The development of indices of welfare for beef cattle in feedlots

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Abstract

The issue of animal welfare is one of increasing importance in many agricultural industries, particularly those based on the intensive management of livestock. While there have been investigations into the welfare status of poultry and pigs kept under intensive conditions, there have been no such studies for intensively managed beef cattle. This study was instigated to develop a set of welfare indices for the beef cattle feedlot industry.

For indices of welfare to be effective they need to be based on a clear ethical framework, pertinent to a model for assessing welfare, scientifically credible and publicly acceptable, i.e., reasonably understandable to the wider community. Moral contractualism was chosen as an appropriate ethical framework for the study.

Methods for assessing animal welfare can be divided into three approaches: (1) examinations of the human-animal interaction, (2) measurements of the mental state or feelings of the animal, and (3) measurements of the physical state of the animal. This study employed the third approach. In this approach, cattle were assumed to have reduced welfare if they were under a significant state of distress. This includes entering a pre-pathological state. This is characterised by physiological changes such as elevated hypothalamus-adrenal-axis (HPA) activity in conjunction with depressed immune functions.

A pre-pathological state is defined in physiological terms, but may well produce behavioural changes. If these could be determined, they would have potential as an aid to the field diagnosis of animals with poor welfare in commercial feedlot situations. Because little is known about the the behaviour of feedlot cattle, three preliminary studies were performed which examined certain behaviours that might be pertinent to identifying distressed cattle.
The first two preliminary behavioural trials were conducted in a large commercial feedlot. These investigated lying behaviour and agonistic and affiliative behaviour. The results of the first trial showed that cattle have a consistent diurnal pattern of lying in commercial feedlot conditions, and that different background environments had no affect on this pattern. The results of the second trial showed that agonistic and affiliative encounters occur frequently in the first 21 days of feedlot life, that there is a clear structure of winners and losers, and a major emphasis on the “head push” as an agonistic behaviour. A third trial was run in an experimental feedlot enclosure. It developed the observational and statistical methodology for determining spatial relationships and grid square occupation for cattle in a feedlot environment. The results of this trial indicated that cattle do have preferences for certain areas in a feedlot.

The experimental part of the study was then initiated. It measured the behavioural and physiological responses of matched groups of cattle to three different environments: (1) pasture, this was regarded as a control, but not with behavioural observations, because group behaviour changes to accommodate different environments, (2) “normal” feedlot, this was characterised by a stocking density of 12 square metres/head and a firm, dry, pen substrate and (3), a “stressed” feedlot which had a stocking density of six square metres/head and a pen substrate which was constantly wet. Inclusive of the physiological responses were two physical measurements of adrenal glands and a panel of 20 immune variables, many of which had not been quantified before on cattle. Two experimental trials were performed.

The results of the experimental trial showed that there was a significantly higher adrenal mass and a higher adrenal cortical volume (strongly indicating increased HPA activity) in both feedlot groups compared to the pasture group. But only two out of the 20 immune variables: serum IgA, and the gamma delta T-lymphocyte WC1, showed decreased activity in the feedlot groups in comparison to the pasture group. It was concluded that support from other immune variables was required before it
could be stated unequivocally that the immune system was compromised and thus that pre-pathological states existed in the feedlot groups. Because pre-pathological individual animals could not be identified, it was not possible to use the behavioural data for determining behavioural correlates. However, the study has provided new information for pasture and feedlot cattle ethograms. Some main findings are: (1) cattle have a very strong motivation for lying behaviour and will adjust their pattern of lying to accommodate chronic wet and crowded conditions, (2) despite high stocking densities, cattle are observed to be by themselves far more than with other animals within one steer’s length of them, indicating that the use of a steer’s length as a measure for determining nearest neighbours should be revised, (3) cattle in all treatments had preferences with regard to the occupation of grid space, (4) the backgrounds of groups of cattle affects their rates of agonistic behaviours.

The conclusions of the main experimental trial are that, although pre-pathological states could not be clearly demonstrated, there were some physiological adjustments taking place to normal and “stressed” feedlot conditions. This suggests that if extra stressors, such as the social mixing of unfamiliar cattle, and/or bacterial or viral pathogens were to be introduced then pre-pathological states could occur, followed in some cases, by pathological states. Further work in this area would benefit by conducting more pre-treatment sampling to clearly establish the baseline for the immune parameters and to “focus” the immune system on a challenge (such as provided by vaccination) midway through the treatments.

In conclusion, this study has determined on an ethical framework and a model for assessing welfare for beef cattle in feedlots. It has provided four physiological variables (relative adrenal weight, adrenal index, serum IgA and WC1 lymphocytes) which are suitable candidates as physiological variables in further welfare studies. It has also provided new findings on the behaviour of feedlot cattle, which have potential for their management and for further welfare investigations.
Acknowledgements

I wish to thank my supervisors, Dr Lloyd Fell of NSW Agriculture, and Professor Robert Whelan and Dr William Buttemer of the University of Wollongong, for their encouragement and assistance during this project. I have appreciated their continued support, and enjoyed the many incisive and constructive discussions we have had regarding the welfare of beef cattle in feedlots. In particular I would like to thank Lloyd for his help and excellent advice throughout the project.

This project would not have eventuated without the financial support of the Cattle and Beef Industries Co-operative Research Centre (CRC) and I appreciate the continued funding of this project through some difficult financial times.

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Finally, trials of this size are impossible to conduct without the assistance of others, and to all of the staff of the Elizabeth Macarthur Agricultural Institute (EMAI) who have participated at one stage or another in the project. I extend my heartfelt thanks.
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Preface

A beef feedlot is defined as: "a confined yard area with watering and feeding facilities where cattle are completely hand or mechanically fed for the purposes of production" (The New South Wales Agriculture Feedlot Manual, 1997).

Beef feedlots in Australia can range in size from a single pen containing a handful of animals to huge dedicated complexes that hold many thousands of animals and consist of hundreds of pens (see overleaf).

The Australian feedlot industry had its beginnings in the Darling Downs area of Queensland in the early 1960's. After years of slow growth there was a period of rapid expansion in the early 1970's. This finished when the Japanese beef market was closed to imports in 1975. Although the market reopened in 1976, growth remained slow for the next 12 years. In 1988, another period of strong growth occurred due to increased demand from the Korean market and enhanced access to the Japanese market. This growth has been maintained. In 1997 beef feedlotting contributed over 150 million dollars to the overall beef market in Australia.

In 1992 a Cooperative Research Centre for the Cattle and Beef Industry was formed. It was a joint venture, established and supported under the Australian Government's Cooperative Research Centres Program. It comprised the following core parties: New South Wales Agriculture, Queensland Department of Primary Industries, the Commonwealth Scientific and Industrial Organisation and the University of New England.

A series of programs were set up inside the CRC. One of these was the Health and Welfare program. It had several aims, one of which was to develop acceptable indices of animal welfare for the intensive beef industry. The work for this thesis was funded primarily by this program of the CRC.
A commercial beef feedlot in northern New South Wales.
Chapter One: Literature Review: Assessing Animal Welfare

1.1 Concepts and definitions of animal welfare

The study of animal welfare encompasses the fields of biology, philosophy, and politics, among others. At this point there is no universal definition of animal welfare and no consensus for means to assess it (Fraser 1995). There are doubts as to whether a universal definition will ever be agreed upon. Two main reasons for this are: (1) that animal welfare is a complex, interdisciplinary subject (Broom and Johnson 1993), and (2) it is intimately connected with contradictory and diverse human values (Tannenbaum 1991; Sandoe and Simonsen 1992; Fraser 1995).

There are a number of definitions of animal welfare in use and they range from the very broad to the very narrow. The broad definitions are useful in that they embrace many of the complexities of animal welfare, but for assessment purposes they are too vague to be applied in practice. For instance, a definition that states that animal welfare is a relationship between animals and humans encompasses many ethical and practical aspects of animal welfare yet is very difficult if not impossible to quantify (Stafleu 1996). The assessment of welfare requires more precise definitions. Consequently, there are a number of practical or working definitions in use. These definitions reflect different approaches to the assessment of animal welfare. They can be placed into three principal categories that emphasise: (1) the human–animal interaction, (2) the measurement of mental states or feelings of the animal, and (3) the measurement of the physical state of an animal (Sandoe and Simonsen 1992; Bekoff 1994; Broom 1996; Duncan 1996). There is a degree of overlap between them, partly due to the fact that multiple rather than single indicators of welfare are advocated (Dawkins 1990, 1997; Broom and Johnson 1993; Broom 1996). Thus an approach which is primarily concerned with evaluating the feelings and preferences of an animal e.g., via behaviour tests, also incorporates indicators of the biological state in the assessment (Dawkins 1990).
These three approaches will be discussed in the next three sections. The first examines the influence of human ethics on animal welfare and outlines four philosophical positions regarding human-animal interactions. The second details the assessment of animal feelings and examines one of the primary issues underlying this approach which is the nature of consciousness in animals. The third details the measurement of biological indices in relation to stress and discusses the issues surrounding the definition and assessment of stress. The overall aim of this review is to arrive at a working definition of animal welfare appropriate for determining indices of welfare for beef cattle in feedlots.
1.2 The human - animal relationship

1.2.1 Introduction

Animal welfare science has been described as a realm of facts and animal welfare ethics a realm of values (Tannenbaum 1991; Sandøe and Simonsen 1992; Mason and Mendl 1993; Fraser 1995). This is because scientific investigations into an animal’s physical or mental well-being generate results, but the interpretation and valuing of these results are processes which are firmly under the influence of human ethics (Rollin 1996).

Fraser (1995) pointed out that this link between animal welfare and diverse and contradictory human ethics and values in effect prevents a universal definition (and universal means of assessment) of animal welfare from being created. Fraser concluded that, in this case, animal welfare scientists should concentrate only on solving individual problems in animal welfare rather than trying to create universal measurements and definitions. The influence of ethics and other human-related factors on animal welfare is illustrated schematically by Figure 1.1. Here animal welfare can be seen as an entity that arises out of a network of contributions from different spheres of activity. The contributions have different weightings that can vary over time and according to political and moral leanings (Fell, in preparation).

Figure 1.1: animal welfare and a network of contributions. No arrows run to or from animal welfare. It arises out of the network.
1.2.2 The animal rights debate and philosophical positions on animal welfare

A good illustration of the diversity of human attitudes to animals is provided by what has been called "the animal rights debate". This debate has a long history. Discussions on this issue were recorded in ancient Greek society. There is no sign of a resolution to this debate, partly because philosophical issues, by their very nature, do not have final answers. The debate itself is best seen, not as a dialogue between two opposing viewpoints, but a continuum of views between two polarised viewpoints. On one pole is the fundamentalist animal liberation viewpoint that supports worldwide vegetarianism. At the other is the view that animals are considered to be little more than machines, allowing wide scope for their exploitation.

The last 20 years have seen this debate become more prominent in the public mind than at any other time (Rowan 1988; Orlans 1993). One of the reasons for this was the publication in 1975 of the book "Animal Liberation", written by the Australian philosopher Peter Singer (Singer 1975, 1990). This brought a huge amount of public attention to the conditions of farm animals, particularly those raised in intensive agricultural systems (Rowan 1988). Singer's work had its antecedents in Ruth Harrison's book "Animal Machines" (1964) and the resulting British parliamentary action, the Brambell Report in 1965. There are two other factors that have contributed to the increase in interest: (1) an increasing gap between consumer and animal producer, leading to a change in consumer perspective (Gee 1986) and (2) the development of a more affluent society that can afford to be more concerned about animal welfare (Jasper and Nelkin 1992).

The topics covered in the animal rights debate are wide-ranging and lengthy. In my opinion, many can be seen as logical arguments based on different moralities or ethical theories. Four different moralities and a selection of arguments from them are as follows:
1. **Theism.** This ethical theory states that morals are created by a divine authority, be that authority a Judaeo Christian God or Buddha, etc. This will not be further addressed because of the lengthy nature and wide variety of theistic theories.

2. **Utilitarianism.** The formation of this theory is attributed to the Scottish philosopher Jeremy Bentham (1748-1832). It states that all actions should be performed so that they create “the greatest good for the greatest number”. Ideally, decisions as to what actions do and do not fulfil this requirement are performed by a neutral arbitrator. This prevents the outcome being biased by personal interests.

3. **Intuitionism.** This ethic states that morals are seen as being outside human identification, but are nonetheless there and can only be seen with the “intellect’s eye” (Leahy, 1994). Good or bad acts are “intuitively” classified as such.

4. **Moral Contractualism.** The formation of this theory is attributed to the sophists from ancient Greece (Rollin 1981). It states that morals are the product of human agreements. Moral agents (humans) enter into a contract. The contract creates ethics under which each agent lives and the system works under a principle of mutual co-operation.

**1.2.3 Utilitarianism**

The most well known proponent of this ethical theory is Peter Singer. His position is summarised in the opening lines of his book “Animal Liberation” : (The book is) *An argument for animal liberation, a plea for vegetarianism, and an end to factory farming*” (Singer 1975 p.1).
The principle of utilitarianism, which previously was concerned with human-human interactions, was transposed by Singer in order to allow the inclusion of human-animal interactions. He achieved this by stating that the capacity of an animal to suffer allows it to be included in a “greatest good for the greatest number” equation. If the suffering of an animal is equal to that of a human then that animal deserves the same compassion and consideration as a human being.

Singer (1975) utilises this concept to criticise livestock industries. He argued that with regard to farm animals, the equation is always lopsided. For example, if humans raise chickens intensively for the purposes of eating chicken then the total suffering of the chickens outweighs the benefits gained by eating them. Therefore, raising chickens for meat-eating purposes should be prohibited. Singer has extrapolated from this position to call for universal vegetarianism, arguing that the suffering involved in raising any animal, intensively or otherwise, outweighs the benefit to humans.

Singer brought the term specieism to prominence. This term, originally coined by the psychologist Richard Ryder (1975), relates to the favouring of humans over animals. Singer maintained that this concept emanates from Judaeo Christian and Greek philosophy, is analogous to racism and sexism and is an insidious and ever-present part of our lives (Singer and Regan 1987).

1.2.4 Intuitionism

A well known proponent of intuitionism is the American philosopher Tom Regan, whose writings are as frequently cited and sometimes confused with Singer’s (Regan 1988). Regan’s arguments are set forth in his book “The Case for Animal Rights” (Regan 1984). He shares with Singer a belief in worldwide vegetarianism and the consequent abolition of all livestock industries. However, Regan maintained that there is a serious flaw in Singer’s utilitarian concept. This is that, in a utilitarian equation, there can never be an impartial arbitrator to carry out the decision-making
process. Humans do the weighing up and humans always run the risk of being influenced by self-interest. Consequently, the potential for bias is high.

Regan's remedy for this deficiency is to supply a non-negotiable quantity for animals which he labels as "inherent value". Essentially any animal that satisfies a certain level of consciousness is the "subject of a life" and possesses "inherent value". He extrapolates from this to state that if an animal has "inherent value" then it has direct moral rights and can make claims on them with humans.

1.2.5 Moral contractualism

Moral contractualism is an ethical theory that permits humans to eat animals but does not permit their mistreatment. A proponent of this theory is the British philosopher Peter Carruthers. Carruthers maintained that animals do not have direct moral rights because they do not satisfy the requirements for creating contracts. This premise is mainly (although not entirely) derived from the inability of animals to use language as humans do (Carruthers 1992).

Animals, according to Carruthers, are not planners, therefore they cannot plan contracts and cannot enter into the moral contract arena. This makes them moral patients rather than moral agents. Carruthers maintains that, nonetheless, there are duties and responsibilities to animals, but these are indirect requirements and they are derived from moral contracts between humans.

1.2.6 Philosophical positions: conclusion

In my opinion none of the theories described covers all of the variations in human-animal relationships. This is possibly because, as the Greek philosopher Aristotle has remarked, morality is a phenomenon that can never be wholly systematised (Clark 1977).
This study is concerned with developing indices for determining the welfare status of beef cattle in feedlots. I feel that moral contractualism is the most applicable philosophical platform for this study because: (1) utilitarianism’s logically undeniable conclusion of vegetarianism and intuitionism’s non-negotiable character are not compatible with a program of this nature, and (2) moral contractualism appears to be the most applicable in that it accommodates the raising of animals for meat-eating purposes, but not their mistreatment. However, moral contractualism has a limitation in that it is a wholly objective approach and it does not accommodate the subjective side of human-animal interactions. Human attitudes to animals are often a blend of objective reasons and subjective feelings, and feelings can frequently prevail over reasons. To some, this issue can be the most dominant one in animal welfare (Midgley 1984).
1.3 Animal welfare and animal feelings

1.3.1 The assessment of animal feelings

This approach maintains that an animal’s welfare is directly concerned with animal feelings (Duncan and Petherick 1991; Wemelsfelder 1993; Duncan 1996; Dawkins 1997). The assessment of these feelings primarily relies on techniques which have animals indicate to the researcher their needs and desires. This assumes that the animals in question possess a degree of consciousness, because needs and desires are seen as conscious states (Dawkins 1997). However, the determination of consciousness in animals is a contentious scientific area. It has been called the deepest mystery in biology (Dawkins 1993).

Consciousness is a large and developing subject and there are many theories regarding its origin and nature. This discussion is concerned only with an outline of some of the properties of consciousness and the argument for consciousness in animals.

1.3.2 Consciousness

As is the case with animal welfare, there is no universal definition of consciousness. In general terms, some properties attributed to it are: (1) the ability to have a sense of oneself or to have an awareness of an ego (2) to think reflectively rather than reflexively, and (3) to have a sense of the past, present, and future (Singer and Regan 1976).

One of the reasons consciousness is difficult to define universally is that it is essentially a private phenomenon that cannot be demonstrated to others. A useful analogy for illustrating this is the feeling of pain. If one person feels pain, he or she can describe it to another person, but they cannot transfer the actual feeling and thus "prove" that they are feeling pain, although their behaviour and description might strongly suggest this (Dawkins 1993; Wemelsfelder 1993).
Although consciousness cannot be directly proven to exist outside oneself, all humans are presumed to experience consciousness because they have a similar physiology and behaviour to oneself (Griffin 1981, 1992; Dawkins 1993). If we accept that animals have varying degrees of similarity in physiology and behaviour to humans then, by inference, this argues that they may have similar states of consciousness. This is one of the principal claims for the presence of consciousness in animals and is called “the argument from analogy” (Dawkins 1993).

An early proponent of this argument was the French philosopher and essayist Voltaire (1694-1778) who stated that, since animals can be taught, they must have minds. The philosopher Donald Hume (1711-1776) stated that animals may be conscious because “like actions between species indicated like causes” and similarities in physiology indicated similarities in emotional and sensory feelings (Regan and Singer 1976). Arthur Schopenhauer (1788-1860) also advocated this theory by arguing that there were more similarities between animals and humans than dissimilarities. Support for this was given by Charles Darwin (1809-1882) who maintained that the difference between animals and man was not one of likeness, but of degree (Regan and Singer 1976). More recently there has been support for the presence of consciousness in animals through numerous and well-documented observations of complex behaviour patterns (Griffin 1981, 1992; Fox 1983).

There are two main criticisms to the argument from analogy. The first is that while some animal behaviour may appear complex, this may not be the case. A set of linked, unconsciously performed, simple behaviours can appear to the observer to be one discrete unit of complex behaviour (Leahy 1993). The nest-building activity of weaver birds illustrates this point. Weavers create very intricate funnel-shaped nests, often characterised by numerous knots. This activity can appear as a complicated task which could be accomplished only by the bird having a mental map of the finished nest. The argument from analogy would in this case proceed as follows: (1) humans have complex behaviours (2) weavers make nests that appear to require complex
behaviour (3) humans have consciousness (4) weavers therefore have consciousness. However, the argument fails in this instance because observations have shown that the birds merely go through a series of stereotypic movements that eventually result in a nest (Kennedy 1992).

Figure 1.2: the nest of the black-capped social weaver. Artwork courtesy of Erin Mayo.

The second criticism is that anthropomorphism can confound interpretations of animal behaviour. Anthropomorphism is the attributing of human mental states to non-human actions. Two criticisms of anthropomorphic interpretations of animal behaviour are that: (1) it can lead to false conclusions, and (2) it is often at variance with the principal of Occam’s Razor, which is to find the simplest explanation for an observation (Kennedy 1992). These points are valid, but at the same time it is worthwhile to recognise that humans can only see the world in anthropomorphic terms. The best strategy for dealing with anthropomorphism with regard to animal behaviour therefore is to acknowledge its influence. Once this is achieved then it has been shown that it can be useful in building models for interpreting animal behaviour. For instance, Moore and Hannon (1994) reported that the most successful approach
for them with their behavioural studies on primates was the use of empathy, a deliberate form of anthropomorphism.

In addition to the argument from analogy there is another issue which receives much debate regarding animal consciousness. This concerns the ability of animals to use language. To some, the lack of language in animals is a major obstacle in attributing consciousness to them (Carruthers 1992; Leahy 1994), but this viewpoint is disputed by others (Clark 1977; Griffin 1991; Dawkins 1993; Wemelsfelder 1993). Cheney and Seyfarth (1982), for instance, showed that Vervet monkeys are able to discriminate between grunts that are indistinguishable to humans. Rollin (1981) has also pointed out that there are many situations in a human context where meaning is conveyed clearly and unambiguously without the use of language, for example, a person hailing a taxi, often does so without speaking, yet the intent and meaning is clear to both the person and the taxi driver.

