus* cattle to these procedures, the fact that this project would be examining the responses of cattle aged 15 to 24months, and the importance of both defining the acute and longer term response to procedures, led to a decision to measure both the bound and unbound cortisol concentrations in Studies I and II.
Typically amputation dehorning induces a marked total plasma cortisol response that lasts 7 to 9 hours (Stafford and Mellor, 2005). Further, the acute cortisol response profile is similar in calves ranging from 6weeks to 6months of age at the time of dehorning. After dehorning, the total plasma cortisol concentrations rapidly increase to reach maximum values after approximately 30 minutes, then decreases to a plateau for 5 to 6 hours before generally returning to pre-treatment concentrations around 8 to 10 hours (Stafford and Mellor, 2005). The timing of the return of cortisol concentrations to pre-treatment levels after dehorning appears to vary according to the age of cattle and whether more than one surgical procedure is performed at the same time. No data could be found on the normal range of bound or unbound cortisol concentrations for *Bos indicus* cattle.

The systemic inflammatory responses to each procedure were defined by profiling the serum concentration of the acute–phase protein, haptoglobin between 0 and 96 hours after the procedures. The acute phase response is part of the innate host defence system against trauma, inflammation and infection. In cattle, the most sensitive acute-phase proteins are haptoglobin and serum amyloid A, concentrations of which increase particularly in response to acute inflammatory conditions (Horadagoda et al. 1999). The normal range for cattle is 25-50 µg/mL (Salonen et al. 1996), however Horadagoda et al. (1999) reported the normal range for cattle was < 0.35mg/mL (note range derived from studies of *Bos taurus* cattle).

The degree of muscle tissue damage (e.g. due to sudden, sustained increase in muscular activity or trauma) in response to each procedure was defined by determining the plasma concentrations of two muscle enzymes, creatinine phosphokinase (CPK) and aspartate aminotransferase (AST) in selected samples collected between 0 and 96 hours after the procedures were performed. Increase in the blood concentration of CPK is a highly specific indicator of muscle cell membrane damage. CPK has a half-life of 4 to 6 hours and, following an initial episode of acute muscle damage, the blood levels of this enzyme may remain significantly elevated for 3 to 4 days. The normal range for cattle is 35 to 280 IU/L (Radostits et al. 2007). The CPK concentrations in housed cattle suddenly turned out onto pasture may increase from 50 IU/L to 5,000 IU/L within a few days, as a result of increased muscular activity. AST is an enzyme found in muscle cells, but also liver and other tissues. It has a longer half-life than CPK and thus, a marked drop in CPK levels and a slow decline in AST levels indicate that no further muscle damage is occurring. The normal range for AST in cattle is 78 to 132 IU/L (Radostis et al. 2007). Note ranges for CPK and AST were derived from studies of *Bos taurus* cattle.

The degree of mobilisation of body fat reserves, which can be indicative of a stress response (Ferguson et al. 2001) in response to each procedure, was defined by measuring the plasma concentrations of non-esterified fatty acids (NEFA) in selected samples collected between 0 and 96 hours. The normal range for NEFA in non-lactating cattle is <0.4 mmol/L (Radostits et al, 2007; note range derived from studies of *Bos taurus* cattle).

The serum and plasma samples collected in Studies I and II were stored at -20˚C at The Animal Research Institute, Brisbane. The fibrin precipitate was removed after thawing and before analysis by centrifuging 1mL of sample at 1,000g for 20 minutes. Plasma samples were analysed for AST, CPK and NEFA using an Olympus Reply Chemistry Analyser. Serum haptoglobin concentrations were determined using the Olympus Reply system. Plasma bound cortisol concentrations were determined using the enzyme-linked immunoassay, Cortisol ELISA kit (DSL Laboratories) and Bio-

Rad 680 plate reader. To measure unbound cortisol concentrations the samples were first filtered (Sartorius 10kDa MWCO filter) and then concentrations determined using an ELISA (Cortisol ELISA EIA Saliva kit; DSL Laboratories).

## 3.8 Assessment of the behavioural responses to procedures

The following behavioural observations were carried out in Study II directly by, or under the supervision of Dr Carol Petherick. A selection of these behaviours was also recorded by Professor Michael McGowan in Study IIIa (Table 4.3-1). No systematic recording of behaviours were performed in Study I, rather an assessment was made of how observations could be conducted and the potential range of behaviours which might be expressed by spayed cattle.

### 3.8.1 Movement through the race and into the crush

In situations where animals are repeatedly exposed to a procedure, it is possible to obtain an indication of the aversiveness of the procedure to the animal by examining, on successive occasions, the length of time it takes the animal, or the amount of "effort" it takes to make the animal approach and enter the place where the procedure takes place (Rushen, 1986, 1996). This technique has been used to assess the aversiveness of branding and handling (Schwartzkopf-Genswein et al. 1997a, b; Goonewardene et al. 1999) and of different kinds of handling when confined in a crush (Petherick et al. in prep.) for beef cattle.

We established a scoring system for both movement along the race and into the crush, and for entry into the headbail, as shown in Table 3.8-1. Each animal was assigned a separate score for both race/crush movement and headbail entry on every occasion that it came into the crush.

**Table 3.8-1 Score and description of "effort" needed to make cows and heifers move along the race, into the crush and into the headbail.**

| Score | Description |
|---|---|
| 1 | Moves along race and into crush/from crush into headbail with vocal/auditory encouragement |
| 2 | Moves using 1. plus 1-4 hits/prods with polypipe |
| 3 | Moves using 1. plus 5-8 hits/prods with polypipe |
| 4 | Moves using 3. plus 1-3 tail-twists |
| 5 | Moves using 3. plus 4-6 tail-twists |
| 6 | Moves using 5. plus 1-3 electric goad applications |
| 7 | Moves using 5. plus 4-6 electric goad applications |
| 8 | Moves only with more-or-less continuous vocal encouragement, pushing and tail-twisting |
| 9 | Refuses to move e.g. lies down |

### 3.8.2 Behaviour in the crush

It was impossible for the females in both the flank spay and electroimmobilisation treatments to perform any behavioural responses to the procedures because they were immobilised. As a consequence, we simply scored the number of vocalisations performed by the animals, as it has been observed that cattle vocalisations are associated with aversive events, at least in abattoirs (Grandin, 2001).

### 3.8.3 Behaviour in the yards and holding paddocks

Many studies have examined the behavioural responses of cattle to husbandry practices that involve varying degrees of tissue damage and which, based on a combination of physiological and behavioural measures and with and without the use of anaesthetics and analgesics, would appear to cause pain (e.g. tail docking, disbudding, dehorning and castration) (e.g. see Robertson et al. 1994;

Petrie et al. 1995; Schwartzkopf-Genswein et al. 1997a; Graf and Senn, 1999; McMeekan et al. 1999). The majority of these studies have used calves and we were unable to find references to work investigating behavioural responses to painful procedures using Bos indicus animals. Furthermore, we could not locate work that had examined the behavioural responses of cows or heifers to spaying, or even to manipulation of the reproductive tract, as occurs during AI and pregnancy diagnosis. This is important because it would appear that different noxious treatments can elicit unique behavioural responses (Mellor et al. 2000). Furthermore, behavioural responses to the same stimuli differ between related species, such as ungulates; lambs, kids and calves castrated using rubber rings show different behaviours (Mellor et al. 2000). As a consequence of this lack of relevant documentation, we used our own experience and knowledge to compile a list of behaviours that we believed may be changed by the procedures conducted in this study (Table 3.8-2). A limited amount of observation (due to time constraints) was conducted on the heifers in Study I, but these revealed few easily observable behaviours shown post-procedures.

The list of behaviours compiled potentially allowed us to detect any changes from “normal” behaviour as well as allowing us to detect a limited number of behaviours that we thought might indicate feelings of pain and discomfort. As noted by Mellor et al. (2000), a behaviour is likely to be a useful indicator of pain if it is seen in treated animals, but not in controls, and it should recede as the pain recedes. Further, all observers understood that their records were not limited to this list, but that additional behaviours could be described and added at any point in the experiment and these additional behaviours needed to be brought to the attention of other observers.

As far as possible, all observations were conducted “blind” i.e., the observers did not know which procedure had been performed on the cattle (were not present when procedures were being done), but simply recorded the ear-tag colour (and number as necessary). However, it was evident which cattle were on the Flank treatment because of the sutured wound in their flank.

**Table 3.8-2 Behaviours recorded during yard and holding paddock observations**

| Behaviour | Description |
|---|---|

| | |
|---|---|
| Standing head down | Head level with or below brisket |
| Standing head up | Head above brisket |
| Standing stiff-tailed | Standing with tail held stiffly away from body |
| Lying sternum | Lying on sternum or partially on sternum with hind-quarters to one side |
| Lying prone | Lying on side, fully-recumbent |
| Locomotion | Walking, trotting |
| Feeding | Taking hay into mouth and/or chewing hay and/or grazing and/or browsing |
| Drinking | Consuming water |
| Ruminating standing | Standing, generally with a relaxed posture with regular chewing and regurgitation movements |
| Ruminating lying | As above, but lying sternum |
| Kicking at belly* | Standing, lifting front or hind leg towards and/or contacting underside |
| Licking standing/lying | Standing or lying on sternum, turning to lick or attempt to lick body (record body region) |
| Rub/scratch | Rubbing/scratching head or body against an object |
| Vocalisation | Bellow or “moo” |
| Teeth-grinding | Noise made by animal grinding molars together |
| Shiver/tremble[#] | Whole of body shivering, shaking or trembling |
| Butt | Butt or attempted butt directed to another animal |
| Charge | Charges at another animal and stops |
| Push | Pushes another animal out of the way |
| Chase | Chases another animal (pursuit continues for some seconds) |
| Retreat | Moves away from butt, charge, push or chase |
| Mount* | Mounts or attempts to mount another animal |
| Receives mount* | Recipient of a mount – may or may not stand for it |
| Grooms another | Licks another animal |
| Receives grooming | Recipient of grooming |

*** not seen during experiment**
**[#] added during experiment**

### 3.8.4 Yard observations on individual animals

On the day that procedures were conducted on the cattle, a single observer (CP) recorded the behaviour (**Table 3.8-2**) of every animal at intervals while they were in their replicate groups. The order in which individuals were recorded was according to the ear-tag being able to be seen and read by the observer. This meant that scans of the group took varying lengths of time, but most scans were conducted at 5-minute intervals.

The observations were opportunistic, in that the observer remained in one location (Store yard, Figure 3.5-2) and recorded the groups as they were moved through pens in the yard complex during the process of repeated blood sampling after the procedures had been performed on them. This meant that not all replicate groups were observed in the same pens as each other and not all groups were observed the same number of times. Also, the number of consecutive scans on a particular group varied because the animals were moved from pens in which the observer could see them into pens that were out of view. Table 3.8-3 shows the times at which scans were conducted; replicates 1 to 5 (heifers) were on Day 1; 6 to 10 (cows) were on Day 2; and 11 to 15 (heifers) were on Day 3.

**Table 3.8-3 Times of day at which scan samples were conducted of behaviour of individual animals within replicate groups on the day that procedures were conducted (Replicates 1-5 on Day 1 – heifers; Replicates 6-10 on Day 2 – cows; Replicates 11-15 on Day 3 – heifers)**

| Replicate | Day | Time of day | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 7:30 | 7:40 | 7:50 | 8:55 | 9:45 | 9:50 | 12:10 | 12:15 | 12:20 | 12:25 | 12:30 | 12:35 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 14:30 | 14:35 | 14:40 | | | | | | | | | |
| 2 | | 8:00 | 8:05 | 8:10 | 10:05 | 10:10 | 10:15 | 10:20 | 11:00 | 11:05 | 11:10 | 11:15 | 14:15 |
| | | 14:20 | 14:25 | 15:15 | | | | | | | | | |
| 3 | | 8:20 | 8:25 | 8:30 | 9:20 | 9:25 | 10:35 | 11:20 | 11:30 | 11:35 | 14:45 | 14:50 | 14:55 |
| | | 15:20 | 15:25 | | | | | | | | | | |
| 4 | | 8:35 | 8:40 | 8:45 | 9:30 | 9:35 | 10:40 | 10:45 | 10:50 | 10:55 | 11:45 | 11:50 | 11:55 |
| | | 15:00 | 15:05 | 15:10 | 15:40 | 15:45 | 15:50 | | | | | | |
| 5 | | 9:00 | 9:05 | 9:10 | 9:55 | 10:00 | 10:25 | 11:25 | 12:00 | 12:05 | 15:30 | 15:35 | 16:15 |
| | | 16:20 | 16:25 | | | | | | | | | | |
| 6 | 2 | 7:30 | 7:35 | 7:40 | 7:45 | 7:50 | 7:55 | 10:20 | 10:25 | 11:20 | 12:15 | 15:00 | 15:05 |
| | | 15:10 | | | | | | | | | | | |
| 7 | | 8:00 | 8:05 | 8:10 | 8:15 | 8:20 | 8:25 | 8:30 | 10:30 | 10:35 | 10:40 | 11:05 | 11:10 |
| | | 11:15 | 11:30 | 11:35 | 11:40 | 15:30 | 15:35 | 15:40 | | | | | |
| 8 | | 8:35 | 8:40 | 8:45 | 9:55 | 10:00 | 10:55 | 11:00 | 12:00 | 14:05 | 14:10 | 14:30 | 14:35 |
| | | 14:40 | 14:45 | 15:50 | 15:55 | 16:00 | 16:05 | 16:10 | | | | | |
| 9 | | 9:00 | 9:10 | 9:15 | 9:20 | 9:25 | 10:05 | 10:10 | 10:15 | 12:20 | 12:25 | 13:10 | 13:15 |
| | | 13:20 | 13:25 | 13:30 | 13:45 | 15:15 | 15:20 | 15:25 | 16:15 | 16:20 | 16:25 | | |
| 10 | | 9:30 | 9:35 | 9:40 | 9:45 | 9:50 | 10:45 | 10:50 | 11:45 | 11:50 | 11:55 | 12:30 | 12:35 |
| | | 12:40 | 13:35 | 13:40 | 13:55 | 14:00 | 14:15 | 14:20 | 14:25 | 14:50 | 14:55 | | |
| 11 | 3 | 7:35 | 7:40 | 7:45 | 7:50 | 9:30 | 9:35 | 9:55 | 10:00 | 11:05 | 11:30 | 11:35 | 12:35 |
| | | 12:40 | 12:45 | 14:45 | 14:50 | 14:55 | | | | | | | |
| 12 | | 7:55 | 8:00 | 8:05 | 8:10 | 8:15 | 9:25 | 10:25 | 10:30 | 11:20 | 11:25 | 12:50 | 12:55 |
| | | 15:00 | 15:05 | 15:10 | 15:15 | 15:20 | 15:25 | 15:30 | | | | | |
| 13 | | 8:20 | 8:25 | 8:30 | 8:35 | 8:40 | 9:50 | 10:45 | 10:50 | 11:50 | 11:55 | 12:20 | 12:25 |
| | | 12:30 | 13:00 | 13:05 | 13:50 | 13:55 | 15:35 | 15:40 | 15:45 | 15:50 | 15:55 | | |
| 14 | | 8:45 | 8:50 | 8:55 | 9:00 | 9:40 | 9:45 | 10:20 | 10:55 | 11:00 | 11:40 | 11:45 | 13:10 |
| | | 13:15 | 13:35 | 13:40 | 13:45 | 16:00 | 16:05 | 16:10 | 16:25 | 16:20 | | | |
| 15 | | 9:05 | 9:10 | 9:15 | 9:20 | 10:05 | 10:10 | 10:15 | 10:35 | 10:40 | 11:10 | 11:15 | 12:05 |
| | | 12:10 | 12:15 | 13:20 | 13:25 | 13:30 | 14:00 | 14:05 | 14:10 | 14:15 | 14:20 | 14:25 | 14:30 |
| | | 14:35 | 16:25 | 16:30 | 16:35 | 16:40 | 16:45 | | | | | | |

It should be noted that, for data analyses, the times of day were converted to time after the procedures had been conducted on the group.

### 3.8.5 Yard observations on groups of animals

At the end of each of the three procedure days, all five replicate groups were combined in a yard (Store yard, see Figure 3.5-2) and the cattle had ad libitum access to hay and water. An observer (CP) scanned the group at 10-minute intervals and tallied the numbers of each ear-tag colour performing the behaviours given in **Table 3.8-2**. These observations were conducted until it became too dark to distinguish ear-tag colours. For Day 1 the observations were between 16:35 and 18:20; for Day 2, 17:05 and 18:25 ; and for Day 3, between 17:15 and 18:25 . These data were converted to proportions for analyses.

### 3.8.6 Holding paddock observations on groups of animals

The cattle were blood-sampled 24 hours after the procedures, held in the yards and then released to the holding paddocks (replicates 1 to 5 in one paddock replicates 6 to 10 in another and replicates 11 to 15 in a third). Observations were conducted on them that afternoon, and in the mornings and afternoons of the following 2 days. Morning observations commenced as soon as it was light enough to discern ear-tag colours (about 7:00) until about 11:00. Afternoon observations were between approximately 15:00 and 18:15, except for the third afternoon for each paddock group, because the cattle were mustered at about 17:00 to be taken to the yards in preparation for blood sampling the next morning.

Observations were usually conducted from quad bikes using binoculars, with care taken to move around the animals as quietly and slowly as possible to minimise disturbance. Data recorded were tallies of the number of each ear-tag colour performing the behaviours given in **Table 3.8-2**. The intervals between the scan samples were quite variable (10 to 30 minutes) because, frequently, the herd was split into a number of sub-groups in different locations in the paddock and it was sometimes necessary to drive between groups to conduct the tallies. Consequently, the number of scans conducted within each time period (a.m. and p.m.) and between days varied (6 to 24). Again, data were converted to proportions for analyses, as not all animals within a treatment group were recorded on every scan-sampling occasion**.**

### 3.8.7 Measurement of flight speed

Flight speed (m/s) was recorded for each animal on each occasion that it exited the crush. The method used was based on that of Burrow et al. (1988), with the first light beam placed at approximately 2 m from the headbail and the second at 1.8 m from the first. We felt that flight speed was an important measure to take because there is increasing evidence that it reflects the innate agitation of cattle (Petherick et al. 2005), and cattle with fast flight speeds may be less able to cope with stressors than those with slower flight speeds (Petherick et al. 2002).

## 3.9 Measurement of production responses to procedures

To define the production responses to procedures, liveweight and fat depth measurements were collected for all trial animals. Full liveweights were recorded during Study I. In Studies II and IIIa liveweights were always recorded the morning after a period of 12 hours without food, but access to water.

For Study I, liveweight and P8 fat depth were recorded at time of backgrounding (11 days prior to procedure) and 21 days and 42 days post procedure. In Study II, liveweight and fat depth were measured at the time of procedure and 96 hours, 21 days and 42 days post-procedure, and in Study III, liveweight and fat depth were recorded at the time of procedure, and at 21 days and 42 days post-procedure.

In all studies, liveweights were electronically recorded using a Tru-test XR3000 data logger connected to HD1010 weigh bars fixed beneath a CIA immobilizer crush / weigh box. Fat Depths were measured using an ULtramac B-10 cattle fat depth meter at the P8 site.

## 3.10 Assessment of morbidity and mortality following procedures

In all studies the general health and well-being of trial animals was visually assessed each time they were mustered into the cattle yards and restrained in the crush. In Studies I and II, general health was also scored using a 4-point scale (1, very poor; 2, poor; 3, good; 4, very good) at 21 days and 42 days post procedure. Flank incision wounds in each study were visually assessed and scored using a 5-point scale (1, wound healed; 2, wound partially healed and clean and dry; 3, wound partially healed with a discharge; 4, little or no healing of wound and clean and dry; 5, little or no healing of wound and with a discharge) at 21 days and 42 days post-procedures (Plate 3.10-1 to **Plate 3.10-5**).

**Plate 3.10-1 Flank Score 1 – wound healed**

**Plate 3.10-2 Flank Score 2 – wound partially healed and clean and dry**

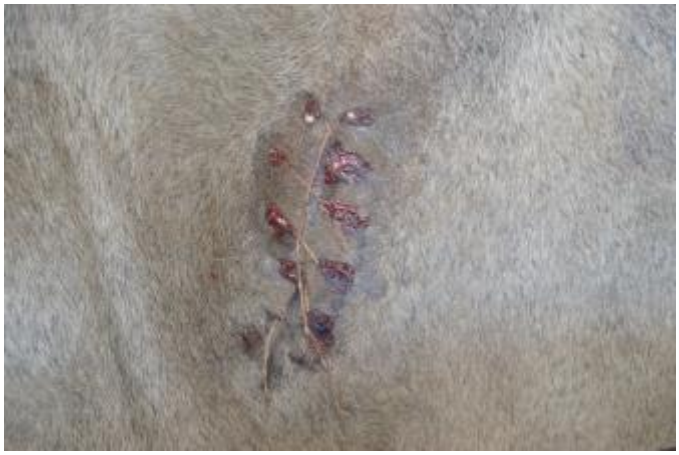


**Plate 3.10-3 Flank Score 3 – wound partially healed with a discharge**

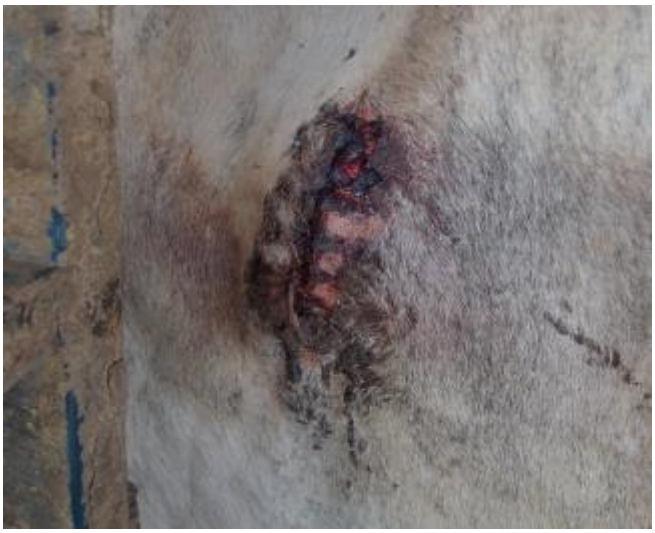


**Plate 3.10-4 Flank Score 4 – little or no healing of wound and clean and dry**


**Plate 3.10-5 Flank Score 5 – little or no healing of wound and with a discharge**

Paddock patrols were done (either on foot or from a motorbike) once daily until day 4 and then at days 21and 42 post procedure in Study I. In Study II paddock patrols were done (from a motorbike) as part of the behavioural observations (see section 3.8.6 for frequency of observations) until day 4 and then during mustering of the paddock at days 21 and 42 post-procedures. In Study IIIa the paddocks in which cattle were held following completion of procedures were patrolled either via motorbike or on horseback. Cattle which appeared clinically unwell (standing away from the mob, not grazing, walking slowly, recumbent) were examined from a distance initially and then in some cases a closer general physical examination was performed (primarily on recumbent cattle). To enable detection of as many cases of morbidity and mortality as possible, paddock patrols from horseback and motorbike were conducted twice per day (early morning and late afternoon) between 36 and 84 h after the procedures were performed, then again on days 7 and 10 post procedures. On days 21 and 42 carcasses were detected using helicopter while conducting muster. Searches of the paddocks were conducted by riding a series of transects across the paddock that were based on 'cattle pads' and rest areas. Bird activity, wedge-tail eagles (*Aquila audax*), crows (*Corvus* spp.) and chicken hawks (*Hiereaatus morphnoides*), were used to identify possible sites of mortalities. The locations of carcasses were recorded using GPS coordinates and time of death estimated. A full post mortem examination was conducted on all carcasses which were not affected by advanced autolysis. Photographs were taken of major findings.

### 3.10.1 Criteria for exclusion of animals temporarily or permanently from studies

Criteria were established prior to the studies for the removal of cattle from the studies. Criteria for temporary removal were:

- In the pound or in the race the animal repeatedly crashes into the yard panels and it is considered very likely that it will cause serious injury to itself or a member of the research team.
- Animal lies down in the race and refuses to move despite multiple use of the jigger and has to be pulled out of the race.
- Animal sustains an injury requiring treatment or causing marked lameness.

Cattle meeting any one of these criteria were drafted off into a yard not being used in the study, and then either brought in for the next sampling time (i.e. would miss one sampling time) or given a longer period to recover, as determined by the principal investigator.

Criteria for permanent removal was:

- Cattle which were found within 3 weeks of the procedures being done, suffering severe clinical disease, or found dead, and for which a definitive diagnosis could be made demonstrating that the disease/death was unrelated to the procedures which had been performed.

Cattle meeting these criteria were examined by the principal investigator and either euthanased or treated according to the clinical diagnosis made.

### 3.10.2 Statistical analyses

As the treatments were applied to each individual animal, and animals can be considered independent, the animal was the experimental unit. As each animal was then successively sampled over time, a repeated-measures analysis of variance (ANOVA) (Rowell and Walters 1976) was adopted, using GenStat (2005). The Greenhouse-Geisser epsilon was estimated to account for the degree of temporal autocorrelation, and the significance levels of the F-tests were appropriately adjusted for this. Residual graphics were utilised to check for non-normality of the residuals and heterogeneity of the variances. For the variables where this was found, the $\log_{10}$ transformation was adopted. Following this transformation, all residuals were approximately normally distributed with homogeneous variances. All variables were measured prior to the treatments being applied, and these measures were used as covariates in the respective analyses. Appendix 9.1 lists an example repeated-measures ANOVA, and shows the degrees of freedom for this design (which ensures adequate power for these analyses), as well as (for this variable) a significant three-way animal class by treatment by time interaction.

Whilst no statistical outliers were identified, it was noted that one animal died from causes unrelated to the treatments. Analyses were rerun omitting this animal, however as the results were virtually unchanged (and did not improve the precision), results from the all-data analyses were retained.

Exploratory spline and regression models over time were investigated, however these tended to smooth out some of the peaks which proved to be of specific interest. Hence the treatment by time means (adjusted for the covariate of initial value) from the repeated-measures ANOVA are presented. ANOVA of areas under the time-curves were also conducted, however these gave very similar results to the analyses of the actual (or log-transformed) values. Areas under the curve can only be interpreted relative to a chosen baseline treatment (such as the control). Actual values can

also be interpreted this way, but in addition are meaningful in their own right and, as such, these are presented in this report.

The time-means for cows and heifers are given separately. Graphically, the cows and heifers mostly appeared to be showing different patterns, and this was confirmed by the statistical tests. For the biochemical data, there were 42 separate F-tests for the 'treatment by class' and 'treatment by class by time' interactions. At a probability level of 0.05 we may expect two of these to be significant due to random chance. Our analyses showed 12 significant results at $P < 0.05$ (and a number of others were close to this significance level), indicating that these interactions are appearing more frequently than expected by random chance alone, and hence are likely to be true effects. Hence, we concluded that cows and heifers did tend to respond differently, and thus separate means for each class are presented in the results section.

For the analyses of $\log_{10}$-transformed data, all back-transformed means ($\log_{10}$ back to the original scale) presented have not used the bias-correction factor (Kendall *et al.* 1983), so these values are the geometric means.

To assist with interpretation, all data variables were re-analysed according to three time periods, namely 0 to 8 hours (acute response period), 8 to 24 hours (immediate post-acute response period), and 24 to 96 hours (early chronic response period). Protected significant-difference testing (using LSD at $P = 0.05$) was used to determine significant differences between the treatment means, as indicated by superscripting. Also, the relative magnitudes of these treatment differences were calculated as percentages relative to the control group.

# 4 Results and Discussion

## 4.1 Study I

### 4.1.1 Physiological responses

Bound and unbound-cortisol were measured as indicators of the overall noxiousness animals experienced (Mellor et al. 2000). Haptoglobin is an indicator of the inflammatory response, while increases in the concentrations of CPK and AST indicate muscle damage due to trauma and /or muscular exertion. Increases in the concentration of NEFA's indicate mobilisation of body fat reserves, which may occur in response to stress.

The actual times cattle were blood sampled are presented in Table 4.1-1. Due to the fact that the procedures took longer to complete than predicted, resulting in a reorganisation of the sampling schedule, three sampling times (1.5, 3.5 and 7 hours samples) were omitted. However, during the first 8 hours after the procedures were performed each heifer was blood sampled 10 times, enabling an accurate definition of the bound cortisol response, our primary measure of the acute pain response. The heifers generally worked well during the first 8 hours after procedures when they were regularly blood sampled, although several heifers towards the end of the day refused to enter the crush and became recumbent. These animals were tail-bled in the race and then subsequently stood and walked through the crush. None of the heifers were either temporarily or permanently excluded from the study. The primary objective of Study I was to determine the blood sampling schedule that would be required to accurately define the acute increase in bound cortisol, and the large number of samplings during the first 8 hours after the procedures were performed generated sufficient data to enable statistical analyses to be conducted.