The arguments regarding the presence of consciousness in animals remain unresolved at this time. Paton (1993) summarised this state of affairs accurately when he stated that while it is quite unlikely to be able to prove that animals are no more than machines, it is as equally unlikely to be able to prove that they are not. Stafleu et al. (1992) maintained that a preferred alternative in their work on laboratory rats was to assume that they possessed consciousness because: (1) there is more evidence in favour of this assumption than against it, and (2) the negative moral consequences of assuming that animals are not conscious are greater than those of accepting that they are. This, in my opinion, is the preferred stance to take on this issue.

1.3.3 Methods of assessment of animal feelings
There are two major methods in use for the assessment of animal feelings. The first one, known as preference testing, determines an animal’s preference for certain objects such as food types, bedding material, housing conditions etc. by arranging them in such a way that the animal can make a choice from two or more alternatives.
This is often performed with the use of Y-shaped walkways which have the two choices placed at the top end of the Y. This test is repeated with many animals and/or with the same animal many times and statistical tests are performed to determine the significance of the choices. Dawkins (1990) has referred to these tests as a first view of the animal's preferences.

Preference tests have been used extensively with farm animals regarding items such as preferences for types of flooring material, sizes of cages and different temperatures and light levels (Mench and Van Tienhoven 1986; Dawkins 1990; Phillips et al. 1992). Once an animal’s preferences are known, the strength of the desire of the animal for a given preference can be measured. To do this an assumption is made that the amount of work the animal engages upon for a certain choice is related to the strength of its desire for that choice. This technique requires training the animal to work for the desired choice and is known as operant training or conditioning. Operant conditioning is a widely used technique and has been successful with many animal species (Alcock 1989).

These techniques have been very successful in demonstrating an animal’s view of the world. They have revealed that there are certain choices for which an animal has a constant, or inelastic demand, in that the animal will work as hard as the test will allow to receive that choice. Other items have an elastic nature in that the animal will work for the choice only up to a certain level. This has allowed the demarcation and calibration of an animal’s needs for some items and desires for others (Friend 1989; Matthews and Ladewig 1994).

1.3.4 Criticisms of the assessments of animal feelings
Preference and operant tests are well-established in animal welfare research and have been influential in many areas of farm animal housing design (Fölsch et al. 1988; Kilgour et al. 1991). However, there have also been conflicting and contradictory results arising from these techniques. Van Rooijen (1983) provided two main reasons
for this: (1) it was unclear to the animal what preferences the researcher was testing for; and (2) other factors not taken into consideration by the researcher influenced the decision of the animal.

One of these factors has been shown to be the previous experience of the animals. Grandin et al. (1994) demonstrated this with an experiment on beef cattle. The animals were required to walk down a Y shaped walkway, both ends of which led to cattle crushes. On one side of the Y the cattle were free to pass through the crush to an open pen. On the other side of the Y the animals were restrained in the crush and then released (see Figure 1.3). The cattle soon developed an aversion to the restraining procedure and preferred to walk to the side of the Y leading to the free crush. However, when the restraining procedure was reversed and performed on the other side of the Y, the cattle still walked through that end, despite the restraining procedure being used on them. Grandin concluded that once having made a choice in the test procedure, cattle were very reluctant to change their preference and this reluctance can confound the interpretation of the results.

The temporal aspect of preference testing must also be taken into consideration. Duncan (1978) has pointed out that animals may not always choose what is best for them in the long term, for example, a feed mixture that has a sweet component such as molasses may be selected over another feed mixture that has a balanced mixture of carbohydrates, fats and protein. The reverse can also apply in that brief events may be important to an animal, but do not show up or have low ratings in preference testing.

A general question of this method of assessment relates to the function of feelings. Feelings presumably direct preferences and it is assumed that feelings help an animal to survive in its environment, but it is possible that some feelings are epiphenomenal in that they do not always have a function in helping the animal live a better life. Therefore, the assessment of some feelings and therefore preferences could be
misleading in terms of an animal’s welfare (Wiepkema and Koolhaas 1992).

Figure 1.3: Y walkway with choices between a restraint or non-restraint procedure.

1.3.5 The assessment of animal feelings: conclusion
Preference testing and operant conditioning techniques have been used extensively on farm and laboratory animals and they have a significant role in the design and planning of farm animal housing. As stated previously, the proponents of this approach state that these tests should not be interpreted in isolation but in conjunction with other indices of welfare. Some potential confounding issues are the nature of consciousness, the influence of time, and the effects of previous experience. This indicates that the results of these tests have to be interpreted with caution (Rushen 1990; Bekoff 1994; Duncan 1996; Wemelsfelder 1997).
1.4 Animal welfare and the assessment of biological states

1.4.1 Introduction

This school of thought maintains that an animal’s welfare can be determined by using a range of physiological and behavioural measures. The principal premise is that if an animal is suffering from significant stress, then it has reduced welfare (Broom and Johnson 1993; Moberg 1996). Accordingly, most physiological and behavioural indices are used to determine whether an animal is suffering from significant stress. The results of these indices are then combined with inputs from other areas such as mental states to establish the welfare level of the animal or animals under study (Wood-Gush, Duncan and Fraser 1975; Moberg 1985; Broom and Johnson 1993; Broom 1988, 1996; Wiepkema and Koolhaas 1993).

There are three challenges with this approach. The first one relates to the relative weighting of the different indices. For example, should a sustained reduction in the level of an immune parameter receive the same weight in evaluation as the development of a stereotypic behaviour? The second one relates to the ambiguity of many indices, for instance, an elevation of heart rate can be due to enjoyable as well as fearful circumstances. The third one relates to calibration of the indices in relation to levels of stress (Duncan and Dawkins 1983). Without calibration there is no way to infer stress from a given level of a chosen biological index. There are at this stage no delineated levels of physiological indices that indicate significant stress and this is because: (1) there are no on-off switches in responses to stress and (2) biological indices associated with poor welfare also have other functions (Gazzaniga 1988).

Barnett and Hemsworth (1990) attempted to tackle this difficult problem by proposing that animals that had sustained levels of plasma glucocorticoids that were 40% above “normal” levels, were under significant stress. However, Rushen (1991) pointed out that this claim has been contradicted by work from other researchers and added to this is the fact that the measurement of corticosteroids in terms of stress is
confounded by many factors such as handling and sampling procedures, the effect of circadian rhythms, and the episodic and brief nature of glucocorticoid expression.

The concept of stress is a crucial issue in this approach and will be discussed next.

1.4.2 The concept of stress

Stress has been called one of the most abused terms in the English language, partly because it is used interchangeably as a noun, a verb and an adjective, e.g., “It seems as if stress, in addition to being itself, and the result of itself, is also the cause of itself” (Jewell and Mylander 1988, in Toates 1996), and partly because there are also different categories of the term in use such as social stress, physical stress and emotional stress. It has a strict definition in physics and engineering, but many definitions in biology.

Numerous papers have been written highlighting the problems in defining biological stress, and there is a consensus that it is a difficult concept to formalise (Friend 1980; Dantzer and Mormede 1983; Freeman 1985; Moberg 1985; Barnett and Hemsworth 1990). Rushen (1986) suggested that the best way to deal with this would be to remove the term altogether. This solution has shortcomings though because the confusion over the concept would still remain and its prominence in physiology and psychology is such that there is a requirement for a sound biological definition (Broom and Johnson 1993). It is also salutary to be reminded that animal welfare research is a young science of around 30 years of age which provides another explanation for the overlapping definitions and consequent misunderstandings (Dawkins 1997).

To provide a perspective of some of the difficulties in defining stress, the following section discusses some of the variability encountered when attempting to measure stress in animals. This will be followed by an outline of common elements in stress definitions and then a discussion on working definitions of stress.
1.4.3 Variability in the measurement of stress

A stress response can be considered as an attempt by an organism to maintain homeostasis following perturbations to its inner and/or outer environment. The interpretation of the response requires several different concepts to be considered in parallel. These are:

1. The concepts of evolution and natural selection.
2. The concepts of coping, learning adaptation, and time. These relate to the development of either resistance or sensitivity to the stressor.
3. The concept of control, which includes the organism’s perception of the stressor.
4. The observer. The response is something which humans observe, measure and deliver judgements on. However, human interpretation can be quite erroneous.

To illustrate this, consider a hypothetical experiment in which groups of rats are placed in two separate cages and are randomly foot shocked through a metal grill floor. The foot shocking is not fatal, but it is unpleasant and the timing unpredictable.

1. From an evolutionary point of view, rats that have a natural resistance at peripheral and/or central locations to foot shocking do not have any great difficulties. This resistance is a function of the genetic makeup of the animal. This natural resistance can take the shape of physical protection, such as a thickened epidermis, some physiological compensatory or reducing mechanism, or have an emotional component.

2. Rats that do not have a natural resistance to foot shocking may develop various physiological and behavioural strategies of avoiding the effects of the shock, perhaps by partially climbing up the sides of the cage, or entering into a tonic, immobile state. As well, they could develop a thickened epidermis over their lifetime. Thus, they adapt to this manner of living. This picture has an addition in
the shape of rats which have a mixture of genetic resistance and physiological and behavioural coping strategies. Conversely some rats can become less able to cope over time, and either eventually die or have an existence that is characterised by sub-optimal physical and/or mental conditions possibly combined with diminished or non-existent reproductive capabilities.

3. Now consider if one of the cages had a lever which would allow a rat to shut off the foot shocking: the shock cannot be prevented, but can be terminated when it occurs. These rats have control over the stressor and can adjust or cope better than rats without the lever. If the shocks are predictable, for example occurring once at sunrise and once at sunset, then this predictability also assists in coping.

4. Assume that there are two researchers observing the rats. One is indifferent to the state of the animals, perhaps due to being raised in an environment where rats were treated as pests. The other is a vegetarian, considering taking up Buddhism and observing the tenet regarding respect for all life forms. Obviously there will be different levels of the observations of rat behaviour and different interpretations of the significance of the behaviour by the two observers.

This example illustrates that differences in experiencing stress and the perceptual bias in the observers can provide sources of variation when interpreting the response of animals to presumed stressors.

1.4.4 Common themes in definitions of stress

There are three themes that are common to many definitions of stress. They are:

1. Homeostasis and perturbations to it. Living systems have a capacity to regulate their internal environment. This capacity is known as homeostasis. Perturbations to homeostasis occur regularly throughout the life of any organism and can take many forms. Some can be directly physical, such as a
high external heat load, or insufficient food and water, others can be more subtle and arise from a combination of emotional and physical sources. The perturbations differ in intensity and duration. The major issue here is what degree of perturbation becomes significant, in a negative sense, to the organism.

2. Perceptual awareness of the perturbations to homeostasis both by the observed and the observer. Significant perturbations to homeostasis are influenced by the perceptions, not only of the observer, but also of the observed, and the two can be quite different. Perception is influenced by the genetic structure of an organism, past history events, cognitive abilities and consciousness capacity (Dantzer and Mormede 1983).

3. Coping and adaptation to the perturbations either positively or negatively over time. Coping is defined as a control of mental and body stability (Broom and Johnson 1993). This is achieved through both physiological adjustments and behavioural strategies.

Many definitions of stress use the framework of control systems theory or first order cybernetics to relate homeostasis, perception and coping to stress (Toates 1996). However, another paradigm, known as second order cybernetics (Fell, Russell and Stewart 1994) has important implications for these themes. Therefore, before discussing some different definitions of stress, these two theories of control systems are outlined.

1.4.5 Control systems: first order cybernetics

The term cybernetics was originated in 1948 by the mathematician Norbert Wiener (1894-1964) who coined it as a label for a theory of control, feedback, and communication within living things and machines. Wiener derived the term from the greek “Kybernetes” meaning steersman (Capra 1996).
The basic principle in cybernetics is that systems, whether inanimate or living are seen as a goal-motivated, that is, self-regulated by internal and/or external feedback and feedforward mechanisms. Cybernetic systems are portrayed through schematic diagrams. For example, the processes at work regarding water consumption in a rat:


1. Body loses water
2. Detectors (volume receptors and osmoreceptors) signal loss of water
3. Drinking occurs
4. Stomach fills with water, sends signal to brain
5. Volume detectors inhibit further drinking.  
   (Adapted from Carlson 1991)

This concept is a very powerful one in aiding the understanding of how living systems function. Animal behaviour, for instance, can be broken down into meaningful components. An animal drinks because it is thirsty and feedback mechanisms control the amount of drinking. Conversely, an animal may anticipate an event such as a long period without water and feedforward mechanisms allow for drinking past normal feedback set points. This theory accounts not only for the functional significance of behaviour of living organisms, but also the tenacity and endurance of this behaviour. In addition, one of the attractions of cybernetic theory is the predictive power of its models.

This procedure of using flow diagrams with feedback lines is now extremely common. It is found in many scientific publications dealing with stress (e.g., Moberg 1987; Sapolsky 1990; Wiepkema and Koolhaas 1993; Jensen 1996; Toates 1996). The idea of goal-directed behaviour also aids in the interpretation of the results of operant conditioning and preference-testing techniques (Toates 1987).
1.4.6 Control systems: second order cybernetics

In the early 1970’s, two biologists, Humberto Maturana and Francisco Varela, proposed a new approach to biological organisation which has had considerable ramifications for cybernetic theory (Bains 1992).

Maturana and Varela developed a theory known as "Autopoeisis". This theory states that biological and other structures have self-referencing properties. They take in inputs from an external environment but process them according to their internal programs.

In second-order cybernetics the organisation of the organism is a central theme (the loss of organisation is equivalent to the death of the organism). The structure of the organism is dedicated to maintaining the organisation. The structure, as delineated by physiological and behavioral adjustments, changes constantly in order to do this (Fell, Russell and Stewart 1994). External perturbations to the organism result in the alteration of the organism’s structure, because the structure changes so that the organisation remains the same. However, since the organisation is self-referencing, the changes in structure will be a function of the internal programming/networking and the external perturbation. Homeostasis, in 2nd order cybernetic terms, refers to the maintenance of the organisation of the self-referencing organism.

The following experiment by Sperry (1945) is seen to be a classic illustration of this theory. One of the features of the eyes of frogs is that they have elongated rather than round pupils. The pupils in effect have a east-west orientation. In this experiment the edge of the eye was cut and the eye rotated 180 degrees during the frog’s tadpole stage. The animal was left to complete its development and metamorphose. Upon maturity it was observed that if the rotated eyeball is covered and a fly placed in front of the frog, it will shoot its tongue out directly at the fly. However, if a fly is presented after the normal eyeball is covered and the rotated one exposed, the frog will shoot its tongue 180 degrees away from the fly. This experiment demonstrates
that the image of the fly on the retina of the frog triggered processes which resulted in the frog’s tongue shooting out to where these internal processes determined the fly to be, rather than where it actually was.

Using this experiment and others, Maturana and Varela have developed extensive theories on communication, organisation, and cognition. There are a number of publications which present and discuss in detail further aspects of second order cybernetics (e.g., Maturana and Varela 1987; Bains 1992; Capra 1996).

The contrast between 1st and 2nd order cybernetics is shown by Figure 1.4:

**Figure 1.4: responses to external perturbations in terms of 1st and 2nd order cybernetics**

Second order cybernetics has application for animal welfare research in that it provides an explanation for the non linear response of many indices to outside perturbations. However, this poses a question. Why attempt to measure physiological
and behavioural indices when their response is potentially so varied and unpredictable? The answer to this question is that a more complete understanding of the function of an index with regard to the maintenance of the organisation of the organism will allow for a more accurate prediction of the response. As well, some indices have a more prominent role with regard to the maintenance of the organism’s organisation than others, therefore they may be expected to show a more consistent response. In essence, a fuller understanding of the roles and functions of the chosen indices allows for a more consistent prediction of their responses to outside perturbations.

The best example of this is provided by the glucocorticoid, cortisol. Cortisol has been measured many times in stress research and as Rushen (1991) has pointed out, the results have been highly variable. Yet a further understanding of the role of cortisol (e.g., Munck et al. 1984) has provided an explanation for many of these variations.

### 1.4.7 Definitions of stress

The following section presents some contemporary definitions of stress that are relevant to its assessment in beef cattle in feedlots. Before discussing these definitions, the contributions of Claude Bernard, Conway Walter Cannon, and Hans Selye to the development of current concepts of stress are briefly presented.

In 1878 Claude Bernard introduced the concept that an animal’s “internal environment” was constant whereas their “external environment” was variable. Conway Walter Cannon (1871-1945) examined the responses of the sympathetic nervous system to stressful situations. In 1914 he introduced the term “homeostasis” and devised the theory of the “flight or fight” syndrome (Allegra and Oliverio 1988).

Hans Selye, at times referred to as the “Father Of Stress” (Jasmin and Proschek 1991) provided the next major contributions to stress research. A medical practitioner who practiced on service personnel during the Second World War. Selye was a prolific and
well-published authority on stress and its effect on people. His most well known thesis was the "General Adaptation Syndrome" (GAS). This proposes that a wide range of stressors evoke non-specific responses in higher organisms, consisting of an increased secretion of adrenal glucocorticoids, suppression of the immune system and the formation of gastro-intestinal ulcers (Broom and Johnson 1993). This response could be divided into three stages:

1. alarm, where the organism first develops a response to a stressor;
2. physiological resistance to the imposition caused by the stressor;
3. exhaustion, the stressor continues to be significant to the organism despite the stage of resistance, leading, ultimately, to the death of the organism.

(Selye 1950).

Selyes model has been extensively modified since then. It has been pointed out that:

1. The response to a stressor is not always non-specific. There are many specific reactions to certain types of stressors (Friend 1980; Freeman 1985; Moberg 1985; Broom and Johnson 1993).
2. Some stressors such as heat, fasting, and exercise do not generate a GAS response (Mason and Mendl 1993).
3. The GAS model places too much emphasis on the physical aspects of stress and not enough on the psychological aspects (Mason 1971).
4. Selye’s hypotheses regarding the secretion of glucocorticoids was that they enhanced the functions of the immune system. If they were expressed in high enough concentrations over a prolonged period they also contributed to "diseases of adaptation" which Selye hypothesised, were the result of the GAS gone awry. The role of glucocorticoids has since been significantly revised. They have a regulatory effect on the immune system, and do not contribute to diseases of adaptation, diseases which, subsequently were extremely hard to replicate in experimental situations (Munck et al. 1984).
These criticisms do not deny that a GAS exists, but they point out that it is not appropriate in all situations. The concept of a generalised response is still of use providing that the preceding caveats are born in mind (Mench and Van Tienhoven 1986).

1.4.8 Operational definitions of stress

Broom and Johnson (1993) defined stress as an environmental effect which creates an overtaxing of control systems so as to reduce fitness (or is likely to do so). The term fitness is used in an evolutionary sense e.g., lifetime reproductive output. This comprehensive definition includes plant and animal systems.

With many farm animals, fitness is inappropriate as a metric for stress because farming practices render them non-reproductive. They are also slaughtered before they reach their full term of life. For this reason Broom and Johnson (1993) argued that if direct signs of fitness are not available then indirect ones must be used. They listed a range of physiological and behavioural indicators which may be used, such as immunosuppression, elevated hypothalamus-adrenal-pituitary (HPA) axis activity, low weight gain, stereotypic behaviour and others. However, this definition is criticised by Jensen (1996). Jensen pointed out that, in accordance with Tinbergen's classification theory, behaviour is analysed on 4 different levels. They are: (1) the evolutionary history of the behaviour, (2) the functional significance of the behaviour, (3) the ontogeny of the behaviour, and (4) the proximate causation of the behaviour (Tinbergen 1963). These levels are considered to be independent of each other and any explanation of a particular phenomenon needs to be kept within one level. Jensen maintained that the inclusion of behaviours which have a proximate cause (perhaps such as stereotypies) in a definition which involves evolutionary fitness is in effect mixing ultimate and proximate levels of analysis.

Jensen (1996) proposed a model of stress which he stated was quite similar to Broom and Johnson's with the exception of the use of fitness. Using a control systems
approach, Jensen depicts stress as a situation that occurs in an organism when its feedback controls cannot compensate against perturbations to it. This principle is demonstrated with Figure 1.5. The hypothetical perturbation to the organism is a heat load resulting from a high ambient temperature.

This concept links stress to feedback and stimulating mechanisms. The animal is not under significant stress when the stimulation (S1) is negated by the feedback adjustments (F1), but when stimulation is greater than the feedback adjustments stress occurs. Jensen defined stress as a state which occurs when S1 is greater than F1, and when the connection between S1 and F1 is open rather than closed e.g., the stressor overrides the feedback adjustments or the feedback adjustments have no effect in maintaining homeostasis.

Using this principle, Jensen maintained that stress can be identified using behavioural and physiological indices similar to those advocated by Broom and Johnson. Concerning the previous diagram, in behavioural terms, if an animal is seen to be panting and wallowing excessively then this indicates that a state of stress is
occurring. However, this brings into play the issue of calibration, which is a consistent theme in stress research: how much panting and wallowing indicates a significantly stressful state? In recognition of this, Jensen proposed that this be accomplished by determining “normal” behaviour in an individual or a species and then using it as a basis for comparison. However, it can be an extremely difficult logistical exercise to determine the normal behaviours of an individual or a species. Further, in terms of housing systems, Duncan and Dawkins (1983) have pointed out that different behaviours can simply mean adaptations to different environments and not be indicative of suffering.