**Table 4.1-1 Study I mean actual blood sampling times for each replicate and each scheduled sampling time (0 to 24h).**

| Replicate | Scheduled sample times | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0h | 0.5h | 1h | 2h | 2.5h | 3h | 4h | 5h | 6h | 8h | 24h |
| 1 | 0 | 0.9 | 1.4 | 1.9 | 2.6 | 3.0 | 3.6 | 5.1 | 6.5 | 8.0 | 24.5 |
| 2 | 0 | 0.6 | 1.2 | 1.9 | 2.4 | 2.9 | 3.6 | 5.0 | 6.3 | 8.0 | 24.0 |
| 3 | 0 | 0.5 | 1.0 | 1.8 | 2.2 | 2.8 | 3.4 | 4.9 | 6.2 | 7.9 | 23.3 |
| 4 | 0 | 0.5 | 1.3 | 1.8 | 2.7 | 3.3 | 4.4 | 5.5 | 6.5 | 7.4 | 23.6 |
| 5 | 0 | 0.4 | 1.2 | 1.7 | 2.6 | 3.2 | 4.4 | 5.5 | 6.5 | 7.4 | 23.6 |

The physiological responses to each procedure are reported below as, **firstly**, the pattern of changes in concentration over time up to 96 hours after each procedure (Figure 4.1-1and Figure 4.1-2), for bound and unbound cortisol data and, **secondly**, the mean values for all biochemical measures, for the following three periods (section 3.7):

**the acute stress response (0 to 8 hours) –**

- Table 4.1-2
- the immediate post-acute response (8 to 24 hours) – Table 4.1-3
- the early chronic response (24 to 96 hours) –Table 4.1-4.

Figures 4.1-1&2 illustrate the patterns of bound and unbound cortisol, whilst the Tables summarise the physiological responses for the 3 periods defined above.

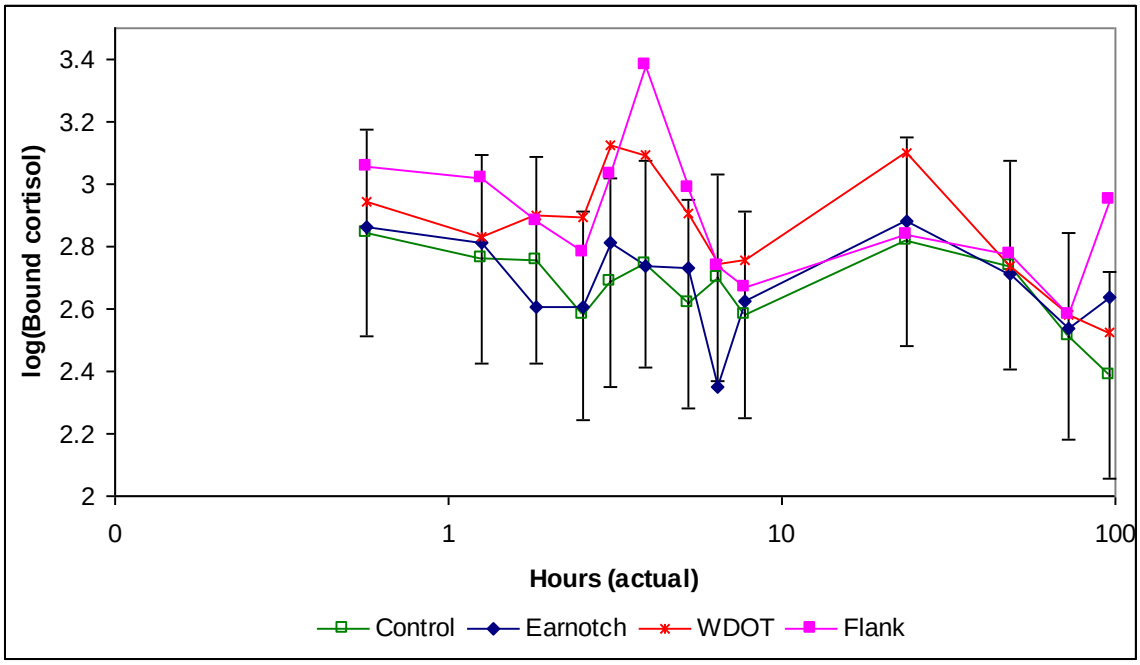



**Figure 4.1-1 Mean ± s.e. changes in bound cortisol concentrations (log transformed) up to 96 hours after procedures were performed.**

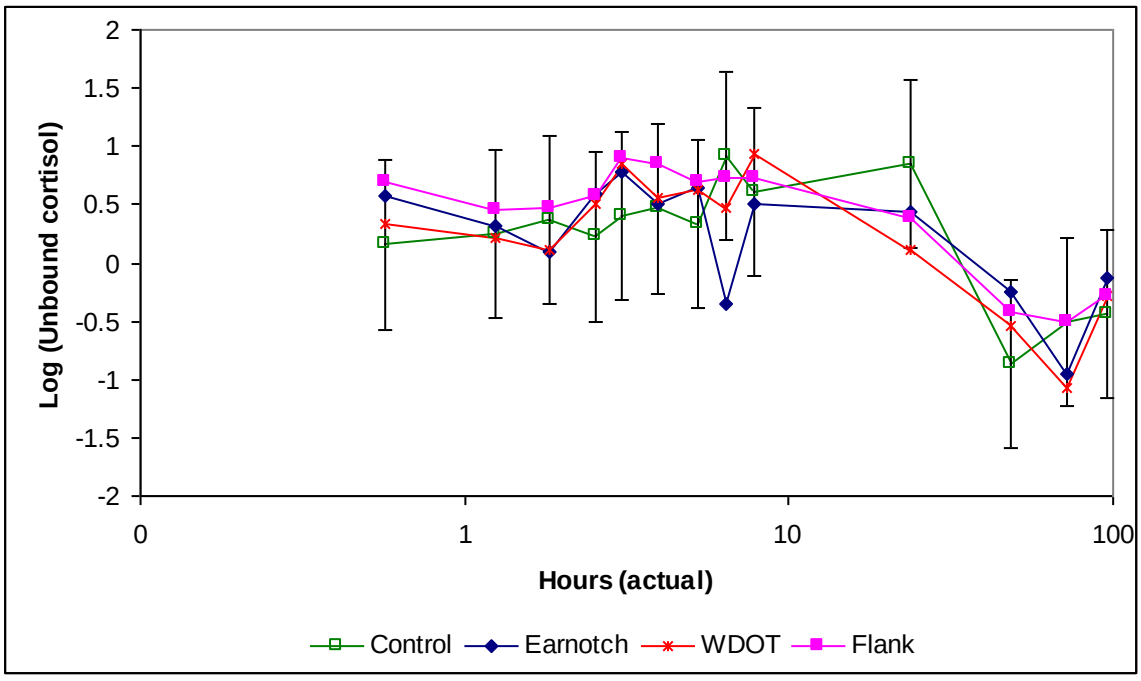



**Figure 4.1-2 Mean ± s.e. changes in unbound cortisol concentrations (log transformed) up to 96 hours after procedures were performed (F= flank, W = WDOT, E = earnotch, C = control, restraint only)**

A distinct peak in concentration of bound cortisol occurred in heifers 3 to 4 hours after the spay procedures were performed. The plasma concentrations of bound cortisol in heifers spayed by both

methods were similar and markedly greater (P>0.05) than the concentration in control heifers at 3 hours and 4 hours. The concentrations of bound cortisol in spayed heifers returned to values similar to controls at 6 hours after the procedures and then followed a similar pattern to the controls to 96 hours. The profile of bound cortisol in the heifers which were ear notched only was very similar to the profile of the control heifers. As has been reported for studies of the response of *Bos taurus* cattle to various surgical husbandry procedures, there was a large amount of variation in bound cortisol profiles between individuals. The profiles of unbound cortisol concentrations for all heifers were similar, albeit the mean concentrations for the flank spayed heifers were significantly (P>0.05) greater than the controls during the first 8 hours.

The flank spayed heifers had a sustained increase in mean serum haptoglobin (greater than the threshold of 0.35mg/ml; Horadagoda et al.1999) from 24 to 96 hours, consistent with a significant systemic inflammatory response to the surgery (Table 4.1-4). The WDOT spayed heifers also showed an increase in mean serum haptoglobin concentrations above the threshold value for this period. There was little or no change in the serum haptoglobin concentrations in the ear notch and control heifers. The NEFA profiles for all groups were similar, with a moderate increase in mean concentrations from 8 to 24 hours after procedures (mean values greater than 0.4mmol/l – Radostits et al, 2007) and then a gradual decline to 96 hours. These increases in NEFA concentrations are consistent with body fat mobilisation in response to the stress associated with the procedures. The CPK and AST profiles were similar for all groups with the CPK and AST concentrations peaking at 8 hours and 24 hours respectively. Within the first 8 hours after the procedures all the heifers had mild increases in CPK concentration (Table 4.1-2). By 96 hours the CPK values had declined to near normal values (35-280IU/L - Radostits et al. 2007 ). The profile of changes in AST concentrations were similar to those described for CPK.

**Table 4.1-2 Mean physiological responses 0 to 8 hrs after each procedure – Study I ( # Not sampled)**

| Physiological measure | Treatment | | | | s.e. |
|---|---|---|---|---|---|
| | Control | Ear Notch | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.692[a] | 2.684[a] | 2.904[b] | 2.953[b] | 0.063 |
| Bound cortisol (nmol/L) | 492.0 | 483.1 | 801.7 | 897.4 | |
| Unbound cortisol (log-scale) | 0.405[a] | 0.444[a] | 0.511[ab] | 0.657[b] | 0.054 |
| Unbound cortisol (nmol/L) | 2.541 | 2.780 | 3.243 | 4.539 | |
| Haptoglobin[#] (mg/ml) | | | | | |
| NEFA (mmol/L) | 0.415 | 0.448 | 0.390 | 0.353 | 0.028 |
| CPK (log-scale) | 3.127 | 3.509 | 3.396 | 3.567 | 0.131 |
| CPK (IU/L) | 1340 | 3228 | 2489 | 3690 | |
| AST (log-scale) | 2.015 | 2.050 | 2.060 | 2.138 | 0.050 |
| AST (IU/L) | 103.6 | 112.1 | 114.8 | 137.5 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

**Table 4.1-3 Mean physiological responses 8 to 24 hrs after each procedure – Study I**

| Physiological measure | Treatment | | | | s.e. |
|---|---|---|---|---|---|
| | Control | Ear Notch | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.703 | 2.75 | 2.949 | 2.747 | 0.082 |
| Bound cortisol (nmol/L) | 504.7 | 562.3 | 889.2 | 558.5 | |
| Unbound cortisol (log-scale) | 0.732 | 0.455 | 0.520 | 0.582 | 0.141 |
| Unbound cortisol (nmol/L) | 5.395 | 2.851 | 3.311 | 3.819 | |

| | | | | | |
|---|---|---|---|---|---|
| Haptoglobin (mg/ml) | 0.283[a] | 0.318[a] | 0.380[ab] | 0.506[b] | 0.047 |
| NEFA (mmol/L) | 0.545 | 0.685 | 0.671 | 0.659 | 0.048 |
| CPK (log-scale) | 3.163 | 3.527 | 3.396 | 3.59 | 0.123 |
| CPK (IU/L) | 1455 | 3365 | 2489 | 3890 | |
| AST (log-scale) | 2.067 | 2.110 | 2.146 | 2.241 | 0.060 |
| AST (IU/L) | 116.7 | 128.7 | 139.8 | 174.2 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

**Table 4.1-4 Mean physiological responses 24 to 96 hrs after each procedure – Study I**

| Physiological measure | Treatment | | | | s.e. |
|---|---|---|---|---|---|
| | Control | Ear Notch | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.623 | 2.689 | 2.75 | 2.776 | 0.054 |
| Bound cortisol (nmol/L) | 419.8 | 488.7 | 562.3 | 597.0 | |
| Unbound cortisol (log-scale) | -0.250 | -0.273 | -0.464 | -0.141 | 0.151 |
| Unbound cortisol (nmol/ml) | 0.562 | 0.533 | 0.344 | 0.723 | |
| Haptoglobin (mg/L) | 0.295[a] | 0.287[a] | 0.467[a] | 0.851[b] | 0.080 |
| NEFA (mmol/L) | 0.396 | 0.65 | 0.604 | 0.612 | 0.068 |
| CPK (log-scale) | 2.833 | 2.984 | 2.821 | 3.061 | 0.114 |
| CPK (IU/L) | 681 | 963 | 663 | 1150 | |
| AST (log-scale) | 2.023 | 2.040 | 2.058 | 2.198 | 0.052 |
| AST (IU/L) | 105.3 | 109.6 | 114.3 | 157.8 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

The mean bound cortisol concentrations for both the WDOT and flank spayed heifers between 0 to 8 hours after surgery were significantly greater than the controls (P<0.05). However, the mean concentration for the ear notched heifers was very similar to that of the controls. The mean unbound cortisol concentration of the flank spayed heifers for this period was also significantly higher than the controls. Further, the mean haptoglobin concentrations of the flank spayed heifers for the period 8 to 96 hours was significantly higher than the controls.

### 4.1.2 Production responses

As outlined previously, changes in liveweight were monitored to detect production responses to treatments. As the trial animals were allocated on liveweight, differences between treatment groups were not detected at allocation (11 days prior to procedure) (grand mean 296.4 ± 6.8 kg s.e.; P=0.64). The mean liveweight of treatment groups were significantly different at 21 days and 42 days, P<0.01 and P<0.05 respectively (

**Table 4.1-5**). Control animals were heavier than the other treatment groups at both 21 days and 42 days (P<0.05). WDOT spayed heifers were recorded to be heavier than the ear notch treatment group at 21 days. As the ear notch was applied to both WDOT and flank spayed heifers, this difference is not likely to be a treatment effect. However, as liveweights were recorded directly after animals were mustered from the paddock, this difference is thought to more likely reflect a variation in gut fill.

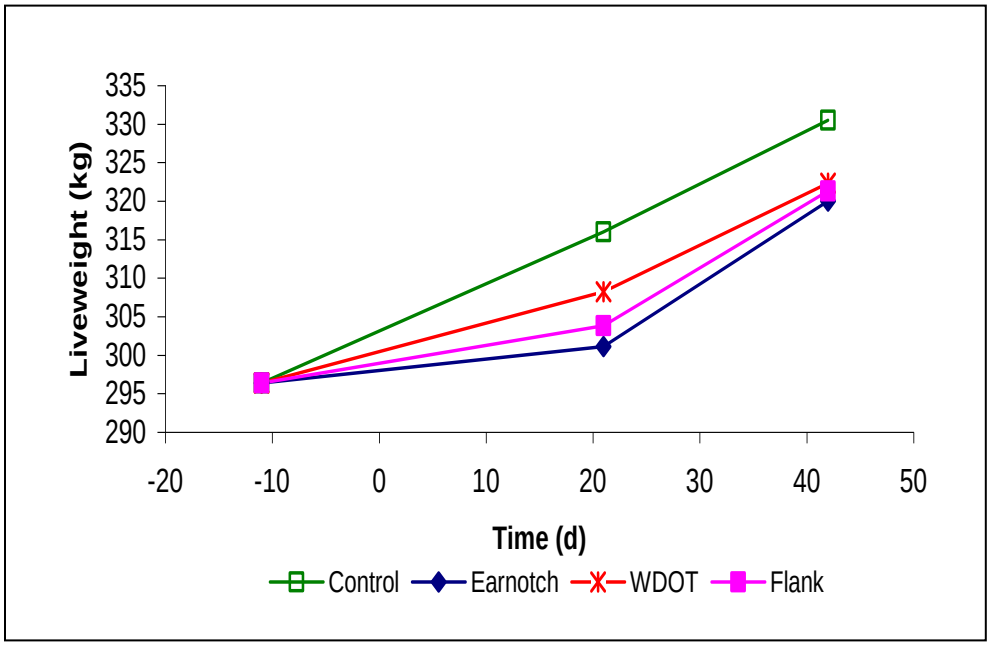



**Figure 4.1-3 Mean liveweight (kg) of Study I heifers grazing at Berrimah Research Farm, NT. (Control = physical restraint only; Ear Notch = restraint + application of spay mark pliers; WDOT = restraint + spayed using dropped ovary technique + application of spay mark pliers; and Flank = restraint, electroimmobilisation + 'web' spayed + application of spay mark pliers)**

**Table 4.1-5 Mean liveweight (kg) and liveweight gain (LWG; kg/day) of Study I heifers. (Control = physical restraint only; Ear Notch = restraint + application of spay mark pliers; WDOT = restraint + spayed using dropped ovary technique + application of spay mark pliers; and Flank = restraint, electroimmobilisation + 'web' spayed + application of spay mark pliers)**

| Variable | Treatment | | | | s.e. |
|---|---|---|---|---|---|
| | Control | Ear Notch | WDOT | Flank | |
| No. of heifers | 5 | 5 | 9 | 5 | |
| Liveweight (kg) (21 d) | 316.0$^{a}$ | 301.1$^{c}$ | 308.2$^{b}$ | 303.8$^{bc}$ | 2.32 |
| Liveweight (kg) (42 d) | 330.5$^{a}$ | 320.0$^{b}$ | 322.3$^{b}$ | 321.3$^{b}$ | 2.56 |
| LWG (kg/d) (-11-21d) | 0.645$^{a}$ | 0.149$^{c}$ | 0.379$^{b}$ | 0.232$^{bc}$ | 0.072 |
| LWG (kg/d) (21-42d) | 0.559 | 0.800 | 0.590 | 0.758 | 0.074 |
| LWG (kg/d) (-11-42d) | 0.607 | 0.433 | 0.462 | 0.471 | 0.046 |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

An increase in liveweight was recorded for all treatment groups. Differences in average liveweight gains (LWG; kg/day) across the entire study (-11 to 42 days) were not detected (P=0.07). However, differences in LWG were recorded for the period -11 to 21 days post procedure (P<0.001). Control heifers recorded the highest LWG during this period and ear notched animals the lowest (0.645 *v.* 0.149 kg/day respectively). As discussed above, the ear-notch was applied to both spay treatment groups, so this effect is difficult to explain. The spayed heifers recorded lower LWGs than control animals (P<0.05) in this same period, but there was no difference in LWG between spay treatments. However, overall the liveweight gain (- 11 to 42 days) for all groups was similar.

### 4.1.3 Morbidity and mortality

When the heifers were released from the yards to the paddock after the 24 hours blood sampling, all heifers were observed to be grazing and moving normally. One 8-week pregnant WDOT heifer aborted 4 days after the procedure. The majority of flank incisions had healed or were partially healed by 42 days (
**Table 4.1-6**). No deaths were recorded during the study period.

**Table 4.1-6 Frequency of wound healing scores at 21 and 42 days after flank spaying**

| Day | n | Wound Score | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 21 | 5 | 0% | 60% | 20% | 0% | 20% |
| 42 | 5 | 40% | 60% | 0% | 0% | 0% |

**(1, Wound healed; 2, Wound partially healed and clean and dry; 3, Wound partially healed and discharge; 4, Little or no healing of wound and clean and dry; 5, Little or no healing of wound and discharge)**

### 4.1.4 Summary of major findings

Examination of the bound cortisol profile for the WDOT spayed heifers demonstrated that it was similar to that of the flank spayed heifers and a blood sampling regime of once hourly to 4 hours after spaying would enable accurate definition of the acute cortisol response to this procedure. Timelines of the percentage changes in each measure of the physiological response relative to the control heifers for each group are presented in Figure 4.1-4, with significant effects (P< 0.05) in bold and underlined. The major findings were as follows:

- During the first 8 hours after flank and WDOT spaying, the bound cortisol concentrations were significantly increased above controls, 82% and 63% respectively. Unbound cortisol levels were also elevated in the flank spayed animals during this period.
- There was no significant difference between the bound cortisol concentrations of flank and WDOT spayed heifers during the first 8 hours after spaying.
- Serum haptoglobin concentrations in the flank spayed heifers were significantly increased (78% and 188%) above controls from 8 to 24 hours, and 24 to 96 hours after spaying, whereas in the WDOT spayed heifers the observed increase in serum haptoglobin concentrations were not significantly greater than controls.
- Ear-notched heifers showed no significant differences to control animals in any of the biochemical parameters.
- Spayed heifers, regardless of method used, had significantly lower liveweight gains compared to controls during the period from 11 days prior to procedures being performed through to 21 days afterwards.

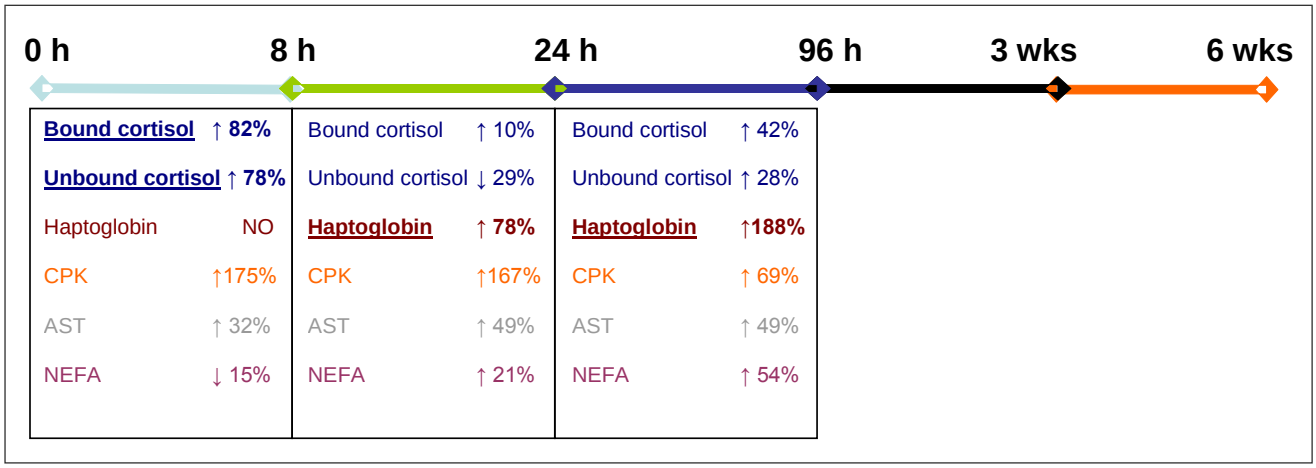



**Figure 4.1-4 Percentage changes in measures of the physiological and production responses of flank spayed heifers relative to control heifers. (NO = Not observed in control group). Underlined bold measurements are significantly (p<0.05) different to controls**

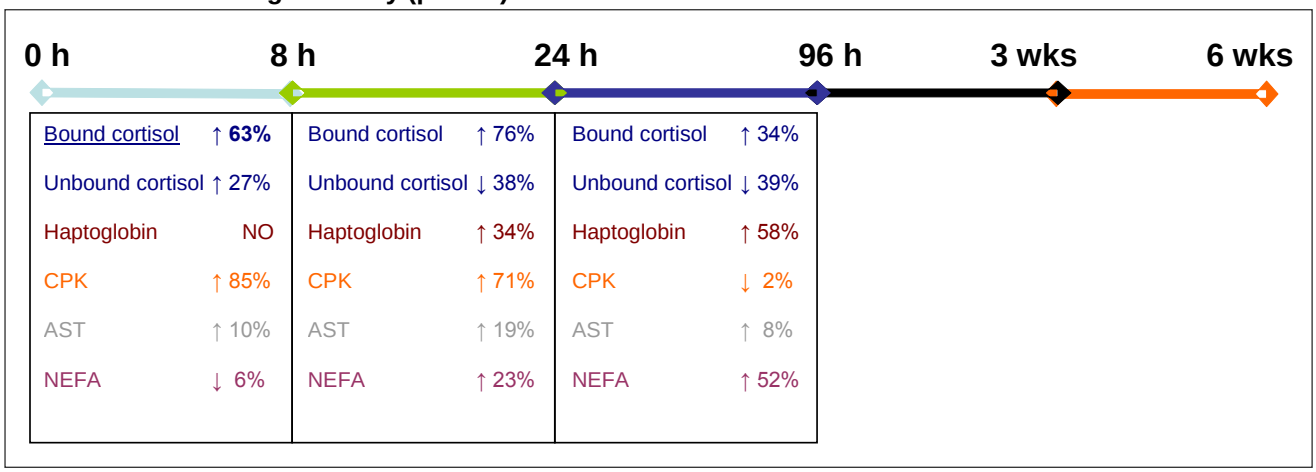



**Figure 4.1-5 Percentage changes in measures of the physiological and production responses of WDOT spayed heifers relative to control heifers. (NO = Not observed in control group).**

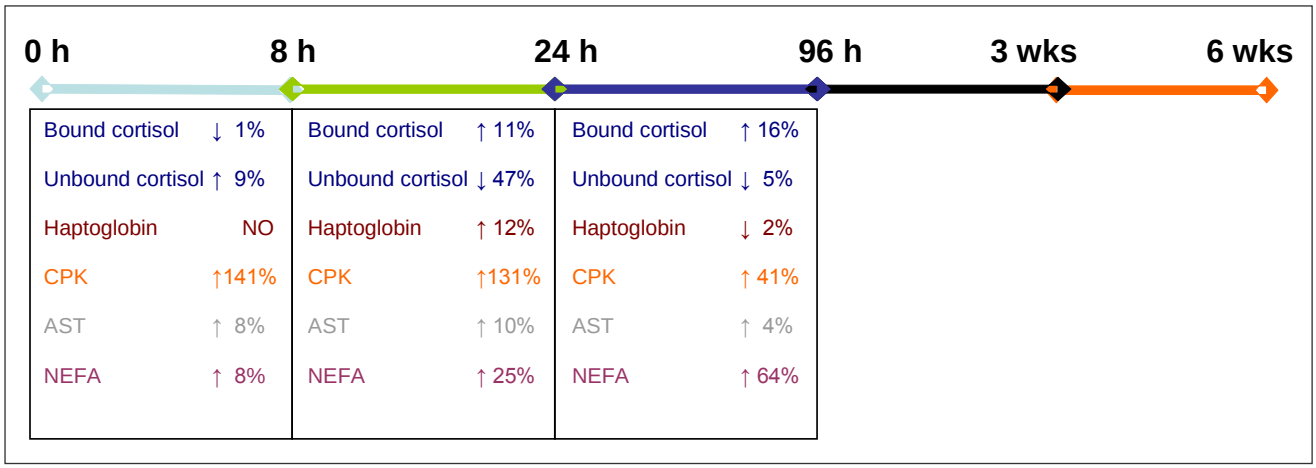



**Figure 4.1-6 Percentage changes in measures of the physiological and production responses of ear notched heifers relative to control heifers. (NO = Not observed in control group).**

## 4.2 Study II

### 4.2.1 Physiological responses

The actual times cattle were blood sampled is presented in
**Table 4.2-1**. Up to 8 hours post procedure the cattle were sampled within ± 0.3 hours of the scheduled time. For all replicates the scheduled blood sampling times were all met. Both the heifers and the cows generally worked well during the first 8 hours after procedures when they were regularly blood sampled and also for the later samplings. One animal was temporarily excluded during the first 8 hours after procedures due to dangerous behaviour, resulting in three samples being missed (Appendix 9.4). One control heifer died at day 12; the post mortem diagnosis was dehorning sepsis.

**Table 4.2-1 Actual blood sampling times for each replicate and each scheduled sampling time – Study II.**

| Replicate | Scheduled sample times | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0h | 1h | 2h | 3h | 4h | 6h | 8h | 24h | 96h | 21d (504 h) | 42d (1008 h) |
| 1 | 0 | 0.9 | 1.9 | 2.9 | 3.8 | 5.8 | 7.8 | 22.9 | 95.6 | 603.4 | 1080.8 |
| 2 | 0 | 1.0 | 2.0 | 3.0 | 4.1 | 6.1 | 8.1 | 23.2 | 95.3 | 555.0 | 1033.0 |
| 3 | 0 | 1.0 | 2.0 | 3.1 | 4 | 6.1 | 8.0 | 24.4 | 96.7 | 556.6 | 1034.5 |
| 4 | 0 | 0.8 | 1.8 | 2.9 | 3.8 | 5.8 | 7.8 | 24.0 | 95.9 | 604.6 | 1082.3 |
| 5 | 0 | 1.1 | 2.0 | 3.0 | 4.1 | 6.1 | 8.0 | 23.6 | 96.1 | 556.7 | 1033.6 |
| 6 | 0 | 0.9 | 1.9 | 3.0 | 4.0 | 5.9 | 7.8 | 24.2 | 96.3 | 604.9 | 1082.5 |
| 7 | 0 | 0.7 | 1.7 | 2.7 | 3.8 | 5.7 | 7.7 | 23.4 | 95.9 | 603.5 | 1081.3 |
| 8 | 0 | 1.1 | 2.1 | 3.1 | 4.1 | 6.1 | 8.1 | 22.9 | 95.2 | 555.2 | 1032.8 |
| 9 | 0 | 0.9 | 1.8 | 2.8 | 3.9 | 5.9 | 7.8 | 22.8 | 95.2 | 603.4 | 1080.8 |
| 10 | 0 | 1.0 | 2.0 | 3.0 | 4.0 | 6.1 | 8.0 | 24.0 | 96.7 | 557.4 | 1034.4 |
| 11 | 0 | 1.2 | 2.2 | 3.2 | 4.2 | 6.3 | 7.2 | 21.8 | 95.3 | 579.3 | 1056.8 |
| 12 | 0 | 0.7 | 2.0 | 3.0 | 4.1 | 6.1 | 8.1 | 22.9 | 96.4 | 580.7 | 1057.7 |
| 13 | 0 | 1.3 | 2.2 | 3.4 | 4.3 | 6.4 | 8.1 | 24.0 | 97.6 | 580.8 | 1058.8 |
| 14 | 0 | 0.9 | 2.3 | 3.1 | 4.2 | 6.2 | 8.1 | 23.8 | 97.0 | 580.7 | 1058.4 |
| 15 | 0 | 1.2 | 2.3 | 3.2 | 4.2 | 6.1 | 8.2 | 22.7 | 96.3 | 579.8 | 1057.9 |

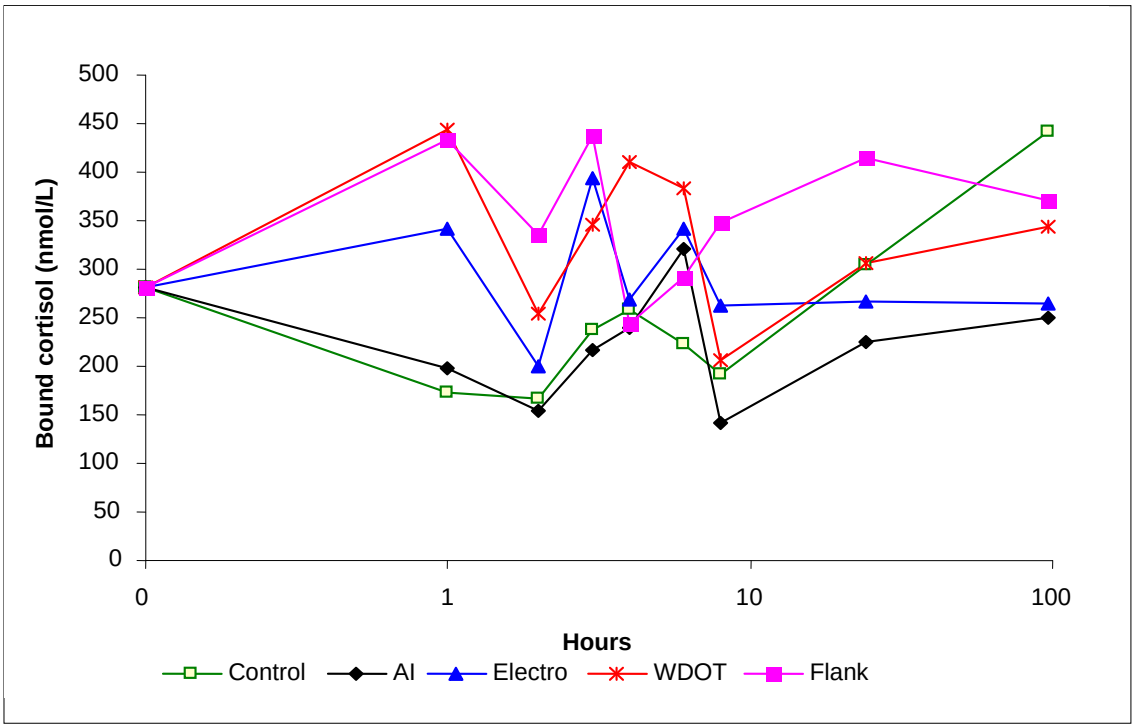
**The physiological responses to each procedure are reported below as,** firstly**, the pattern of changes in concentration over time up to 96 hours after each procedure for cows and heifers respectively ( Figure 4.2-1 to Figure 4.2-7), and,** secondly**, the mean values for the following three periods for cows and heifers respectively:**

- the acute stress response (0 to 8 hours) (Table 4.2-2 and Table 4.2-3)
- the immediate post-acute response (8 to 24 hours) (Table 4.2-4 and Table 4.2-5)

**the early chronic response (24 to 96 hours) (Table 4.2-6 and**

- Table 4.2-7)

a)




b)

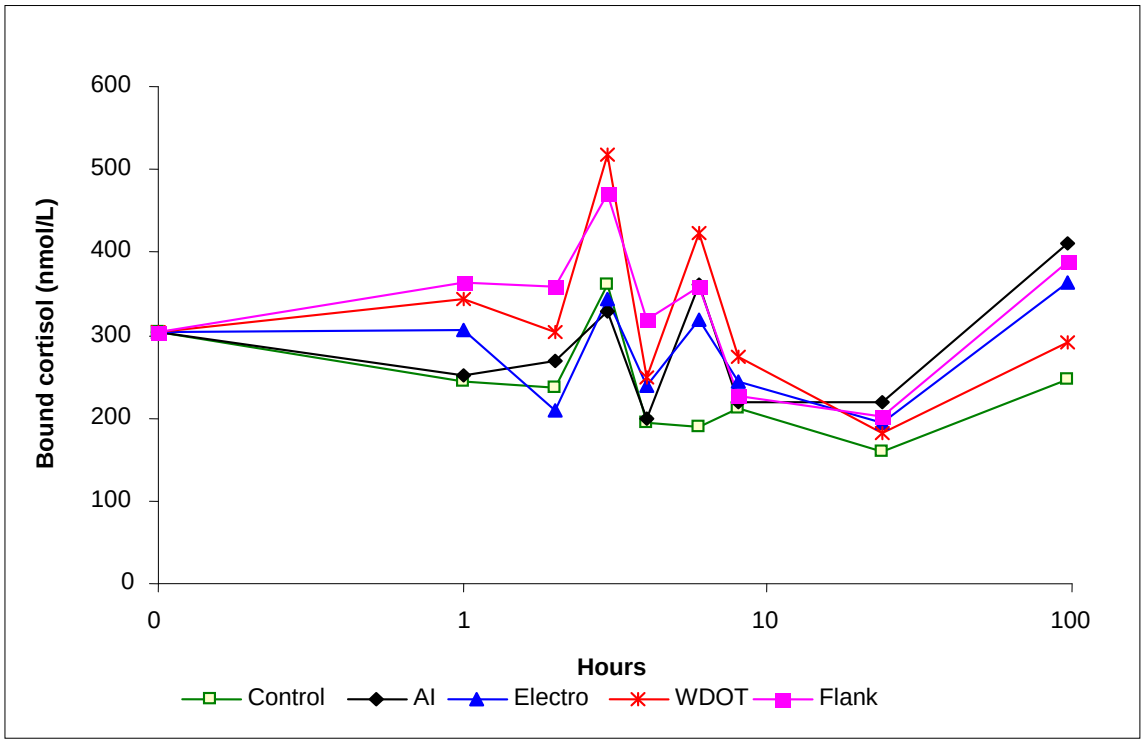
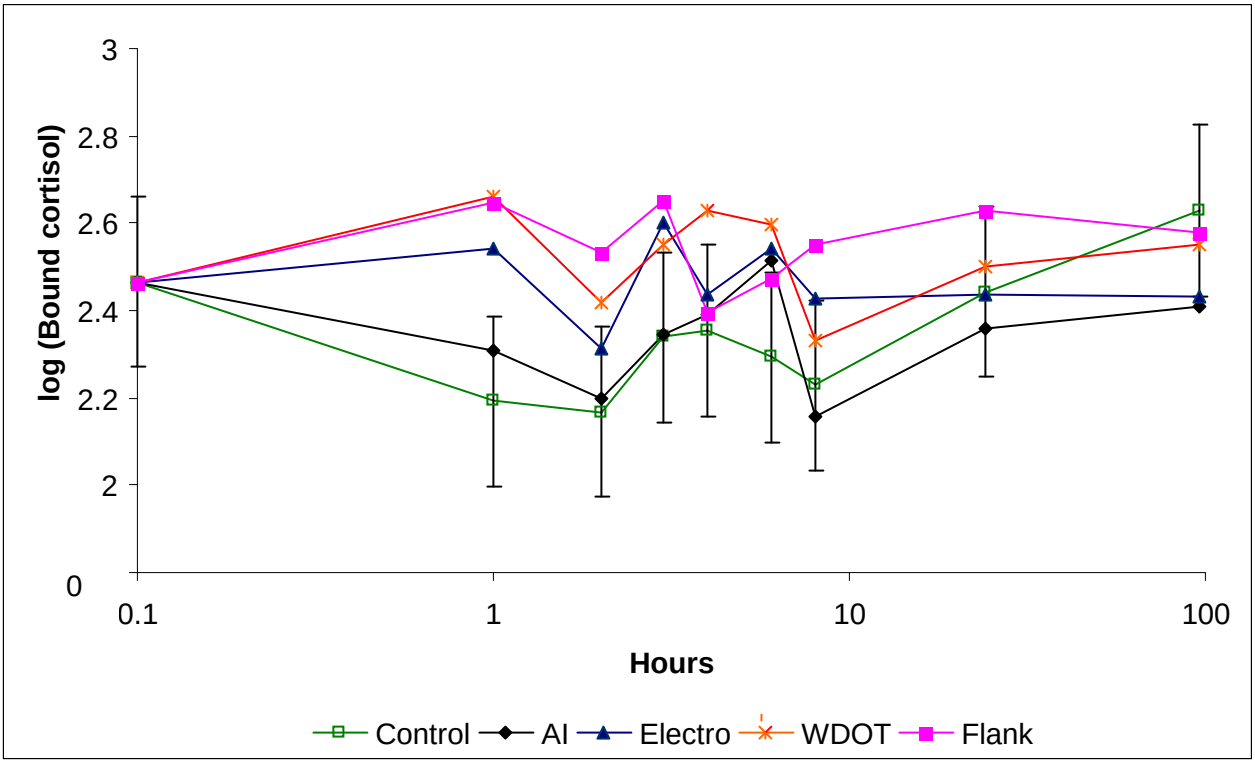
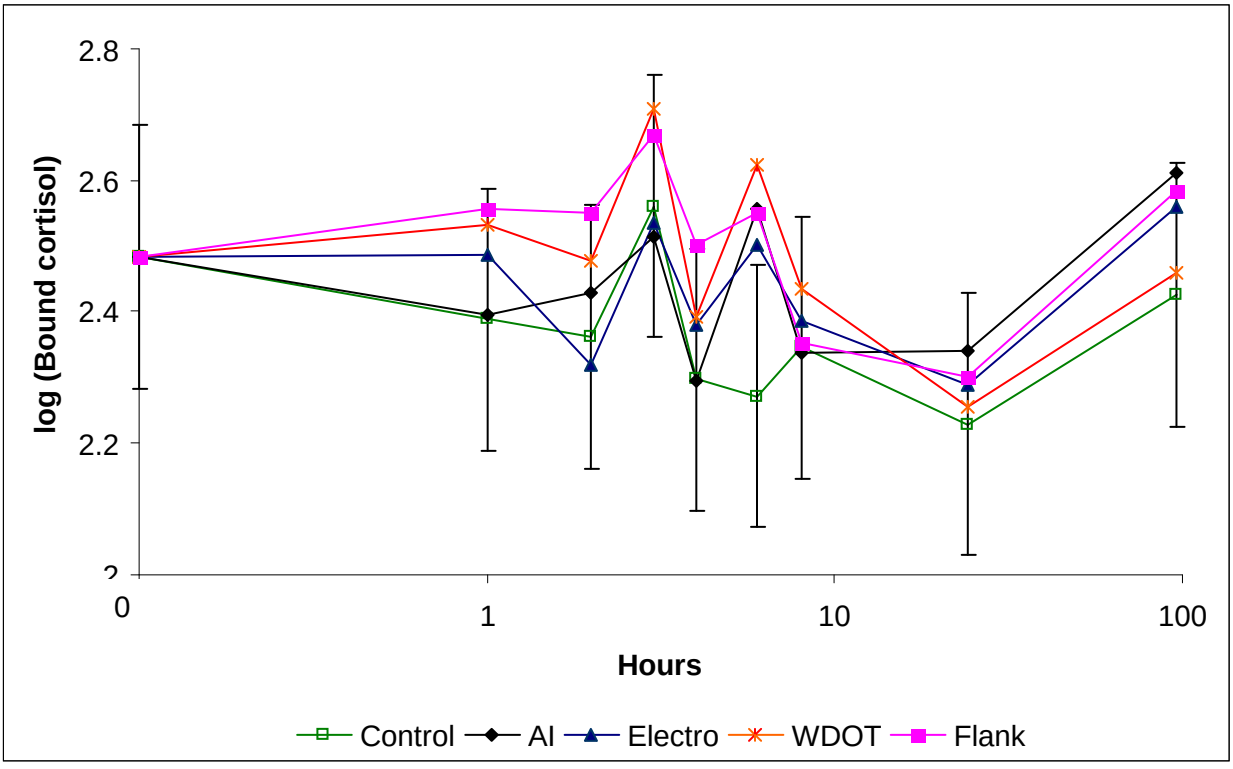



**Figure 4.2-1 Mean changes in bound cortisol concentrations (nmol/L) between 0 to 96 hours after procedures were performed for a) cows and b) heifers**



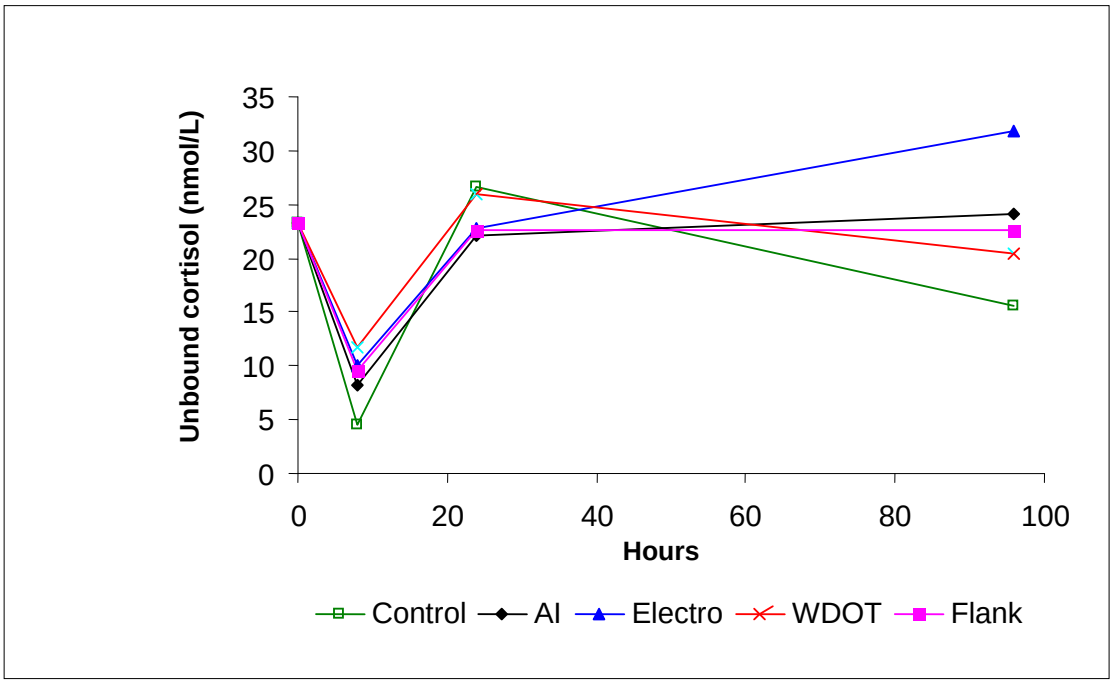

a)




b)

**Figure 4.2-2 Mean ± s.e. changes in bound cortisol concentrations (log transformed) between 0 to 96 hours after procedures in a) cows and b) heifers.**

a)




b)

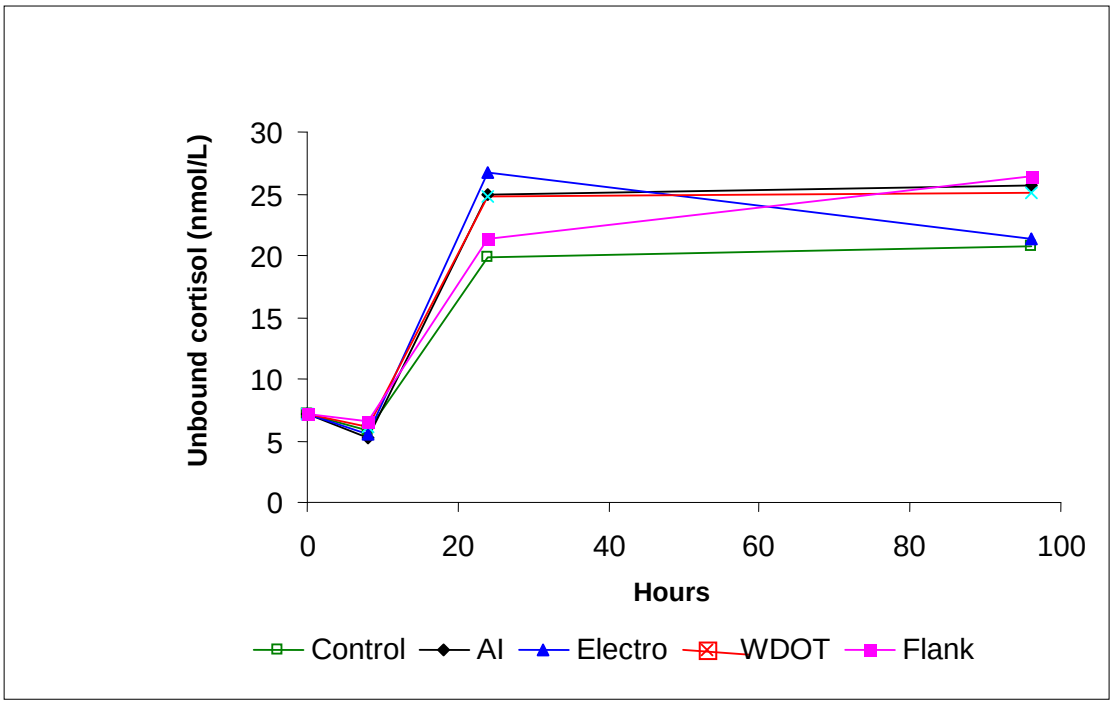
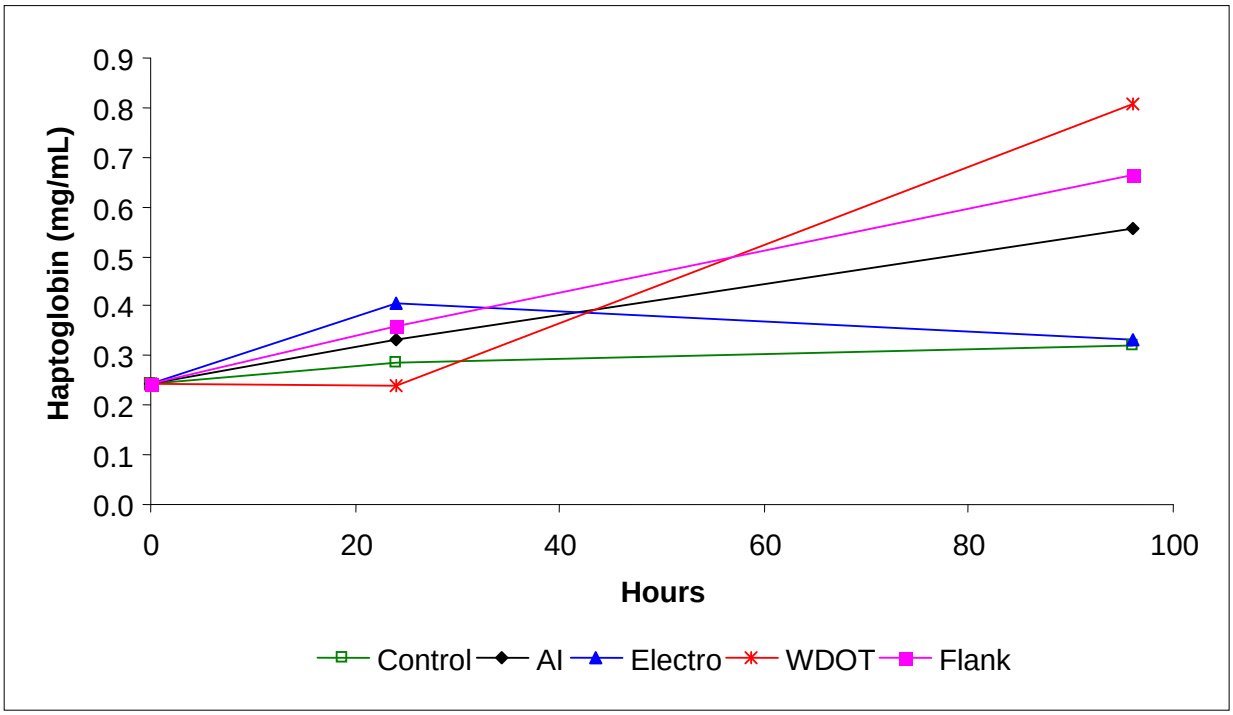
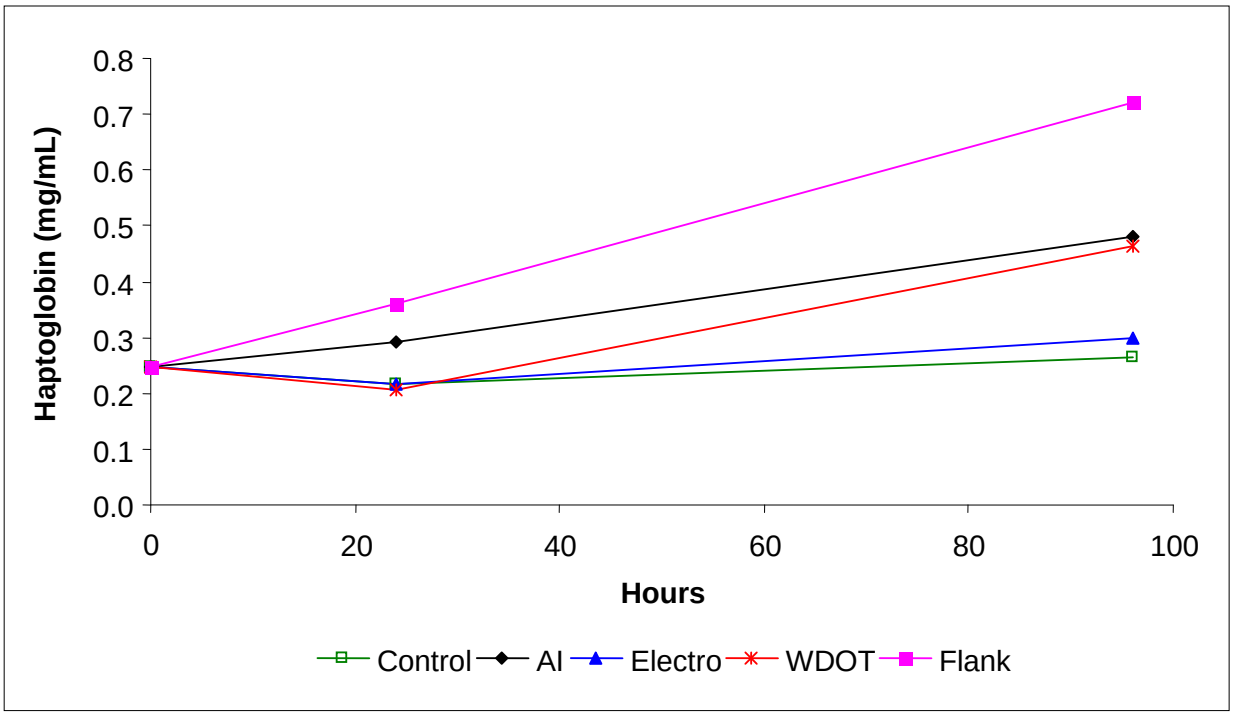



**Figure 4.2-3 Mean changes in unbound cortisol concentrations (nmol/L) between 0 to 96 hours after procedures were performed in a) cows and b) heifers.**

a)



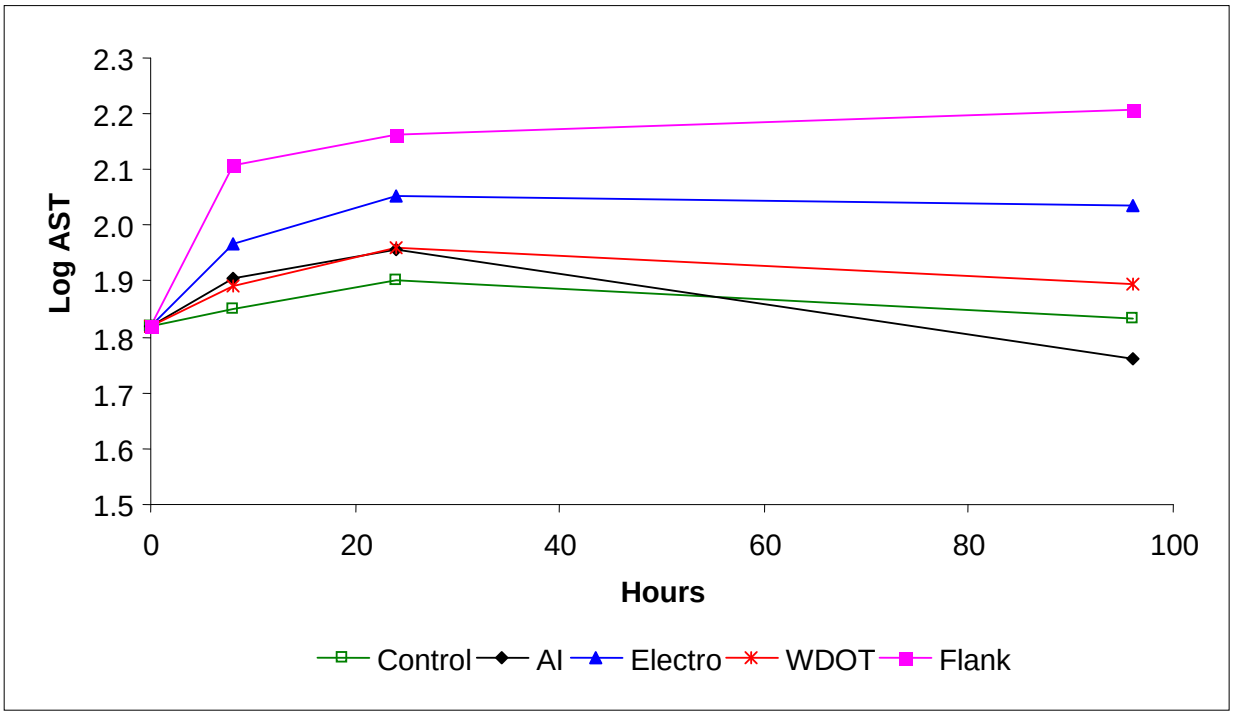

b)




B)

**Figure 4.2-4 Mean changes in haptoglobin concentrations (mg/mL) between 0 to 96 hours after procedures were performed in a) cows and b) heifers.**

a)




b)