Another approach to defining stress was proposed by Moberg (1985, 1996). He used the term “pre-pathological state” as a way of qualifying a “stressed” condition. This state is attained when the organism, in response to a perturbation(s) to homeostasis, in attempting to cope with the perturbation(s) becomes predisposed to metabolic declines and opportunistic infections (see Figure 1.6). It is identified physiologically by the presence of an elevated hypothalamus-pituitary-adrenal axis in conjunction with a depressed immune system. This definition answers in part the problem of determining what levels of a biological index are physiologically pertinent with regard to stress.

![Figure 1.6: the pre-pathological state model (adapted from Moberg 1996).](image)
This definition is either associated with, or incorporated into, other approaches to quantifying a state of significant stress. Kirkwood et al. (1994) for example, pointed out similarities in the descriptions of stress by Sanford et al. (1986) and Wiepkema and Koolhaas (1993). Sanford et al. for example, described three states:

1. A state of stress: where there is a graded response to normal fluctuations against homeostasis.
2. A state of overstress: where the organism puts substantial expenditure into combating the perturbation.
3. A state of distress: where the organism is not able to combat the perturbation.

In this instance a state of "overstress" is similar to a pre-pathological state and a state of "distress" can be identified as a pathological state as evidenced by disease onset, etc. Pathological states are not suitable as stress indicators, because by the time of their onset, the organism has suffered serious consequences from the stress. Pathological states therefore can be seen as "lag" rather than "lead" indicators.

1.4.9 A definition of stress for beef cattle in feedlots

For beef cattle in feedlots, the definition of Moberg (1996) appears to be the most applicable for the following reasons:

1. A pre-pathological state can be supported on the frameworks of either first or second order cybernetics in that it is a result or a function of internal processes, for example, a pre-pathological state could arise when stimulation is greater than feedback control in a first order cybernetics system, it could also be evidence that a self-referencing organism is undergoing significant structural change in order to maintain its organisation.

2. Moberg’s definition is similar to Selye’s and Broom and Johnson’s definitions in that physiological indicators such as immunosuppression and elevation of
hypothesis-pituitary-adrenal axis activity are utilised. It therefore possesses a degree of non-specificity with regard to different types and intensities of stressors.

A pre-pathological state is defined physiologically. However, behavioural indicators of stress, which at this stage are unknown for beef cattle in feedlots could possibly be elucidated by studying animals defined as being in a pre-pathological state.

1.4.10 The positive side of stress

On a final note, the positive side to stress should be mentioned. Stress has been shown to help an organism survive in its environment. Zulkifli and Siegel (1995) for instance, pointed out that chickens raised in a “low stress” environment have lower resistance to environmental pathogens. They postulated that early stressful experiences which, they emphasised, are not too stressful, may be critical in helping animals to survive in certain environments. Wiepkema and Koolhaas (1993) also stated that good welfare can be linked to the occurrence of short-lasting stress responses (e.g., conflict behaviour), which may prevent boredom and optimise the alertness and vigilance of the animal.
1.5 A definition of animal welfare for beef cattle in feedlots

For the purposes of this thesis a definition of animal welfare is required which leads to practical assessment methods. However, concern has been raised that “practical” definitions tend to lose sight of, and subsequently erode, other social, political and ethical components of animal welfare (Stafleu et al. 1995).

To partly address this concern, I propose to use a working definition of animal welfare which I feel is the most appropriate for feedlot cattle. This will be accompanied by an acknowledgement that the results of the work arising from this definition will not represent the final word on discussions of the welfare of beef cattle in feedlots but will represent a significant input into those discussions.

Definitions which emphasise the animal’s mental state, may to some extent avoid the ambiguity and problems of calibration that arise from physiological and behavioural indices of welfare. They also include a quality of life component in that it is not enough for an animal to be just coping with its environment, it must be experiencing positive feelings as well. However, the determination of consciousness and the measurement of an animal’s feelings are areas of deep complexity and concerning beef cattle the findings have been difficult to interpret (Grandin 1994). In my opinion it would be insufficient to determine indices of welfare for beef cattle in feedlots by emphasising the assessment of mental states.

The definitions which emphasise the physical state of the animal appear the most suitable for this work. A definition of animal welfare proposed by Moberg (1996) is particularly appropriate. This definition states that an animal’s welfare is reduced when it is suffering from a significant amount of stress and this state is identified by the presence of a pre-pathological state. Therefore, the first task in the assessment of welfare levels of beef cattle in feedlots is to determine whether or not significant numbers of beef cattle experience pre-pathological states in a feedlot environment.
Chapter Two: Literature Review: Indices of Welfare

2.1 Introduction

The indices of stress and welfare relevant to Moberg’s definition are concerned with the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the function of the immune system. This section will outline the principles and major features of these systems. The inter-relatedness of the nervous, immune and endocrine systems will also be outlined, followed by a discussion on stress and immunity. A pre-pathological state is defined by physiological indices. However, it is possible that some behaviours are associated with this state and therefore some behavioural aspects of stress will also be detailed.

2.2 The HPA axis

Perturbations to homeostasis (stressors) result in numerous behavioural, physiological and biochemical changes. These changes are conceptualised as components of an integrated “stress system” (Johnson et al. 1992). The HPA axis and the Sympathetic Nervous System (SNS) are major elements of this system. Products of the HPA axis and the SNS exert stimulatory and inhibitory effects in an integrated and complementary fashion on virtually every tissue type in the body (Cohn 1991). The HPA axis is regulated by a complex collection of pathways (Dohms and Metz 1991). A simplified schematic version is shown by Figure 2.1:
perturbation (stressor)

perception of stressor by organism

corticotrophin releasing hormone (CRH) synthesised in parvocellular cells of the para ventricular nucleus (PVN) of the hypothalamus, becomes the principal effector for adrenocorticotrophic hormone (ACTH) release.

adrenocorticotrophic hormone (ACTH) synthesised and released from anterior pituitary

biosynthesis and release of corticosteroids (glucocorticoids and mineral corticoids) in the adrenal cortex

increase in plasma corticosteroids

target tissues

Figure 2.1: the HPA axis
The magnitude of the response of the HPA axis to external stressors depends on a variety of factors such as circadian rhythms, stress history, season etc. Harbuz and Lightman (1992) stated that the response is in proportion to the severity of the stressor, but only over a small range. This limits the idea of using the size of the response as an indication of the magnitude and identification of the stressor.

The corticotrophin releasing hormone (CRH, also called corticotrophin releasing factor, CRF) that is secreted by the hypothalamus during stress is the principal biological effector of the HPA axis. CRH expression is influenced by many factors apart from external stressors. It is stimulated by products of the immune system such as the cytokines, interleukin-1 (IL-1), and interleukin-2 (IL-2) and inflammatory mediators such as Platelet Activating Factor (PAF) and Tumour Necrosis Factor (TNF). Products of the sympathetic nervous system; adrenaline (A) and noradrenaline (NA) exert negative control over CRH expression as do circulating glucocorticoids, increased blood pressure, ACTH, β-endorphin and CRH itself. CRH expression has also been shown to have a diurnal rhythm in some mammals (Johnson et al. 1992). Receptor sites for glucocorticoids have been located centrally in the hippocampus, the anterior thalamus, the septum and mesencephalic reticular formation sites of the brain suggesting that suppression of CRH by glucocorticoids takes place at these sites as well as in the pituitary (Kannan 1988).

CRH is the most effective stimulator of ACTH production, but ACTH is also regulated by other peptides such as arginine vasopressin, oxytocin, angiotensin II, and serotonin. Lymphocytes have been shown to produce ACTH as well (Toates 1995). ACTH is derived from propiomelanocortin (POMC). POMC is located principally in the brain and pituitary but is also found in other tissues (Johnson et al. 1992).

ACTH stimulates the production of glucocorticoids in the zona fasciculata of the adrenal cortex. The effect of glucocorticoids on bodily functions such as metabolism, inflammation, and immunity is profound (Harbuz and Lightman 1992). Corticosterone and cortisol are glucocorticoids which have been studied extensively
with regard to indices of stress (Barnett 1987, Shutt, Fell and Connell 1987; Pitman et al. 1988; Flores et al. 1990). The HPA axis applies to most vertebrates, but the glucocorticoids produced may vary, for example, corticosterone is the predominant glucocorticoid in birds, reptiles, seals, amphibians and rodents. Cortisol is found in most other mammals.

Glucocorticoids exert catabolic effects on some tissues and anabolic effects on others. In general terms they inhibit anabolic functions such as growth, immunity, digestion and reproduction while stimulating protein catabolism and the synthesis and release of glucose, amino acids and free fatty acids from muscle, fat, tissue and liver. These effects are receptor mediated; glucocorticoids bind to cellular receptors on target tissues and then to cellular DNA which results in the alteration of gene expression of that tissue (Cohn 1991).

Glucocorticoids are particularly known for their suppressive effects on immune and inflammatory cell functions. (Munck et al. 1984; Buckingham 1996). This effect varies with different species. The mouse, rat, and rabbit for example have been found to have immune systems relatively sensitive to glucocorticoids whereas other mammals such as humans, dogs, and pigs are comparatively more resistant (Cohn 1991). Glucocorticoids have a greater effect on cell-mediated immune mechanisms than on humoral ones, and this varies as well, for instance immature CD4-/CD8+ thymocytes are the most sensitive elements to cortisol and mature CD4+/CD8- thymocytes are relatively resistant (Hässig et al. 1996). Some other effects include the reduction in numbers and efficacy of circulating macrophages and B and T lymphocytes. They affect leucocyte functions and increase random rather than target-directed movements of neutrophils (Cohn 1991). They also decrease the production and effects of inflammatory mediators and they impair phagocytic immunity by preventing phagocytes from reaching sites of inflammation or infection.
2.3 The immune system

Immunosuppression is an important aspect of animal stress and welfare and there are many studies which link chronic stress to depressed immune function in humans and animals (Maier et al. 1985; Martin 1987; Biondi and Zanino 1997). A diagram outlining the essential features of the immune system is presented with Figure 2.2

The immune system has traditionally been divided into two systems, the innate and the adaptive (or acquired) system. Invertebrates and some vertebrates possess only an innate system. This system is non-specific and non-adaptive in that the immune cells do not specifically recognise different antigens, nor do they possess a memory of those antigens after exposure to them. Higher vertebrates possess an innate system and an adaptive system. These systems do not act separately but work together in a highly integrated manner (Davey 1989).

The activities of cells of the innate system can be divided into three categories: (1) the engulfing of foreign bodies (phagocytosis), (2) the destruction of foreign cells and organisms by disrupting their cell membranes (cytotoxicity), and (3) the generation of an inflammatory response around the site of an infection. These activities are effected in higher vertebrates by two groups of leucocyte cells: the granulocytes such as the neutrophils, basophils, mast cells and eosinophils which are found in the bloodstream, and the monocytes which differentiate into macrophages and are found mostly in lymphoid tissue (Tizard 1996).

A number of antigens are initially destroyed by the cells of the innate system. However, some persist past this stage and in higher vertebrates, a second phase immune response occurs. The first stage of this response involves the suitable presentation of the antigen to the adaptive system immune cells. Macrophages and dendritic cells are among some of those that perform this function. The second stage of this response involves the activity of the adaptive system cells.
Adaptive immunity is divided into two sub-systems: the humoral and cell-mediated immunity (CMI) systems. Cells from both of these systems originate from stem cells in the bone marrow. CMI system cells, known as T cells, migrate from the bone marrow and mature in the thymus. Humoral cells, known as B cells mature in the bone marrow or in the case of birds, the bursa Fabricius (Tizard 1996).

Humoral and CMI cells have specific epitopes or antigen binding sites on their cell membranes which makes them highly specific for one or two distinct antigens only. After recognition and binding to an antigen, clonal expansion takes place which allows the proliferation of large families of these specific cells. These are divided into memory cells and cells which facilitate the destruction of the antigen. There are millions of T and B cells with individual epitopes in an individual human being (Davey 1989).

Humoral cells, which work in concert with T cells, are characterised by their capacity to secrete antibodies – the immunoglobulins (Ig)A, IgG, IgM, and IgE. Activation of B cells results in a proliferation of these cells into plasma cells and memory cells. Plasma cells secrete large amounts of antibody, which bind to the antigen. Some antigens such as the cell walls of bacteria such as *Streptococcus pneumoniae* can directly stimulate the production of antibodies without help from T cells. B cells require help from T cells and antigen presenting cells before maturation to antibody producing plasma cells occurs (Maier et al. 1985).

T cells are divided into three groups: cytotoxic, helper and suppressor cells. Cytotoxic cells destroy infected body cells. Helper cells are essential for the activation of cytotoxic and suppressor T cells and most B cells. Suppressor cells inhibit all T cells, most B cells and sometimes also cells of the innate system.
Figure 2.2: the essential features of the immune response. Adapted from Tizard, 1996
Cytotoxic T cells differ from B cells in that they recognise fragments of antigens that are on the surface of a cell membrane in association with a particular group of molecules. These molecules are the products of a set of genes called the major histocompatibility complex. This allows for the destruction of antigens hidden in cells such as viruses. Infected host cells, macrophages, B cells, and phagocytic cells are capable of presenting this complex to these cells (Tizard 1996). There is a degree of specificity in this process, for example, cytotoxic T cells such as CD8+ recognise antigen in the context of MHC class 1, whereas CD4+ cells recognise antigen in the context of MHC class 11.

The action of the T helper cells is facilitated by the secretion of glycoproteins known as cytokines. Activated T cells secrete over 20 different cytokines. Apart from promoting the effector cells of the immune response, cytokines also perform a number of important functions regarding communication with the nervous and endocrine systems (Toates 1995).

Other components of the innate response are natural killer (NK) cells. These are large white blood cells that are non-specific in their action, but only react to a limited range of targets such as host cells infected with viruses and tumours (Tizard 1996).

2.4 The immune, nervous and endocrine systems

Ursin (1994) wrote that in the early 1980's the response of the scientific community to the theory that there was a complementary and coordinated relationship between the nervous, endocrine and immune systems was very reserved. He quotes a paper on this subject which was stalled for two years before eventually being published in the Scandinavian Journal of Psychology in 1984. This approach has changed significantly since that time. Today there is widespread acceptance and evidence for these systems being viewed as one integrated system (Blalock 1994; Ursin 1994; Falaschi et al. 1994; Husband 1995; Toates 1996). Some of the reasons for this are:
1. Neuroendocrine-nervous-immune system interactions are known to share common signal molecules and receptors. For example glucocorticoid, ACTH and CRH receptors are found on leucocytes and interleukin-2 (IL-2) binding sites are widely distributed in the nervous system. Receptors for interleukin-1 (IL-1) are also found in the pituitary, and in brain sites such as the hippocampus and hypothalamus (Falaschi et al. 1994).

2. Hormones and neuropeptides can alter functional activities of immune system cells. The immune system and its products such as cytokines in turn modulate neuroendocrine functions. One example of this is that the circadian rhythm of peripheral blood lymphocyte counts is inversely related to cortisol levels in plasma (Toates 1996).

3. Sympathetic Nervous System (SNS) pathways terminate in lymphoid organs such as the spleen, thymus, and bone marrow at the surface of lymphocytes. This suggests that sympathetic activation exerts either an inhibitory or stimulatory effect on the immune system.

4. Electrical stimulation or interference with brain sites has been shown to alter immune function. Immune responses have also been shown as able to be conditioned, indicating that the CNS influences the memory cells of the immune system (Toates 1996).

5. Lymphocytes of mice infected with the virus responsible for Newcastle Disease can produce ACTH. This indicates a possible “short cut” with regard to HPA axis stimulation.

Blalock (1994) and Husband (1995) have suggested that this complex inter-relatedness lends support to the theory that the immune system is not just a response-based effector mechanism, but is also a sensory organ which recognises non-cognitive stimuli such as bacteria, viruses and tumours that are not distinguished by the nervous
system. The nervous system is thought to recognise only cognitive, physical, emotional and chemical stimuli. They suggest that the role of the endocrine system is seen as one of regulation. It is triggered by the immune system (amongst many other triggers) and in turn counter-regulates it. It is interesting to note that these ideas are in keeping with the network theory of second order cybernetics.

If this is the case, then the results from some studies indicate that this triggering and counter-regulation process may be quite intricate and complex. Mormede et al. (1988) for example, in an experiment on foot-shocked rats found that some immune responses did not appear to be affected by the HPA axis. Flores et al. (1990) also found that, in rats, elevation of the HPA axis as stimulated by restraint stressors, did not have any effects on NK cell activity and mitogen stimulated lymphocyte proliferation.

2.5 Stress and immunity

There is a wide range of tests used to examine the immune responses of animals suffering from chronic stress. Until animal welfare regulations limited its application, one commonly used method was to deliberately infect experimental animals and then measure their morbidity and mortality rates. Currently a range of more subtle and specific methods are employed. Some of these are detailed in the following section. The results of many of these tests have been inconsistent in terms of expected responses to stressors and possible reasons for these inconsistencies are also presented.

2.5.1 Tests of cell-mediated immunity

A commonly used method is to measure the activity of T and B cells by determining their rate of proliferation of these cells in \textit{in vitro} culture systems. The proliferation can either be unstimulated or stimulated with mitogens such as phytohaemagglutinin (PHA), concanavalin A (Con A), and lipopolysaccharide (LPS).
Ballieux and Heijnen (1987) stated that these tests should be interpreted cautiously. The test calls for a cooperative interaction between monocytes, non lymphoid mononuclear white blood cells and the different types of T lymphocytes. A diminished response could be due, not only to stress, but possibly also to the following reasons:

1. The monocytes failed to produce sufficient growth factor for the T cells or produced a suppressive amount of prostaglandins.
2. The T cells changed in sensitivity to growth factors put out by T cells or monocytes.
3. PHA induced responsiveness differs depending on whether defibrinated whole blood cultures or isolated blood lymphocytes in control plasma are used.

One other factor that can affect these results is that, in many studies, lymphocyte proliferation assays are performed on peripheral blood samples. Peripheral blood does not contain the whole pool of lymphocytes, but is thought to constitute a representative sample in that lymphocytes move from tissue to tissue via the bloodstream. Some studies have shown that this is not always the case (Hou et al. 1996; Dhabhar 1995; Tarcic et al. 1995). However, with a proper experimental design (control and test) false conclusions will be minimised since these caveats would apply to all in vitro examinations of immune function.

Studies relating stress to lymphocyte subsets have not produced clear-cut results. For instance, Morrow-Tesch et al. (1996) investigated the relation of heat stress to a subset of T cells labelled as gamma-delta (γδ) T- lymphocytes in cattle. These cells constitute more of the total lymphocyte population in ruminants and pigs than in any other species. Their data were not conclusive although changes in cell numbers did occur after varying intervals of heat stress. Hessing et al. (1995) were able to relate social structure to total numbers of CMI cells. They found that dominant pigs (as determined by ranking at feed troughs and agonistic victories) had higher levels of CMI cells than subordinate pigs. Pigs classified as active or passive in their coping
strategies to handling and intensive housing also had significant differences in humoral cell levels with passive pigs having a higher level. These findings could have a relationship to stress if subordinate pigs are presumed to be more stressed than dominant pigs. Changes in the numbers and percentages of the lymphocyte subsets CD5, CD4, CD8, and the CD4: CD8 ratio have been associated with acute stress in humans (Kiecolt-Glaser et al. 1992; Manuck et al. 1991; Naliboff et al. 1991).

2.5.2 Tests of humoral immunity

It has been theorised that relatively slow-reacting indicators such as the immunoglobulins would be better markers for chronic stress than acute stress (Broom and Johnson 1993). However, both positive and negative relationships between immunoglobulins and stress have been found (Ursin 1994). For instance, Kugler et al. (1996) reported that competition stress in soccer coaches increased salivary IgA and salivary cortisol, but Skandakumar (1995) found that stress in dogs was related to lower salivary IgA and higher cortisol levels. Kelley et al. (1980) also found that in cattle, stress resulting from hot air and high humidity conditions reduced serum IgG levels, but cold conditions resulted in only slightly reduced levels of serum IgG and had no effect on serum IgM. Perhaps different types of stress elicit different types of physiological response (e.g., Mason 1971).

2.5.3 Tests of innate immunity

Some measurements of the innate system have been linked to chronic stress in humans. For example, Zakowski et al. (1992) found that natural killer cell activity, and lymphocyte responsiveness was significantly lower in bereaved women than in non-bereaved women. Other measurements have been linked to acute stress. Murata et al. 1987, for example, found that blood samples of calves collected after a period of road transportation had higher neutrophil counts than did samples collected before the period of transportation.
2.5.4 Stress and immunity: conclusion

There are many reasons that can partly account for the inconsistencies seen in studies that relate stress to the immune system. Some of these are:

1. In many cases, a certain technique such as immobilisation through restraint or forced swimming in cold water or social mixing of animals is arbitrarily classified as imposing a significant stress to the animal. Immune system assays are performed with the expectation that the results will reflect this. A problem with this assumption is that it is possible that the putative stressor was not perceived by the animal as being stressful. This can lead to results differing from those expected.

2. If a network approach (based on second order cybernetics) is adopted then the inconsistencies can be seen as a result of an as yet incomplete understanding of the immune network.

3. The presence or non-presence of pathogens can confound the interpretation. As Ursin (1994) points out, low immunoglobulin levels, for instance, may not necessarily mean low immune function, rather it could be that the animal has not been challenged by infectious agents and could be in a state of good health.