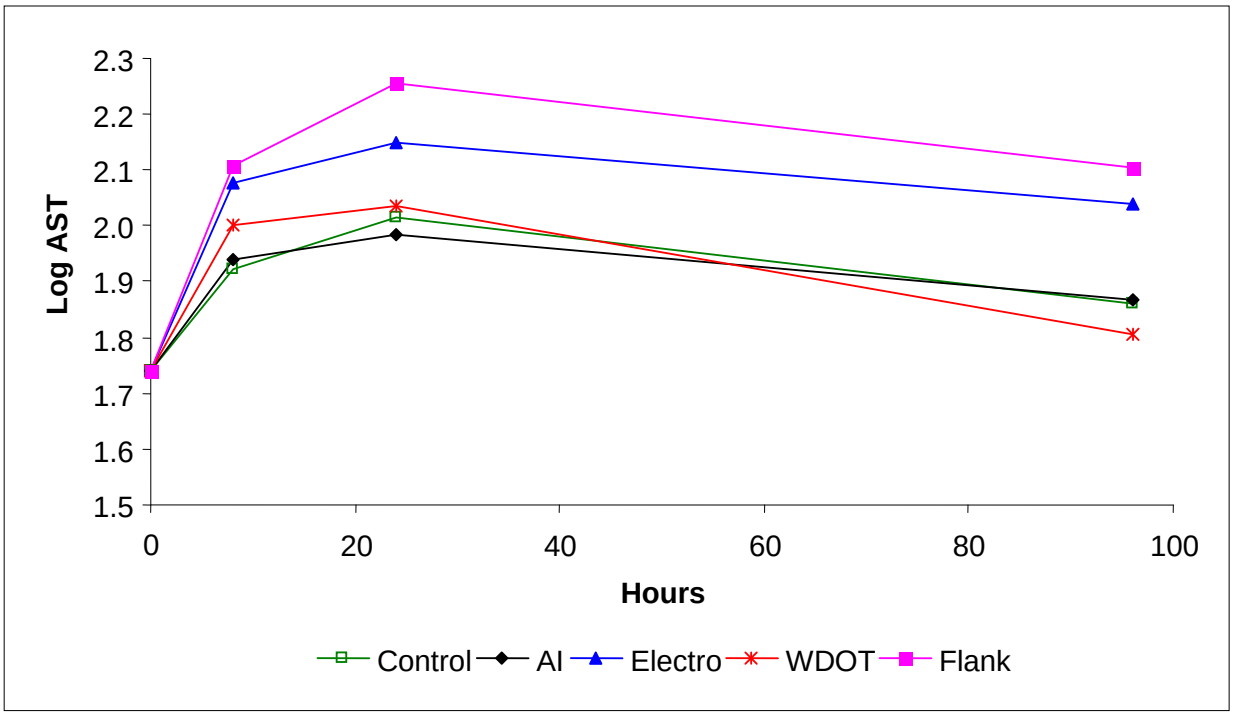
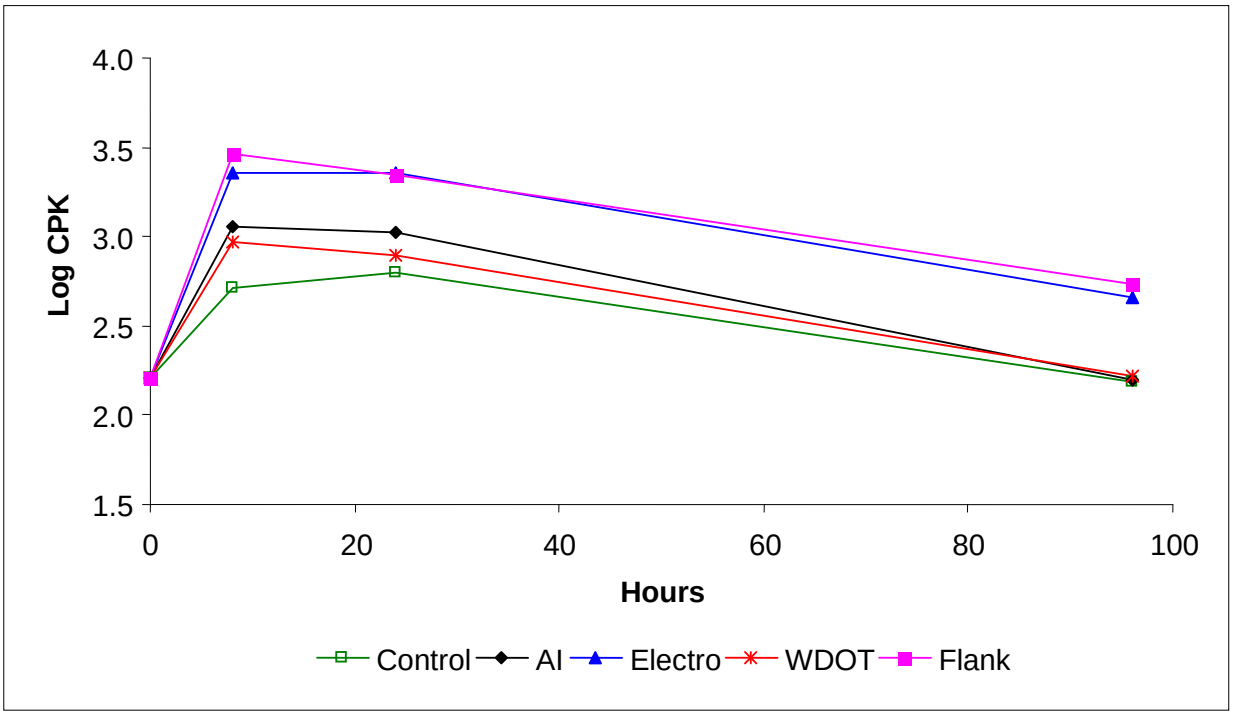



**Figure 4.2-5 Mean changes in log AST concentrations between 0 to 96 hours after procedures were performed in a) cows and b) heifers.**

a)




b)

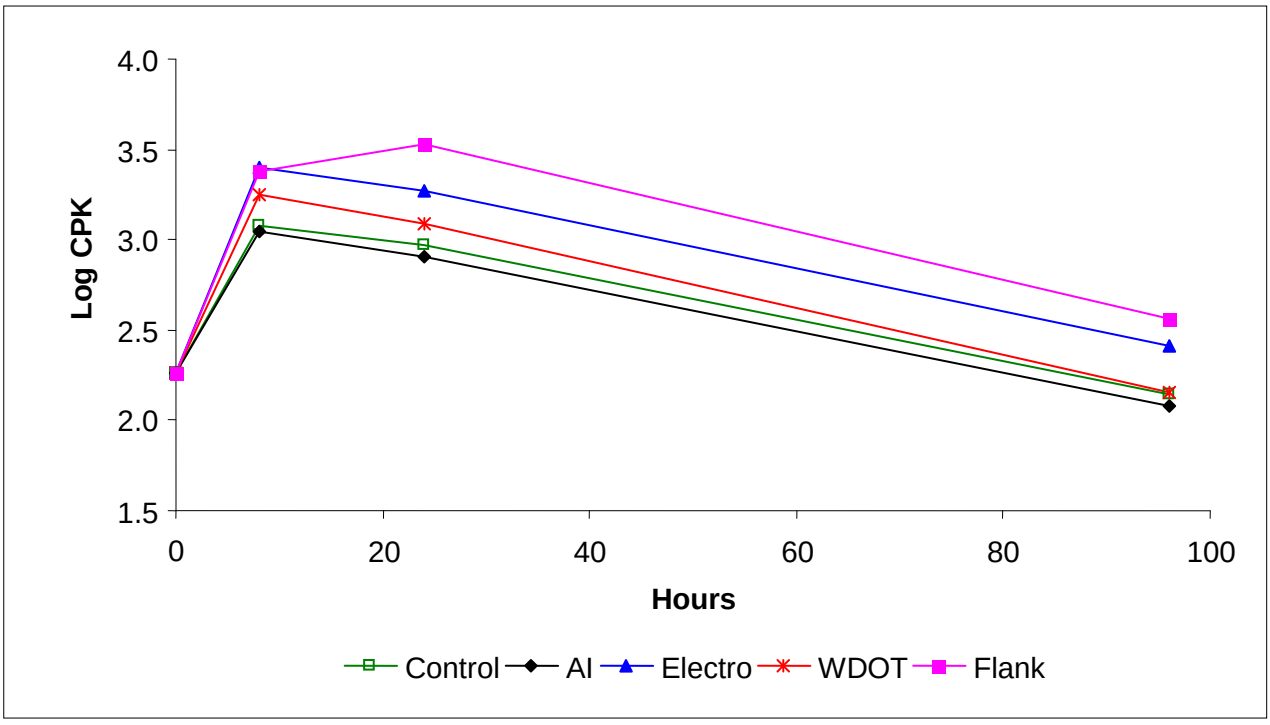
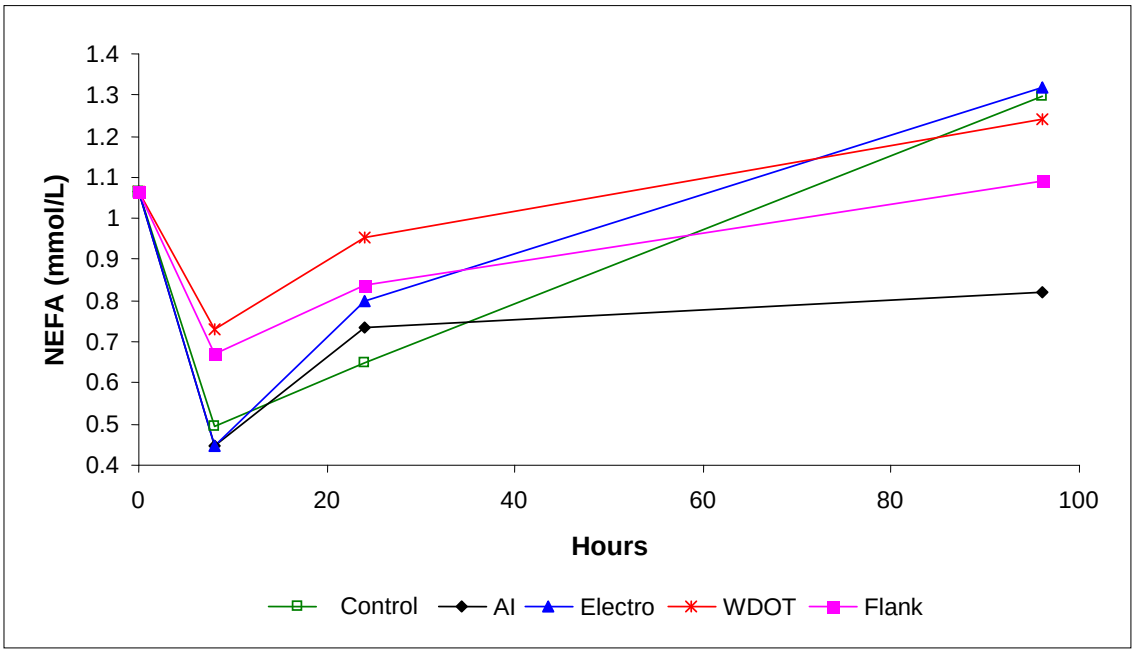



**Figure 4.2-6 Mean changes in log CPK concentrations between 0 to 96 hours after procedures were performed in a) cows and b) heifers.**

a)




b)

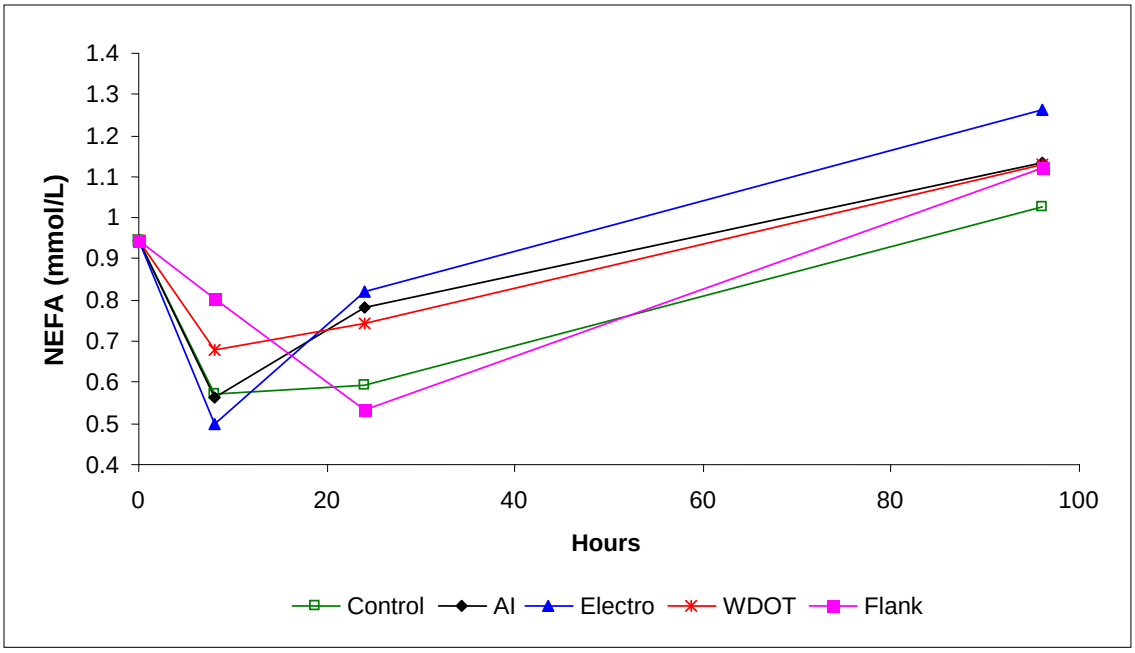



**Figure 4.2-7 Mean changes in NEFA concentrations between 0 to 96 hours after procedures were performed in a) cows and b) heifers.**

*Changes in cortisol concentrations in response to procedures*

The bound cortisol profiles of all heifers (except controls) were characterised by biphasic increases in concentration at 3 and 6 hours after procedures. A peak in mean bound cortisol concentrations occurred in all groups at 3 hours after procedures, but there was no significant difference in the mean concentration between groups (Figure 4.2-1B). However, at 4 hours the mean concentration in the flank spayed heifers was significantly ($P< 0.05$) greater than controls and at 6 hours the mean concentrations in the flank and WDOT spayed heifers and AI heifers were significantly ($P< 0.05$) greater than controls. At 24 hours there was no significant difference between the mean concentrations of each group. However, examining the changes in mean unbound cortisol concentrations (Figure 4.2-3.B) there was an approximate 3-fold increase in concentrations for all groups at 24 hours which was sustained through to 96 hours. Further, at 96 hours the mean concentrations of bound cortisol for the flank spayed and AI heifers were significantly ($P< 0.05$) greater than controls, however the concentrations of the WDOT spayed heifers were similar to controls. The profile of bound and unbound cortisol concentrations in the control heifers which were blood sampled and only restrained indicates that all heifers experienced an acute and chronic stress response to the handling and sampling but superimposed on this were the stress (pain) responses to the procedures. Similar findings have been reported for positively and negatively handled gilts and dairy heifers (reviewed by Hemsworth and Barnett, 2000). The spayed heifers showed an acute stress response at 3 hours which was then sustained through to 8 hours in contrast to the controls, which showed a sustained decline after the initial acute response associated with the first episode of restraint in the crush.

The cows also showed a biphasic increase in bound cortisol concentrations, albeit less discrete than in the heifers. There was an initial increase in concentration at 1 hour and then a second increase at 3 to 4 hours. The controls and AI cows only showed one increase and it coincided with the timing of the second increase in bound cortisol shown by the heifers.

**The repeated measures ANOVA means, presented in Table 4.2-2 to**

Table 4.2-7, enables the treatments to be ranked according to the physiological responses measured. During the first 8 hours after the procedures the mean bound cortisol concentrations of heifers and cows spayed by either technique was very similar (Table 4.2-2). The mean bound cortisol concentrations for the spayed heifers was significantly ($P < 0.05$) greater than the control heifers , but not significantly different to the mean concentrations for the AI and electroimmobilised heifers. For the cows during the same period the mean bound cortisol concentration of the spayed animals was significantly ($P< 0.05$) greater than both the controls and AI animals but not the electoimmobilised animals. During the period 8 to 24hours after the procedures were conducted there was no significant difference between the heifer groups, however for the cows the flank spayed animals had significantly ($P< 0.05$) greater mean bound cortisol concentrations than any of the other groups, and the mean unbound cortisol concentration of the WDOT cows was significantly ($P<0.05$) greater than the controls. These findings, suggest that the impact of spaying is greater in cows compared to heifers. In addition the bound cortisol concentrations of the electroimmobilsed cattle were consistently elevated (in some cases significantly) compared to the controls, indicating that this procedure also induces a marked stress response. The significantly ($P< 0.05$) increased mean bound cortisol concentration in the AI heifers between 24 to 96 hours cannot be readily explained.

*Changes in other physiological measures in response to procedures*

There were no significant differences in mean NEFA concentrations between groups for the three time periods. The mean haptoglobin concentrations for flank spayed heifers was significantly ($P<0.05$) increased from 8 to 96 hours, and significantly increased for WDOT and flank spayed cows from 24 to 96 hours indicative of a marked inflammatory reaction to the surgical procedures. Only the flank spayed heifers showed a sustained increase above the threshold of 0.35mg/ml

(Horadagoda et al. 1999). The mean concentrations of CPK and AST were significantly (P< 0.05) increased for the three periods in the electroimmobilised and flank spayed heifers. The response in the cows was similar, except the CPK was also significantly increased for the AI and WDOT spayed heifers between 8 to 24 hours and the mean CPK concentrations at 24 to 96 hours were not significantly greater than the controls.

**Table 4.2-2 Mean physiological responses 0 to 8 hrs after each procedure – Study II heifers (n=99)**

| Physiological measure | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.408[a] | 2.450[ab] | 2.469[ab] | 2.556[b] | 2.560[b] | 0.042 |
| Bound cortisol (nmol/L) | 255.6 | 281.5 | 294.4 | 359.7 | 363.2 | |
| Unbound cortisol (log-scale) | 0.858 | 0.841 | 0.884 | 0.916 | 0.918 | 0.039 |
| Unbound cortisol (nmol/L) | 7.21 | 6.93 | 7.66 | 8.24 | 8.28 | |
| Haptoglobin[#] (mg/ml) | | | | | | |
| NEFA (mmol/L) | 0.650 | 0.573 | 0.490 | 0.610 | 0.647 | 0.069 |
| CPK (log-scale) | 3.019[a] | 3.053[a] | 3.557[b] | 3.179[a] | 3.490[b] | 0.087 |
| CPK (IU/L) | 1045 | 1130 | 3606 | 1510 | 3090 | |
| AST (log-scale) | 1.917[a] | 1.957[a] | 2.189[b] | 1.988[a] | 2.124[b] | 0.029 |
| AST (IU/L) | 82.5 | 90.6 | 154.4 | 97.3 | 133.1 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

**Table 4.2-3 Mean physiological responses 0 to 8 hrs after each procedure –Study II cows (n= 49)**

| Physiological measure | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.198[a] | 2.249[a] | 2.416[b] | 2.472[b] | 2.476[b] | 0.042 |
| Bound cortisol (nmol/L) | 157.6 | 177.5 | 260.4 | 296.3 | 299.4 | |
| Unbound cortisol (log-scale) | 0.558[a] | 0.767[b] | 0.855[bc] | 0.890[c] | 0.820[bc] | 0.039 |
| Unbound cortisol (nmol/L) | 3.61 | 5.85 | 7.16 | 7.76 | 6.61 | |
| Haptoglobin[#] (mg/ml) | | | | | | |
| NEFA (mmol/L) | 0.518[abc] | 0.422[a] | 0.483[ab] | 0.722[c] | 0.641[bc] | 0.069 |
| CPK (log-scale) | 2.687[a] | 2.968[b] | 3.339[c] | 3.045[b] | 3.361[c] | 0.087 |
| CPK (IU/L) | 486 | 929 | 2183 | 1109 | 2296 | |
| AST (log-scale) | 1.857[a] | 1.885[ab] | 1.959[bc] | 1.914[abc] | 1.982[c] | 0.029 |
| AST (IU/L) | 71.9 | 76.8 | 91.1 | 82.1 | 95.9 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

**Table 4.2-4 Mean physiological responses 8 to 24 hrs after each procedure –Study II heifers (n=99)**

| Physiological measure | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.328 | 2.371 | 2.368 | 2.377 | 2.359 | 0.049 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Bound cortisol (nmol/L) | 212.8 | 235.0 | 233.3 | 238.2 | 228.6 | |
| Unbound cortisol (log-scale) | 1.035 | 1.057 | 1.105 | 1.118 | 1.041 | 0.038 |
| Unbound cortisol (nmol/L) | 10.84 | 11.40 | 12.74 | 13.12 | 10.99 | |
| Haptoglobin# (mg/ml) | 0.181[a] | 0.210[a] | 0.214[a] | 0.280[a] | 0.421[b] | 0.049 |
| NEFA (mmol/L) | 0.651 | 0.677 | 0.666 | 0.677 | 0.669 | 0.071 |
| CPK (log-scale) | 3.032[a] | 2.973[a] | 3.331[bc] | 3.159[ab] | 3.475[c] | 0.087 |
| CPK (IU/L) | 1077 | 940 | 2142 | 1442 | 2984 | |
| AST (log-scale) | 1.984[a] | 1.971[a] | 2.126[bc] | 2.031[ab] | 2.199[c] | 0.038 |
| AST (IU/L) | 96.3 | 93.6 | 133.7 | 107.4 | 158.1 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

**Table 4.2-5 Mean physiological responses 8 to 24 hrs after each procedure – Study II cows (n=49)**

| Physiological measure | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.267[ab] | 2.187[a] | 2.370[b] | 2.351[b] | 2.524[c] | 0.049 |
| Bound cortisol (nmol/L) | 184.9 | 153.8 | 234.4 | 224.4 | 334.2 | |
| Unbound cortisol (log-scale) | 1.050[a] | 1.086[ab] | 1.161[bc] | 1.198[c] | 1.116[abc] | 0.038 |
| Unbound cortisol (nmol/L) | 11.22 | 12.19 | 14.49 | 15.78 | 13.06 | |
| Haptoglobin# (mg/ml) | 0.273 | 0.333 | 0.401 | 0.243 | 0.358 | 0.049 |
| NEFA (mmol/L) | 0.553 | 0.518 | 0.616 | 0.794 | 0.665 | 0.071 |
| CPK (log-scale) | 2.666[a] | 2.910[b] | 3.390[c] | 2.957[b] | 3.313[c] | 0.087 |
| CPK (IU/L) | 463 | 812 | 2454 | 906 | 2056 | |
| AST (log-scale) | 1.869[a] | 1.908[a] | 2.037[b] | 1.939[ab] | 2.037[b] | 0.038 |
| AST (IU/L) | 74.0 | 80.8 | 108.9 | 86.8 | 108.9 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

**Table 4.2-6 Mean physiological responses 24 to 96 hrs after each procedure –Study II heifers (n= 99).**

| Physiological measure | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.342[a] | 2.524[b] | 2.448[ab] | 2.403[ab] | 2.483[b] | 0.050 |
| Bound cortisol (nmol/L) | 219.8 | 334.2 | 280.5 | 252.9 | 304.1 | |
| Unbound cortisol (log-scale) | 1.309 | 1.355 | 1.344 | 1.365 | 1.367 | 0.033 |
| Unbound cortisol (nmol/L) | 20.37 | 22.65 | 22.08 | 23.16 | 23.28 | |
| Haptoglobin# (mg/ml) | 0.241[a] | 0.386[ab] | 0.258[a] | 0.337[a] | 0.541[b] | 0.061 |
| NEFA (mmol/L) | 0.831 | 0.941 | 1.028 | 0.916 | 0.792 | 0.076 |
| CPK (log-scale) | 2.564[a] | 2.490[a] | 2.835[bc] | 2.608[ab] | 3.056[c] | 0.091 |
| CPK (IU/L) | 366 | 309 | 684 | 406 | 1138 | |
| AST (log-scale) | 1.939[a] | 1.924[a] | 2.091[b] | 1.913[a] | 2.184[b] | 0.047 |
| AST (IU/L) | 86.8 | 83.9 | 123.4 | 81.8 | 152.7 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

**Table 4.2-7 Mean physiological responses 24 to 96 hrs after each procedure – Study II cows (n= 49).**

| Physiological measure | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| Bound cortisol (log-scale) | 2.451[ab] | 2.330[a] | 2.360[a] | 2.445[ab] | 2.542[b] | 0.050 |
| Bound cortisol (nmol/L) | 282.5 | 213.8 | 229.1 | 278.6 | 348.3 | |
| Unbound cortisol (log-scale) | 1.388 | 1.433 | 1.493 | 1.433 | 1.425 | 0.033 |
| Unbound cortisol (nmol/L) | 24.43 | 27.10 | 31.12 | 27.07 | 26.61 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Haptoglobin[#] (mg/ml) | 0.286[a] | 0.445[ab] | 0.367[ab] | 0.522[b] | 0.511[b] | 0.061 |
| NEFA (mmol/L) | 0.924 | 0.783 | 1.038 | 1.096 | 0.990 | 0.076 |
| CPK (log-scale) | 2.495 | 2.607 | 3.009 | 2.558 | 2.989 | 0.091 |
| CPK (IU/L) | 313 | 405 | 1021 | 361 | 975 | |
| AST (log-scale) | 1.876[a] | 1.870[a] | 2.057[b] | 1.941[ab] | 2.191[c] | 0.047 |
| AST (IU/L) | 75.1 | 74.2 | 113.9 | 87.2 | 155.4 | |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

### 4.2.2 Behavioural responses

As noted previously, there are no studies that have recorded effects of the procedures investigated on behaviour with which we can compare our findings. Mellor et al. (2000) list vocalisations, temperament, postural and locomotory changes in response to noxious stimuli and the descriptions include “standing drooping”, “depressed” and “miserable”. Based on our own experiences of monitoring cattle, these descriptions fit the behaviour of cattle that are sick or in pain. It has been suggested that decreases in feed consumption and a lack of interest in food and water can indicate pain (Loeffler, 1986; Cook 1996), although, of course, there could also be many other reasons for a reduction in feeding and drinking. A study on dehorning of calves found that control animals stood ruminating, as did calves given an anaesthetic prior to dehorning. However, after about 2 hours, the amount of ruminating decreased in the calves given an anaesthetic and their behaviour became more like the dehorned calves (Sylvester et al. 2004). These results suggest that a decrease in rumination is also an indicator of pain in cattle.

#### 4.2.2.1 Behavioural responses to 8 hours post-procedures

The data used for analysis for the period of 0 to 8 hours post-procedures being conducted on the cattle comprised the scan-samples of the individual animals conducted in the yard complex. There were too few data for many of the behaviours, the only ones having sufficient data for analyses were feeding, locomotion, standing head up, standing head down and lying on sternum (

Table 4.2-8). Although there were significant effects of the treatments on feeding behaviour it should be noted that not all pens within the yard complex provided an opportunity for cattle to feed. However, as all treatments were included in each replicate group, any unreliability of the data would result only from certain replicate groups being in the pens containing grass/hay more frequently than other replicate groups and the former groups not being representative of all groups.

**Table 4.2-8 Behaviours (mean percentages of animals and s.e.) shown by heifers and cows on the treatments during the period 0 to 8 hours after treatments were applied (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only)**

| Behaviour | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| *Heifers* | | | | | | |
| Feeding∝ | 1.10$^{c}$ | 0.72$^{bc}$ | 0.54$^{abc}$ | 0.30$^{ab}$ | 0.00$^{a}$ | 0.20 |
| Locomotion | 8.33 | 11.39 | 10.37 | 5.61 | 6.77 | 2.06 |
| Standing head up | 74.98$^{c}$ | 65.90$^{b}$ | 72.26$^{bc}$ | 68.21$^{bc}$ | 55.41$^{a}$ | 3.19 |
| Standing head down | 3.59$^{a}$ | 8.96$^{ab}$ | 7.77$^{ab}$ | 9.76$^{b}$ | 26.26$^{c}$ | 2.13 |
| Lying sternum | 7.47 | 6.25 | 2.43 | 10.45 | 7.97 | 1.91 |
| *Cows* | | | | | | |
| Feeding∝ | 0.00$^{a}$ | 0.00$^{a}$ | 0.36$^{b}$ | 0.00$^{a}$ | 0.93$^{c}$ | 0.12 |
| Locomotion | 6.38 | 11.79 | 16.56 | 13.38 | 6.85 | 3.39 |
| Standing head up | 71.43 | 70.45 | 70.60 | 62.26 | 65.31 | 4.70 |
| Standing head down | 2.77$^{a}$ | 6.82$^{a}$ | 1.65$^{a}$ | 1.56$^{a}$ | 16.47$^{b}$ | 2.02 |
| Lying sternum | 5.89 | 1.76 | 0.54 | 1.49 | 1.84 | 1.52 |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

**∝ Not all pens contained feed, but as all treatments were represented in replicate groups, data are likely to be reliable**

The behavioural responses of heifers and cows to the treatments during the period up to 8 hours after the procedures had been conducted were different, as can be seen from

Table 4.2-8. The proportion of heifers observed feeding was zero for the Flank treatment, which was not statistically different to the proportion recorded in the WDOT and Electro treatments. Although higher, the proportion feeding in the AI treatment was not different to either the Electro or Control. The Control treatment had the highest proportion of heifers feeding, but it did not differ statistically to AI and Electro. In contrast, for the cows, the Control, AI and WDOT all showed zero feeding, the Electro showed a higher percentage and the Flank was significantly greater than all other treatments. This would suggest that the heifers were affected by the treatments more than the cows, with the greatest impacts observed in the Flank group, and lesser impacts observed in the WDOT and Electro treatment groups.

Standing head down showed a similar pattern in heifers and cows, with by far the greatest levels shown by the Flank treatment. For cows there was no significant difference in the levels shown by the other treatments, but for heifers the WDOT treatment showed significantly higher levels than the Control animals. The AI and Electro treatments were not significantly different to either the WDOT or Control treatments.

Standing head up was the predominant behaviour in this period for both heifers and cows. It showed the reciprocal pattern to standing head down in the heifers, with the significantly lowest levels shown by the Flank treatment and highest levels in the Control animals. The levels shown by the AI, Electro and WDOT treatments did not differ from each other significantly, but AI levels were significantly lower than the Controls, whilst Electro and WDOT did not differ from the Controls. Treatment did not affect standing head up in the cows, or locomotion and sternum lying in either class.

Overall, these results indicate that the Flank treatment had the greatest negative impact on both cows and heifers during this period, but considering the relative proportions showing behavioural changes (26.26% of heifers showing standing head down vs. 16.47% of cows), it would appear that the impact was greater on the heifers than the cows. An alternative interpretation is that the heifers were somewhat able to cope with the pain and stress via behavioural mechanisms, in contrast to the cows that responded physiologically. There is some evidence that the WDOT procedure also had a negative impact on the heifers, which was not seen in the cows. Also, for the heifers it would appear that there was some pain/discomfort associated with the AI and Electro treatments. Again this was not evident in the cows.

4.2.2.2 Behavioural responses between 8 and 24 hours post-procedures

The data used for analysis of the responses during the period 8 to 24 hours after the procedures comprised the scan-samples made at the end of the day when the replicates were combined into a single yard. There were too few data for many of the behaviours, the only ones having sufficient for analyses were feeding, locomotion, standing head up, standing head down, lying on sternum and drinking (Table 4.2-9).