4. The quality, quantity and duration of the stressor may vary (Booth 1997).

5. Other factors such as diurnal rhythms also play a role; for example, studies in hens showed that granulocyte counts and activities of phagocytes exhibited clear diurnal rhythms (Kondo et al. 1992; Cahyanningsih et al. 1990).

2.6 Stress and behaviour

The study of animal behaviour is closely linked to the issue of animal welfare. Frequently behavioural signs of distress will occur before an animal enters a pathological state (Broom and Johnson 1993; Gonyou 1994). However, there are many difficulties in the interpretation of animal behaviour. For this reason, behavioural indices are often combined with physiological indices, although one of the problems arising from this is that the collection of samples for physiological
assays can interfere with the behaviour of the animals under examination (Ladewig and Von Borell 1988). Some factors that can confound the interpretation of behaviour are:

2. An incomplete understanding of “normal” behaviour (Broom 1987).
3. Individual variation (Martin and Bateson 1993).
4. Differing interpretations of the motivation of certain behaviours (Jensen and Toates 1993).

Anthropomorphism has been discussed previously in section 1.3.2 and will not be detailed further in this review. Each of the other points will be considered below.

2.6.1 Normal and abnormal behaviour

To be able to recognise abnormal behaviour it is essential to know what constitutes normal behaviour in a species and in an individual. One primary strategy has been to examine the behaviours of animals in wild or semi-captive environments, and use the ethogram (inventory of behaviour patterns) derived from them for comparison with animals in captive environments. For example, the behaviour of pigs kept in open range systems has been compared to those of pigs in intensive piggeries (Gonyou 1994). This process has been taken further with comparisons being made between animals in normal and artificially stressful captive environments. In these studies, allowances have to be made for the different behavioural adaptations animals may make in different environments before determining that a particular behaviour is indicative of reduced welfare (Duncan and Dawkins 1983). The interpretation of these differences in behaviour requires careful consideration, for example, in a study on wild and captive giraffes, it was concluded that it was possible that some behaviours correlated with, rather than caused, enhanced welfare. In other words, they were a symptom of good welfare, rather than a contribution to it (Veasey et al. 1996).
2.6.2 Individual variation

There can be a wide range in individual behaviour within any group of animals. This is related to many factors such as: (1) ontogenic or developmental processes (2) genetic influence e.g., “active”, and “passive” strains of pigs (3) the animal’s position in the social structure of the group and (4) the immediate environment (Hinde 1982). A number of these factors are also subject to temporal variation.

The results of group behaviour studies also have the potential to mask individual variation. Martin and Bateson (1993) provided an example of this with a graph of the mean growth rate derived from a group of kittens. The line of this graph does not represent any single kitten in the group (see Figure 2.3). This can be important in terms of animal welfare. A group that is considered to be on average having good welfare, can still have individuals that have a relatively high degree of suffering.

![Figure 2.3](image)

*Figure 2.3: two graphs of the growth rate in kittens. The graph on the left represents an individual kitten, the graph on the right the change in mean weight derived from the group. Adapted from Martin and Bateson 1993.*

2.6.3 Motivational aspects of behaviour

Ethologists examine behaviour on four different levels in order to understand why an animal behaves as it does. These four levels have been previously mentioned in section 1.4.8. They are: (1) ontogenic or developmental aspects of behaviour; (2) function; the beneficial consequences of the behaviour from an evolutionary fitness
viewpoint; (3) phylogeny; the evolution of the behaviour; and (4) causation; the immediate or proximal causes of the behaviour (Tinbergen 1963). Behavioural aspects of animal welfare can encompass all of these levels, but it is predominantly associated with causation (Duncan 1995).

One of the major findings arising from the study of the behaviour of farm animals is that they may have essential behavioural needs. Needs vary in type and intensity according to the species and the individual. This has led to some broad classifications, Poole (1992), for example, maintained that all vertebrates had ethological needs, and mammals had ethological and psychological needs although this division can be debated because it assumes that all mammals have conscious states and that non-mammals lack consciousness.

The motivational processes associated with needs are major components in an animal’s well-being. If these processes are thwarted or frustrated over a period of time, then welfare can be reduced (Friend 1989). Much work has gone into determining the nature of the processes. However, Jensen and Toates (1993) have pointed out that this can be a problematical exercise, highly prone to error. They maintain that it is more fruitful to measure the strength of the motivational process, through techniques such as preference testing and operant conditioning, rather than to try and elucidate their nature.

An important distinction is made between the internal processes that motivate a certain behaviour and the behaviour itself. If a different behaviour satisfies the process, then, it would appear logical that welfare is not reduced. Further, the process may be satisfied by the actual performance of the behaviour and not the outcome of the behavioural event itself. Two examples of this are dust-bathing and nest building activity in hens. Open range birds will dust-bathe on a regular basis. Birds kept on wire floors have no access to dirt and dust, but nonetheless also regularly engage in this behaviour (Gonyou 1994). Similarly, hens will attempt to build nests in
environments that are already provided with suitable nests (Hughes and Duncan 1988).

If a behaviour is driven more by an external stimulus than an internal motivation, predator-avoidance behaviour for instance, then the outcome of the behaviour and not its performance would seem to be more important to the animal than the performance of the behaviour alone, and consequently captive environments may not be required to accommodate this behaviour. It is argued, however, that the lack of this behaviour creates a void, which would need to be filled, and the presence of a void in the behavioural repertoire constitutes a welfare problem (Veasey et al. 1996).

2.6.4 Behavioural indices of welfare

One of the most distinctive behavioural features associated with poor welfare is stereotypic behaviour. In terms of animal welfare, stereotypies are repetitive, unvarying behavioural patterns that appear to have no immediate function or objective. A range of factors appear to be responsible for their onset, but these seem to be specific to the relationship between the animal and its environment. This makes it difficult to predict if a certain captive management system will produce this behaviour. Past traumatic events have been associated with stereotypies but in general, their onset is indicative of a sub-optimal environment (Mason 1991).

Individual, anomalous behaviours have been interpreted as signs that an animal lacks control over its environment and is experiencing difficult conditions. Examples of these are feather pecking in hens, tail biting and aggressive behaviour in boars, “dog sitting” in tethered sows etc. (Gonyou 1994; Wechsler et al.1997).

Fraser (1984) described the behavioural indicators of animal depression. These were: (1) low or null responses to stimuli that are normally positive or reinforcing to the animal, and (2) a reduction and a hierachical fragmentation in the normal behavioural repertoire in conjunction with the appearance of new, unusual behaviours.
Frequency and duration changes to the behavioural repertoire of an animal have been used as an indicator of poor welfare. Dybkjær (1992) for example, compared piglets from two levels of environmental and social stressors and found that the piglets from the more stressful environment developed significant differences in the frequency and duration of behaviours such as chain-chewing, belly nosing and passive sitting. However, these findings must be interpreted cautiously since the behavioural differences could merely be a function of the response to a different environment rather than a response to stress.

Changes and disruptions to sleep patterns and other cyclic behaviours have been related to stress (Przekop et al. 1985; Brock et al. 1996). This has significance for cattle as it has been demonstrated that they have a strong motivation for rest (Ruckebusch 1972; Metz 1985). Munksgaard and Simonsen (1996) for example, found that dairy cattle that had disrupted rest patterns and others that were socially isolated had a greater number of transitions from one behaviour to another than did dairy cattle kept under normal conditions.
2.7 The selection of indices of welfare for beef cattle in feedlots

2.7.1 Introduction
The previous section outlined the operation of the immune system and the HPA axis. It detailed ways in which stress may be related to immunity and to behaviour. The following section presents a set of physiological and behavioural indices that I consider to be suitable for the determination of pre-pathological states in feedlot animals.

2.7.2 Relative adrenal mass and measurement of the zona fasciculata
Elevation of HPA activity in cattle results in the increased manufacture and expression of the glucocorticoid cortisol. Cortisol is manufactured solely in the zona fasciculata of the adrenal cortex (see Figures 2.4 and 2.5). Chronic increased secretion of ACTH increases the production of cortisol and also stimulates the expansion of the zona fasciculata and an increase in adrenal mass. Therefore, the weight of the adrenal glands, relative to body mass, and the size of the zona fasciculata, have been used as indicators of the chronic activation of the HPA axis, and by extrapolation, of a chronic stress response (Appleby and Sohrabi 1978; Dede et al. 1989; Pliška et al. 1992; Van Rijswijk and Vorster 1995).

2.7.3 Cortisol
In many animal studies it is impractical or impossible to obtain adrenal glands. Serum, plasma, saliva, and urine samples have therefore been analysed for cortisol levels. Serum and plasma samples are measured for total cortisol. This is comprised of protein-bound and free cortisol. Saliva and urine samples contain free cortisol only (Carlstead et al. 1992; Greenwood and Shutt 1992). However, interpretation of the results requires careful consideration because many stress-related experiments have shown a wide variation in cortisol response (Rushen 1991). There are several reasons for this:
Figure 2.4: adrenal gland from an angus steer

Zona glomerulosa

Zona fasciculata

Zona reticularis

Figure 2.5: histological cross section of an adrenal gland showing the cortex and medulla and the 3 zones of the cortex.
1. Cortisol is secreted from the adrenal gland episodically. These episodes can be of brief duration. Therefore, large numbers of samples are required in order to ascertain a representative picture of cortisol expression (Ladewig and Smidt 1989).

2. Plasma cortisol levels may be different at the time of sampling to normally occurring values. This may result from an artificial elevation of cortisol due to the stress of the sampling techniques or to decline due to its rapid clearance from the bloodstream.

3. Cortisol has a circadian pattern of expression.

4. The difference between free and bound cortisol may be important. Bound cortisol varies between species but is typically 90% of total cortisol. It is bound to cortisol-binding globulin (CBG) and albumin. Only the free cortisol binds to target cells. The total cortisol therefore, is considered as an indirect measure of free cortisol. However, conditions of pregnancy, liver malfunction and dehydration can alter the proportion of bound versus free cortisol. Also, after a long period of cortisol expression, the CBG's become saturated with cortisol and further rises comprise only the free fraction of cortisol (Cook 1997).

5. The HPA axis may not have been affected by the putative stressor.

2.7.4 Immune parameters: lymphocyte proliferation tests
This assay, in principle, tests the proliferation of lymphocytes in the presence of mitogens, such as the plant lectin concanavalin A (Con A) or phytohaemaglutinin (PHA) among others. Stressful conditions are associated with reduced proliferation rates. For example, Mormede et al. (1988), showed that rats which were subjected to inescapable footshocks, had a significantly reduced activity of lymphocytes (isolated from the spleen) in comparison with control rats. Interestingly, the same rats, when
provided with warning of the shocks, did not differ from control rats in splenocyte reactivity. The caveats mentioned on page 46 need to be borne in mind when interpreting the results of these tests.

2.7.5 **Immune parameters: lymphocyte subsets.**
The inconsistent results from studies linking stress and lymphocyte sub-sets have been discussed in section 2.5.1. However, it is important to note that these studies have not been applied to feedlot cattle. Therefore at this stage it would seem appropriate to include them in an immune study on stress in feedlot cattle.

2.7.6 **Immune parameters: total numbers of leucocytes**
Total numbers of leucocytes have been frequently used as an indicator of depressed immune function, but they need to be considered in conjunction with counts of white cell subsets such as neutrophils, eosinophils etc. This is because a stress response can be characterised by an increase in the number of neutrophils. This is due to several factors, principally the increased availability of neutrophils for sampling, due in part, to rises in glucocorticoids which can decrease the adherence of neutrophils to endothelium, and to rises in catecholamines which may increase vascular pressure (Tizard 1996). Furthermore, this rise in neutrophils can occur simultaneously with a reduction in other white cell counts such as lymphocytes. Therefore total white cell numbers need to be examined both qualitatively and quantitatively.

Other factors apart from stress can also alter numbers of white cell counts. Sapolsky (1993) for example, found that in wild baboons, subordinate males had significantly lower levels of circulating lymphocytes than did dominant males. However, Sapolsky stated that these results could be confounded by his sporadic sampling techniques (a result of the difficulty in capturing and sampling wild baboons), and the presence in the diet of individual baboons of arthropods, the ingestion of which can produce high and variable leucocyte counts, presumably because the arthropods have an antigenic effect on the immune system.
2.7.7 Immune parameters: ratios of white cells

The ratios of granulocytes to lymphocytes have been used as indicators of stress in poultry (Mitchell et al. 1992). McFarlane and Curtis (1989) for example, found in poultry, that corticosterone levels were unaffected by any combination of a range of stressors associated with poor housing. However, heterophil to lymphocyte ratios became elevated (the numbers of heterophils rose in comparison to the lymphocytes) in response to the individual stressors of aerial ammonia, intermittent electric shocks and heat stress. They concluded that this ratio could be more reliable than corticosterone levels as indicators of stress. However, the factors previously mentioned in cortisol sampling may have influenced their corticosterone results.

2.7.8 Immune parameters: humoral immunity

The levels of immunoglobulins and their relationship to stressors have been discussed in the previous section. To date they have given mixed results as indices of stress. However, because plasma levels of immunoglobulins represent the activity of the humoral immune system, it is important to include them in a panel of immunological tests.

2.7.9 Erythrocyte parameters

Cockram et al. (1996) found that packed cell volume levels of lambs that were transported by truck, were higher than those of control lambs. This coincided with increased cortisol and heart rate levels. Mitchell et al. (1988), also found that haematocrit values rose in stressed cattle but Van Der Walt et al. (1993) found that over a 14 day period of regular blood sampling of feedlot oxen (which was assumed to be a stressful procedure), packed cell volume levels decreased significantly from initial levels. This also corresponded to lower cortisol levels. Packed cell volume is an indicator of dehydration (Schalm 1986), and it is possible that the stressors used in these studies resulted in altered degrees of dehydration to the animals concerned which may account for the variable findings. In catecholamine–driven situations packed cell volume measures can also increase due to the splenic release of red blood cells.
2.7.10 Production measures

Two production measures that have been traditionally used to gauge welfare status are weight gain and disease incidence (Broom and Johnson 1993). Animals that have increased disease incidence and/or low weight gain in comparison with other animals in the same situations have been considered to be under stress. These measures need to be used with caution because other factors associated with the epidemiology of disease onset, and the selective breeding of animals for rapid weight gain can confound their interpretation. However, they should not be ignored. Fraser (1993) pointed out that they have been underestimated in identifying welfare problems, (such as starvation) in free range systems.

2.7.11 Behavioural indices

The total inventory of maintenance and social behaviour is known as an ethogram Fraser (1980). Feedlot environments necessitate several major changes in cattle ethograms. This is due to increased stocking densities, increased human handling, increased mixing of unfamiliar cattle, a different nutritional intake and feeding regime. If some behavioural activities can be distinguished from these necessary changes and related or correlated to pre-pathological states then it is possible they would be of value in future diagnoses of animals with reduced welfare.

Cattle behaviour can be divided into two main categories, maintenance behaviour and social behaviour. Maintenance behaviour includes activities such as eating, drinking, resting, ruminating, walking and body-care (rubbing, scratching etc). Social behaviour includes activities that influence or are associated with the social structure in a group of cattle. Activities such as agonistic and affiliative behaviour and the frequency and duration of the occupation of pen and paddock locations, can provide some idea of individual positions within a social structure. Maintenance and social behaviours are integrated. For example Stricklin and Gonyou (1981) found that highly dominant cattle (a social feature) when placed in a competitive feeding situation, had fewer meals but tended to spend more time per day eating (a maintenance behaviour), than subordinate cattle.
2.7.12 Maintenance behaviour

Resting behaviour is very important for cattle, and the frequency, duration and circadian patterns of rest may be of value as indices of welfare, particularly as links have been demonstrated between disrupted sleep and depressed immune function (Krueger and Karnovsky 1995; Moldovsky 1995; Toth 1995). Veissier et al. (1989) looked at the circadian pattern of lying as a measure of the rate of adaptation of just-weaned heifers to a new environment. They found that normal patterns of lying resumed within 2-4 days.

In terms of production measures, feeding behaviour is linked to weight gain. Therefore the frequency and duration of feeding behaviour could have potential as an index of welfare.

2.7.13 Social behaviour: affiliative behaviour

Studies have shown that cattle form bonds with other cattle that are not immediately related to them. This conclusion is based on affiliative behaviours such as licking (see Figures 2.6 and 2.7). Social licking can occur frequently in cattle, for example Sato (1984) recorded an average of 15 episodes of social licking per hour in a group of heifers and steers. Each episode lasted between 37.8 and 40 seconds on average. All animals in the group were licked by others but only 72.3% of the animals licked other animals. Animals that were close to each other in terms of a social hierarchy tended to lick each other more frequently than remote ones. In this study, weight gain and time receiving licking were positively correlated. From this and other subsequent studies, Sato concluded that social licking has a tension reducing and bonding effect. This may lead to psychological stability, which would improve their welfare (Sato and Maeda 1991; Sato et al. 1993).
Figures 2.6 and 2.7: licking behaviour in a commercial feedlot
2.7.14 Social behaviour: dominance

Social dominance has been well investigated in cattle (Craig 1986; Bouissou 1980). A dominance hierarchy may be of interest in welfare investigations if certain positions in a social structure are associated with pre-pathological conditions in feedlot animals. An established definition of dominance states that one animal inhibits the behaviour of the subordinate (Beilharz and Zeeb 1982). Early studies of cattle (for example, Beilharz and Mylrea, 1963 and Wagnon et al. 1966) were based on the results of agonistic encounters. These were classified into categories such as: threat and avoidance behaviour; bunting to various parts of the body; charging; pushing, etc. Hierarchies determined from these encounters could be linear, but more commonly, were non-linear (see Figure 2.8).

![Figure 2.8: different configurations of dominance in cattle. The arrows indicate the direction of dominance](image)

Some dominance hierarchies have been correlated with body mass and the presence of horns (Stricklin et al. 1980; Bouissou et al. 1972). However, Bouissou (1980) maintained that experience is the most significant factor in dominance hierarchies. Stricklin et al. (1980) found that dominance orders were established soon after weaning, and the order remained stable, even when the groups were moved to other
pens. Bouissou (1980) agreed with this finding, and showed that young, same-sex cattle took a long time to form a dominance order but once it was formed, it remained stable for many years. Wagnon et al. (1966) also found a strong, but not perfect, linear relationship of dominance in a herd of Angus Hereford and Shorthorn cows that was quite stable. Once established, a dominance hierarchy was frequently maintained using postures and avoidance behaviour rather than physical contact.

Feeding patterns have also been linked to dominance, as have zones of avoidance, that is, dominant animals do not appear to spend as much time on their own as do subordinate animals. They have a smaller zone of avoidance or personal space. They also associate with larger numbers of different animals (Bennett and Holmes 1987). The use of agonistic encounters to determine dominance hierarchies has been criticised by Bennett and Holmes (1987). They stated that many studies of dominance were confounded by the presence of mock fighting in cattle. Mock fighting, they surmised, is a form of play, and encounters can take place between any individuals in a group. However, it is sometimes difficult to distinguish mock fighting from aggressive fighting (see Figures 2.9 and 2.10). They claimed that only experience in observing cattle can aid in determining this. Bennett and Holmes also maintained that agonistic interactions with unpredictable results always took place with cattle that were no more than 3 or 4 positions apart in a dominance hierarchy. Accordingly, they preferred to use measurements of zones of avoidance over agonistic interactions to assign ranks in a dominance hierarchy.

Agonistic encounters in cattle can occur often in feedlots, particularly during the initial settling in period (see Figure 2.9). One of the features of feedlot environments is that they have smaller space, therefore zones of avoidance are constantly being violated, which should create more physical encounters. Also, cattle are normally mixed with strange cattle when initially placed in feedlots. Tennessen (1985) found that mixing of strange cattle leads to more frequent agonistic behaviours. However, this trend did not persist after 10 days of being in the feedlot. Bouissou and Hövels (1976) also found that if two or more groups of cattle were placed together, the
Figures 2.9 and 2.10: agonistic behaviour in feedlot and pasture environments
frequencies of agonistic interactions was higher for cattle emanating from different
groups than for cattle emanating from the same group.

It could be surmised that these increased agonistic encounters during the initial phases
of feedlot life form part of the restructuring of the dominance hierarchy, but, it is also
possible that feedlots have too many animals for a stable dominance hierarchy to be
established. It could be that dominance hierarchies become more dynamic, complex
and transitional in nature when animals are placed in large numbers such as in feedlot
conditions.

An awareness of dominance hierarchies may be important in welfare investigations.
However, their identification is complicated by the problems of distinguishing
mock-fighting from real fighting, and the possibility that there are too many animals
in a feedlot pen for a stable hierarchy to be established.

2.7.15 **Social behaviour: spatial relationships**
The determination of the frequency and duration of an animal’s nearest neighbours,
and the frequency and duration of their use of particular locations in a feedlot pen
may be of value with regard to welfare status. Fraser (1980) reported that cattle have
preferences for certain areas when kept in confined conditions. These positions were
located along the perimeters of the pens rather than the central area. Stricklin (1979)
also found that pen-shape affected mean distance between nearest neighbours and
concluded that in some pen designs, the size of the perimeter should be increased as
much as possible. The use of pen space may be linked to dominance, and/or an
animal’s preference for certain areas with regard to activities such as lying,
ruminating or standing. However, once again, it is important to be able to distinguish
necessary changes in behaviour in order to adapt to feedlot conditions from behaviour
which correlates with individual animals under a pre-pathological state.
2.7.16 Selection of appropriate indices for identifying pre-pathological conditions: summary

Assays relating to HPA axis activity and depressed immune function are appropriate for the evaluating pre-pathological status. Suitable measurements and assays are: (1) the determination of adrenal gland weight, and adrenal index (the ratio of the zona reticularis to the combined zona fasciculata and glomerulosa); (2) the measurement of plasma and salivary cortisol; and (3) assays on cell mediated, humoral, and innate immunity.

I argue that an examination of the frequency, duration and circadian pattern of expression of essential maintenance activities, and observations and measurements of social behaviour, such as agonistic and affiliative encounters, dominance, and the use of pen space are worth exploring to see if some aspect of these behaviours are correlated with animals in pre-pathological conditions.
2.8 Summary

The problems associated with defining and assessing animal welfare have been presented. Three approaches which emphasise different aspects of the assessment of animal welfare have also been discussed. The approach that emphasises the physical state of the animal has been chosen as the most suitable for this study. A definition of animal welfare has been selected from this approach and it is accompanied by an acknowledgement of the influence of human ethics on animal welfare. This definition states that an animal has reduced welfare when it is suffering from significant stress. A state of significant stress is identified by the presence of a pre-pathological state. The physiological systems and selected assays relevant to a pre-pathological state have been detailed. The possibility of certain behaviours being linked to a pre-pathological state has been raised and some previous studies on behaviour and welfare and behaviour in cattle have been discussed.

With regard to beef cattle in feedlots, there are gaps in the knowledge regarding their behaviour, their adrenal status and their immune responses. The next two trials presented in this study examine the following aspects of their behaviour: diurnal lying patterns (this follows the thread between disrupted rest patterns and immune responses) and agonistic and affiliative behaviour. The third trial determines the statistical and behavioural methodology for examining spatial relationships and feedlot location preferences. The aim of these trials is to see if these behaviours are significant enough to be considered as possible pre-pathological state correlates. The principal aim of the main trial in this study is to examine a range of selected physiological and behavioural responses of matched groups of cattle to three treatments: pasture, normal feedlot and stressed feedlot with regard to pre-pathological states.
Chapter 3: Preliminary Trial Number 1: Diurnal Patterns of Rest in Two Groups of Cattle at a Commercial Feedlot

3.1 Introduction

Resting behaviour, as mentioned in the literature review (see pages 51 and 58), is an important component of cattle maintenance behaviour. The frequency, duration and circadian pattern of rest may or may not be related to the welfare status of a feedlot animal. There has been little research into the lying behaviour of feedlot cattle (Hicks et al. 1989). This trial was designed to establish whether or not a diurnal pattern of rest exists in commercial feedlots in the first instance and to see if this diurnal pattern differed between cattle undergoing different weaning treatments at the Elizabeth Macarthur Agricultural Institute, Camden NSW and those from commercial steers purchased under normal feedlot buying arrangements.

3.2 Methodology

This trial was run in conjunction with another trial, the aim of which was to determine the effectiveness of different weaning treatments with regard to successful adaptation to feedlot environments. The weaning trial was run over 3 years. This trial on diurnal patterns of rest was run in conjunction with the second year of the weaning trial. Chapters 4 and 5 detail trials that were run in conjunction with the third year of the weaning trial. This accounts for the slight difference in numbers in the second and third trials.

Weaning trial details: 196 Hereford and Angus × Hereford castrated male beef calves of 7-8 months of age were separated from their mothers. They were placed into 3 groups and put through separate weaning treatments: (1) paddock weaning (2) pen weaning with a round bale feeder, and (3) pen weaning with feed placed in feedlot style feedbunks. This last treatment was associated with a field test designed to identify “shy” and “confident” animals. The weaning procedures took 10 days. After a
period of 9 months on pasture at the Elizabeth Macarthur Agricultural Institute (EMAI) they were transferred to a large commercial feedlot in northern New South Wales. At the feedlot they were placed in a single pen with 196 other steers (designated as commercial steers). These steers were made up of smaller groups of animals purchased from different locations around Australia. They were predominantly a mixture of Hereford, Angus and Angus × Hereford.

For the purposes of examining rest patterns the EMAI steers were treated as one group and the commercial steers treated as another.

The steers were placed in the commercial feedlot in February 1995. The pen area was 4,350 square metres, which resulted in a standard stocking density of 11.1 square metres per head. The weather during the observation period (29/01/1995- 09/02/1995) was warm (21° C- 30° C) and dry. Observations conducted to record lying behaviour were made from a tower placed in the middle of the feedlot. The tower was approximately 3 metres high and allowed for full viewing of the feedlot pen (see Figure 3.1).

The protocol for the observations on lying behaviour was as follows: at 60 minute intervals, every animal in the pen was observed, and either recorded as lying or not lying. The scans started on 29/01/1995 which was one day after the EMAI cattle and 5 days after the commercial cattle arrived in the feedlot. The scans started at 0700 hours (after sunrise) and finished at 1900 hours (just before sunset). This procedure was repeated for 11 consecutive days. Some hourly observations were not made when steers were disturbed by outside human activity, such as the arrival of pen riders, and staff who cleaned water troughs etc. This often caused all the steers in the pen to stand. The feed trucks delivered feed to the feedbunks at 0800 and 1600 hours daily. This interruption was not excluded from the observations as they constitute a regular component of feedlot life.
Figure 3.1: layout of the commercial feedlot pen and tower.

3.3 Results

Figure 3.2 shows the mean percent number of animals observed lying at each hourly observation over 11 days of observations. These observations were tested for significant differences using a Mann-Whitney U test. The mean percentage of cattle standing during any given hourly observation period did not differ significantly between the two groups (p<0.05).
Figure 3.2: mean diurnal pattern of lying for EMAI and commercial steers in a feedlot. All error bars are ± SEM

3.4 Conclusion

The results of the trial demonstrated that a regular pattern of resting behaviour was exhibited over the observation periods. They also indicated that there were no significant differences between experimental and commercial cattle, and therefore, no effect of the weaning treatments, with regard to the pattern of lying behaviour. The results also indicate that there was a major peak in lying behaviour between 1400 and 1800 hours and a minor peak of lying behaviour between 1000 and 1200 hours. The consistency of this pattern supports the point that cattle have a strong motivation for
lying behaviour. This could partly be attributed to rumination status. However, during this trial, cattle were often observed ruminating while standing.

For the purposes of this thesis, the consistency of the pattern of lying and the links between disrupted lying and depressed immune function indicate that lying behaviour is a significant component of a feedlot cattle ethogram and is an important inclusion in investigations into the occurrence of pre-pathological states of beef cattle in feedlots.

Figure 3.3: the observation tower and pen at the commercial feedlot.
Chapter 4: Preliminary Trial Number 2: Agonistic and Affiliative Behaviour of Beef Cattle in a Commercial Feedlot.

4.1 Introduction

Agonistic and affiliative behaviour in cattle, as discussed in the literature review (see pages 58-62) may be linked to dominance hierarchies or social structures or they may have some other role, some agonistic encounters for example, may be examples of ritualised or play behaviour.

These behaviours have received a degree of investigation in open range and feedlot conditions (Bouissou 1980; Fell and Clarke 1993; Sato et al. 1993). Their potential as pre-pathological correlates is unknown at this stage.

The aims of this preliminary trial were to:

1. Examine the rates of wins, draws and losses for individual animals with regard to agonistic encounters.
2. Identify and record specific types of agonistic behaviours.
3. Distinguish and document animals that have strong pairing tendencies, as determined by numbers of affiliative encounters with the same animal.

4.2 Methodology

This trial was run in conjunction with the third phase of a weaning trial previously discussed in the introduction to chapter 3 (see page 66). A total of 206 experimental cattle (with different experimental weaning histories) were put into a commercial feedlot pen with 155 commercial cattle. The animals were continuously observed from 0700 hours to 1800 hours for 9 days (days 1-3, 9-11 and 16-18 after entry to feedlot, 7-9/11/1996, 15-17/11/1996, 22-24/11/1996). Agonistic interactions were recorded for experimental animals only. The outcome of these interactions were scored as wins, losses or draws and were classified as follows:
1. **Threat**: a situation whereby one animal forces another to give ground by non-contact threatening behaviour.

2. **Head push**: one animal pushes another animal by direct head to head contact

3. **Head stand**: neither animal is pushed or gives ground from head to head contact yet the heads appear to be pushing against each other.

4. **Bunt**: an action where one animal uses its head to push at any other part of the anatomy of another animal in a short sharp manner. This is further divided into head bunts, side bunts and rear bunts.

5. **Push**: an action where an animal primarily uses its body against another animals, but may use its head in an auxiliary fashion.

6. **Charge**: an action whereby one animal charges at another animal. This is further divided into contact and non-contact charges.

7. **Mixed**: an action which incorporates more than one of the above categories.

Affiliations were recorded when one animal was observed licking, rubbing or scratching against another animal. The approximate duration and the parts of the anatomy involved were also recorded.

### 4.3 Results

#### 4.3.1 Agonistic behaviour

*4.3.1.1 Numbers of agonistic encounters per individual: wins and losses per individual.*

These results are concerned with the experimental cattle only. There were 206 experimental cattle in the feedlot pen. A total of 25 (12 %) of these animals were observed not to have any agonistic encounters. Of the remaining 184 animals, 47 (25 %) had no wins at all, and 46 (25 %) had no losses at all. The remaining 93 animals had a mean of 2.52 wins, standard error = 0.18, a mean of 2.37 losses, standard error = 0.18, and a mean of 1.78 draws, standard error = 0.20.
4.3.1.2 Different forms of agonistic behaviour.

Figure 4.1 shows the breakdown of the different forms of agonistic behaviour for all animals (experimental and commercial cattle) observed over the 9 day period. It can be seen that the most frequent form of encounter was the head push.

![Graph showing different forms of agonistic encounters](image)

Figure 4.1: overall percentages of the different forms of agonistic encounters observed in a commercial feedlot during the hours of 0700 to 1800 for 9 days.

4.3.2 Affiliations

These results are concerned with the experimental cattle only. Out of 206 animals, 192 (93%) were observed engaging in affiliative behaviours either with other experimental animals or with other commercial animals. A total of 49 (23.8%) animals were observed having 2 or more affiliative behaviours with the same animals. A total of 5 (2.4%) animals were observed having 3 or more affiliative behaviours with the same animals.
4.4 Discussion and Conclusion

4.4.1 Agonistic interactions

The total of 915 encounters over the observation period indicates that agonistic interactions are a significant feature of feedlot life in the first 3 weeks. Future analyses will examine the frequency of these interactions against time in order to determine whether agonistic behaviour decreases as cattle become adapted to feedlot life.

The results of the study show that animals could be divided into 4 groups. Those that had no agonistic encounters, those that had all wins, those that had all losses, and those that had mixed results. The significance of these groupings is unknown at this stage. The analysis of types of agonistic encounters show that head pushes account for nearly half of the pen encounters. Further work will examine the interactions between types of encounters and the number of wins, losses and draws for the experimental animals and also consider the agonistic encounters of commercial animals. This may help to clarify the role of head pushes in feedlot conditions and perhaps add to the work of Bennett and Holmes (1987) regarding “mock” fighting in cattle.

For the purposes of this thesis, the results of the experimental animals indicate that agonistic encounters are a significant feature in the first few weeks of feedlot life. They may have potential for determining an animal’s position in a group structure and this in turn may be affected by pre-pathological states.

4.4.2 Affiliative interactions

The high percentage of animals observed engaged in affiliative behaviour supports the work of Sato (1984) who also recorded high percentages of affiliative behaviour in heifers and steers. The number of animals that had two or more interactions with the same animal (23.8 %) also indicates that there is a degree of selection and the behaviour is not totally random. Further work will address the frequency of within
experimental animal affiliative behaviours and experimental-commercial animal affiliative behaviours.

For the purposes of this thesis, the results of the experimental animals indicate that affiliative behaviours are a significant social feature in the first few weeks of feedlot life. For this reason affiliative behaviour appears to be a worthwhile behaviour to investigate with regard to pre-pathological states.
Chapter 5: Preliminary Trial Number 3: Behaviour of Calves Undergoing a Feedlot-Based Weaning Treatment: Affiliations and the use of Pen Space

5.1 Introduction

The use of pen space and the type and frequency of associations between cattle in a feedlot has received some investigation (Stricklin 1979; Fraser 1980). Their potential as behavioural correlates to pre-pathological states is unknown at this stage. As stated in the literature review (see page 62) the use of pen space may be linked to an animal’s position in the social structure of the group and/or be related to topographic features and the direction of the sun. Associations may be linked to affiliations between animals and consequently to social structure.

The general aim of the trial detailed in this chapter was to determine suitable methodologies regarding these behaviours for use in a future experimental trial. This trial was run opportunistically in conjunction with the third year of a separate weaning experiment, the details of which are in the introduction to the first preliminary trial (see page 65).

The specific aims of this trial are to establish the methodology for ascertaining if cattle vary in their occupation of pen space in a feedlot environment and to develop the procedures for quantifying cattle associations in a feedlot pen.

5.2 Methodology

This trial was run in conjunction with the weaning phase of the third year of the weaning trial. The yard weaning procedure enabled an opportunity for examining the use of pen space and associative behaviour in the confined calves.
5.2.1 Experimental site and pen design

The trial was conducted at the experimental feedlot pens of the Elizabeth Macarthur Agricultural Institute (EMAI), Camden, NSW between 07/05/1996 and 13/05/1996. The pen used measured 13 × 13 metres and had the following features:

1. It was enclosed by a rubber lining from ground level to a height of 1.5 metres. The purpose of this was to minimise the effects of any outside disturbances.
2. A circular hay feeder was placed in the centre of the pen and a drinking trough was located at the south-eastern corner.
3. The surface of the pen was composed of a blue metal base overlayed with a hard packed mixture of cattle manure and mud. This is a typical composition for a commercial feedlot “pad”.

An observation tower was placed outside the pen at the western end. For observation purposes the pen was divided into a 5 × 5 grid. The grids measured 2.6 metres by 2.6 metres (see Figure 5.1).

5.2.2 Experimental animals

A total of 52 castrated male calves were used in the trial. The calves were predominantly a mixture of Hereford and Hereford × Angus. They were between 7 and 9 months of age. There were 27 animals from the EMAI breeding stock and 25 animals from a commercial property in Braidwood NSW. The animals had been separated from their dams 2 days before the start of the observations. They were weighed and bled on the day preceding the trial as part of the weaning procedure. They were confined to the observation pen for 7 days.
Figure 5.1: pen design and grid pattern for an observational trial on the use of pen space and proximity to each other of weaned calves.
5.2.3 Behavioural observations

A total of 8 “focal” animals were randomly selected from the 52 calves (which represented one group in the weaning trial) for the observations. The observations were conducted between 0900 and 1100 hours and 1200 and 1500 hours for days 2-7. The observations consisted of scan samplings taken at 10 minute intervals. There was a total of 196 scans throughout the 5-day observation period. At each scan, the activity, location, and nearest neighbours of the focal animals were recorded. The activity categories recorded were as follows:

<table>
<thead>
<tr>
<th>Primary activity</th>
<th>Secondary activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lying</td>
<td>1. Ruminating (animal observed chewing the cud)</td>
</tr>
<tr>
<td>2. Standing</td>
<td>2. Self grooming**</td>
</tr>
<tr>
<td>3. Walking</td>
<td>3. Allogrooming</td>
</tr>
<tr>
<td>4. Feeding</td>
<td></td>
</tr>
<tr>
<td>5. Standing at the water trough*</td>
<td></td>
</tr>
</tbody>
</table>

* Standing at the water trough was used as a substitute for drinking. This was because we were unable to determine if an animal was drinking or simply standing with its head close to the water.

** Grooming was defined as an activity whereby the animal was scratching, rubbing or licking itself. Allogrooming related to these activities being performed on another animal.

The nearest neighbour was defined firstly as any animal that was located on any grids adjacent to the focal animal. Their positions were marked on a recording sheet. At a later date a scaled equivalent of a 2 metre radius circle was drawn on the sheet using the focal animal as the centre of the circle. Nearest neighbours were then defined as those animals wholly within the circle.
5.3 Results

5.3.1 Use of pen space

The null hypothesis for this analysis was that there would be no significant differences in the occupation of the pen squares by each of the focal animals. The most appropriate techniques for testing this hypothesis were as follows:

1. For every animal determine the overall numbers or frequencies of scans where an animal was observed on the grid squares. Then calculate the average frequency of occupation of each grid for every animal (Martin and Bateson 1994).

2. Determine the duration of occupation of the grids for every animal. Then calculate the average duration of occupancy of each grid for every animal (Martin and Bateson 1994).

3. Calculate mean bout length of grid occupation for every animal by dividing average duration by average frequency (Martin and Bateson 1994).

Comparisons of mean frequency, duration and bout length of grid occupation were performed using a Kruskal-Wallis non-parametric analysis. This analysis showed that for all focal animals there was at least one grid that was occupied significantly more than one other grid (p < 0.05). This applied for mean frequency, mean duration and mean bout length. For the purposes of illustration, Figure 5.2 shows a graph of mean bout lengths with standard error bars for focal animals 2 and 7 only. These animals were selected only because they illustrate well the differences between individual animals.
5.3.2 Associations between focal animals and other pen animals

There are two null hypotheses which were evaluated in terms of cattle associative behaviour. The first hypothesis was that the time a focal animal spent alone was not significantly different to the time it spent with any other animals in the pen. The second hypothesis was that the time a focal animal spent alone was not significantly different to the time it spent with any particular animal in the pen. The appropriate analyses for testing these hypotheses is similar to those used in the pen space analysis. The first hypothesis was not tested formally as the number of scans in which a focal animal was observed alone were very low in comparison to the number of scans they were observed in the company of other animals i.e., within 2 metres. This is an
expected result for a pen of 13 metres by 13 metres holding 52 calves. The second hypothesis was tested formally. In this case a Mann Whitney U-test non parametric analysis on the mean frequency of scans, and the mean duration and mean bout length of time for all focal animals was applied. Table 5.1 shows the results of this analysis. For the purposes of illustration only, Figure 5.2 shows the mean bout lengths and standard errors for the associations of focal animals 12 and 43.

Table 5.1: Results of a Mann Whitney U-test on the comparisons of the mean frequency of scans, and the mean duration and mean bout length of time spent alone for a focal animal versus the time it spent with any particular animal. \( P < 0.05 \) for significant results.

<table>
<thead>
<tr>
<th>focal animals</th>
<th>mean frequency</th>
<th>mean duration</th>
<th>mean bout length</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>significant</td>
<td>not significant</td>
<td>significant</td>
</tr>
<tr>
<td>7</td>
<td>not significant</td>
<td>significant</td>
<td>not significant</td>
</tr>
<tr>
<td>9</td>
<td>not significant</td>
<td>significant</td>
<td>not significant</td>
</tr>
<tr>
<td>10</td>
<td>not significant</td>
<td>significant</td>
<td>not significant</td>
</tr>
<tr>
<td>11</td>
<td>not significant</td>
<td>significant</td>
<td>significant</td>
</tr>
<tr>
<td>12</td>
<td>not significant</td>
<td>not significant</td>
<td>not significant</td>
</tr>
<tr>
<td>31</td>
<td>not significant</td>
<td>significant</td>
<td>not significant</td>
</tr>
<tr>
<td>43</td>
<td>significant</td>
<td>significant</td>
<td>significant</td>
</tr>
</tbody>
</table>
Figure 5.3: the mean bout length and standard error for associations of focal calves 12 and 43 with 25 other calves in a feedlot pen. Animal 12 = pen animal 6 in the top graph and animal 43 = pen animal 8 in the bottom graph. The arrows indicate these calves and the bar represents the mean bout length of the time these calves were observed by themselves, i.e., time spent alone. All error bars are ±SEM. For reasons of space only 25 animals are shown rather than the total of 51.
5.4 Discussion and Conclusion

5.4.1 The use of pen space

The results of the trial indicated that the focal animals had preferences for different areas of the pen. This result must be interpreted with caution since the observations took place only during daylight hours. Nocturnal locations may well have been different. Further, the figures for frequencies and durations were directly influenced by the lying behaviour of the animals, e.g., an animal lying in a grid square occupied it for a longer period than when it was standing. A future trial would need to consider the interaction between the occupation of grid squares and the different behavioural categories. Once this was achieved rankings of grid squares could be made and compared between animals.

5.4.2 Associative behaviour

The results of the trial showed that the focal animals spent more time with other animals than they did alone and that there were some differences between them for frequency of scans and duration of time spent with any particular animal in the pen. Focal animal 43, for instance, spent much less time alone than did focal animal 12. These results are of interest, but the same criticisms and recommendations apply to these results as with the analysis of pen space use. Therefore, their interpretation must necessarily be conservative.

The conditions of this trial were such that concrete conclusions about cattle behaviour in feedlots could not be drawn. However, it was successful in developing behavioural methodologies and it did provide some background information with regard to these aspects of feedlot cattle behaviour.

Chapters 3-5 have presented results of trials which investigated certain aspects of behaviour in beef cattle feedlots. These behaviours appear to be significant components of a feedlot cattle ethogram. With regard to this thesis, a main question
is: could they be useful as indicators of pre-pathological states in cattle? The investigation of this question is presented in the main trial of this thesis. This is presented in the following chapter.
Chapter 6: Main Experiment: The Evaluation of Potential Indices of Welfare for Beef Cattle in Feedlots

6.1 Introduction

A principal question facing this study is: is there a significant incidence of pre-pathological states in beef cattle kept in standard feedlot conditions? This experiment was designed to answer this question. Pre-pathological states are evaluated by using selected physiological indices (outlined previously). The previous preliminary trials investigated some behavioural aspects of feedlot life to determine if they had potential as co-indices with the pre-pathological determinants.