**Table 4.2-9 Behaviours (mean percentages of animals and s.e.) shown by heifers and cows on the treatments during the period 8 hours to 24 hours after treatments were applied (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only)**

| Behaviour | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| *Heifers* | | | | | | |
| Feeding | 78.65$^{c}$ | 70.22$^{c}$ | 73.60$^{c}$ | 56.74$^{b}$ | 40.11$^{a}$ | 3.72 |
| Locomotion | 9.55 | 7.30 | 7.87 | 3.93 | 8.47 | 2.16 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Standing head up | 9.55[a] | 16.29[ab] | 15.73[ab] | 24.16[b] | 35.59[c] | 3.48 |
| Standing head down | 0.56[a] | 1.12[a] | 1.12[a] | 6.18[b] | 5.08[b] | 0.91 |
| Lying sternum | 0.00[a] | 1.69[b] | 0.00[a] | 2.81[bc] | 3.39[c] | 0.48 |
| Drinking | 1.12[a] | 0.56[a] | 1.12[a] | 1.12[a] | 3.95[b] | 0.66 |
| *Cows* | | | | | | |
| Feeding | 56.41[b] | 55.13[b] | 26.92[a] | 30.77[a] | 22.67[a] | 5.68 |
| Locomotion | 5.13 | 16.67 | 15.38 | 16.67 | 12.00 | 4.16 |
| Standing head up | 29.49[ab] | 24.36[a] | 52.56[b] | 43.59[b] | 50.67[b] | 4.70 |
| Standing head down | 1.28[a] | 0.00[a] | 0.00[a] | 1.28[a] | 9.33[b] | 0.96 |
| Lying sternum | 5.13[b] | 0.00[a] | 0.00[a] | 1.28[a] | 0.00[a] | 0.51 |
| Drinking | 2.56[ab] | 0.00[a] | 1.28[ab] | 1.28[ab] | 2.67[b] | 0.93 |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

Feeding was the predominant behaviour during this period and the proportions were higher for the heifers than for the cows, which could demonstrate that the heifers were hungrier, having less body and rumen reserves than the cows, or that the cows were experiencing more pain/discomfort than the heifers at this time. The cows and heifers that were not feeding appeared to be standing head up and the proportions of animals were reciprocals of each other i.e. a treatment with a large proportion of animals feeding had a small proportion standing head up and a treatment with a small proportion feeding had a large proportion standing head up.

The effects of treatment on feeding showed a similar pattern in heifers and cows with the significantly highest levels in the Control and AI groups and significantly lowest levels in the Flank treatment. However, for the heifers there was a significant difference between the WDOT and the other treatments, and Electro was no different to the Control and AI treatments. For the cows, there was no significant difference between Electro, WDOT and Flank.

As pointed out above, standing head up mainly reflected what non-feeding animals were doing. Consequently, for the heifers, standing head up was greatest in the Flank treatment and then the WDOT treatment, with least in the Control animals. There was no significant difference between the Control, AI and Electro treatments, or between the AI, WDOT and Electro treatments. For the cows, standing head up was greatest in the Flank, WDOT and Electro groups and least in the AI and Control, although there was no statistical difference between the Control, Electro, WDOT and Flank treatments.

Standing head down was greatest in the Flank treatment for cows, and there was no difference between the other treatments in the level shown. For heifers the levels were no different in the Flank and WDOT treatments and these were significantly higher than the other treatments, between which there were no statistical differences.

The pattern for sternum lying differed between heifers and cows. For the cows the highest level was shown by the Control animals and there was no difference between the other treatments. In the heifers, by contrast, the highest levels were in the Flank and WDOT treatments, then the AI treatment (there was no significant difference between WDOT and AI) and none was shown by the Control and Electro treatments.

The pattern for drinking was also inconsistent between heifers and cows. For heifers, drinking was significantly higher in the Flank treatment, with no significant difference between any of the others. Drinking was also highest in the Flank treatment cows, but there was no significant difference between this level and those of the Control, Electro and WDOT treatments which themselves did not differ significantly. The AI treatment animals showed no drinking.

Treatment had no significant effect on locomotion for either the heifers or cows.

Looking at the overall trends, particularly in relation to the large proportions feeding, suggests that the pain/discomfort was greater in this time period for the cows than for the heifers. However, for both classes of cattle the greatest negative impacts were from both the Flank and WDOT procedures. There was some evidence that the Electro treatment caused some pain/discomfort to the cows during this period, which was not evident in the heifers. Given that the mechanism of electro-immobilisation is to cause muscle contraction/tetany (Molony, 1986; Rushen, 1986) it is probable that cows were more adversely affected than heifers as a consequence of their greater muscle mass. For both cows and heifers, the Flank treatment resulted in relatively high levels of drinking (although it was not significantly different to the Control groups in the cows). This suggests a loss of body fluids (e.g. internal bleeding), with the impact of this on the heifers being greater than on the cows, as a result of their smaller body size and reduced fluid reserves.

4.2.2.3 Behavioural responses between 24 and 96 hours post-procedures

The data used for analysis for the period of 24 to 96 hours post-procedures being conducted on the cattle comprised the scan-samples of the counts of animals in each treatment performing the behaviours when they were in the holding paddocks. There were too few data for many of the behaviours; the ones having sufficient for analyses were feeding, locomotion, standing head up, standing head down, lying on sternum, drinking, ruminating and standing self-licking (Table 4.2-10).

**Table 4.2-10 Behaviours (mean percentages of animals and s.e.) shown by heifers and cows on the treatments during the period 24 hours to 96 hours after treatments were applied (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only)**

| Behaviour | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| *Heifers* | | | | | | |
| Feeding | 64.82 | 65.90 | 69.66 | 63.44 | 61.10 | 2.78 |
| Locomotion | 8.91 | 7.11 | 6.46 | 8.48 | 7.47 | 1.42 |
| Standing head up | 8.97 | 8.94 | 6.98 | 9.68 | 9.97 | 1.28 |
| Standing head down | $0.065^{a}$ | $0.086^{ab}$ | $0.213^{b}$ | $0.216^{b}$ | $0.639^{c}$ | 0.047 |
| Lying sternum | 10.92 | 11.43 | 11.30 | 12.66 | 14.99 | 1.30 |
| Drinking | 0.457 | 0.486 | 0.612 | 0.393 | 0.449 | 0.091 |
| Ruminating | $4.82^{b}$ | $4.55^{b}$ | $3.62^{ab}$ | $4.16^{ab}$ | $2.76^{a}$ | 0.53 |
| Standing self-licking | $0.653^{ab}$ | $1.016^{b}$ | $0.604^{a}$ | $0.358^{a}$ | $1.791^{c}$ | 0.139 |
| *Cows* | | | | | | |
| Feeding | 19.16 | 24.73 | 18.04 | 21.21 | 21.79 | 3.05 |
| Locomotion | 24.11 | 22.13 | 20.16 | 21.14 | 20.10 | 2.77 |
| Standing head up | 24.47 | 27.10 | 31.26 | 29.25 | 26.77 | 2.54 |
| Standing head down | $0.093^{a}$ | $0.203^{a}$ | $0.455^{b}$ | $0.194^{a}$ | $0.803^{c}$ | 0.072 |
| Lying sternum | 23.86 | 17.94 | 24.11 | 21.77 | 22.45 | 2.25 |
| Drinking | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Ruminating | 7.08 | 6.91 | 5.72 | 5.85 | 7.04 | 0.91 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Standing self-licking | $0.529^{bc}$ | $0.349^{b}$ | $0.000^{a}$ | $0.259^{ab}$ | $0.799^{c}$ | 0.120 |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

Judging from the behavioural responses, the negative effects of the procedures had declined during this period compared to the previous periods. However, there were still detectable differences in some behaviours, suggesting that there were still some negative effects on animals. The treatments had effects only on standing head down and standing self-licking for both classes of cattle, and for ruminating in the heifers.

Standing head down was seen at the highest level in the Flank treatment for cows and heifers. For the heifers the lowest level was seen in the Control group, with intermediate levels in the other treatments, although there was no significant difference between Control and AI. For the cows, the second highest level was seen in the Electro group, with no significant difference between the other treatments.

Standing self-licking was seen most often in the Flank group for both heifers and cows and the observations revealed that many licks were directed at the wound area (32.9% for all treatments and 78.1% for Flank). In the heifers, intermediate levels of self-licking were seen in the Control and AI groups and lowest in the Electro and WDOT, although there was no significant difference between the Control, Electro and WDOT groups. For the cows, the lowest level was seen in the Electro group (none performed). There was no significant difference in the level shown in the Control and Flank groups, or between the WDOT and Electro groups.

Treatment affected ruminating only in the heifers, with the Flank groups showing the least and the Control and AI groups showing the most. The levels shown by the Electro and WDOT groups were intermediate and not statistically different to either the Flank group or the Control and AI groups.

The levels of feeding, locomotion and standing head up were markedly different between the heifers and cows. The difference in feeding was probably a consequence of two factors; the greater body and rumen reserves of the cows compared to the heifers as a result of their greater body size and differences in food availability in the paddocks. The area in which the cows were held had less pasture and the cows were given hay from feeders adjacent to the yard complex. This meant that the cows walked between the hay and camping/grazing areas, which probably accounts for the high levels of locomotion recorded compared to the heifers. It would appear that the relatively high levels of standing head up in the cows compared to the heifers simply reflects that the cows were feeding less than the heifers.

Overall, the results indicate that the negative impacts on the cattle had declined by this time, but that behaviours indicative of pain/discomfort was still evident in the Flank treatment for both cows and heifers.

#### 4.2.2.4 Movement through the race and into the crush, and into the headbail, and vocalisations

As described previously, the amount of effort it takes to get animals to return to a place where they have had procedures conducted on them may be indicative of how aversive the animals found those procedures. The scores recorded for both movement through the race and into the crush (race score) and movement into the headbail (headbail score) are given in Table 4.2-11 and Table 4.2-12 respectively.

**Table 4.2-11 Scores (means and s.e.) for heifers and cows for ease of movement (on a scale of 1, easiest, to 9, hardest) along the race and into the crush on the day treatments were applied and at 96**

**hours post-treatment (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only) - significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

| Score | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| *Heifers* | | | | | | |
| Treatment day | 1.95 | 1.54 | 1.78 | 1.94 | 2.13 | 0.20 |
| 96 h | 4.40$^{ab}$ | 2.62$^{a}$ | 3.30$^{ab}$ | 3.00$^{ab}$ | 3.52$^{ab}$ | 0.51 |
| *Cows* | | | | | | |
| Treatment day | 1.65 | 1.73 | 1.43 | 1.50 | 1.34 | 0.20 |
| 96 h | 4.37$^{c}$ | 3.35$^{bc}$ | 1.81$^{a}$ | 2.24$^{ab}$ | 2.21$^{ab}$ | 0.51 |

**Table 4.2-12 Scores (means and s.e.) for heifers and cows for ease of movement (on a scale of 1, easiest, to 9, hardest) from the crush into the headbail on the day treatments were applied and at 96 hours post-treatment (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only)**

| Score | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| *Heifers* | | | | | | |
| Treatment day | 1.59 | 1.38 | 1.38 | 1.73 | 1.61 | 0.17 |
| 96 h | 2.84 | 1.59 | 2.96 | 2.69 | 2.92 | 0.50 |
| *Cows* | | | | | | |
| Treatment day | 1.69 | 1.32 | 1.15 | 1.37 | 1.46 | 0.17 |
| 96 h | 3.35 | 3.88 | 3.11 | 2.72 | 2.72 | 0.50 |

As can be seen, there were no differences between the treatments on the day of treatment (day 0), but at 96 hours there were differences and the cows and heifers responded differently. For the heifers, the Control was significantly higher than the AI treatment, but there was no difference between the Control and the other treatments, or between AI and the other treatments. For the cows, the lowest score was for Electro, although it was not significantly different to the WDOT and Flank treatments. The highest score was for the Control cows and was not significantly different to the AI treatment. The AI treatment was no different to the WDOT and Flank treatments. These findings would indicate that the heifers found all the treatments similarly aversive. For the cows, it would appear that there was, again, little difference, although the Electro treatment cattle were the least aversive and the Control the most, which is counter-intuitive.

Table 4.2-11 also shows that the score for all treatments was higher at 96 hours than on day 0, suggesting that the animals had found their treatments (and subsequent restraint and blood sampling) aversive. This effect was significant for heifers (combined means for all treatments on day 0 and at 96 hours were 1.77 and 3.37 respectively (t-test = 3.6; P<0.01)), but not for cows (combined means for all treatments on day 0 and at 96 hours were 2.64 and 2.80 respectively (t-test = 0.4; n.s.)).

Similar to the race scores, the headbail scores were, in most cases (except AI for heifers), much higher at 96 hours than on the day of treatment, indicating a greater reluctance of both cows and heifers to enter the headbail, suggesting the animals had found the treatments and blood sampling aversive. The combined means for all treatments were 1.59 and 2.60 for day 0 and 96 hours respectively for the heifers (t-test = 4.2; P<0.01) and 1.14 and 3.16 for the cows (t-test = 8.4;

P<0.01). The degree of aversiveness would appear to be similar for all treatments, as there were no significant differences.

Table 4.2-13 shows the count of vocalisations by heifers and cows during the treatments and when subsequently restrained for blood sampling. Effect of treatment was almost significant (P = 0.056) on the day of treatment. For the heifers, vocalisations on Electro and Flank were 104.3% and 81.5% respectively higher than the Controls and for cows, vocalisations on the Flank treatment were 143.1% higher than the Controls.

**Table 4.2-13 Counts (means and s.e.) for heifers and cows of vocalisations during physical restraint on the day treatments were applied and at 96 hours post-treatment (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only)**

| Score | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| *Heifers* | | | | | | |
| Treatment day | 1.60 | 1.73 | 3.26 | 1.28 | 2.90 | 0.86 |
| 96 h | 0.71 | 1.24 | 0.14 | 0.50 | 0.35 | 0.40 |
| *Cows* | | | | | | |
| Treatment day | 1.94 | 1.31 | 1.34 | 2.10 | 4.72 | 0.86 |
| 96 h | 1.12 | 1.30 | 1.63 | 0.80 | 0.80 | 0.40 |

The numbers of vocalisations were high initially i.e. at the time that the procedures were conducted and declined over time, as can be seen in Figure 4.2-8 and Figure 4.2-9. T-test comparisons between the mean vocalisations at the time of the procedures and those for the remainder of day 0 were significantly different for all treatments and for the Flank and Electro treatments (these were selected for investigation because of their high values) for both heifers and cows (Table 4.2-14).

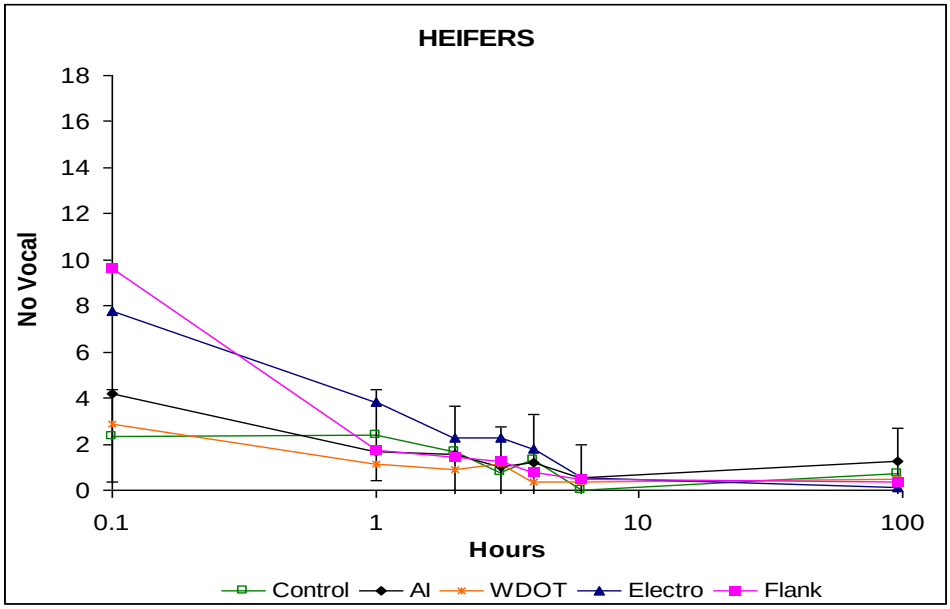



**Figure 4.2-8 Number of vocalisations for heifers from the time treatments were applied (0.1 on the graph) and when animals were restrained for blood sampling (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro =**

**electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only)**

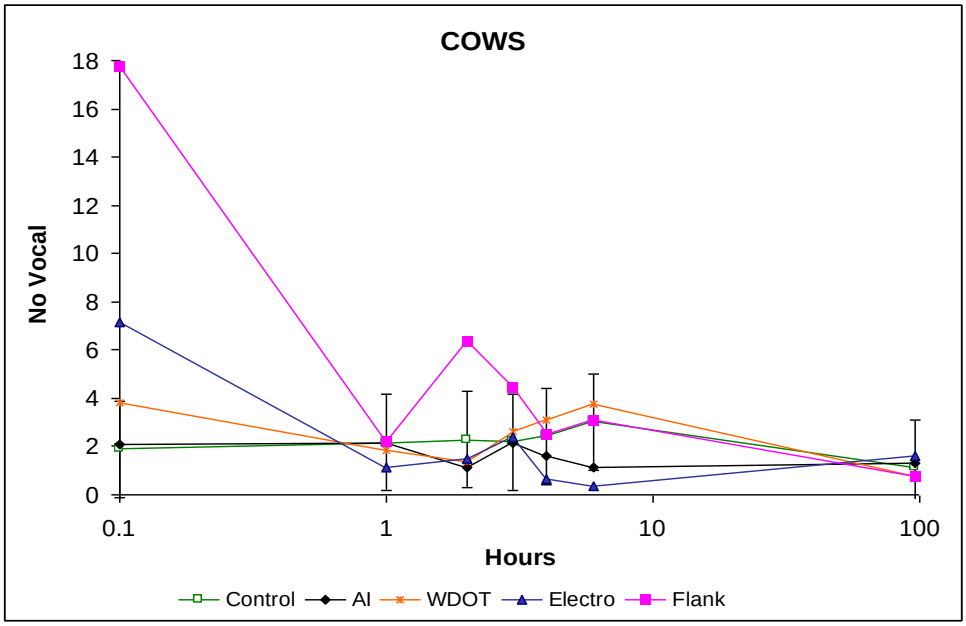



**Figure 4.2-9 Number of vocalisations for cows from the time treatments were applied (0.1 on the graph) and when animals were restrained for blood sampling (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only)**

**Table 4.2-14 Comparison of the mean number of vocalisations for heifers and cows between the time treatments were applied (time 0) and for the remainder of that day (hours 1-6) when animals were restrained for blood sampling only (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = physical restraint only)**

| Vocals | Heifers | | | Cows | | |
|---|---|---|---|---|---|---|
| | Time 0 mean | Hours 1-6 mean | t-test | Time 0 mean | Hours 1-6 mean | t-test |
| All treatments | 5.36 | 1.27 | 5.6** | 6.55 | 2.31 | 5.8** |
| Flank | 9.60 | 1.14 | 5.5** | 17.78 | 3.73 | 9.2** |
| Electro | 7.80 | 2.15 | 3.7** | 7.15 | 1.21 | 3.9** |

**** P< 0.01**

Based on Grandin's (2001) statement that vocalisation is associated with aversive events, it would appear that heifers and cows found electroimmobilisation and flank spaying aversive. As flank spaying also involved electroimmobilisation it is not possible to separate the effects of the two procedures, but given that numbers of vocalisations were greater for the Flank than Electro treatments it would appear the the effects of the two procedures were cumulative. This is evidence that Electro treatment does not provide effective analgesia.

4.2.2.5 Flight speed

Table 4.2-15 shows the flight speed (FS) recordings for both heifers and cows on the day that treatments were imposed and at 24 hours and 96 hours post-procedures. There was no effect of

treatment, but a comparison between the mean FS on day 0 and the mean FS at 96 hours for all treatments combined showed that FS decreased for both heifers and cows (heifers: 2.81 v. 2.48 m/s, t = 3.7, P<0.01; cows: 2.62 v. 2.33 m/s, t = 3.2, P<0.01). Petherick et al. (2005) have reported a similar finding, which they suggest is due to the animals becoming accustomed to the handling routine and learning to move out from the crush.

**Table 4.2-15 Flight speed (m/s) (means and s.e.) of heifers and cows on the day treatments were applied and at 24 hours and 96 hours post-treatment (Flank = flank spayed with electroimmobilisation; WDOT = dropped ovary technique of spaying; Electro = electroimmobilisation; AI = mock artificial insemination; and Control = restraint only).**

| Score | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | AI | Electro | WDOT | Flank | |
| *Heifers* | | | | | | |
| Treatment day | 2.66 | 2.76 | 2.90 | 2.46 | 2.63 | 0.10 |
| 24 h | 2.34 | 2.66 | 2.52 | 2.25 | 2.41 | 0.14 |
| 96 h | 2.21 | 2.44 | 2.33 | 2.16 | 2.46 | 0.13 |
| *Cows* | | | | | | |
| Treatment day | 2.26 | 2.43 | 2.53 | 2.47 | 2.43 | 0.10 |
| 24 h | 2.14 | 2.43 | 2.53 | 2.47 | 2.37 | 0.14 |
| 96 h | 2.19 | 2.23 | 2.42 | 2.39 | 2.38 | 0.13 |

The fact that treatment per se did not affect FS lends further support to the hypothesis that flight speed reflects the innate agitation of cattle and that it is difficult to modify through handling and procedures imposed on the animals.

### 4.2.3 Production responses

Liveweight and fat depth were monitored to detect production responses to the procedures. Animals were allocated on liveweight and therefore, no differences were detected between treatment groups at 0 hours (Heifers: grand mean 208.3 ± 4.3 kg s.e., P=0.899; Cows: grand mean 439.4 ± 14.5 kg s.e., P=0.903). Across treatments all cattle experienced modest weight loss during the study, however there were no significant differences in the magnitude of loss between groups.

**Table 4.2-16 Production responses of Heifers and Cows to procedures during Study II. (Control = restraint only; A.I. = restraint + mock artificial insemination; Electro. = restraint + electroimmobilisation; WDOT = restraint, spayed using dropped ovary technique and application of spay mark pliers; and Flank = restraint, "web" spayed with electroimmobilisation and application of spay mark pliers)**

| Variable | Treatment | | | | | s.e. |
|---|---|---|---|---|---|---|
| | Control | A.I. | Electro. | WDOT | Flank | |
| *Heifers* | | | | | | |
| Liveweight (kg) (4 d) | 205.5 | 206.3 | 208.1 | 203.8 | 206.9 | 1.4 |
| Liveweight (kg) (21 d) | 208.8 | 208.7 | 209.7 | 209.7 | 208.7 | 1.3 |
| Liveweight (kg) (42 d) | 208.4 | 207.1 | 207.3 | 205.6 | 206.7 | 1.3 |
| LWG (kg/d) (0-4d) | -0.74 | -0.63 | -0.08 | -1.17 | -0.33 | 0.36 |
| LWG (kg/d) (0-21d) | 0.014 | -0.008 | 0.055 | 0.053 | 0.018 | 0.07 |
| LWG (kg/d) (0-42d) | -0.009 | -0.045 | -0.033 | -0.074 | -0.044 | 0.09 |
| Fat Depth at P8 site (mm) (21d) | 1.05 | 1.00 | 1.20 | 1.00 | 1.29 | 0.10 |
| Fat Depth at P8 site (mm) (42d) | 1.12 | 1.30 | 1.25 | 1.15 | 1.36 | 0.13 |
| *Cows* | | | | | | |
| Liveweight (kg) (4 d) | 441.2 | 435.8 | 436.9 | 437.7 | 441.0 | 3.1 |
| Liveweight (kg) (21d) | 448.8 | 446.0 | 441.9 | 438.0 | 435.3 | 3.6 |
| Liveweight (kg) (42d) | 443.7 | 442.4 | 437.2 | 433.8 | 427.3 | 4.3 |
| LWG (kg/d) (0-4 d) | -0.29 | -1.62 | -1.40 | -1.20 | -0.36 | 0.76 |
| LWG (kg/d) (0-21d) | 0.304 | 0.146 | -0.056 | -0.207 | -0.321 | 0.18 |
| LWG (kg/d) (0-42d) | 0.025 | -0.030 | -0.117 | -0.200 | -0.359 | 0.10 |
| Fat Depth at P8 site (mm) (21d) | 16.3 | 14.0 | 11.7 | 19.6 | 8.8 | 3.2 |
| Fat Depth at P8 site (mm) (42d) | $17.1^{a}$ | $7.6^{bc}$ | $9.6^{bc}$ | $14.4^{ab}$ | $6.3^{c}$ | 2.4 |

**Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different**

As outlined previously, fat measurements were recorded at 21 and 42 days post procedure at the P8 site. No treatment differences were detected in mean P8 fat measurements at both 21 and 42 days for heifers (P=0.152 and P=0.703 respectively). Similarly for the cows no difference in fat measurements were detected between treatment groups at 21 days post procedure (P=0.170). However, a significant difference between treatment groups was recorded in cows at 42 days (P=0.013). The WDOT and Control recorded greater fat depths than AI, Electro and Flank treatment groups at 42 days (P<0.05).

### 4.2.4 Morbidity and mortality

The health status of the cattle was visually assessed twice daily from day 1 to 4, then daily until day 7 then at days 21 and 42 after procedures. Immediately after release from the crush, three flank spayed cows showed signs of mild to moderate left radial nerve palsy. They were monitored throughout the day and all had significantly improved by 8 hours after procedures, although one cow still had an abnormal gait which persisted.

A summary of the wound healing scores is presented in
**Table 4.2-17**. Sixteen percent of flank spayed heifers showed signs of delayed or abnormal wound healing (wound scores 3 and 4), but all the flank incisions in the cows had either healed or were partially healed without any evidence of wound infection.

**Table 4.2-17 Frequency of wound healing scores at 21 and 42days after flank spaying**

| Class | Day | n | Wound Score |
|---|---|---|---|

| | | | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| Heifers | 21 | 18 | 0% | 72% | 22% | 6% | 0% |
| | 42 | 19 | 68% | 16% | 11% | 5% | 0% |
| Cows | 21 | 8 | 0% | 63% | 25% | 0% | 13% |
| | 42 | 8 | 88% | 13% | 0% | 0% | 0% |

**(1, Wound healed; 2, Wound partially healed and clean and dry; 3, Wound partially healed and discharge; 4, Little or no healing of wound and clean and dry; 5, Little or no healing of wound and discharge)**

Only one mortality directly associated with the procedures was confirmed: one flank heifer died at day 5 with acute diffuse peritonitis. Two control cows and one flank heifer were missing at days 21 and 42. No carcasses were found in the holding paddock. As the paddock was mustered by helicopter it is concluded these cattle had strayed into an adjoining paddock.

### 4.2.5 Summary of major findings

For both heifers and cows, changes in behaviour throughout the experimental period would indicate that the Flank treatment caused the greatest pain/discomfort. For the heifers, the WDOT treatment ranked second for the indicators of pain/discomfort throughout the experiment, as evidenced by behavioural responses, although by the 24 to 96 hours phase there was some evidence of pain/discomfort in the Electro group, which may have arisen from muscle soreness and stiffness. In the 0 to 8 hours phase there was some evidence that the AI treatment caused some pain/discomfort.

For the cows, there was little difference between the treatments, other than Flank, although in the 8 to 24 hours phase the WDOT and Electro appeared to be causing more pain and discomfort compared to the AI and Control and in the 24 to 96 hours phase, as with the heifers, there was some evidence of pain/discomfort in the Electro group, probably resulting from muscle soreness and stiffness.

The heifers and cows in all groups appeared to find the procedure of physical restraint, treatment application and blood sampling aversive, as indicated by their greater reluctance to move through the race, into the crush and headbail at 96 hours compared to the day of treatment. Judging from this reluctance, it would appear that there was little difference in the perception of the aversiveness of the different treatments, including the Control. However, the number of vocalisations indicated otherwise, with higher numbers of vocalisations in the Flank and Electro treatments compared to the others which suggests cows and heifers found these treatments aversive.

Standing head down met Mellor et al.' s (2000) description of a useful indicator of pain, as it was seen to a greater extent in treated animals than in controls, and its incidence declined over time as the pain/discomfort receded.

Flight speed was unaffected by treatments lending further support to the suggestion that it is a measure of a trait that is largely innate and difficult to modify through handling and procedures imposed on cattle.
Timelines of the percentage changes in each measure of the physiological and behavioural response relative to the control heifers and cows for each group are presented in Figure 4.2-10 to Figure 4.2-17, with significant effects ($P< 0.05$) shown in underlined, bold text. The reader is cautioned to ensure they interpret the percentage changes in the occurrence of specific behaviours relative to the controls for each treatment, in light of the number of animals displaying these behaviours during the specified period (data presented in Tables 4.2-8 to 4.2-10). Summary Tables

show comparisons between specific procedures: Table 4.2-18 shows Flank v. WDOT; Table 4.2-19 shows Flank v. Electro; Table 4.2-20 shows WDOT v AI; and Table 4.2-21 shows AI v Control. These comparisons are shown because it is important to see how animals responded to the two spaying procedures, how they responded to the spaying procedure and its particular "control" treatment (i.e., Flank v Electro and WDOT v AI) and how they responded to AI, which may be regarded as the least invasive and inducing the smallest increases in indicators of pain and discomfort, in comparison to the physical restraint control.