The aim of this experiment was to compare the physiological and behavioural responses of beef cattle to three treatments. They are: pasture conditions, “normal” feedlot conditions and “stressed” feedlot conditions. The pasture treatment was designed as a control treatment against which the standard and stressed feedlot treatments would be compared. It was hypothesised that if pre-pathological states were promoted by feedlot conditions then the comparison of selected physiological indices of the steers in the pasture treatment against those in the two feedlot treatments would show this. This approach addresses a major issue in identifying pre-pathological states and that is to provide a baseline permitting identification of the levels of adrenal activity and immune function which signify a departure from it. This is an ongoing problem of animal welfare research and applies to other indices apart from those involved with pre-pathological states (Duncan and Dawkins 1983).

The normal feedlot conditions were designed to replicate a commercial feedlot operating under optimal conditions. The base of the pen, the feedlot “pad” was always firm and dry, there was a low stocking density, and the animals were fed under commercial feedlot guidelines. The feeding behaviour was carefully monitored and feeding rates were adjusted according to it.
The stressed feedlot condition differed from the standard feedlot condition in that the stocking density was doubled, thus creating competition for lying room and feed bunk space, and the surface of the pen was kept continually wet, with the aim of interfering with the lying behaviour of the animals. The principle behind these changes was firstly to replicate the conditions of a commercial feedlot under poor conditions, and secondly to introduce stressors such as overcrowding and reduced lying behaviour. As stated in chapter two, cattle show a strong motivation for lying, and links have been established between disrupted sleep periods and reduced immune function in humans. It was considered, therefore, that the disruption of lying behaviour could represent a significant stressor.

6.2 Methodology

6.2.1 Introduction

Two trials were performed. Each trial was of 42 days duration. The first trial ran from 9/4/96 to 6/5/96. The second trial ran from 9/10/96 to 18/11/96. The trials were run in the same experimental area in order to match geographical and topographical factors. As far as possible they were run in similar climactic conditions. A total of 42 beef steers were used in each trial with 14 steers per treatment. The steers were selected for uniformity of breed and background. At 14 day intervals the animals were collected, inspected by a veterinarian, weighed and sampled for blood and saliva (see page 96 for methodology). It has been documented that the initial two weeks of feedlot life includes a number of acute stressors (Fell et al. 1997). Therefore, since this experiment was concerned with chronic stress in feedlots, behavioural observations commenced after the first 14 days of feedlot conditions.

The experimental design was approved by the Animal Care and Ethics Committee of the Elizabeth Macarthur Agricultural Institute before the commencement of the experiment. A photocopy of the approved submission (Reference number: 95/34) is located in the appendix.
6.2.2 Experimental animals: trial one

The animals were purchased at 18 months of age, at an average weight of 370 kilograms from the Candamine property of Provos Proprietary Limited. They were screened for a range of bacteriological and viral pathogens and then run on the property of the Elizabeth Macarthur Agricultural Institute, Woodbridge Road, Menangle, NSW for two months before entering the trial. They consisted of 37 Angus and 18 Hereford / Angus steers.

In total, 42 steers were chosen for the trial from a group of 55. They were allocated to the different groups on the basis of breed and weight. There were 10 Angus and four Hereford / Angus crosses in each treatment. The initial mean weight of the animals used for each treatment was as follows:

1. Pasture: 376.9 kilograms (standard error: 6.0)
2. Normal feedlot: 380.6 kilograms (standard error: 6.4)
3. Stressed feedlot: 384.2 kilograms (standard error: 7.0)

6.2.3 Experimental animals: trial two

The animals were purchased at 15 months of age at an average weight of 315 kilograms from the Belltrees property of Anthony White. They were screened for a range of bacteriological and viral pathogens and then run on the property of the Elizabeth Macarthur Agricultural Institute, Woodbridge Road, Menangle, NSW for six months before entering the trial. They consisted of 61 Angus steers. In total, 42 steers were chosen for the trial from a group of 61. They were allocated to the different groups on the basis of weight. The mean weight of the animals used for each treatment in trial two was as follows:

1. Pasture: 351.7 kilograms (standard error: 4.4)
2. Normal feedlot: 361.2 kilograms (standard error: 3.4)
3. Stressed feedlot: 359.1 kilograms (standard error: 4.5)
6.2.4 **Treatments: pasture conditions: trial one**

A total of 14 animals were rotated as a group between four 300 × 50 metre paddocks (1.5 hectares) for the duration of the trial. The paddocks were covered in a 70/30 ratio of kikuyu and white clover. The pasture density was light, therefore the animals were moved from one paddock to another at the following intervals: two periods of seven days, then two periods of 14 days. A water trough was placed centrally on one side of each paddock.

6.2.5 **Treatments: pasture conditions: trial two**

A total of 14 animals were rotated in two of the same 300 × 50 metre paddocks as used in the trial one. These were sown with ryegrass in the intervening months after the completion of trial one. This resulted in an 80/20 ratio of ryegrass to kikuyu grass with a medium to heavy density of cover. Due to this growth and density the animals were rotated from one paddock to the other only at 14 day intervals.

6.2.6 **Treatments: standard feedlot conditions: both trials**

A total of 14 animals were placed for the duration of the trials in a single 13 × 13 metre experimental feedlot pen. The stocking density of the pen was 12.07 square metres per head. This figure is within the recommended industry standards (The New South Wales Agriculture Feedlot Manual, 1997). The base of the pen was similar to a typical feedlot pad: a blue metal base overlayed with packed dirt and cow manure. The 1.5 metre high walls of the pens were lined with a rubber and canvas material. This was designed to minimise outside disturbances. A water trough was situated in one corner. The animals were fed from feedbunks twice daily. The feedbunks allowed 900mm feeding room per head, which is above recommended industry standards. The feeding regime is discussed further below.

6.2.7 **Treatments: stressed feedlot conditions: both trials**

A total of 14 animals were placed in a similar pen as used in the standard feedlot pen with the exception that the animals were kept in only half of the pen, resulting in a stocking density of 6.03 square metres per head, a figure which is less than half of
the recommended minimum industry standard of 12-15 square metres per head and considerably less than the recommended minimum of 9 square metres per head. The base of the pen was also continually kept wet, initially through twice daily watering. After 7 days, the accumulation of urine and faeces maintained this condition to a point where no further watering was needed. Figures 6.1, 6.2 and 6.3 show the three different environments.

Figure 6.1: the pasture treatment; the observation tower is in the background. The yellow lines partly indicate the boundaries of the paddocks which the cattle were rotated on for the trial.
Figures 6.2 and 6.3: the normal feedlot treatment and the stressed feedlot treatment. The positions of the cameras and lights in the stressed feedlot are also shown.
6.2.8 Feedlot feeding regime

The animals were fed at 0800 hours and at 1600 hours each day. The feed was a mixture of chaff (lucerne, clover and oats) and a commercial pelleted feed concentrate. In accordance with normal feedlot practice the animals were introduced to the mixture in a graded fashion. The procedure for both trials was as follows:

<table>
<thead>
<tr>
<th>Days</th>
<th>Pellets</th>
<th>Roughage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td>5-8</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>9-11</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>12-14</td>
<td>70%</td>
<td>30%</td>
</tr>
<tr>
<td>15-43</td>
<td>80%</td>
<td>20%</td>
</tr>
</tbody>
</table>

The chaff was harvested from the paddocks of EMAI. It was analysed by the Feeds Evaluation Service of EMAI and yielded the following results:

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter:</td>
<td></td>
</tr>
<tr>
<td>88.29 %</td>
<td>91.96 %</td>
</tr>
<tr>
<td>Digestible dry matter</td>
<td></td>
</tr>
<tr>
<td>51.4 %</td>
<td>59.4 %</td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
</tr>
<tr>
<td>2.24 %</td>
<td>2.15 %</td>
</tr>
<tr>
<td>Crude protein</td>
<td></td>
</tr>
<tr>
<td>14 %</td>
<td>15.8 %</td>
</tr>
<tr>
<td>Available dietary fibre</td>
<td></td>
</tr>
<tr>
<td>46.16 %</td>
<td>37.39 %</td>
</tr>
<tr>
<td>Estimated energy</td>
<td></td>
</tr>
<tr>
<td>7.7 MJ / kg</td>
<td>8.9 MJ / kg</td>
</tr>
</tbody>
</table>

The pellets were supplied by a commercial feed company and were called a “standard dairy pellet”. They were analysed by the Feeds Evaluation Service of EMAI and yielded the following results:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter:</td>
<td></td>
</tr>
<tr>
<td>88.98 %</td>
<td></td>
</tr>
<tr>
<td>Digestible dry matter</td>
<td></td>
</tr>
<tr>
<td>83.2 %</td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
</tr>
<tr>
<td>3.44 %</td>
<td></td>
</tr>
</tbody>
</table>
Crude protein 21.5 %
Available dietary fibre 11.43 %
Estimated energy 12.5 MJ/ Kg

The amount fed to the animals was calculated as 3% of liveweight per day. This amount was closely monitored. Feed left in the feedbunks from previous feeding times was collected and weighed. The following amount of feed was then reduced relative to the amount left in the bunks.

*Figure 6.4: feedlot feeding procedure*
### 6.2.9 Physiological indices used to compare the effects of feedlot conditions on the incidence of pre-pathological states

The background to the selection of indices has been detailed in the literature review (see pages 52-64). The following is a list of indices selected for this experiment.

<table>
<thead>
<tr>
<th><strong>Production parameters:</strong></th>
<th>Carcass weight, average daily weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence of morbidity and mortality</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Adrenal function:</strong></th>
<th>Relative adrenal weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adrenal index</td>
</tr>
<tr>
<td></td>
<td>Plasma cortisol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Humoral immune variables:</strong></th>
<th>Salivary IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum IgA</td>
</tr>
<tr>
<td></td>
<td>Serum IgG</td>
</tr>
<tr>
<td></td>
<td>Total numbers of B cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Cell mediated immune variables:</strong></th>
<th>CD4 +</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD 5 +</td>
</tr>
<tr>
<td></td>
<td>CD8 +</td>
</tr>
<tr>
<td></td>
<td>WC 1 +</td>
</tr>
<tr>
<td></td>
<td>CD4 / CD8 ratio</td>
</tr>
</tbody>
</table>

| **Cytokines:** | Interleukin 2 RA |

<table>
<thead>
<tr>
<th><strong>Immune cell function:</strong></th>
<th>Lymphocyte proliferation assay (unstimulated, stimulated with Concanavalin A and Phytohaemagglutinin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutrophil myeloperoxidase assay</td>
</tr>
</tbody>
</table>
6.2.10 Production parameters: methodology
The animals were collected, weighed on a commercial cattle scale, and inspected individually by a veterinarian for signs of illness at 14 day intervals. They were also visually inspected on a twice daily basis from outside the yards by the feeding staff. Carcass weights at the time of slaughter were collected from the commercial abattoirs.

6.2.11 Relative adrenal weight: methodology
The adrenal glands were removed from the animals at a commercial abattoir. The glands were removed by abattoir staff who I had previously trained in this procedure. The glands were tagged and placed in plastic bags, kept at room temperature and removed to the post mortem room of EMAI. They were then trimmed of external fat and weighed on a top pan balance to the nearest 0.01 gram. The relative adrenal weight was determined by expressing the glands as a percentage of carcass weight. The carcass weight was measured by the scales of the commercial abattoir.

6.2.12 Adrenal index: methodology
After the weighing process, each gland was sectioned and placed in a solution of 4.0 % phosphate buffered formalin. They were then prepared for histological examination using standard histological procedures (processing and staining for a haematoxylin-eosin stain). The resulting sections were examined under 40 times magnification on a Nikon “Optiphot” binocular microscope. A calibrated ocular (1 ocular unit = 25
microns) was used to measure the width of the zona glomerulosa, and the combined zona fasciculata and reticularis (see Figure 2.5 on page 51). The measurement for the zona glomerulosa was then divided into the measurement of the combined zona fasciculata and reticularis. The result was called the adrenal index (Van Rijswijk and Forster 1995). A total of 6 readings were made per gland. These came from 2 or 3 different sections taken from the same plane of the gland. The histology of cattle adrenal glands is characterised by irregular indentations into and extrusions of the cortex. The readings were made in areas that were not affected by these factors. To limit any possible human bias in the procedure, the numbers on the slides were covered during the measuring process.

6.2.13 Sampling regime

The blood and saliva samples were collected at the following intervals:

1. On day 1 of each trial. This is a pre-treatment sample.
2. On day 14 of each trial
3. On day 28 of each trial
4. On day 42 of each trial (the last day)

6.2.14 The collection and preparation of blood and saliva samples for plasma and salivary cortisol, salivary IgA and serum IgG

The blood samples for cortisol assays were collected in 10 ml heparinised vacutainer tubes (with 18 gauge needle) from the jugular vein. The vacutainer tubes were centrifuged at 2800 rpm for 20 minutes. The plasma was then poured off into sterile 5ml sample tubes. These were labelled and frozen until the time they were required for the assay procedure.

The saliva samples were collected from the area of the parotid gland in the mouth cavity. Cotton wool was wrapped around a pair of artery forceps and the forceps were inserted into the mouth of the restrained animal. The cotton wool was then removed from the forceps and placed into a 20 ml sterile container. These containers were then
centrifuged at 1500 rpm for 15 minutes. The supernatant was then decanted into sterile 5 ml sample tubes and frozen until required for the assay procedure.

6.2.15 Methodology for remaining physiological indices
The methodologies for the assay of these indices were supplied by Dr Ian Colditz, and Mr Brian Anderson of the laboratories of the Commonwealth Scientific Industrial Research Organisation (CSIRO) of Australia (“Chiswick”, Armidale, NSW). These are based on standard assays, but have been modified for use on cattle. To my knowledge, in many instances, this is the first time they have been used on feedlot cattle. These methodologies are described in the appendix.

6.2.16 Methodology for behavioural observations: introduction
The observations took place over 28 days, beginning on day 14 and concluding on day 42. They consisted of scan samplings with an interval of 10 minutes. Every animal within the 3 treatment groups was observed at the scan and the following information was recorded until the finish of the trial (see tables 6.1 and 6.2).

1. Location
2. Nearest neighbours: designated as any animal within one steer’s length of the observed animal.
3. Primary or maintenance behaviour i.e., feeding, standing, standing at the water trough, and lying; lying behaviour was further subdivided into lateral or sternal recumbency (see Figures 6.5 and 6.6).
4. Secondary behaviour i.e., ruminating, grooming, allogrooming

Agonistic and affiliative behaviours were recorded whenever they were observed during the scans. The types of agonistic behaviour recorded have been listed in chapter two. They are repeated here.

1. Threat: a situation whereby one animal forces another to give ground by non-contact threatening behaviour.
2. **Head push**: one animal pushes another animal by direct head-to-head contact

3. Head stand: neither animal is pushed or gives ground from head-to-head contact yet the heads appear to be pushing against each other.

4. **Bunt**: an action where one animal uses its head to push at any other part of the anatomy of another animal in a short sharp manner. This is further divided into head bunts, side bunts and rear bunts.

5. **Push**: an action where an animal primarily uses its body against another animals, but may use its head in an auxiliary fashion.

6. **Charge**: an action whereby one animal charges at another animal. This is further divided into contact and non-contact charges.

7. **Mixed**: an action which incorporates more than one of the above categories.

Affiliative behaviour was recorded whenever one animal was observed either licking or rubbing another animal.

A system of continuous scan sampling for 28 days was not performed due to logistical constraints. Therefore scan samplings were performed in 4 hour blocks and these blocks were run in a set pattern inside larger 48 hour blocks. The configuration of the pattern was such that the set of 4 hour blocks covered an entire day/night cycle. A block started at 0800 hours, which coincided with feeding time, and finished at 0800 hours 48 hours later. The 0800 start time was chosen because it was hypothesised that the start of a day and feeding time would be significant periods for feedlot cattle. This procedure was performed for the remaining 4 weeks of the trial.

At hourly intervals during the observation periods, measurements of temperature, barometric pressure, cloud cover and humidity were made from the observation tower. There were breaks in the observations immediately before, during, and after bleeding sessions. This was, firstly, to allow for a feeding curfew (a period of 12 -14 hours without access to food preceding the weighing sessions) so that recorded bodyweights were classed as "empty" bodyweights and secondly to allow the cattle to
resume normal behaviour patterns after the disruption caused by the sampling process. Tables 6.1 and 6.2 shows the timetable for behavioural observations for both trials. The following is an overview of the methodology for the behavioural observations:

**Pasture animals**

These animals are observed manually by staff placed in an observation tower. Staff use binoculars or an infra-red “Nightscope” with an infra-red spotlight. All staff trained in observational techniques to the satisfaction of the author.

**Feedlot animals**

These animals are recorded by closed-circuit video cameras with infra-red spotlights. Data taken off videotapes by staff trained in observational techniques to the satisfaction of the author.

A system of four-hour blocks of observations commences at 0800 hours on day 14 of the study. These observations are called scan samplings. Scan samplings consist of recording the location, activity and nearest neighbour of every animal in all groups. Scan samplings are performed every 10 minutes inside the four-hour block. Agonistic and affiliative behaviours are also recorded whenever they occur within the four-hour block. Weather data also recorded every 60 minutes within the four-hour block.

The four-hour blocks are run alternately, that is, four hours on, four hours off. They are continued non-stop (with the exception of when physiological sampling curfews etc. take place) for the next 28 days.

For analysis, the alternate four-hour blocks are grouped into larger 48 hour blocks. These larger blocks represent a complete day/night period.
Figure 6.5 and 6.6: lateral and sternal recumbency in a commercial feedlot
Table 6.1: the timetable for observations of behaviour of cattle in the first trial of an experiment which had 3 treatments: pasture, normal and stressed feedlot. N=14 per treatment. The periods of observations represent simultaneous observations of the cattle in the 3 treatments.

<table>
<thead>
<tr>
<th>Date</th>
<th>4 hour blocks of scan samplings at 10 minute intervals</th>
<th>48 hour block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0800-1200</td>
<td>1200-1600</td>
</tr>
<tr>
<td>9/4/96 - 6/5/96</td>
<td>9/4/96 = 1st bleed of all animals. No observations until 19/4/96</td>
<td></td>
</tr>
<tr>
<td>16/4/96</td>
<td>Recovery period</td>
<td></td>
</tr>
<tr>
<td>17/4/96</td>
<td>Recovery period</td>
<td></td>
</tr>
<tr>
<td>18/4/96</td>
<td>Recovery period</td>
<td></td>
</tr>
<tr>
<td>19/4 - 20/4</td>
<td>Obs.</td>
<td>Obs. 1</td>
</tr>
<tr>
<td>20/4 - 21/4</td>
<td>Obs.</td>
<td>Obs. lost data</td>
</tr>
<tr>
<td>21/4 - 22/4</td>
<td>Obs.</td>
<td>Obs. 2</td>
</tr>
<tr>
<td>22/4 - 23/4</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>23/4 - 24/4</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>24/4 - 25/4</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>25/4 - 26/4</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>26/4 - 27/4</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>27/4 - 28/4</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>28/4 - 29/4</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>29/4 - 30/4</td>
<td>Obs.</td>
<td>curfew begins</td>
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<tr>
<td>30/4 - 1/5</td>
<td>3rd bleed of all animals</td>
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</tr>
<tr>
<td>1/5 - 2/5</td>
<td>Obs.</td>
<td>Obs. 6</td>
</tr>
<tr>
<td>3/5 - 4/5</td>
<td>Obs.</td>
<td>Obs. lost data</td>
</tr>
<tr>
<td>4/5 - 5/5</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>5/5 - 6/5</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>6/5 - 7/5</td>
<td>Obs.</td>
<td>lost data</td>
</tr>
<tr>
<td>7/5 - 8/5</td>
<td>Obs.</td>
<td>Obs. 7</td>
</tr>
<tr>
<td>8/5 - 9/5</td>
<td>Obs.</td>
<td>Obs.</td>
</tr>
<tr>
<td>9/5 - 10/5</td>
<td>Obs.</td>
<td>Obs. lost data</td>
</tr>
<tr>
<td>10/5 - 11/5</td>
<td>Obs.</td>
<td>Obs.</td>
</tr>
<tr>
<td>11/5 - 12/5</td>
<td>Obs.</td>
<td>Obs. lost data</td>
</tr>
<tr>
<td>12/5 - 13/5</td>
<td>Obs.</td>
<td>Obs.</td>
</tr>
<tr>
<td>13/5 - 14/5</td>
<td>Obs.</td>
<td></td>
</tr>
<tr>
<td>14/5</td>
<td>Obs.</td>
<td></td>
</tr>
<tr>
<td>15/5</td>
<td>4th and final bleed of all animals abattoirs</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.2: The timetable for observations of behaviour of cattle in the second trial of an experiment which had 3 treatments: pasture, normal and stressed feedlot. 

*N*=14 per treatment. The periods of observations represent simultaneous observations of the cattle in the 3 treatments.