The findings summarised in the summary Figures and Tables demonstrate that the flank spay procedure induces significant increases in the physiological indicators of acute pain/discomfort, inflammation and muscle damage, albeit the latter response was considered mild. It also impacted on behaviour, reducing feeding in the acute phase and ruminating in the longer term. Standing head down, which appears to reflect pain, was markedly increased up to 96 hours and animals showed higher self-grooming, which was mainly targeted to the wound site. When compared to the responses for the other procedures it is clear that flank spaying induces the greatest physiological and behavioural response of all the procedures examined.

As the mean muscle enzyme responses in the group which were physically restrained and then had the electroimmobiliser applied for 1 minute was similar to the responses in the group which were spayed by the flank procedure (Figure 4.2-10**,** Figure 4.2-11, Figure 4.2-13 and Figure 4.2-14), it is reasonable to conclude that electroimmobilisation alone is contributing significantly to the observed sustained mild increase in muscle enzymes in the flank spayed cattle. In addition, the significant percentage increases in bound and unbound cortisol for the electroimmobilsed cows indicates that electroimmobilisation may be contributing significantly to the observed significant increase in cortisol in flank spayed cows.

WDOT spaying induced significant increases in the physiological indicators of acute pain/discomfort and standing head down, indicative of short-term (up to 24 hours) pain and discomfort particularly in cows. Interestingly cows, but not heifers, showed a marked inflammatory response in the 24 to 96 hours period.

AI had little impact on physiological and behavioural responses, other than an acute (0 to 8 hours) cortisol response by the cows and a later (24 to 96hours) cortisol response in the heifers.

Generally there was good agreement between the direction and magnitude of physiological and behavioural responses to each procedure.

The comparison between Flank and WDOT (Table 4.2-18) clearly illustrates that the impacts were greater in Flank than WDOT and that these impacts were greater in heifers than cows. A comparison between Flank and Electro (Table 4.2-19) supports what has been stated above; in the cows there were many similarities in responses between the procedures, although flank spaying evoked greater cortisol release and standing head down. These results suggest that electroimmobilisation makes a significant contribution to the adverse responses seen in the flank spayed cows. In contrast, for the heifers, flank spaying resulted in a greater inflammatory response and more animals standing head down indicating that it was more painful and stressful than electroimmobilisation. The comparison between WDOT and AI (Table 4.2-20) shows that there were few differences for the heifers, although there was some behavioural evidence of pain in the 8 to 24 hours period. In contrast, the cows showed greater physiological (cortisol) responses to WDOT than AI, suggesting that WDOT was the more stressful/painful procedure. The comparison between AI and the restraint control (Table 4.2-21) shows negligible differences between the procedures for heifers, but cows showed an acute cortisol response and signs of muscle damage to AI.


**Figure 4.2-10 Percentage changes in measures of the physiological, behavioural and production responses of flank spayed heifers relative to control heifers. (NO = not observed in control group). Underlined bold measurements are significantly ($p<0.05$) different to controls.**

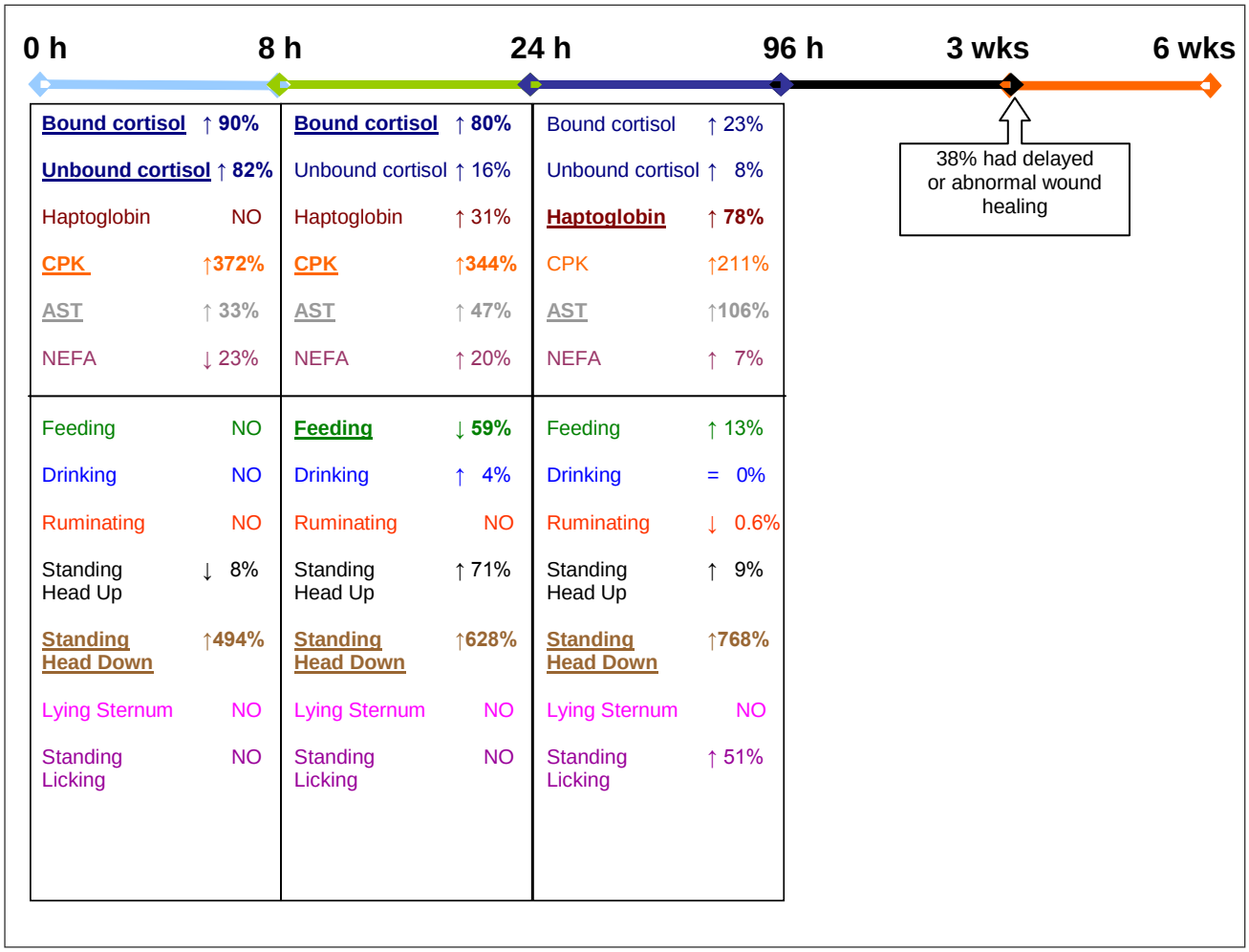



**Figure 4.2-11 Percentage changes in measures of the physiological, behavioural and production responses of flank spayed cows relative to control cows. (NO = not observed in control group). Underlined bold measurements are significantly (p<0.05) different to controls.**

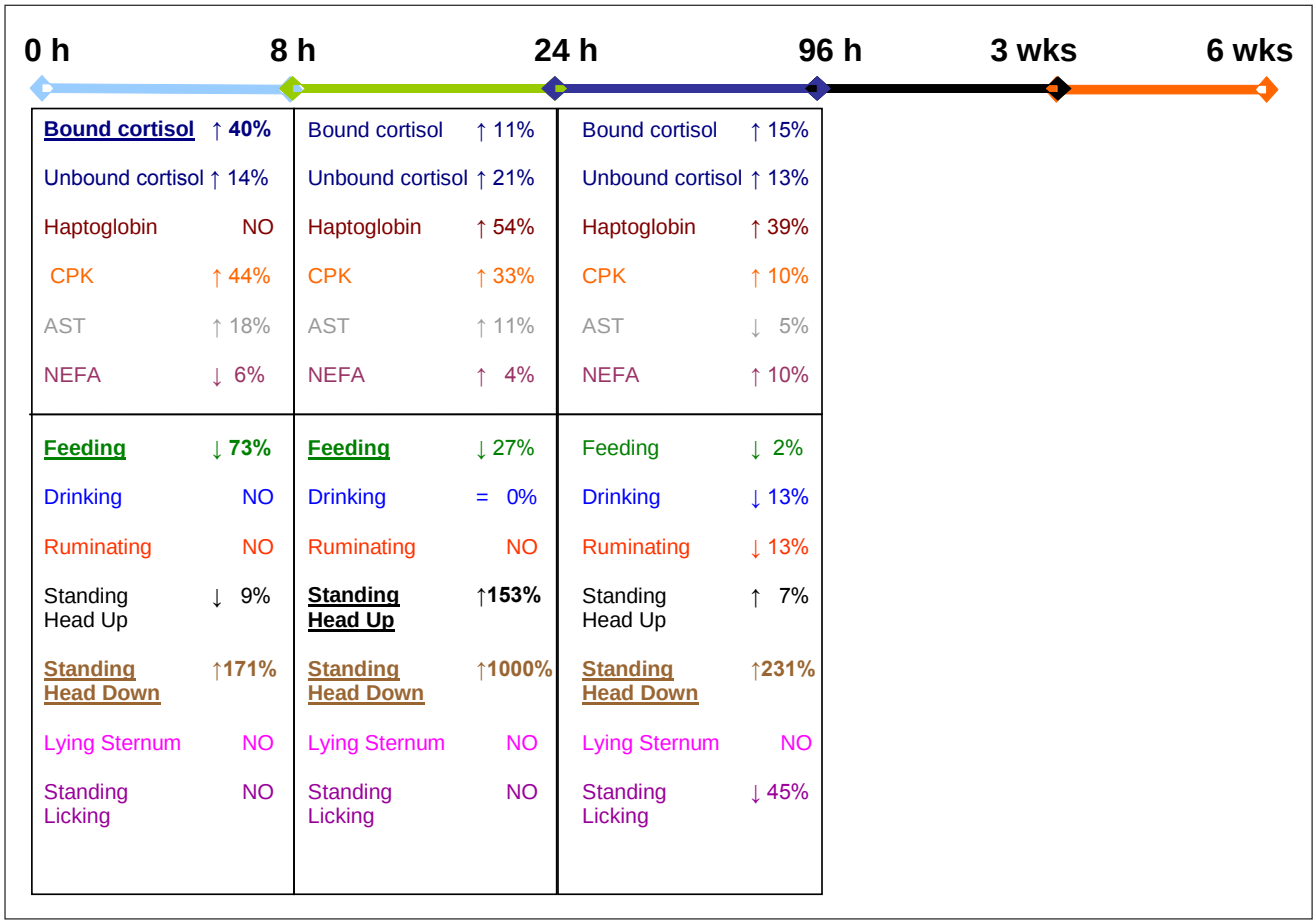



**Figure 4.2-12 Percentage changes in measures of the physiological, behavioural and production responses of WDOT spayed heifers relative to control heifers. (NO = not observed in control group). Underlined bold measurements are significantly ($p<0.05$) different to controls.**

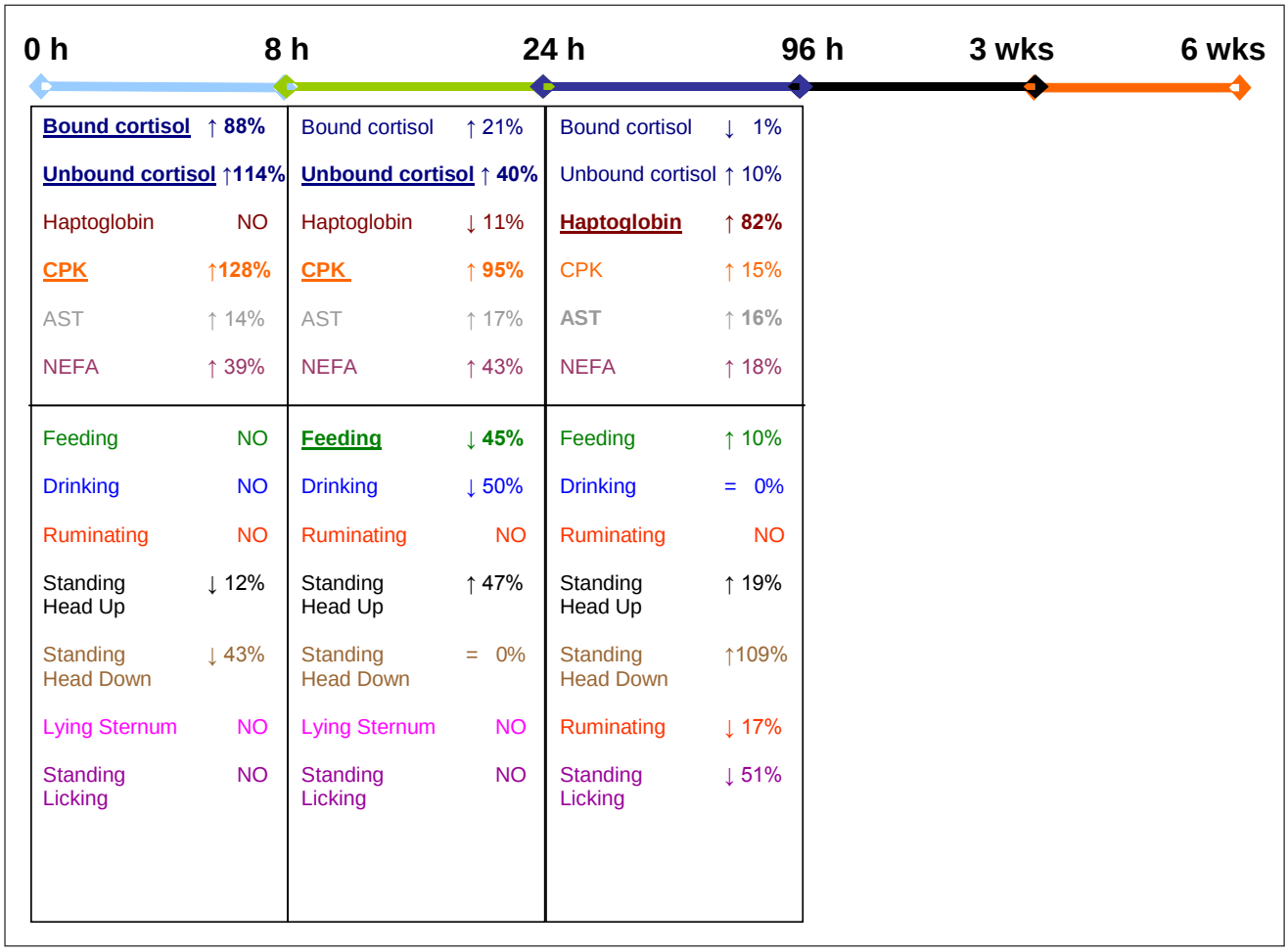



**Figure 4.2-13 Percentage changes in measures of the physiological, behavioural and production responses of WDOT spayed cows relative to control cows (NO = not observed in control group). Underlined bold measurements are significantly ($p<0.05$) different to controls.**

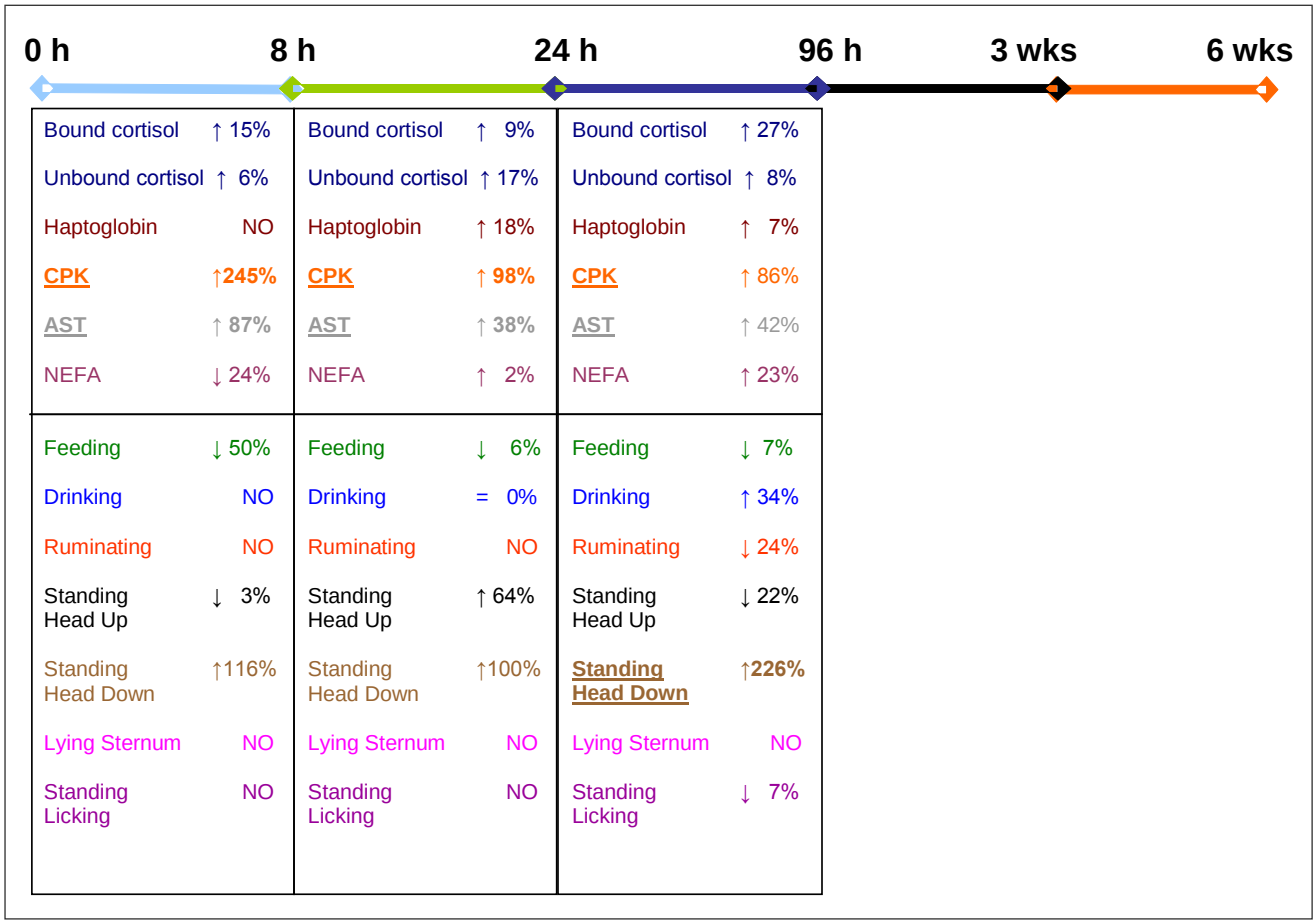



**Figure 4.2-14 Percentage changes in measures of the physiological, behavioural and production responses of electroimmobilised heifers relative to control heifers. Underlined bold measurements are significantly ($p<0.05$) different to controls.**

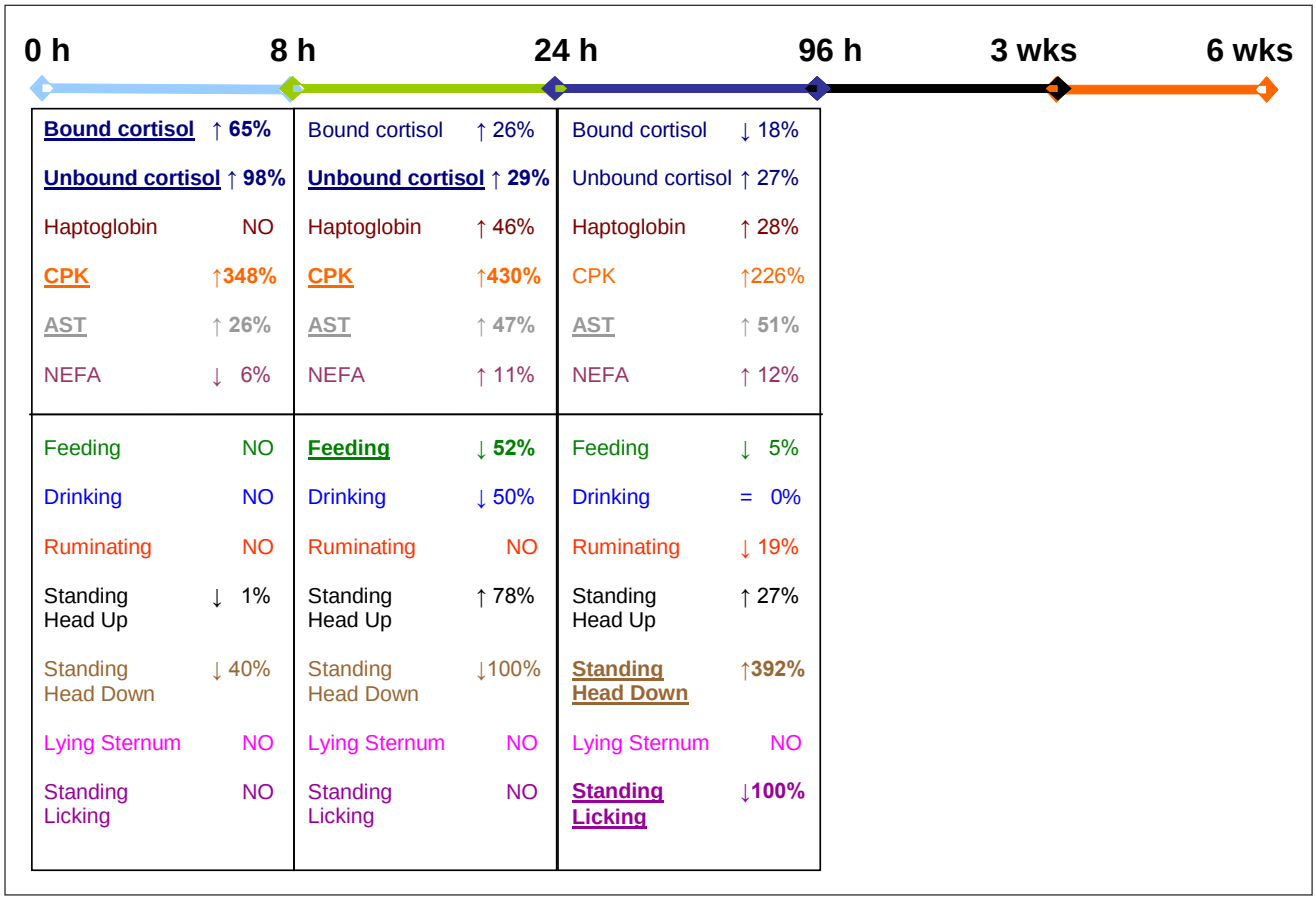



**Figure 4.2-15 Percentage changes in measures of the physiological, behavioural and production responses of electroimmobilised cows relative to control cows. (NO = not observed in control group). Underlined bold measurements are significantly ($p<0.05$) different to controls.**

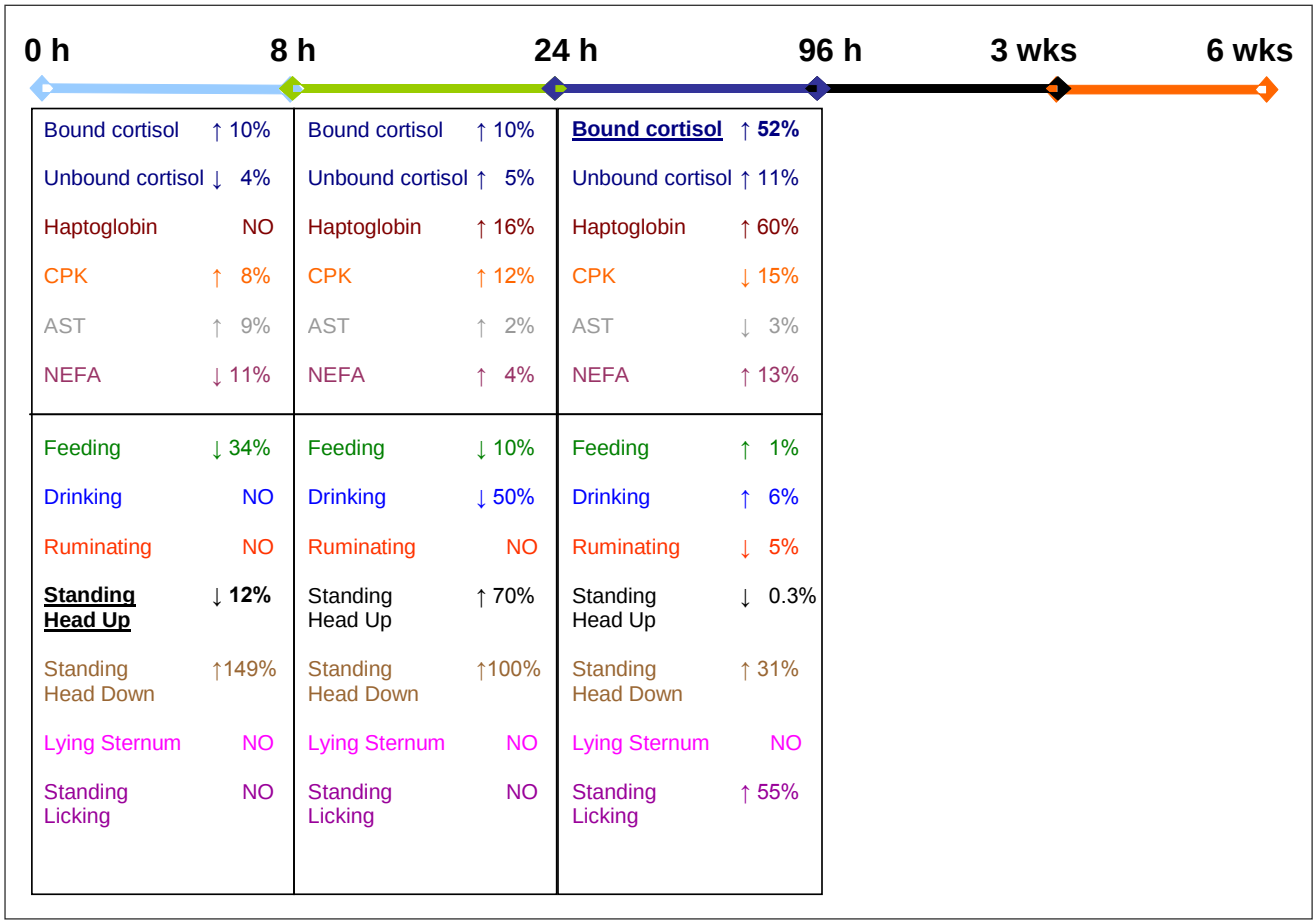



**Figure 4.2-16 Percentage changes in measures of the physiological, behavioural and production responses of artificially inseminated heifers relative to control heifers. (NO = not observed in control group). Underlined bold measurements are significantly ($p<0.05$) different to controls.**

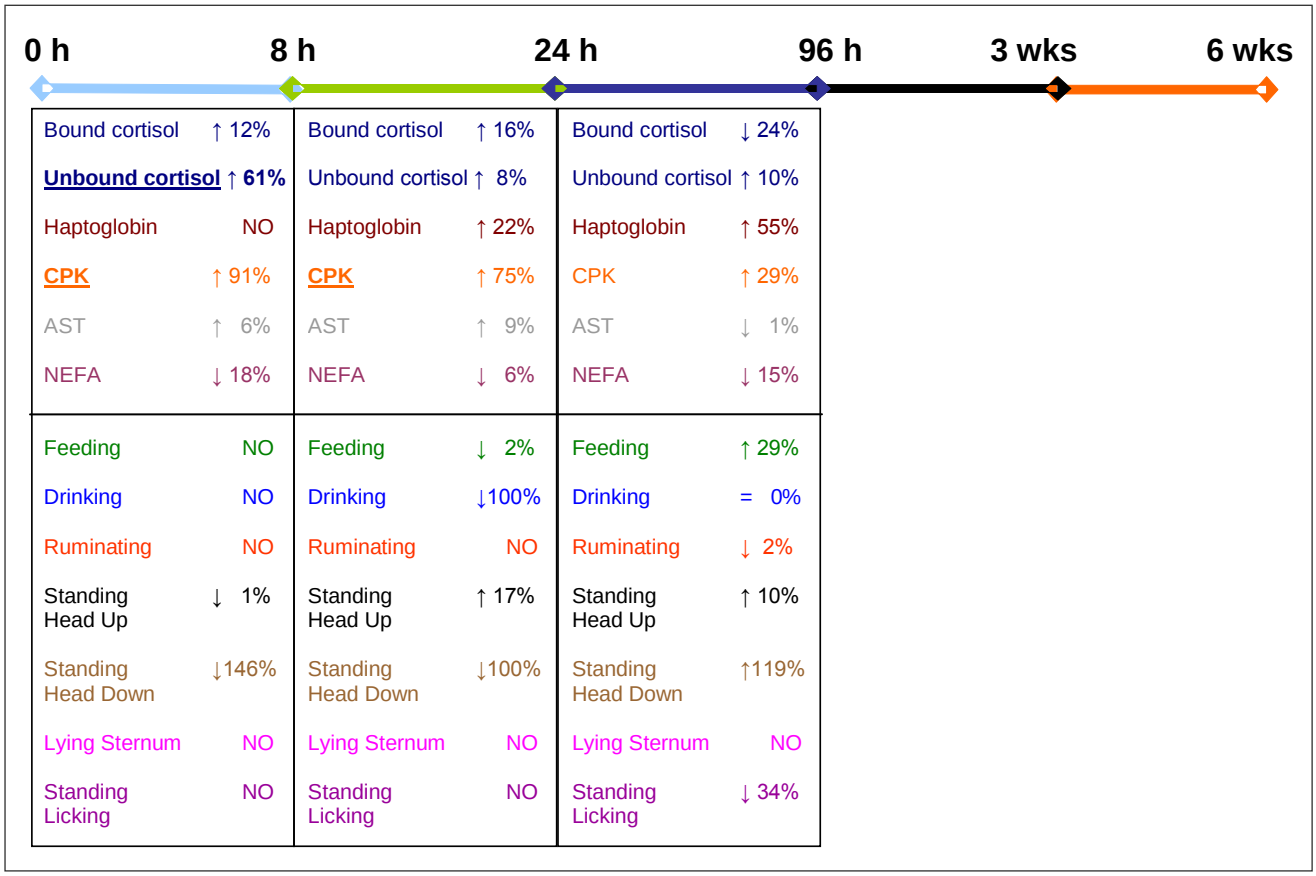



**Figure 4.2-17 Percentage changes in measures of the physiological, behavioural and production responses of artificially inseminated cows relative to control cows. (NO = not observed in control group). Underlined bold measurements are significantly (p<0.05) different to controls.**

**Table 4.2-18 Summary comparison between WDOT and flank spaying for parameters for which there was a significant effect of treatment (ns = no significant difference (P>0.05) between flank and WDOT; ↑ indicates significantly (P< 0.05) higher; ↓ indicates significantly (P< 0.05) lower**

| Parameter | Heifers | | | Cows | | |
|---|---|---|---|---|---|---|
| | 0-8 h | 8-24 h | 24-96 h | 0-8 h | 8-24 h | 24-96 h |
| Bound cortisol | ns | | ns | ns | flank↑ | ns |
| Unbound cortisol | | | | ns | ns | |
| Haptoglobin | | flank ↑ | flank↑ | | | ns |
| CPK | flank↑ | flank↑ | flank↑ | flank↑ | flank↑ | |
| AST | flank↑ | flank↑ | flank↑ | ns | ns | flank↑ |
| NEFA | | | | ns | | |
| Feeding | ns | flank↓ | | flank↑ | ns | |
| Drinking | | flank↑ | | | ns | |
| Ruminating | | | ns | | | |
| Standing head up | flank↓ | flank↑ | | | ns | |
| Standing head down | flank↑ | ns | flank↑ | flank↑ | flank↑ | flank↑ |
| Lying sternum | | ns | | | ns | |
| Self-licking | | | flank↑ | | | flank↑ |

**Table 4.2-19 Summary comparison between electroimmobilisation and flank spaying for parameters for which there was a significant effect of treatment (ns = no significant difference (P>0.05) between flank and electroimmobilisation; ↑ indicates significantly (P< 0.05) higher; ↓ indicates significantly (P< 0.05) lower**

| Parameter | Heifers | | | Cows | | |
|---|---|---|---|---|---|---|
| | 0-8 h | 8-24 h | 24-96 h | 0-8 h | 8-24 h | 24-96 h |
| Bound cortisol | ns | | ns | ns | flank↑ | flank↑ |
| Unbound cortisol | | | | ns | ns | |
| Haptoglobin | | flank ↑ | flank↑ | | | ns |
| CPK | ns | ns | ns | ns | ns | |
| AST | ns | ns | ns | ns | ns | flank↑ |
| NEFA | | | | ns | | |
| Feeding | ns | flank↓ | | flank↑ | ns | |
| Drinking | | flank↑ | | | ns | |
| Ruminating | | | ns | | | |
| Standing head up | flank↓ | flank↑ | | | ns | |
| Standing head down | flank↑ | flank↑ | flank↑ | flank↑ | flank↑ | flank↑ |
| Lying sternum | | flank↑ | | | ns | |
| Self-licking | | | flank↑ | | | flank↑ |

**Table 4.2-20 Summary comparison between AI and WDOT spaying for parameters for which there was a significant effect of treatment (ns = no significant difference (P>0.05) between AI and WDOT; ↑ indicates significantly (P< 0.05) higher; ↓ indicates significantly (P< 0.05) lower**

| Parameter | Heifers | | | Cows | | |
|---|---|---|---|---|---|---|
| | 0-8 h | 8-24 h | 24-96 h | 0-8 h | 8-24 h | 24-96 h |
| Bound cortisol | ns | | ns | WDOT↑ | WDOT↑ | ns |
| Unbound cortisol | | | | WDOT↑ | WDOT↑ | |
| Haptoglobin | | ns | ns | | | ns |
| CPK | ns | ns | ns | ns | ns | |
| AST | ns | ns | ns | ns | ns | ns |
| NEFA | | | | WDOT↑ | | |
| Feeding | ns | WDOT↓ | | ns | WDOT↓ | |
| Drinking | | ns | | | ns | |
| Ruminating | | | ns | | | |
| Standing head up | ns | ns | | | WDOT↑ | |
| Standing head down | ns | WDOT↑ | ns | ns | ns | ns |
| Lying sternum | | ns | | | ns | |
| Self-licking | | | WDOT↓ | | | ns |

**Table 4.2-21 Summary comparison between Control and AI for parameters for which there was a significant effect of treatment (ns = no significant difference (P>0.05) between Control and AI; ↑ indicates significantly (P< 0.05) higher; ↓ indicates significantly (P< 0.05) lower**

| Parameter | Heifers | | | Cows | | |
|---|---|---|---|---|---|---|
| | 0-8 h | 8-24 h | 24-96 h | 0-8 h | 8-24 h | 24-96 h |
| Bound cortisol | ns | | ns | ns | ns | ns |
| Unbound cortisol | | | | AI↑ | ns | |
| Haptoglobin | | ns | ns | | | ns |
| CPK | ns | ns | ns | AI↑ | AI↑ | |
| AST | ns | ns | ns | ns | ns | ns |
| NEFA | | | | ns | | |
| Feeding | ns | ns | | ns | ns | |
| Drinking | | ns | | | AI↓ | |
| Ruminating | | | ns | | | |
| Standing head up | AI↓ | ns | | | ns | |
| Standing head down | ns | ns | ns | ns | ns | ns |
| Lying sternum | | AI↑ | | | AI↓ | |
| Self-licking | | | ns | | | ns |

## 4.3 Study III

### 4.3.1 Behavioural responses

The incidence of only a limited number of behaviours were able to be determined following spaying in Study IIIa (Table 4.3-1). The most obvious difference between groups was the occurrence of a persistently elevated tail in nearly 1 in 10 of WDOT spayed heifers (Table 4.3-1). This behaviour was expressed immediately after the procedure and then intermittently up to 48 hours after the procedure in a small number of heifers. This behaviour has been reported by others following WDOT spaying (Habermehl,1993; Jubb et al , 2003). A small amount of loose hay was in the holding yards and a higher proportion of control heifers compared to spayed heifers were observed eating.

**Table 4.3-1 Average incidence of different behaviours during each observation period of 0 to 6 hours after spaying – Study IIIa .**

| Behaviour | Treatment | | | Chi-square | Probability |
|---|---|---|---|---|---|
| | Control (n=200) | WDOT (n=200) | Flank (n=199) | | |
| Tail elevated | 0% | 9% | 0% | 37.10 | <0.001 |
| Lying down | 8% | 11% | 12% | 1.87 | 0.392 |
| Drinking | 3% | 2% | 3% | 0.51 | 0.773 |
| Feeding | 11% | 6% | 2% | 13.71 | 0.001 |

### 4.3.2 Production responses

Liveweight and fat depth were monitored to detect production responses to the procedures in Study IIIa only. The treatment groups were similar in liveweight at the time of procedures (grand mean = 233.7 ± 2.1 kg s.e., P=0.862). The mean liveweight of treatment groups were found to be different at 21 and 42 d post procedure (P<0.001 and P<0.001 respectively). The control animals were heaver than both spay treatment methods at 21 and 42 d (P<0.05 and P<0.05 respectively). At d 42, the control heifers were recorded as being approximately 10 kg heavier than the spayed heifer. No differences in liveweight were detected between spaying methods (P>0.05).

**Table 4.3-2 Mean liveweight (kg) and Liveweight Gain (LWG; kg/d) of Study IIIa heifers, Pigeon Hole Station NT. (Control = restraint only; WDOT = restraint, spayed using dropped ovary technique and application of spay mark pliers; and Flank = restraint, "web" spayed with electroimmobilisation and application of spay mark pliers)**

| Variable | Treatment | | | s.e. |
|---|---|---|---|---|
| | Control | WDOT | Flank | |
| Liveweight (kg) (21 d) | 245.2[a] | 242.8[b] | 241.9[b] | 0.56 |
| Liveweight (kg) (42 d) | 249.9[a] | 238.7[b] | 238.3[b] | 0.55 |
| LWG (kg/d) (0-21 d) | 0.552[a] | 0.435[b] | 0.391[b] | 0.027 |
| LWG (kg/d) (21-42 d) | 0.223[a] | -0.202[b] | -0.173[b] | 0.022 |
| LWG (kg/d) (0-42 d) | 0.390[a] | 0.124[b] | 0.111[b] | 0.013 |

Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different

All treatment groups recorded an increase in liveweight across the duration of the study. The WDOT and Flank spayed heifers had significantly lower liveweight gains than control groups during 0-21 d, 21-42 d and 0-42 d (P<0.001, P<0.001 and P<0.001 respectively) and whilst the WDOT treatment group usually recorded higher liveweight gains than the flank group, differences were not statistically significant. These findings are similar to those reported by Jubb et al. (2003) who reported that during the two months following procedures heifers spayed using the WDOT lost less weight than flank spayed heifers, but more than control heifers. These authors also reported that in the subsequent two months, flank spayed heifers grew similarly to the WDOT and control heifers and by the end of the trial (about 12 months after treatment) there were no significant differences in liveweight between the WDOT and flank spayed animals. Unfortunately, the crucial comparison with the control animals was not possible as most of these animals were pregnant by the end of the study.

There appears to be limited information on whether spayed heifers regain lost weight or display any compensatory gain. Jeffery et al. (1997) reporting work conducted in northern Australia found no effect of spaying on daily weight gains up to 8 weeks post spaying, but at the end of the experiment (about 15 months after treatment) the non-spayed heifers were significantly heavier than the flank

spayed heifers and the overall weight gains of non-spayed heifers was significantly higher than that of spayed heifers. These findings are similar to other studies outlined below which were conducted in USA where entire heifers recorded higher growth rates than spayed heifers: For example work by Cain et al. (1986) reported reduced liveweight gains in spayed heifers (flank and a technique similar to WDOT) compared to controls during both a 0-90 d and 91-150 d. Garber et al. (1990) found that WDOT spayed heifers were 20 kg lighter at the conclusion of the study (lot fed for at least 128 d) than the non-spayed animals and the WDOT spayed heifers grew 0.14 kg/d slower than non-spayed heifers. However, these differences were not found to be significant. In a study conducted in southern Australia using Hereford and Hereford cross heifers that were flank spayed using a local anaesthetic and treated with benzathine penicillin, Saul et al. (1982) found no differences in growth rates of flank spayed and entire heifers.

Garber et al. (1990) reported a non-significant tendency for the spayed heifers to have better feed conversion efficiencies than control animals, suggesting some compensatory weight gain. However, under northern grazing regimes compensation may occur; Kidd and McLennan (1998) suggest that growing cattle usually only achieve a portion of their potential compensatory weight gain due to the wet season and pasture growth period being too short to support complete compensation. It therefore seems unlikely that during a normal season, spayed heifers would recover the weight lost resulting from spaying particularly if heifers were not supplemented during the dry season.
The treatment groups were similar in fat depth at the time of procedures (grand mean = 2.08 $\pm$ 0.05, P= 0.864) and 21 d after procedures (P>0.05) (Table 4.3-3). At 42 d after treatment, control animals were found to have less fat at the P8 site than the WDOT and flank spayed heifers (P<0.05). This finding should be interpreted with some caution as the depth of fat in the study heifers was frequently at the limit of detection of the device used. Other research has indicated that spaying did not influence fat depth or carcass characteristics (Jeffery et al, 1977 and Saul et al, 1982).

**Table 4.3-3 Mean Fat depth (mm) of Study IIIa heifers, Pigeon Hole Station NT. (Control = restraint only; WDOT = restraint, spayed using dropped ovary technique and application of spay mark pliers; and Flank = restraint, "web" spayed with electroimmobilisation and application of spay mark pliers)**

| Fat Depth (mm) | Treatment | | | | | | s.e. |
|---|---|---|---|---|---|---|---|
| | Control | | WDOT | | Flank | | |
| | n | Mean | n | Mean | n | Mean | |
| 0 d | 197 | 2.11 | 195 | 2.08 | 195 | 2.04 | 0.09 |
| 21 d | 194 | 1.87 | 185 | 2.01 | 189 | 1.92 | 0.07 |
| 42 d | 117 | 1.78[a] | 82 | 2.08[b] | 63 | 2.08[b] | 0.06 |

Significant differences at P<0.05 are shown by superscripts; means followed by a common letter within rows are not significantly different

### 4.3.3 Morbidity and mortality

Overall in Study IIIa a higher proportion of flank versus WDOT spayed heifers showed clinical signs consistent with mild to severely reduced health status within 84 hours of spaying, although differences were not statistically significant (Table 4.3-4). There was considerable variation in the final outcome of heifers observed to be recumbent, with some over a period of several days without intervention recovering completely whilst others succumbed within 24 hours. By day 42, 95% of flank wounds had either fully or partially healed, however 5% showed signs of a wound infection (Table 4.3-5).

**Table 4.3-4 Morbidity* rate within 84 hours of spaying - Study IIIa**

| Time | Control | | WDOT | | Flank | | Chi-square | Probability |
|---|---|---|---|---|---|---|---|---|
| | $n^{\propto}$ | Morbidity rate (%) | $n^{\propto}$ | Morbidity rate (%) | $n^{\propto}$ | Morbidity rate (%) | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0-12 h | 75 | 0 | 75 | 2.7 | 75 | 6.0 | 4.44 | 0.108 |
| 0-36 h | 125 | 0 | 125 | 0.8 | 125 | 1.6[#] | 2.02 | 0.365 |
| 12-60 h | 200 | 0 | 200 | 2.0 | 200 | 1.5[#] | 3.76 | 0.153 |
| 36-84 h | 200 | 0.5 | 200 | 0.5 | 200 | 0 | 1.00 | 0.606 |

*** depression, recumbency, scouring, slow, restricted gait/rising**
**[∝] n varies because observations were conducted as animals were pooled post conduct of procedures**
**[#] 1 heifer later died**

**Table 4.3-5 Frequency of wound healing scores at 21 and 42days after flank spaying – Study IIIa**

| Time (d) | n | Wound score | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 21 | 193 | 4% | 41% | 43% | 11% | 1% |
| 42 | 194 | 69% | 26% | 5% | 0% | 0% |

**1 Wound healed.**
**2 Wound partially healed and clean and dry.**
**3 Wound partially healed and discharge.**
**4 Little or no healing of wound and clean and dry.**
**5 Little or no healing of wound and discharge.**

In Study IIIa there was a tendency (Chi-square (2 d.f.) = 4.81; P= 0.09) for mortality rate to differ between groups (Table 4.3-6). The mortality rate in the WDOT spayed heifers was the same as that reported by Jubb et al (2003), but the rate in the flank spayed heifers was higher than that observed by these workers. Two of the deaths in the flank spayed group were due to acute surgical haemorrhage which can be avoided if the 'webbing technique' is used (P Letchford pers.comm.), however as the WDOT involves excision of the ovaries, for the purposes of comparison ovariectomy via the flank was performed in Study IIIa. An unexpected finding was the occurrence of deaths between 11 and 21 days after spaying. Based on the limited number of published reports and the extensive experience of the veterinarian performing the procedures it was expected that deaths due to severe surgical haemorrhage would occur within the first 24 to 48 hours after spaying, and deaths due to acute diffuse peritonitis secondary to inadvertent surgical laceration of the intestines would occur between 1 to 7 days later. As a result, no monitoring of the heifers took place between 11 and 21days. Further, due to the fact that the heifers were held in a small paddock which could be systematically patrolled, it is unlikely that these deaths were missed during patrolling up to day 10. Also, at day 10 all the heifers appeared in good health and any further deaths were considered very unlikely. The carcasses were readily identified from the air by the helimuster pilot, but were too decomposed to enable any reliable diagnosis of the cause of death to be made. However, an indication of a possible explanation for these deaths was provided by observations on day 21 of one heifer showing signs of muscle twitiching and 'flicking' of the third eyelid; this heifer was found dead the following day. These are signs are consistent with a diagnosis of tetanus. The heifers had only been vaccinated for botulism; routine 5-in-1 vaccination of young stock is not practiced in the Victoria River District.

**Table 4.3-6 Mortality rate determined from monitoring twice daily to Day 4, then on Days 7, 10, 21 and 42 – Study IIIa**

| Treatment | n | Mortality Rate (%) | Comments |
|---|---|---|---|
| Control | 200 | 0.0 | |
| WDOT | 200 | 1.5 | Day 12*: 3 deaths**<br>(1 heifer missing at 21 and 42 days, no carcass found**)** |
| Flank | 200 | 2.5 | Day 1: 2 surgical haemorrhage deaths<br>Day 5: 1 death*<br>Day 12*: 1 death*<br>Day 22: 1 death - suspect tetanus |

*** estimated day of death; **carcasses in an advanced state of decomposition when located, therefore cause of death could not be determined**

In Study IIIb the overall mortality rate in WDOT spayed heifers was 0.5% (three deaths from 574 animals). There was some variation between the two mobs which were all similar age, breed, body condition heifers, and spayed by the same veterinarian on consecutive days, however this variation was consistent with the random nature of low-level mortalities. The mortalities were:

- Mortality of 1.0% in the 199 animals spayed (197 not detectably pregnant, two, 8 to 10 weeks pregnant) on 23/8/06 at Coles yards. Two deaths were detected on day 1; one case of peracute diffuse peritonitis and one case of surgical hemorrhage (pregnant). Four heifers were observed to be depressed or slow to rise or walking with a stiff gait on days 1 to 4 after spaying. The vulva mucous membranes of one heifer was observed to be markedly pale consistent with significant blood loss, however this heifer had fully recovered by day 14.

- Mortality of 0.3% in the 375 animals spayed on 24/8/06 at No.12 yards. One death was detected on day 4; a case of acute diffuse peritonitis due to large bowel puncture.

### 4.3.4 Summary of major findings

Approximately one in ten WDOT spayed heifers showed behavioural signs of some pain and discomfort within 6 hours of spaying. Twice as many flank versus WDOT spayed heifers showed behavioural signs of mild to severely reduced health status within 36 hours of spaying. The mortality rate following WDOT spaying varied somewhat (0.3%, 1.0% and 1.5%) between mobs of the same cattle spayed by the same veterinarian during the same season, with an overall rate of 0.8% (six deaths from 774 animals). Deaths may be continuing to occur in mobs of spayed females after the generally recognised period of highest risk.

## 4.4 Comparison of responses to surgical spaying with responses to other surgical husbandry procedures

As there have been no other published studies reporting the physiological and behavioural responses of *Bos indicus* cattle to surgical husbandry procedures, caution needs to be exercised in comparing the findings from this study with findings from other studies, most of which report the responses of young (< 6 months of age) *Bos taurus* cattle (mainly dairy breeds) to various husbandry procedures. Further, as the methods used to determine the concentrations of bound and unbound cortisol in this project (ELISA) were different to those used by many other studies (primarily radioimmunoassay), direct comparisons between cortisol concentrations should not be made. Possibly the only valid comparison that can be made is between the relative changes in cortisol concentrations reported following conduct of different surgical husbandry procedures.

Using data from a review of 'dehorning and disbudding distress and its alleviation in calves' by Stafford and Mellor (2005b) the areas under the curve for total cortisol concentrations for dehorned and control calves (their Figure 1) between 0 to 8 hours after procedures, was estimated. Total cortisol concentrations were increased by 339% in dehorned calves compared to controls. Comparing the peak total cortisol concentration in dehorned calves to controls demonstrated that dehorning caused a 222% increase in total cortisol concentration. In our Studies I and II, the observed percentage increase in bound cortisol concentrations compared to controls for spayed females for the period 0 to 8 hours ranged between 40% and 90%. Although these percentage increases are derived from the repeated measures ANOVA, comparison of the results for repeated measures ANOVA and area under the curve showed that the results were very similar. Although it is tempting to suggest that spaying may cause less distress to cattle than dehorning, a more valid conclusion would be that spaying is unlikely to cause more distress to cattle than dehorning.

However, such a conclusion is very tentative given the differences between the experimental animals in terms of age, liveweight and genotype, as well as influences resulting from the experiences particularly relating to the type and frequency of handling. Further as amputation dehorning and flank spaying both result in significant wounds both are associated with a degree of chronic pain and distress due to the inflammatory response to the surgery. The data from Study II shows that although the acute pain/distress response is similar for flank and WDOT spaying, flank spaying causes a significantly greater chronic pain/distress response than WDOT spaying particularly for heifers (Table 4.2-18).

# 5 Success in Achieving Objectives

All of the objectives were met within the timeframe of the project. The project has generated an internationally unique database of the responses of *Bos indicus* cattle to a range of routine husbandry procedures. Further the study designs and methodologies employed in this project provide a model for the assessment of the impact of other husbandry and management practices on the health, welfare and productivity of tropically adapted cattle. Using internationally recognised measures, the physiological responses of flank (n= 35) and WDOT (n= 39) spayed females were defined and compared with the responses of cattle which were only restrained (controls; n = 33) or had a procedure performed which is a component of the spay procedure (n =60). The incidence of a range of behaviours indicative of the welfare of cattle was determined for flank (n= 230) and WDOT (n = 230) spayed females and compared with that observed in control females (n= 228). We identified a behavioural response (standing head down) indicative of pain/discomfort in cattle that has never been previously documented. The health and productivity of flank (n = 230) and WDOT (n = 239) spayed females was compared with that of control females (n= 233). In addition the project has generated the first data on the physiological and behavioural responses of *Bos indicus* cattle to electroimmobilisation. Except for Study I, all studies were conducted under genuine industry conditions with the procedures performed to best practice. In addition, the establishment of a collaborative research team provided an excellent opportunity for training of early career researchers in the approach to conducting the required rigorous field research to address a major industry issue. An edited video recording all the procedures performed and the range of immediate responses to these procedures was produced.

# 6 Impact on Meat and Livestock Industry – now and in five years time

Few question the need to prevent unwanted pregnancies in cattle in northern Australian beef herds as these can lead to poor welfare and productivity outcomes e.g. the slaughter of pregnant animals, calves born “out of season” when poor nutrition can have severe adverse effects on both the dam and calf; and the rate of genetic improvement of herds can be slowed through the breeding of females with undesirable traits.

The findings from this project clearly demonstrate that spaying by either of the commonly used methods (via the flank and the WDOT) results in acute pain and stress in both heifers and cows, although the pain and stress was less in heifers spayed using the WDOT. The Industry needs to determine: (a) whether it wishes to continue using surgical spaying as a management tool to prevent unwanted pregnancies and, (b) whether it wishes to address the issue that spaying causes pain and stress. If the industry wishes to retain spaying and practice pain management, then further research will be necessary because there have been no published studies on pain alleviation in surgically spayed cattle. There have been studies on the responses of cattle to other common surgical

husbandry procedures, but we would suggest that it is inappropriate to extrapolate. As an example, the findings of a series of studies on alleviation of pain caused by castration and dehorning (reviewed by Stafford and Mellor 2005a, b) on young (a few weeks to a few months of age), *Bos taurus* calves indicate there may be methods of reducing the acute pain associated with spaying. These authors reported that administration of a non-steroidal anti-inflammatory drug (NSAID; ketoprofen) 15 to 20 minutes before amputation dehorning reduced the acute cortisol response 3-fold, from a marked response to a mild response. They further reported that administration of ketoprofen did not significantly affect the initial peak in plasma cortisol concentration, but it prevented the establishment of a sustained cortisol response, with concentrations returning to pre-treatment levels at 2 hours rather than 8 hours after dehorning.

With respect to the issue of flank spaying without use of local anaesthesia, it is likely that the general public would find it difficult to understand why spaying of dogs and cats requires anaesthesia but performing the same procedure in much larger animals, such as cattle, does not. Also, it is worthy of mention that the leading embryo transfer veterinarian in northern Australia routinely transfers cattle embryos via a flank laparotomy (similar to that used for flank spaying) performed under local anaesthesia. Further, although there can be little doubt that in situations where cattle handling facilities and/or property staff are suboptimal, use of electroimmobilisation by an appropriately trained operator will reduce the risk of injury to cattle and operators, it must be recognised, as demonstrated in Study II, that electroimmobilisation significantly adversely impacts on cattle welfare.

The installation of appropriately designed squeeze crushes in cattle yards routinely used for spaying would reduce the need for electroimmobilisation and could also facilitate the use of local anaesthesia. Recent developments in needle-less injection technology (currently being trialled for use with mulesing) may enable very rapid administration of a local anaesthetic to the site for the flank incision. However the issue of the timing of administration relative to that of the procedure must also be considered, because animals would either need to be restrained for prolonged periods or double-handled. It may be that this restraint and additional handling are, in themselves, stressors for animals unaccustomed to them.

The main alternative to surgical spaying, primarily use of long-acting deslorelin implants, although shown to inhibit ovulation consistently for periods of 200 to 300 days in female cattle, is currently too expensive. It is possible that ongoing research into other chemical methods of spaying may yield practical cost effective alternatives to surgical spaying. However, in the short to medium term, surgical spaying is likely to continue to be the primary method used by the industry to permanently prevent female cattle from becoming pregnant. Therefore, research should be immediately initiated to develop practical, cost effective and safe methods of significantly reducing the pain and stress of surgical.

# 7 Conclusions and Recommendations

### Conclusions

1. Flank spaying and WDOT spaying cause similar acute (0-8 hours) pain/stress responses in female *Bos indicus* cattle, however flank spaying also induces a significant response indicative of a ($P<0.05$) chronic (at least 96 h) pain/stress response.
2. In contrast to the WDOT spayed females, the inflammatory, muscle damage and pain/stress responses in flank spayed cattle are still significantly increased 4 days after the procedure.

3. A comparison of flank spaying and electroimmobilisation indicates that electroimmobilisation makes a substantial contribution to the adverse impacts on flank spayed animals, particularly in cows.
4. A comparison between WDOT and AI indicates that there are no significant physiological differences between the procedures for heifers, but cows show a significantly greater response indicative of pain and stress (0-24hours) to WDOT than to AI. Behavioural responses indicative of pain and stress to WDOT are apparent in both heifers and cows to 24 hours post-procedure.
5. A comparison between AI and control (physical restraint alone) treated animals suggest that both heifers and cows respond similarly to these procedures, although during the first 24 hours measures of pain/stress and muscle damage were significantly increased in AI cows compared to control cows.
6. In terms of overall impact of procedures on the welfare of cattle when compared to physical restraint alone, the ranking (from highest to lowest) is flank spaying, WDOT spaying, electroimmobilisation alone and AI.
7. The mortality rate following WDOT spaying varies (0.3%, 1.0% and 1.5%) between mobs of the same class of cattle spayed by the same veterinarian during the same season, with an overall rate of 0.8%.
8. Deaths may occur in mobs of spayed females after the generally recognised period of highest risk (< 7 days) for both flank and WDOT methods.
9. There are dangers of increased numbers of deaths from acute haemorrhage post-surgery using standard flank ovariectomy spaying and in some instances this could be reduced by use of the “webbing” technique.

**Recommendations**

1. Research should be immediately initiated to develop effective, practical and safe treatments to reduce both the acute and chronic effects of spaying by either technique. The model for defining the impact on animal welfare developed in the present project should be used to define the responses to selected treatments.
2. In the immediate short-term it is recommended that if spaying is to be conducted then it should be done by WDOT on yearling heifers, with in the longer term it being done with appropriate analgesia.
3. Due to the demonstrated marked impact of electroimmobilisation on cattle welfare, appropriately designed and constructed cattle crushes should be installed at the sites where cattle are routinely spayed so that cattle can be adequately restrained without the use of electroimmobilisation.
4. Accurate estimates of the mortality rates following spaying by either technique should be obtained, as this work was conducted on a single property and spaying was performed by a veterinarian highly-skilled in both spaying techniques. Current industry estimates may be underestimating losses as there is only limited surveillance for a short period of time after spaying in most cases. As demonstrated in this project, helicopter surveillance of the paddock in which the recently spayed cattle are held can provide an accurate estimate of number of deaths. This surveillance may be able to be readily incorporated into existing helimustering schedules. This should be done as part of an epidemiological study to define the factors (e.g. disease, water availability, pasture quality, monitoring) associated with increased risk of mortalities after spaying.
5. Research should be conducted to determine whether modifications to the design of the WDOT ovariotome could be made to enable it to be routinely used on cows without the observed increased risk (compared with heifers) of mortality due to severe surgical haemorrhage.
6. The observations from Study III strongly support the practice of ensuring cattle are walked only a short distance after spaying to a paddock with easy access to water and good grazing

# Acknowledgements

The project research team wishes to gratefully acknowledge the contributions of staff from Heytsbury Beef, NTDPIFM's Berrimah and Katherine Research Stations and the DPI&F's Animal Research Institute who assisted in the development and execution of this project.