<table>
<thead>
<tr>
<th>4 hour blocks of scan samplings at 10 minute intervals</th>
<th>48 hour block</th>
</tr>
</thead>
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<td>0800-1200 1200-1600 1600-2000 2000-2400 0-0400 0400-0800</td>
<td></td>
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<table>
<thead>
<tr>
<th>Date</th>
<th>9/10/96 = 1st bleed of all animals. No observations until 25/10/96</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/10/96</td>
<td>23/10/96 = 2nd bleed of all animals.</td>
</tr>
<tr>
<td>23/10/96</td>
<td>Recovery period</td>
</tr>
<tr>
<td>24/10/96</td>
<td></td>
</tr>
<tr>
<td>25/10 - 26/10</td>
<td>1st bleed of all animals.</td>
</tr>
<tr>
<td>26/10 - 27/10</td>
<td>Obs</td>
</tr>
<tr>
<td>27/10 - 28/10</td>
<td>Obs</td>
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<tr>
<td>28/10 - 29/10</td>
<td>Obs</td>
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<td>29/10 - 30/10</td>
<td>Obs</td>
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<td>30/10 - 31/10</td>
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<td>31/10 - 1/11</td>
<td>Obs</td>
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<td>1/11 - 2/11</td>
<td>Obs</td>
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<tr>
<td>2/11 - 3/11</td>
<td>Obs</td>
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<tr>
<td>3/11 - 4/11</td>
<td>Obs</td>
</tr>
<tr>
<td>4/11 - 5/11</td>
<td>Obs</td>
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<td>5/11 - 6/11</td>
<td>Obs</td>
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<td>6/11 - 7/11</td>
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<td>7/11 - 8/11</td>
<td>Obs</td>
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<tr>
<td>8/11 - 9/11</td>
<td>Obs</td>
</tr>
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<td>9/11 - 10/11</td>
<td>Obs</td>
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<tr>
<td>10/11 - 11/11</td>
<td>Obs</td>
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<tr>
<td>11/11 - 12/11</td>
<td>Obs</td>
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<td>12/11 - 13/11</td>
<td>Obs</td>
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<tr>
<td>13/11 - 14/11</td>
<td>Obs</td>
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<tr>
<td>14/11 - 15/11</td>
<td>Obs</td>
</tr>
<tr>
<td>15/11 - 16/11</td>
<td>Obs</td>
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<td>16/11 - 17/11</td>
<td>Obs</td>
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<tr>
<td>17/11 - 18/11</td>
<td>Obs</td>
</tr>
<tr>
<td>18/11 - 19/11</td>
<td>Obs</td>
</tr>
<tr>
<td>19/11</td>
<td>4th and final bleed of all animals</td>
</tr>
<tr>
<td>20/11</td>
<td>Abattoirs</td>
</tr>
</tbody>
</table>
6.2.17 Pasture observations

The paddocks were divided into 8 grid rectangles. These grids were 75 metres long and 25 metres wide. An observation tower was placed either immediately within or just outside the paddock the animals were in. This tower was moved when the animals were relocated from one paddock to another. The tower was 5 metres high and constructed of aluminium tubing. The structural design of the tower met building industry standards. The viewing platform had a solid plywood base and a waterproof canvas cover. Figure 6.7 shows the grid design, and the placement of the tower and water troughs (see Figure 6.1 on page 90 for a further illustration of the tower location).

![Diagram of grid design and tower placement]

Figure 6.7: the layout of 4 paddocks used for cattle observations in a trial which had 3 treatments: pasture, normal and stressed feedlot.
Observations were made on the animals by staff who operated in 4 hour shifts. Observations by day were assisted with the use of 10 × 50 magnification binoculars (Nikon 10 x 50, 6.0° “Sport II”). Night time observations were performed with the use of a high powered infra red image intensifier (Litton “Pocketscope” Model M911A: Miltec Systems, Sydney, New South Wales). This was assisted with a battery powered spotlight fitted with an infra red filter. (Lightforce Portable Lighting Systems, Adelaide, South Australia). There were no observed disturbances to the animals from these devices. As far as I am aware, this is the first time that cattle have been extensively observed at night with this non invasive system. Figure 6.8 shows the nightscope, binoculars and weather recording system.

Figure 6.8: the binoculars, weather gauge and nightscope.
6.2.18 Feedlot pen observations

The feedlot cattle were monitored by an infra-red video surveillance system. This consisted of 2 video surveillance cameras (GEC Panasonic: CCD wv- BP 500) mounted on 5 metre poles or towers per pen. The cameras were situated at both ends of the pen. The cameras were linked to a time-lapse video recorder (GEC Panasonic: TL Ag-6024) which was situated in a central tower. Infra-red lighting (GEC Video Systems: IR 550) was used for the night time observations. At a later date the videos were analysed for those 4 hour blocks which coincided with the pasture observations. Figure 6.3 shows the location of the video cameras in the stressed feedlot.

There were periods when data was not recorded. This was due to a variety of reasons ranging from mechanical problems with a generator which supplied power for the video system to the presence of fog in the early hours of the morning. The analysis of the results was therefore restricted to the 48 hour blocks that were mostly intact.

6.2.19 Statistical methodology

There were a total of 28 animals for each treatment. There were two independent trials of each treatment. Differences between the trials in terms of time and weather conditions requires that the experimental error for formal statistical tests will be the pooled among-animal within-group within-trial error.

A multivariate analysis was initially performed on all adrenal results and all immune test results in order to provide some protection against falsely assuming a significant result when many univariate tests are performed. With regard to the immune results, and this analysis, because there are four bleeds, the adopted approach was to use the differences between the first and fourth bleed for the three groups. Adjustment for missing values is required in a multivariate analysis. A few variables with high numbers of missing values were removed (greater than 6 missing values). Other variables which had less than 6 missing values were imputed with the respective group by trial by bleed mean. This imputation slightly overestimates the size of the group and trial effects.
With regard to the univariate analyses, the results present graphs of means with standard error bars of the 3 groups for the tested variables. Where a difference between the groups appears significant at a particular bleed, ANOVA’s are presented.

A second analysis was also performed on the data (except for adrenal data and carcass weights). This analysis makes the 1st pre-treatment bleed a covariate of the 4th and final bleed (an analysis of covariance or ANCOVA). The reason for using the 4th bleed was that it was considered to be more indicative of a chronic treatment affect than the 2nd and 3rd bleeds. Where the results of the analysis were significant they are reported as a second set of results.

6.2.20 Location of raw data
The raw data for the physiological assays and behavioural observations are located at the Department of Biological Sciences, University of Wollongong, Northfields Rd, Wollongong, N.S.W. Australia, and at the Office of the Co-operative Research Centre for Beef Cattle, University of New England, Armidale, NSW. Australia.
6.3 Results

For a number of reasons (such as lack of antibodies, staff shortages and physical limitations to numbers of samples for certain analyses), some tests have results from one trial only, or for one trial have only half the numbers from each group sampled.

6.3.1 Initial multivariate analysis and combination of trial results

The multivariate analysis showed a very significant group-within-trial effect ($p=0$). This provided sufficient justification for proceeding with a univariate analysis of the tested variables.

Where there is no treatment by trial interaction, trial results have been combined. This is to provide some idea of the results using statistical tests of a higher power. The restrictions on the interpretation of two trial, rather than replicate, results remain.

Note:
Error bars for all graphs in this section are ± SEM.
6.3.2 Carcass Weight and Average Daily Gain

The trials were not combined because of a group by trial interaction (ANOVA, $F = 90.15$, $p = 0$, $df = 2$). This was partly due to the improvement in pasture conditions in the second trial, which allowed the pasture animals to gain weight at the same rate as the feedlot treatments. In the first trial, the pasture treatment mean was significantly lower than the feedlot treatment means (ANOVA, $F = 20.73$, $df = 2$, $p = 0$). In the second trial there was no significant difference between the treatments.

![Bar chart showing mean carcass weights for cattle placed in an experiment with 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 experimental trials.](image)

**Figure 6.9:** mean carcass weights for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. $N=14$ per treatment. Results shown from 2 experimental trials.
Average daily gain results are presented as kg/head/day and are as follows:

**Trial 1:**
- **Pasture:** average = -0.09 kg  
  standard error: 0.05
- **Normal feedlot:** average = 1.60 kg  
  standard error: 0.05
- **Stressed feedlot:** average = 1.62 kg  
  standard error: 0.08

**Trial 2:**
- **Pasture:** average = 1.30 kg  
  standard error: 0.06
- **Normal feedlot:** average = 1.55 kg  
  standard error: 0.08
- **Stressed feedlot:** average = 1.44 kg  
  standard error: 0.06

The pasture animals did not quite maintain weight in the first trial yet had a comparable weight gain to the feedlot animals in the second trial. This was due to improvements made to the pasture and good rainfall in the interval between the trials.
6.3.3 Relative Adrenal Weight

Both adrenal glands were collected. It made no difference to the results whether the weight of the heavier gland only was used or the combined weight of both glands. This analysis used the results of the heavier gland only. There was no significant difference between trials in terms of mean relative adrenal weights for each treatment. This allowed them to be combined (see Figure 6.12). An analysis of the combined results showed that the pasture treatment mean was significantly less than the means of both feedlot treatments (p< 0.05). An ANOVA table is presented overleaf.

Figures 6.10 and 6.11: mean relative adrenal weights for cattle placed in an experiment which had 3 treatments: pasture, normal and stressed feedlot. N = 14 per treatment. Results shown for 2 experimental trials. Figure 6.10 shows the results when the total weight of both adrenal glands are used and Figure 6.11 when the weight of the heavier gland only is used.
Figure 6.12: mean relative adrenal weights (using the weight of the heavier gland only) for cattle placed in a trial which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=28 per treatment. Results shown from the combination of 2 trial replicates.

ANOVA Table: combined replicates, heavier gland only.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum of Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor (group)</td>
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<td>4.40</td>
<td>2.20</td>
<td>4.21</td>
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<td>Factor (trial)</td>
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<td>3.77</td>
<td>7.23</td>
<td>0.01</td>
</tr>
<tr>
<td>(trial): (group)</td>
<td>2</td>
<td>0.12</td>
<td>0.06</td>
<td>0.11</td>
<td>0.89</td>
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<tr>
<td>Residuals</td>
<td>73</td>
<td>38.105</td>
<td>0.5220</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6.3.4 Adrenal Index

One outlier (animal number 11 from the stressed treatment, trial 2) was removed from the analysis because of its high value. There was a treatment by trial interaction, therefore the trials were not combined (p < 0.05). In trial 1, there was no significant difference between the pasture and normal feedlot means. Both were significantly less than the stressed feedlot treatment mean (p < 0.001). In trial 2 the pasture treatment mean was less than both feedlot treatment means. This was not quite significant (p = 0.07). ANOVA tables are presented overleaf.

Figure 6.13: mean adrenal index values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 experimental trials.
**ANOVA Table: replicate one**

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<th>Pr (F)</th>
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</thead>
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**ANOVA Table: replicate two**

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<th>Pr (F)</th>
</tr>
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<td>70.01</td>
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</table>
6.3.5 Adrenal function: plasma cortisol

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.14: mean plasma cortisol values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.6 **Humoral immune responses: serum IgA**

The pasture treatment mean was consistently higher than the feedlot treatment means after the first bleed in both trials. The difference was significant at bleed 4 in the first trial and bleeds 2, 3, and 4 in the second trial. In the second trial the 4th bleed for the stressed feedlot treatment was significantly lower than the 1st bleed (T test, df = 39, T statistic = -2.28, p < 0.05). The analysis which used the 1st bleed as a covariate of the 4th found that there was a significant difference between the feedlot groups and the pasture group in both trials (p<0.05). ANOVA and ANCOVA tables are presented overleaf.

![Graph](image)

**Figure 6.15:** mean serum IgA values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
### ANOVA Table: replicate one: bleed 4

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### ANOVA Table: replicate two: bleed 2

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### ANOVA Table: replicate two: bleed 4

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<th>Pr (F)</th>
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</thead>
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<tr>
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<td>4846.63</td>
<td>2423.31</td>
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<tr>
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### ANCOVA Table

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<th>F-stat</th>
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<tr>
<td>Residuals</td>
<td>77</td>
<td>18740.38</td>
<td>243.381</td>
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</table>
6.3.7 **Humoral immune responses: salivary IgA**

The data showed a high degree of variability. The analysis which uses the 1st bleed as a covariate of the 4th found that after the removal of some outliers there was a large group effect present. This showed that the pasture group was lower across both trials than the feedlot groups. ANCOVA table overleaf. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

![Graph showing salivary IgA values for cattle placed in an experiment with three treatments: pasture, normal feedlot, and stressed feedlot.](image)

**Figure 6.16:** mean salivary IgA values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
## ANCOVA table

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<tr>
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6.3.8 **Humoral immune responses: serum IgG**

There were no consistent trends or differences between groups after the pre-treatment sample.

![Graph](image)

**Figure 6.17:** mean serum IgG values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 experimental trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.9 **Humoral immunity: B cells**

Only samples from trial 2 were tested, therefore it is difficult to determine on any consistent trends. The pasture treatment means were lower than the feedlot treatment means at bleeds 3 and 4. This was significant at bleed 4 (p < 0.05). The means of the feedlot treatments at the 4th bleed were not significantly different from their means at the 1st (before treatment) bleed. In the analysis which used the 1st bleed as a covariate of the 4th, the feedlot groups had a significantly higher count than the pasture group (p<0.05). ANOVA and ANCOVA tables overleaf.

---

**Figure 6.18:** mean B cells values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from trial 2 only. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
ANOVA Table: trial two: bleed 4

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ANCOVA table

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6.3.10 Cell mediated immune responses: CD4 lymphocytes

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.19: mean CD4+ lymphocyte values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.11 Cell mediated immunity: CD8 lymphocytes

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.20: mean CD8+ lymphocyte values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.12: CD4:CD8 Ratio

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.21: mean CD4:CD8 ratio values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.13 Cell mediated immunity: CD5 lymphocytes

The analysis which used the 1st bleed as a covariate of the 4th found that the stressed group was significantly higher than the pasture and normal feedlot groups in both trials \( p<0.05 \). An ANCOVA table is presented overleaf. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

![Graph of CD5+ lymphocyte values](image)

**Figure 6.22**: mean CD5+ lymphocyte values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. \( N=14 \) per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
### ANCOVA table

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6.3.14 Cell mediated immunity: WC1 lymphocytes

At the final bleed for both trials, the pasture treatment mean was higher than the feedlot treatment means. This difference was only significant in the second trial and between the pasture and normal feedlot means only (T-test, df = 18, t statistic = 2.61, p < 0.05). The feedlot treatment means became lower after the first (pre treatment) bleed, indicating some effect of the treatments. This is not entirely consistent. In the first trial, the stressed group mean was lower at the 4th bleed than at the 1st bleed. In the second trial, it is the normal feedlot mean which was lower at the 4th bleed compared to the 1st bleed. The analysis which used the 1st bleed as a covariate of the 4th found that the pasture group was significantly higher than the feedlot groups for both trials (p < 0.01). ANCOVA table overleaf.

Figure 6.23: mean WC1 lymphocyte levels for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds.
Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

**ANCOVA table**

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6.3.15 Cytokines: interleukin 2 RA

Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.24: mean interleukin (2RA) values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 1 trial only (see section 6.4.1). The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.16 Immune cell function: lymphocyte proliferation assay

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 50% of animals in all groups.

Figure 6.25: mean values for unstimulated lymphocyte proliferation assay results for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 experimental trials. The second trial has bleeds 2 and 3 missing (see page 105). The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.17 Immune cell function: ConA stimulated lymphocyte proliferation assay

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 50% of animals in all groups.

Figure 6.26: mean ConA stimulated lymphocyte proliferation assay results for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 experimental trials. The second trial has bleeds 2 and 3 missing (see page 105). The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.18 Immune cell function: PHA stimulated lymphocyte proliferation assay

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 50% of animals in all groups.

Figure 6.27: mean PHA stimulated lymphocyte proliferation assay values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The second trial has bleeds 2 and 3 missing (see page 105). The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.19 Immune cell function: Con A lymphocyte stimulation index

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 50% of animals in all groups.

Figure 6.28: mean Con A lymphocyte stimulation index values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The second trial has bleeds 2 and 3 missing (see page 105). The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.20 Immune cell function: PHA stimulation index

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 50% of animals in all groups.

Figure 6.29: mean PHA lymphocyte stimulation index values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The second trial has bleeds 2 and 3 missing (see page 105). The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.21 Immune cell function: neutrophil myeloperoxidase assay

The pasture and normal feedlot treatment means were higher than the stressed feedlot mean at the 4th bleed. However, this was not significant. In addition, the stressed feedlot mean was not significantly different at the 4th bleed from the 1st bleed. Trial 1 = 50% of animals in all groups.

Figure 6.30: mean neutrophil myeloperoxidase assay values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 1 trial (see page 105). The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.22 Natural killer cell activity assay

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups.

Figure 6.31: mean natural killer cell activity assay values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 1 trial only (see p105). The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.23 Haematology: leucocyte parameters: total leucocyte count

After the 1st bleed, the pasture means were consistently lower than the feedlot means. However, this is significant only at bleed 3 in the first replicate ($p < 0.01$) and at bleed 2 in the second replicate ($p < 0.05$). Replicate 1 = 50% of animals in all groups. Replicate 2 = 100% of animals in all groups.

---

**Figure 6.32**: mean total leucocyte counts for cattle placed in a trial which had 3 treatments: pasture, normal feedlot, and stressed feedlot. $N=14$ per treatment. Results shown from 2 replicates of the trial. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
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6.3.24 Haematology: leucocyte subsets: lymphocytes

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.33: mean lymphocyte count values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.25 Haematology: leucocyte subsets: monocytes

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.34: mean monocyte count values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.26 Haematology: leucocyte subsets: neutrophils

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.35: mean neutrophil count values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.27 Haematology: leucocyte subsets: eosinophils

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.36: mean eosinophil count values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.28 Haematology: erythrocyte parameters: total erythrocyte count

The pasture means were consistently higher than the feedlot means. This is significant at bleeds 2, and 3 in both trials. \( p = 0.027, 0.054 \). However, at bleed 4 in the second trial, the pasture and stressed feedlot treatment means were significantly higher than the normal feedlot treatment mean \( p = 0.003 \). The feedlot treatments do not appear to have lowered the erythrocyte counts. The pasture treatment means have risen in both trials, thus creating the significant differences.

Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

ANOVA tables overleaf.

---

**Figure 6.37**: mean erythrocyte values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. \( N=14 \) per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
**ANOVA Table: trial one: bleed 2**

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**ANOVA Table: trial two: bleed 4**

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6.3.29 Haematology: erythrocyte parameters: haemoglobin
There is a resemblance to the results of the total erythrocyte count in that the pasture means rose after the first bleed. As with total erythrocyte counts, at the 4th bleed in the second trial, the stressed feedlot and pasture treatment means were significantly higher than the normal feedlot treatment mean (p = 0.005). Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups. ANOVA table overleaf.

Figure 6.38: mean haemoglobin values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14-day interval between bleeds. The first bleed represents a before treatment sample.
### ANOVA Table: trial two: bleed 4

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6.3.30 Haematology: erythrocyte parameters: haematocrit

The pasture treatment means were higher than the feedlot treatment means, except at the 4th bleed in trial 2. However, there were no significant differences between the groups for any bleed in trial 1 and only at bleeds 3 and 4 in the second trial (p = 0.027, 0.0025). Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups. ANOVA table overleaf.

Figure 6.39: mean haematocrit values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
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6.3.31 Haematology: erythrocyte parameters: mean corpuscular haemoglobin

There were no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.40: mean corpuscular haemoglobin values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.32 Haematology: erythrocyte parameters: mean corpuscular haemoglobin concentration

The pasture treatment values were higher in both trials. However, no statistical analysis was performed because of the variability between trials. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

![Graph](image_url)

**Figure 6.41:** mean corpuscular haemoglobin concentration values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.33 Haematology: erythrocyte parameters: platelet count

There are no consistent trends or differences between groups. Trial 1 = 50% of animals in all groups. Trial 2 = 100% of animals in all groups.

Figure 6.42: mean platelet count values for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot, and stressed feedlot. N=14 per treatment. Results shown from 2 trials. The 4 points on the x-axis represent bleeds. There was a 14 day interval between bleeds. The first bleed represents a before treatment sample.
6.3.34 Health
There were no health problems during the trials, and no disease-related lesions in any animals were seen at post-mortem.

6.4 Physiological results: summary and discussion

The principal aim of the experiment was to measure the physiological and behavioural responses of cattle to three treatments: pasture, normal feedlot and stressed feedlot, with regard to determining the existence of pre-pathological states.

Table 6.3 (overleaf) presents a summary of the P values derived from ANOVA’s for all the tested variables.

In many of the physiological indices measured, the initial values of the three groups in the second trial are different to those of the first trial. It is possible that this difference is a function of the different histories of the animals (in an immune and HPA sense) prior to the trial.

The turnover rates and nature of expression of most of the indices were such that the sampling regime could be considered a representative one. However, for two of the measured indices it is possible that the sampling regime was such that non-representative levels were gained. Salivary IgA results were more variable than serum IgA results. This could be due to the fact that cattle can produce upwards of 30 litres of saliva per day, and the amount collected (although all attempts were made to minimise any variation) may not have been representative. The variability observed in the plasma cortisol response can be attributed in part to the properties of cortisol secretion previously discussed in the literature review e.g., its brief episodic nature of release and the rapidity with which it disappears from the blood stream. The time an animal spent in the cattle race before sampling could in this case be one of several factors contributing to the variable
Table 6.3: P values in relation to ANOVA analyses of a range of variables tested in a trial which examined the effects of 3 treatments: pasture, normal feedlot and stressed feedlot, on 3 matched groups of cattle (N=14 per group). Values are for separate bleeds (except where stated otherwise) in 2 trials. Values below 0.05 are highlighted.