# 8 Bibliography

Breuer K, Coleman GJ, Hemsworth PH. 1998. The effect of handling on the stress physiology and behaviour of nonlactating heifers. Proceedings 29th Annual Conference of the Australian Society for the Study of Animal Behaviour, pp.8-9.

Burrow HM, Seifert GW, Corbet NJ. 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod. 17: 154-157.

Cook C. 1996. Pain in farm animals: awareness, recognition and treatment. In: R Baker, R Einstein and D Mellor (Eds.) Farm Animals in Biomedical and Agricultural Research. ANZCCART, Glen Osmond. pp. 75-82.

de Witte K, Jubb T, Letchford P. 2006. The Dropped Ovary Technique for Spaying Cattle. Australian Cattle Veterinarians Manual. 14-20.

D'Occhio MJ, Fordyce G, Whyte TR, Jubb TF, Fitzpatrick LA, Cooper NJ, Aspden WJ, Bolam MJ, Trigg TE. 2002. Use of GnRH agonist implants for long-term suppression of fertility in extensivel managed heifers and cows. Anim. Reprod. Sci. 74:151-162.

Ferguson DM, Bruce HL, Thompson JM, Egan AF, Perry D, Shorthose WR. 2001. Factors affecting beef palatability – farmgate to chilled carcass. Aust. J. Expt. Agric. 41: 897-891.

Garber MJ, Roeder RA, Combs JJ, Eldridge L, Miller JC, Hinman DD, Ney JJ (1990) Efficacy of vaginal spaying and anabolic implants on growth and carcass characteristics in beef heifers. J. Anim. Sci. 68: 1469-1475.

Gayrard V, Alvinerie M, Toutain PL. 1996. Interspecies variations of corticosteroid-binding globulin parameters. Dom.Anim Endocrin.13:35-45.

GenStat 2005. GenStat for Windows, Release 8.1, Eighth Edition. VSN International Ltd., Oxford.

Goonewardene LA, Price MA, Okine E, Berg RT. 1999. Behavioral responses to handling and restraint in dehorned and polled cattle. Appl. Anim. Behav. Sci. 64: 159-167.

Graf B, Senn M. 1999. Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. Appl. Anim. Behav. Sci. 62: 153-171.

Grandin T. 2001. Cattle vocalizations are associated with handling and equipment problems at beef slaughter plants. Appl. Anim. Behav. Sci. 71: 191-201.

Hemsworth PH, Barnett, JL. 2000. Human-animal interactions and animal stress. . In: GP Moberg and JA Mench (Eds.) The Biology of Stress. Basic Principles and Implications for Animal Welfare. CABI Publishing, Wallingford. pp.309-334.

Jeffery M, Loxton I, Van der Mark S, James T, Shorhose R, Bell K, D'Occhio MJ (1997) Liveweight gains, and carcass and meat characteristics of entire, surgically spayed or immunologicalled spayed beef heifers. Aust. J. Expt. Agric. 37: 719-726.

Jubb TF, Fordyce G, Bolam MJ, Hadden DJ, Cooper NJ, Whyte TR, Fitzpatrick LA, Hill F, D'Occhio MJ. 2003. Trial introduction of the Willis dropped ovary technique for spaying cattle in northern Australia. Aust. Vet. J. 81: 66-70.

Jubb T, Letchford P. 1997. Cattle spaying and electroimmobilisers – their use in extensive beef cattle herds in the Kimberley region. Proceedings Australian Cattle Veterinarians Annual Conference, pp.31 - 35

Kendall M, Stuart A, Ord JK. 1983. The Advanced Theory of Statistics (Volume 3, 4th edition). Griffin, London.

Kidd J, McLennan SR (1998) Relationship between liveweight change of cattle in the dry season in northern Australia and growth rate in the following wet season. Animal Production in Australia 22: 363.

Loeffler K. 1986. Assessing pain by studying posture, activity and function. In: IJH Duncan and V Molony (Eds.) Assessing Pain in Farm Animals. Commission of the European Communities, Luxembourg. pp. 49-57.

McMeekan C, Stafford KJ, Mellor DJ, Bruce RA, Ward RN, Gregory N. 1999. Effects of local anaesthetic and non-steroidal anti-inflammatory analgesic on the behavioural responses of calves to dehorning. N.Z. Vet. J. 47: 92-96.

Mellor DJ, Cook CJ, Stafford KJ. 2000. Quantifying some responses to pain as a stressor. In: GP Moberg and JA Mench (Eds.) The Biology of Stress. Basic Principles and Implications for Animal Welfare. CABI Publishing, Wallingford. pp. 171-198.

Molony V. 1986. The Electrimmobilisation and Electroanaesthetisation of Farm Animals: A Review. Report to the Farm Animal Welfare Council (UK). 43 pp.

Petherick JC, Doogan VJ, Venus, BK, Quinn PL, Holroyd RG. 2005. Does quality and quantity of handling change cattle temperament? Proc. Aust. Anim. Prod. Mini-conference (ASAP Central Queensland Sub-Branch), 5-6 July 2005, Rockhampton. pp. 19-22.

Petherick JC, Holroyd RG, Doogan VJ, Venus BK. 2002. Productivity, carcass and meat quality of lot-fed *Bos indicus* cross steers grouped according to temperament. Aust. J. Expt. Agric. 42: 389-398.

Petherick JC. 2005. Animal welfare issues associated with extensive livestock production: The northern Australian beef cattle industry. Appl. Anim. Behav. Sci. 92: 211-234.

Petrie NJ, Stafford KJ, Mellor DJ, Bruce RA, Ward RN. 1995. The behaviour of calves tail docked with a rubber ring used with or without local anaesthesia. Proc. N.Z. Soc. Anim. Prod. 55: 58-60.

Radostits OM,Gay CG, Hinchcliff KW, Constable PD. 2007 Veterinary Medicine – A textbook of the diseases of cattle, horses, sheep, pigs and goats - 10th edition. Published by Saunders, Philadelphia.

Robertson IS, Kent JE, Molony V. 1994. Effect of different methods of castration on behaviour and plasma cortisol in calves of three ages. Res. Vet. Sci. 56: 8-17.

Roux CW, Chapman GA, Kong WM, Dhillo WS, Jones J, Alaghband-Zaedh J. 2003. Free cortisol index is better than serum total cortisol in determining hypothalamic-pituitary-adrenal status in patients undergoing surgery. J. Clin. Endocrin. & Metab. 88:2045-2048.

Rowell JG, Walters RE. 1976. Analysing data with repeated observations on each experimental unit. J Agric Sci 87: 423-432.

Rushen J. 1986. Aversion of sheep to electro-immobilization and physical restraint. Appl. Anim. Behav. Sci. 15: 315-324.

Rushen J. 1996. Using aversion learning techniques to assess the mental state, suffering, and welfare of farm animals. J. Anim. Sci. 74: 1990-1995.

Salonen, M., Hirvonen J, Pyörälä S, Sankari S, and Sandholm M. 1996. Qualitative determination of bovine serum haptoglobin in experimentally induced Escherichia coli mastitis. Res.Vet.Sci. 60: 88-91.

Saul GR, Baud SR, McDonald JW, McRae C, Clark AJ (1982) A comparison of the growth rate and carcase characteristcs of steers and heifers when entire, spayed, pregnant or fitted with an intravaginal device. Aust. J. Expt. Agric. & Anim. Hus. 22: 258-263.

Schwartzkopf-Genswein KS, Stookey JM, Welford R. 1997a. Behavior of cattle during hot-iron and freeze branding and the effects on subsequent handling ease. J. Anim. Sci. 75: 2064-2072.

Schwartzkopf-Genswein KS, Stookey JM, Janzen ED, McKinnon J. 1997b. Effects of branding on weight gain, antibiotic treatment rates and subsequent handling ease in feedlot cattle. Can. J. Anim. Sci. 77: 361-367.

Stafford KJ, Mellor DJ. 2005a. The welfare significance of the castration of cattle. New Zealand Vet. J. 53:271-278.

Stafford KJ, Mellor DJ. 2005b. Dehorning and disbudding distress and its alleviation in calves. The Vet. J. 169:337-349.

Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA, Ward RN. 2004. Behavioural responses of calves to amputation dehorning with and without local anaesthesia. Aust. Vet. J. 82: 697-700.

# 9 Appendices

## 9.1 Repeated-measures analysis of variance for bound cortisols (log-transformed).

```
Box's tests for symmetry of the covariance matrix
-------------------------------------------------

Chi-square 55.15 on 34 degrees of freedom: probability 0.012

F-test 1.62 on 34 and 130334 degrees of freedom: probability 0.012


Greenhouse-Geisser epsilon = 0.8989


Analysis of variance (adjusted for covariate)
=============================================

Variate: logBound_Cort[1,2,3,4,6,8,24,96]
Covariate: logBound_Cort[0]

Source of variation    d.f.     s.s.    m.s.    v.r.   F pr.

Block stratum
Covariate                 1   8.690   8.690   14.59   0.002
Residual                 13   7.741   0.595    2.44

Block.Subject stratum
Treatment                 4   8.115   2.028    8.32   <.001
Treatment.Class           4   1.289   0.322    1.32   0.266
Covariate                 1  24.768  24.768  101.52   <.001
Residual                123  30.008   0.243    3.31    1.81

Block.Subject.Time stratum
(d.f. correction factor 0.8989)
Time                      7   7.665   1.095   14.87   <.001
Time.Treatment           28   4.192   0.149    2.03   0.002
Time.Class                7   3.131   0.447    6.08   <.001
Time.Treatment.Class     28   3.657   0.130    1.77   0.011
Residual                949  69.875   0.073

Total                  1165 159.544
```

## 9.2 Study III Fecal NIRS

| Date | Dietary CP Content (%) | Fecal N | DMD (%) | Proportion Non-grass (%) | Fecal Ash |
|---|---|---|---|---|---|
| 13/07/2006 | 6.6 | 1.49 | 53 | 47 | 20 |
| 21/08/2006 | 4.1 | 1.05 | 49 | 31 | 17 |
| 23/08/2006 | 4.5 | 1.00 | 49 | 33 | 21 |

## 9.3 Project Weather Data

### 9.3.1 Study I Berrimah Research Farm



Notes:
* Data Drill for Lat, Long: -12.45 130.95 (DECIMAL DEGREES), 12 27'S 130 57'E
* Extracted from Silo on 20070412
* Please read the documentation on the Data Drill at http://www.nrm.qld.gov.au/silo
* As evaporation is read at 9am, it has been shifted to the day before ie The evaporation measured on 20 April is in row for 19 April
* The 6 Source columns Smx-Svp indicate the source of the data to their left
* Relative Humidity has been calculated using 9am VP, T.Max and T.Min RHmaxT is estimated Relative Humidity at Temperature T.Max, RHminT is estimated Relative Humidity at Temperature T.Min
* FAO56 = Potential Evapotranspiration calculated using the FAO Penman-Monteith formula as in FAO Irrigation and Drainage paper 56, http://www.fao.org/docrep/X0490E/X0490E00.htm
* As the evapotranspiration has been calculated from other data, particularly, Tmax, Tmin, Rad, and VP, its accuracy and source code are dependant on the source and accuracy of the data in those columns.
* The accuracy of the data depends on many factors including date, location, and variable for consistency data is supplied using one decimal place, however it is not accurate to that precision.
* Further information is available from http://www.nrme.qld.gov.au/silo

**Table 9.3-1**

| Date (ddmmyyyy) | Max. Air Temp (°C) | Min. Air Temp (°C) | Rainfall (mm) | Evaporation (mm) | Radiation (MJ/m$^2$) | Vapour Pressure (hPa) | Av. Relative Humidity (%) | FAO56 (mm) |
|---|---|---|---|---|---|---|---|---|
| 1/05/2006 | 31.5 | 23 | 0 | 6.4 | 16 | 24 | 68.7 | 4.2 |
| 2/05/2006 | 29.5 | 21 | 0 | 6.8 | 22 | 17 | 54.8 | 5.1 |
| 3/05/2006 | 29 | 18.5 | 0 | 6.4 | 22 | 15 | 54 | 5 |
| 4/05/2006 | 30 | 18.5 | 0 | 4.8 | 24 | 16 | 56.45 | 5.3 |
| 5/05/2006 | 30.5 | 19.5 | 0 | 6.6 | 23 | 18 | 60.3 | 5.1 |
| 6/05/2006 | 31 | 19 | 0 | 5.4 | 23 | 17 | 57.6 | 5.2 |
| 7/05/2006 | 31.5 | 19.5 | 0 | 7.2 | 23 | 18 | 59.15 | 5.2 |
| 8/05/2006 | 30.5 | 19.5 | 0 | 5.6 | 19 | 15 | 50.3 | 4.9 |
| 9/05/2006 | 30 | 18 | 0 | 6.8 | 18 | 14 | 50.45 | 4.7 |
| 10/05/2006 | 31 | 20 | 0 | 6 | 23 | 17 | 55.25 | 5.2 |
| 11/05/2006 | 32.5 | 22.5 | 0 | 6.6 | 21 | 22 | 62.85 | 5 |
| 12/05/2006 | 32.5 | 23.5 | 0 | 7.2 | 22 | 21 | 57.75 | 5.3 |
| 13/05/2006 | 32 | 22 | 0 | 5.4 | 22 | 20 | 58.9 | 5.2 |
| 14/05/2006 | 32.5 | 22 | 0 | 6.8 | 19 | 23 | 67 | 4.6 |
| 15/05/2006 | 32 | 21.5 | 0 | 7.4 | 21 | 19 | 57.05 | 5.1 |
| 16/05/2006 | 32 | 21.5 | 0 | 7.4 | 20 | 22 | 66.05 | 4.7 |
| 17/05/2006 | 31.5 | 21 | 0 | 7 | 20 | 21 | 64.95 | 4.7 |
| 18/05/2006 | 31.5 | 20.5 | 0 | 6 | 21 | 21 | 66.25 | 4.8 |
| 19/05/2006 | 31 | 20.5 | 0 | 6.4 | 18 | 20 | 63.75 | 4.4 |
| 20/05/2006 | 31 | 20 | 0 | 5.2 | 17 | 21 | 68.3 | 4.2 |
| 21/05/2006 | 32 | 23 | 0 | 6.8 | 19 | 26 | 73.65 | 4.4 |
| 22/05/2006 | 32 | 23.5 | 12.2 | 5.6 | 17 | 28 | 77.8 | 4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 23/05/2006 | 32 | 23.5 | 0 | 6 | 21 | 28 | 77.8 | 4.5 |
| 24/05/2006 | 31 | 22 | 0 | 5.6 | 21 | 22 | 66.1 | 4.7 |
| 25/05/2006 | 31 | 20.5 | 0 | 5.8 | 21 | 21 | 66.9 | 4.6 |
| 26/05/2006 | 30.5 | 20 | 0 | 4.8 | 20 | 16 | 52.55 | 4.8 |
| 27/05/2006 | 30 | 19 | 0 | 7.4 | 21 | 15 | 51.85 | 4.9 |
| 28/05/2006 | 29 | 17.5 | 0 | 8.4 | 22 | 10 | 37.5 | 5.2 |
| 29/05/2006 | 27.5 | 16 | 0 | 6.6 | 22 | 10 | 41.1 | 4.9 |
| 30/05/2006 | 28.5 | 15 | 0 | 6.6 | 20 | 10 | 42.2 | 4.8 |
| 31/05/2006 | 29.5 | 15 | 0 | 7.2 | 20 | 10 | 41.5 | 4.9 |
| 1/06/2006 | 30 | 15.5 | 0 | 5.2 | 17 | 13 | 52.25 | 4.5 |
| 2/06/2006 | 30 | 18 | 0 | 6.6 | 21 | 16 | 57.65 | 4.7 |
| 3/06/2006 | 29 | 17.5 | 0 | 6.2 | 20 | 12 | 45 | 4.8 |
| 4/06/2006 | 27 | 16.5 | 0 | 7 | 21 | 12 | 48.85 | 4.5 |
| 5/06/2006 | 28.5 | 16.5 | 0 | 7 | 21 | 10 | 39.5 | 4.9 |
| 6/06/2006 | 28.5 | 16.5 | 0 | 5.8 | 20 | 11 | 43.45 | 4.7 |
| 7/06/2006 | 29 | 15.5 | 0 | 7.2 | 20 | 10 | 40.9 | 4.8 |
| 8/06/2006 | 28.5 | 15.5 | 0 | 5.6 | 19 | 9 | 37.1 | 4.8 |
| 9/06/2006 | 30 | 16 | 0 | 4.6 | 20 | 12 | 47.15 | 4.8 |
| 10/06/2006 | 30.5 | 18 | 0 | 7.4 | 19 | 14 | 50 | 4.7 |
| 11/06/2006 | 28.5 | 19 | 0 | 7.4 | 19 | 10 | 35.6 | 4.9 |
| 12/06/2006 | 26.5 | 15 | 0 | 6.4 | 21 | 8 | 35 | 4.7 |

### 9.3.2 Study II - Blackgin Yards – Mt Sanford Station

Notes:
* Data downloaded from automated weather station located at Mt Sanford Station – Blackgin, approximately 5 km from Blackgin yards.

**Table 9.3-2**

| Date | Max Air Temp | Min Air Temp | Total Rainfall (mm) | Av Evap (mm) | Av Wind Speed (km/h) | Max Wind Speed (km/h) | Av Solar Radiation (MJ/m2) | Av Relative Humidity (%) |
|---|---|---|---|---|---|---|---|---|
| 24/05/2006 | 28.5 | 12.9 | 0 | 6.294 | 7.8 | 23.2 | 205 | 59.2 |
| 25/05/2006 | 27.6 | 10.1 | 0 | 6.713 | 6.2 | 19.2 | 208 | 49 |
| 26/05/2006 | 28.2 | 10.8 | 0 | 6.553 | 5.6 | 17.7 | 202 | 45 |
| 27/05/2006 | 29.2 | 10.6 | 0 | 7.532 | 6.2 | 19.9 | 191 | 32.5 |
| 28/05/2006 | 29.2 | 10.9 | 0 | 7.829 | 6.9 | 20.2 | 211 | 35.4 |
| 29/05/2006 | 25.4 | 8.5 | 0 | 7.504 | 9.4 | 26.1 | 208 | 47.7 |
| 30/05/2006 | 24 | 6.1 | 0 | 7.361 | 8.1 | 26.1 | 213 | 38.4 |
| 31/05/2006 | 25.6 | 7.3 | 0 | 7.2 | 6.5 | 22.2 | 212 | 36.1 |
| 01/06/2006 | 27.1 | 7.7 | 0 | 7.631 | 6.8 | 23.1 | 206 | 32.6 |
| 02/06/2006 | 28.3 | 10.7 | 0 | 7.83 | 7 | 21.7 | 207 | 33.6 |
| 03/06/2006 | 28.4 | 10.4 | 0 | 8.077 | 8.9 | 21.9 | 204 | 37.5 |
| 04/06/2006 | 25.2 | 9.5 | 0 | 7.701 | 9.7 | 24.4 | 202 | 42.8 |
| 05/06/2006 | 25.7 | 8.6 | 0 | 7.736 | 9.6 | 23.7 | 203 | 39.2 |
| 06/06/2006 | 24.5 | 7.6 | 0 | 7.697 | 10.1 | 29 | 204 | 41.5 |
| 07/06/2006 | 24.2 | 8.7 | 0 | 6.965 | 8.5 | 26 | 197 | 39.3 |
| 08/06/2006 | 24.2 | 7.8 | 0 | 7.446 | 9.2 | 22.8 | 201 | 36.1 |
| 09/06/2006 | 24.8 | 8.1 | 0 | 7.449 | 8.8 | 26.7 | 197 | 38.7 |
| 10/06/2006 | 25.9 | 7.6 | 0 | 7.216 | 7.4 | 21.9 | 204 | 34.7 |
| 11/06/2006 | 25.1 | 8 | 0 | 7.998 | 10.1 | 24.1 | 199 | 33.6 |
| 12/06/2006 | 21.6 | 8.2 | 1.8 | 7.741 | 13.1 | 34 | 202 | 39.7 |
| 13/06/2006 | 21.1 | 5.9 | 3.6 | 7.233 | 12.2 | 32.9 | 204 | 43 |
| 14/06/2006 | 20.1 | 7.8 | 6 | 6.79 | 10.5 | 30.4 | 202 | 39.7 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15/06/2006 | 26.6 | 12.4 | 0 | 6.466 | 7.4 | 19.8 | 198 | 49.5 |
| 16/06/2006 | 29.8 | 13.7 | 0 | 6.644 | 8 | 21.2 | 195 | 53.3 |
| 17/06/2006 | 28.8 | 11.2 | 0 | 6.783 | 7.3 | 23 | 196 | 50.6 |
| 18/06/2006 | 28.8 | 11.5 | 0 | 6.857 | 8.6 | 25.4 | 199 | 53 |
| 19/06/2006 | 25.9 | 9.9 | 0 | 7.013 | 8.8 | 25.7 | 194 | 46.8 |
| 20/06/2006 | 26.3 | 8.8 | 0 | 7.675 | 9.5 | 24.7 | 201 | 42.9 |
| 21/06/2006 | 25.7 | 6 | 0 | 7.414 | 8.3 | 23.7 | 204 | 40.2 |
| 22/06/2006 | 25.5 | 9.2 | 0 | 7.607 | 8.6 | 22.2 | 205 | 35.8 |
| 23/06/2006 | 26.5 | 9.7 | 0 | 7.265 | 8.7 | 24.6 | 203 | 46.9 |
| 24/06/2006 | 27.3 | 11 | 0 | 7.11 | 8.7 | 24.2 | 201 | 48 |
| 25/06/2006 | 27.6 | 11.5 | 0 | 7.306 | 9.1 | 29.5 | 200 | 46.3 |
| 26/06/2006 | 27.4 | 13.8 | 0.2 | 7.196 | 9.3 | 27.7 | 197 | 48.9 |
| 27/06/2006 | 28.6 | 12.7 | 0 | 7.528 | 9.7 | 24.4 | 195 | 49.7 |
| 28/06/2006 | 26.8 | 9 | 0 | 7.491 | 8.8 | 27.3 | 199 | 45.9 |
| 29/06/2006 | 27.7 | 12.6 | 0 | 6.091 | 5.4 | 19.5 | 191 | 47 |
| 30/06/2006 | 25.2 | 15.1 | 0 | 4.64 | 6.5 | 19.2 | 90 | 47.6 |
| 01/07/2006 | 29.9 | 11.6 | 0 | 7.214 | 8.3 | 21.2 | 194 | 49.6 |
| 02/07/2006 | 28.3 | 10.2 | 0.6 | 6.526 | 5.2 | 17.3 | 199 | 42.7 |
| 03/07/2006 | 28.8 | 9.2 | 0.2 | 7.181 | 6.4 | 20.5 | 201 | 34.8 |
| 04/07/2006 | 26.5 | 9.3 | 0 | 7.464 | 8.9 | 25.2 | 168 | 34.4 |
| 05/07/2006 | 24.3 | 7.3 | 0 | 7.088 | 9 | 27.2 | 201 | 42.8 |

### 9.3.3 Study III - Coles Yards – Pigeon Hole Station

**Table 9.3-3**

| Date (ddmmyyyy) | Max. Air Temp (°C) | Min. Air Temp (°C) | Rainfall (mm) | Evaporation (mm) | Radiation (MJ/m$^2$) | Vapour Pressure (hPa) | Av. Relative Humidity (%) | FAO56 (mm) |
|---|---|---|---|---|---|---|---|---|
| 4/07/2006 | 25 | 9 | 0 | 5.2 | 20 | 9 | 53.4 | 4 |
| 5/07/2006 | 26 | 6 | 0 | 5.2 | 20 | 8 | 54.7 | 4.1 |
| 6/07/2006 | 27 | 6.5 | 0 | 6.2 | 20 | 7 | 46 | 4.4 |
| 7/07/2006 | 27 | 8.5 | 0 | 5.2 | 20 | 8 | 47.25 | 4.3 |
| 8/07/2006 | 27.5 | 8 | 0 | 4.8 | 20 | 11 | 65 | 4.1 |
| 9/07/2006 | 27.5 | 6.5 | 0 | 5 | 20 | 10 | 63.6 | 4.1 |
| 10/07/2006 | 29 | 5.5 | 0 | 4.2 | 20 | 8 | 54.3 | 4.6 |
| 11/07/2006 | 30.5 | 8.5 | 0 | 4.6 | 19 | 11 | 62.2 | 4.5 |
| 12/07/2006 | 32 | 12.5 | 0 | 3.8 | 17 | 17 | 67.9 | 4.1 |
| 13/07/2006 | 30.5 | 18 | 1.3 | 3.8 | 15 | 21 | 74.05 | 3.6 |
| 14/07/2006 | 25.5 | 13 | 0 | 4.6 | 17 | 11 | 53.6 | 3.8 |
| 15/07/2006 | 24 | 8.5 | 0 | 5 | 20 | 10 | 61.85 | 3.7 |
| 16/07/2006 | 24 | 9.5 | 0 | 5 | 19 | 11 | 64.8 | 3.5 |
| 17/07/2006 | 22.5 | 6.5 | 0.3 | 5.2 | 20 | 10 | 68.35 | 3.4 |
| 18/07/2006 | 22.5 | 4.5 | 0 | 4.6 | 21 | 9 | 66.5 | 3.5 |
| 19/07/2006 | 24 | 6 | 0 | 4.6 | 21 | 11 | 68.45 | 3.6 |
| 20/07/2006 | 26 | 4.5 | 0.1 | 4.2 | 21 | 10 | 64.9 | 4 |
| 21/07/2006 | 28 | 6 | 0 | 4.4 | 21 | 10 | 63.25 | 4.3 |
| 22/07/2006 | 30 | 9 | 0 | 7.8 | 21 | 14 | 66.5 | 4.3 |
| 23/07/2006 | 29.5 | 10 | 0 | 4.6 | 20 | 17 | 70.6 | 3.8 |
| 24/07/2006 | 29.5 | 12 | 0 | 4.4 | 20 | 13 | 62.15 | 4.4 |
| 25/07/2006 | 28 | 10 | 0 | 5 | 21 | 10 | 54 | 4.5 |
| 26/07/2006 | 26.5 | 7.5 | 0 | 5 | 21 | 9 | 56.45 | 4.3 |
| 27/07/2006 | 28 | 5.5 | 0 | 5 | 21 | 9 | 61.75 | 4.5 |
| 28/07/2006 | 30.5 | 6.5 | 0 | 4.8 | 21 | 9 | 56.8 | 4.9 |
| 29/07/2006 | 31 | 8.5 | 0 | 4.8 | 21 | 11 | 61.85 | 4.8 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 30/07/2006 | 32.5 | 9.5 | 0 | 5.6 | 21 | 12 | 62.25 | 5 |
| 31/07/2006 | 33.5 | 11.5 | 0 | 5 | 22 | 13 | 60.5 | 5.3 |
| 1/08/2006 | 32.5 | 14 | 0 | 5.2 | 22 | 12 | 49.8 | 5.3 |
| 2/08/2006 | 32.5 | 12.5 | 0 | 6 | 22 | 14 | 62.65 | 5.1 |
| 3/08/2006 | 33 | 11.5 | 0 | 6.4 | 21 | 13 | 60.85 | 5.1 |
| 4/08/2006 | 29.5 | 12 | 0 | 7.6 | 22 | 6 | 28.7 | 5.3 |
| 5/08/2006 | 27.5 | 6 | 0 | 5.8 | 22 | 7 | 47 | 4.8 |
| 6/08/2006 | 27 | 5 | 0 | 6.2 | 22 | 7 | 49.95 | 4.7 |
| 7/08/2006 | 27 | 5 | 0 | 7.6 | 22 | 6 | 42.8 | 4.8 |
| 8/08/2006 | 28 | 5.5 | 0 | 6.4 | 22 | 6 | 41.2 | 5 |
| 9/08/2006 | 28.5 | 6.5 | 0 | 6.8 | 22 | 8 | 51.65 | 4.9 |
| 10/08/2006 | 29 | 9.5 | 0 | 7.4 | 22 | 9 | 49.15 | 4.9 |
| 11/08/2006 | 29.5 | 9 | 0 | 6.2 | 22 | 8 | 44.55 | 5.1 |
| 12/08/2006 | 29.5 | 8.5 | 0 | 6.6 | 22 | 10 | 57.25 | 4.9 |
| 13/08/2006 | 30 | 9 | 0 | 5.6 | 23 | 9 | 49.8 | 5.2 |
| 14/08/2006 | 31 | 11 | 0 | 6 | 22 | 12 | 59.1 | 5.1 |
| 15/08/2006 | 31.5 | 11.5 | 0 | 6 | 22 | 13 | 62 | 5.1 |

## 9.4 Animals Removed from Study II

| Anim. ID | Sampling time missed | Reason for removal |
|---|---|---|
| MSF_P15 | 4 h | animal repeatedly crashed into the yards and it was considered likely that it would cause injury to itself or a member of the research team |
| MSF_P15 | 6 h | animal repeatedly crashed into the yards and it was considered likely that it would cause injury to itself or a member of the research team |
| MSF_P15 | 8 h | animal repeatedly crashed into the yards and it was considered likely that it would cause injury to itself or a member of the research team |