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
</tr>
<tr>
<td>relative adrenal weight</td>
<td>0.02</td>
<td>(1 result from combined trials)</td>
</tr>
<tr>
<td>adrenal index</td>
<td>0.00</td>
<td>0.07</td>
</tr>
<tr>
<td>plasma cortisol</td>
<td>0.33</td>
<td>0.91</td>
</tr>
<tr>
<td>serum IgA</td>
<td>0.96</td>
<td>0.35</td>
</tr>
<tr>
<td>salivary IgA</td>
<td>0.69</td>
<td>0.13</td>
</tr>
<tr>
<td>serum IgG</td>
<td>0.01</td>
<td>0.80</td>
</tr>
<tr>
<td>total B cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 lymphocytes</td>
<td>0.23</td>
<td>0.27</td>
</tr>
<tr>
<td>CD8 lymphocytes</td>
<td>0.45</td>
<td>0.04</td>
</tr>
<tr>
<td>CD4 to CD8 ratio</td>
<td>0.74</td>
<td>0.00</td>
</tr>
<tr>
<td>CD5 lymphocytes</td>
<td>0.17</td>
<td>0.00</td>
</tr>
<tr>
<td>WC 1 lymphocytes</td>
<td>0.29</td>
<td>0.30</td>
</tr>
<tr>
<td>Interleukin 2RA</td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>lymphocyte proliferation assay (LPA) unstimulated</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Con A LPA</td>
<td>0.51</td>
<td>0.17</td>
</tr>
<tr>
<td>Con A stimulation index</td>
<td>0.20</td>
<td>0.01</td>
</tr>
<tr>
<td>PHA LPA</td>
<td>0.74</td>
<td>0.29</td>
</tr>
<tr>
<td>PHA stimulation index</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td>neutrophil myeloperoxidase activity</td>
<td>0.97</td>
<td>0.48</td>
</tr>
<tr>
<td>natural killer cells</td>
<td>0.59</td>
<td>0.93</td>
</tr>
<tr>
<td>total leucocytes</td>
<td>0.55</td>
<td>0.85</td>
</tr>
<tr>
<td>% lymphocytes</td>
<td>0.97</td>
<td>0.34</td>
</tr>
<tr>
<td>% monocytes</td>
<td>0.48</td>
<td>0.00</td>
</tr>
<tr>
<td>% neutrophils</td>
<td>0.88</td>
<td>0.47</td>
</tr>
<tr>
<td>% eosinophils</td>
<td>0.79</td>
<td>0.48</td>
</tr>
<tr>
<td>total red blood cell count</td>
<td>0.48</td>
<td>0.02</td>
</tr>
<tr>
<td>haemoglobin</td>
<td>0.65</td>
<td>0.00</td>
</tr>
<tr>
<td>haematocrit</td>
<td>0.53</td>
<td>0.14</td>
</tr>
<tr>
<td>mean corpuscular haemoglobin (MCH)</td>
<td>0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>MCH concentration</td>
<td>0.39</td>
<td>0.32</td>
</tr>
<tr>
<td>platelet count</td>
<td>0.29</td>
<td>0.88</td>
</tr>
</tbody>
</table>
results. Although all attempts were made to standardise the sampling procedures, the time in the race was not uniform for all animals.

Variables indicative of elevated adrenal activity were relative adrenal weight and adrenal index. In both cases the feedlot groups had higher values than the pasture group. Adrenal index results are not as clear-cut as relative adrenal weight results in that the difference was significant in the first replicate (p<0.05), but not quite significant in the second replicate (p = 0.07). However, in terms of trends and differences between groups, adrenal index results can be seen as supportive of relative adrenal weight results. The one variable that did not indicate elevated adrenal activity was plasma cortisol, the possible reasons for which have been discussed on page 150.

The variables that did not clearly indicate depressed immune function were: the humoral immune variables salivary IgA and serum IgG, all but one of the cell mediated variables, and all of the cell function assays.

Variables such as total erythrocyte count, haemoglobin, and haematocrit (p137-139) showed significant differences between the pasture and the feedlot treatments. However, it is important to note that these differences were a function of an elevation in pasture values compared to the first bleed. The feedlot values did not change significantly from their pre-treatment bleed values.

The variables that were indicative of the presence of a depressed immune function were serum IgA and the gamma delta T-lymphocyte WC1 (using the analysis which made the first pre-treatment bleed a covariate of the final bleed). The serum IgA results (Figure 6.15, p 113) showed differences between replicates. Importantly, though, with regard to the feedlot treatments, the post-treatment bleeds showed declining values, indicating that the feedlot treatments had an effect in the direction that would be expected if immune function was depressed.
There is the possibility that the other variables measured were too difficult to interpret in the light of pre-pathological states, yet could still indicate their presence. For example, in terms of network theory, it has been argued that it is the pattern of the immune response that is diagnostic of the state of the immune system (Varela and Couthino, 1991). Therefore, the levels of the T-cell sub-sets for example, could represent a pattern that is indicative of depressed immune competence. However, the knowledge of the immune system is not advanced enough at this stage to apply this theory to these results.

In summary, for the two feedlot treatments there was a significant increase in adrenal activity compared to the pasture treatment and a decrease in serum IgA levels and WC1 lymphocyte levels compared to the pasture treatment (this is with regard to the present understanding of the functions of the immune system). For these three indices, there were no significant differences between the two feedlot treatments.

As stated previously, pre-pathological states are defined physiologically in an organism by the presence of increased adrenal activity, in conjunction with a depressed immune system. The results of the adrenal gland analyses indicate an increase in chronic adrenal activity in the feedlot groups. The question then arises: do the results of the indices of serum IgA and the gamma delta T-lymphocyte WC1 in themselves represent a depressed immune system?

WC1 lymphocytes are associated with epithelial immunity but at this stage have not been definitely linked to stress in other studies (e.g., Morrow-Tesch et al. 1996). There is evidence that depressed secretory IgA levels are strongly linked to reduced mucosal immunity (e.g., Drummond and Hewsen - Bower 1997, Kagnoff and Kiyono 1996). However, the role of serum IgA is less clear. For example, Benjamini et al (1996) states that currently its function is unknown. However, changes in serum IgA levels are often linked with exposure to infectious agents in other species (e.g., Campbell 1991). In conclusion, these results are not definitive and support from
other immune indices is required in order to state unequivocally that the immune system was depressed in this study.

Examination of the correlations between variables, within individuals, in the feedlot groups with regard to individual relative adrenal weight, serum IgA, and WC1 lymphocyte values showed that they did not co-vary. However, these correlations are based on a relatively small sample size of 14 animals per group, therefore the interpretation of the result is limited.

To summarise, in terms of the results of this trial, although physiological changes were occurring in the feedlot animals, no inferences can be clearly made to the presence of pre-pathological states in the feedlot groups and no pre-pathological animals can be identified. Nonetheless relative adrenal gland weight, serum IgA and the gamma delta T-lymphocyte WC1 do appear to be good candidates as indices of pre-pathological states in feedlot cattle in further studies.

With regard to the behavioural results, because pre-pathological states cannot be clearly detected in individual animals in the first instance, then the behavioural results cannot be linked to animals identified as being in a pre-pathological state.

Group behaviour does not add anything to the physiological results. As stated in the literature review and throughout this thesis, this is because it is not possible to clearly distinguish between adaptive behaviour to the treatment conditions and pre-pathological state derived behaviour. For example, the greater percent of time spent feeding by the pasture animals is influenced by the fact that they do not receive a feed ration as the feedlot animals do. However, outstanding consistent individual behaviour within the groups could be of interest in the final analysis.

The following section presents the results of the behavioural observations. These results are presented firstly in terms of group behaviour. This provides an overall perspective of the behavioural differences between the groups (particularly with
regard to lying behaviour which was considered as a potential stressor for the stressed feedlot treatment), then in terms of individual behaviours within that group. Except where stated, these results are derived from five observation blocks in replicate one (blocks 1,3,8,9 and 10) and six blocks in replicate two (blocks 1,4,5,6,8 and 10). Each block represents a day-night cycle.
6.5 Behavioural results

6.5.1 Time budgets

The pasture animals had a lower mean percentage of lying and standing behaviour compared to the feedlot groups. This is a result of an increase in feeding, or grazing, time. It can be seen that, with regard to lying behaviour, the stressed feedlot was only slightly different to the normal feedlot despite the treatment effects.

**Trial 1**

<table>
<thead>
<tr>
<th></th>
<th>Mean % of sample points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasture</td>
<td>39% 18% 41%</td>
</tr>
<tr>
<td>Normal f/lot</td>
<td>10% 34% 54%</td>
</tr>
<tr>
<td>Stress f/lot</td>
<td>11% 38% 51%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>L.R = 0.14%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W = 1.87%</td>
</tr>
<tr>
<td></td>
<td>L.R = 0.09%</td>
</tr>
<tr>
<td></td>
<td>W = 1.59%</td>
</tr>
<tr>
<td></td>
<td>L.R = 0.28%</td>
</tr>
<tr>
<td></td>
<td>W = 0.83%</td>
</tr>
</tbody>
</table>

**Trial 2**

<table>
<thead>
<tr>
<th></th>
<th>Mean % of sample points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasture</td>
<td>33% 20% 44%</td>
</tr>
<tr>
<td>Normal f/lot</td>
<td>12% 38% 48%</td>
</tr>
<tr>
<td>Stressed f/lot</td>
<td>8% 42% 49%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>L.R = 0.08%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W = 7.44%</td>
</tr>
<tr>
<td></td>
<td>L.R = 0.06%</td>
</tr>
<tr>
<td></td>
<td>W = 1.73%</td>
</tr>
<tr>
<td></td>
<td>L.R = 0.14%</td>
</tr>
<tr>
<td></td>
<td>W = 0.75%</td>
</tr>
</tbody>
</table>

*Figure 6.43: mean activity time budgets for cattle placed in a trial which had 3 treatments: pasture, normal and stressed feedlot. N = 14 per treatment. Results derived from 5 day/night blocks in trial 1 and 6 day/night blocks in trial 2.*
6.5.2 Circadian pattern of lying

The results were similar for both trials. The diurnal section of these graphs are similar to those in the trial detailed in Chapter 3. There was a consistent period of reduced lying for all 3 groups between 1500 and 1800 hours. The pasture

**Figure 6.44**: mean circadian pattern of lying for cattle placed in a trial which had 3 treatments: pasture, normal and stressed feedlot. N=14 per treatment. Results derived from 5 day/night blocks in trial 1 and 6 day/night blocks in trial 2.
animals had a longer duration of reduced lying than did the feedlot animals. For both replicates, the stressed feedlot group showed more lying activity during the daylight hours and less lying activity during the nocturnal hours than the two feedlot groups. This could be a compensatory adjustment to the wet surface conditions.

6.5.3 Lying behaviour: frequency and duration

For both replicates, there was a greater range in the duration of lying for both feedlot treatments compared to the pasture treatment. The range of the pasture animals was from 550-650 minutes per block, whereas for the feedlot groups it was 550 – 950 minutes per block (see Figure 6.45). This indicates that there is a greater variability in the feedlot groups compare to the pasture group. For the stressed feedlot group this could be attributed to competition for lying space. But this does not account for the normal feedlot group results. Interestingly, the frequency of lying behaviour was similar between the three groups for both replicates. This suggests that the pasture cattle lie down as frequently as the feedlot cattle but not as long.

6.5.4 Agonistic behaviour: numbers of interactions

Figure 6.46 presents the total numbers of agonistic interactions per group per replicate. There was a significantly higher number of interactions in replicate two than in replicate one (Mann-Whitney U test statistic = 63.000, P = 0.009, Chi-square approximation = 6.812 with 1 df). This could be due to the different background of the 2nd replicate animals. The pasture groups had a higher incidence of agonistic interactions in both trials.

6.5.5 Agonistic behaviour: winners and losers

Figure 6.47 shows the percentages of wins, draws and losses for each animal in the trial. There appeared to be no outstanding individual differences in the study. These results do provide an insight to the social structure of the groups. However that subject is outside the scope of this thesis.
Figure 6.45: average duration values for lying behaviour for cattle in a trial which had 3 treatments: pasture, normal and stressed feedlot. Average durations are shown for 5 observation blocks of 48 hours in trial 1 and 6 observation blocks in trial 2. The lines in each graph represent individual animals.
6.5.6 Affiliative behaviour

Figure 6.48 shows the results of the affiliative interactions. There were no significant differences between the trials with regard to numbers of interactions per observation block (Mann-Whitney U test statistic = 116.000, P = 0.491, Chi-square approximation = 0.475 with 1 df). There were also no significant differences between the groups of each trial with regard to numbers of interactions.
Figure 6.4, total number of agonistic interactions won, lost and drawn for cattle in an experiment which had 3 treatments: pasture, normal feedlot and stressed feedlot. N= 14 per treatment. Results are shown for each animal in each of the two trials in the experiment.
Figure 6.47: percentage of agonistic encounters won, lost and drawn for cattle placed in an experiment which had 3 treatments: pasture, normal feedlot and stressed feedlot. N=14 per treatment. Results are shown for each animal in each of the two trials in the experiment.
Figure 6.48: number of affiliative interactions for cattle in an experiment which had 3 treatments: pasture, normal feedlot and stressed feedlot. N=14 per treatment. Results are shown for pasture group, trial 1.
Figure 6.49: number of affiliative interactions for cattle in an experiment which had 3 treatments: pasture, normal feedlot and stressed feedlot. N=14 per treatment. Results are shown for normal feedlot group, trial 1.
Figure 6.50: number of affiliative interactions for cattle in an experiment which had 3 treatments: pasture, normal feedlot and stressed feedlot. N=14 per treatment. Results are shown for stressed feedlot group, trial 1
Figure 6.51: number of affiliative interactions for cattle in an experiment which had 3 treatments: pasture, normal feedlot and stressed feedlot. N=14 per treatment. Results are shown for pasture group, trial 2.
Figure 6.52: number of affiliative interactions for cattle in an experiment which had 3 treatments: pasture, normal feedlot and stressed feedlot. N=14 per treatment. Results are shown for normal feedlot group, trial 2.
Figure 6.53: number of affiliative interactions for cattle in an experiment which had 3 treatments: pasture, normal feedlot and stressed feedlot. N=14 per treatment. Results are shown for stressed feedlot group, trial 2.
6.5.7 Nearest neighbours

The results showed that, with regard to both frequency and duration, every animal from every group, including the stressed feedlot group, was observed to be “by itself” far more times than it was observed to be within one steer’s length of another animal. This applied to both trials. The magnitude of the difference was very large. For example, the mean duration of time observed by itself for animal 1 in the pasture group for the first trial was 1,110.2 minutes (18.5 hours). The most time it spent with another animal was with animal number 3. The mean duration of time spent with animal 3 was 40 minutes. This would seem not surprising in a pasture situation. The stressed feedlot group had a double stocking density. The mean duration of time animal 1 in the 1st trial of the stressed feedlot group was observed by itself was 840.6 minutes (14.01 hours). The most time it spent with another animal was animal number 14. The mean duration of time with animal 14 was 114 minutes (1.9 hours).

These results do not provide any evidence of pair bonding, clustering or zones of inhibition. Possible reasons for this finding are discussed further in the behaviour summary.

6.5.8 Grid space occupation

The complete results for this analysis are located in the appendix. These results present frequency and duration values for grid space location. These are broken up into activities such as lying behaviour, feeding behaviour, standing behaviour etc. in keeping with the conclusion to the preliminary trial number 4 (see page 84). All results are for the three groups, plus every individual within the group. They are presented in graphical form as average values for each animal with standard error bars.

The most noticeable feature of these results was the fact that the occupation of different parts of the pen was not evenly distributed across the grid spaces. Most animals appeared to show a definite preference for certain grid spaces and were found infrequently or not at all in other grid spaces.
This finding applied for lying behaviour, standing behaviour, and to a slightly lesser extent, feeding behaviour.

With regard to lying behaviour, in the normal feedlot group, the grid squares most commonly occupied were those furthest from the feedbunk. Those closest to the feedbunk were least occupied. In the stressed feedlot group lying was more common on the higher side of the pen where the mud was not as deep. In all groups the pattern for frequency of occupation of grid spaces was not the same as the pattern for duration, the frequency showing a wider spread.

With regard to feeding behaviour, nearly all animals showed preferences for a certain feedbunk position or positions. There was only a small number of exceptions and these were in the stressed feedlot group: in trial 1, there were 3 animals that appeared to have no preference for a feeding position in terms of frequency of feeding, and 2 animals that appeared to show no preference in terms of duration of feeding. In trial 2, also in the stressed feedlot group, there were 3 animals that did not appear to have an even distribution of feeding position in terms of both frequency and duration.

6.6 Behavioural results: summary and discussion

6.6.1 Group behaviour

The behavioural differences between the groups do not aid in the identification of pre-pathological states, as has been mentioned previously. However, this experiment has provided some important results. Some of these are:

1. Figure 6.3 (activity time budgets for the three groups) on page 156 shows that there was little difference in time spent feeding for the pasture animals in both trials, yet the grass cover was much higher and denser in the second trial. This indicates that when there is adequate grass cover, cattle eat for as long as when there is low cover, but perhaps are more selective in their feeding.
2. For pasture animals the time spent feeding has had the affect of reducing the time spent standing.

3. For the feedlot animals there was little difference between them with regard to time spent feeding, despite the increased competition for feedbunk space for the stressed feedlot animals.

4. With regard to lying behaviour there was very little difference between the feedlot groups and the pasture group had a lower lying percentage overall. It was hypothesised that a wet substrate and double stocking density in the stressed feedlot would disrupt and reduce lying behaviour and thus act as a stressor. An examination of the circadian pattern of lying shows that the stressed groups had higher lying activity than the other groups during the day and lower lying activity during the night. This suggests that lying behaviour was disrupted but not reduced. These results indicate that cattle have a very strong motivation for lying, a finding that agrees with the work of Ruckebusch (1972) and Metz (1985).

5. The difference in rates of agonistic behaviours between the first and second replicates may demonstrate the effect of different backgrounds of groups of cattle on these behaviours.

6.6.2 Individual behaviour

There were differences between individuals for some behaviours. For example, with regard to feedbunk occupation in the feedlot treatments, most of the animals exhibited a preference for certain feedbunk locations. A small number did not, yet at this stage it is unknown as to whether this would constitute a pre-pathological state correlate or index.

The nearest neighbour analysis showed that all animals were observed to be by themselves far more than with other animals. Two possible reasons for this are:
1. Cattle have a personal space that they maintain, and to this end, they avoid having other animals within one steer's length of them.

2. The criterion for determining what is a nearest neighbour (any animal within one steer’s length of the animal being observed) is too conservative. Being herd animals, it is conceivable that cattle have a large personal space and perhaps the whole group may be classed as “nearest neighbours”.

6.7 Main trial: conclusion

The results of this study showed that there was a significant elevation of adrenal cortical activity (strongly suggesting increased HPA activity) in the feedlot groups compared to the pasture group. Out of the 20 immune variables tested in this study, the variables serum IgA and the gamma delta T-lymphocyte WC1 showed decreased activity in the feedlot groups compared to the pasture group. The other variables may represent a physiological pattern of response, which could be indicative of depressed immune function, but this can not be adequately interpreted at this stage.

The group behavioural results showed that, with regard to the duration of lying behaviour, the stressed feedlot animals were not significantly affected by a wet substrate and double stocking density. However, competition for lying space, feedbunk access and increased social interaction, plus a degree of discomfort from the wet substrate may still have acted as stressors. The pasture group had a lower percentage of lying and standing behaviour compared to the feedlot groups. This was a result of increased feeding/grazing activity.

The individual behavioural results showed a strong degree of similarity within the groups. The agonistic and affiliative results may indicate a well-established dominance hierarchy and social structure. These behaviours will be investigated further, outside of this thesis.
The results for the nearest neighbour analysis showed that each animal in all groups was observed "by itself" far more than with any other animal. There was at times a greater than tenfold difference involved.

The results for pen and pasture grid space occupation showed that all pasture and feedlot animals had major differences (which seems to indicate preferences) in the occupation of grid squares. These differences were evident for lying and standing behaviour. With regard to feeding behaviour, all animals from the pasture and normal feedlot groups had major differences in grid square occupation. For the stressed feedlot group there were 5 animals in the 1st replicate and 3 animals in the 2nd that seemed to have no preferences for feedbunk locations.

In conclusion, under the conditions of this study, pre-pathological states could not be unequivocally identified in animals in the feedlot treatments. There was support for the presence of pre-pathological states through the indices: relative adrenal weight, adrenal index, the gamma delta T-lymphocyte WC1 and serum IgA. Relative adrenal weight and adrenal index are a clear indication of elevated HPA activity. These variables also did not co-vary within individuals and at present, it is an open question as to whether the two immune indices represent a depressed immune system. Behavioural correlates to pre-pathological states were not able to be determined, due to the comparative uniformity of behaviour of animals within the three treatments and the inability to identify pre-pathological animals in advance.
Chapter Seven: Discussion

7.1 Introduction
The principal aim of this study was to develop a model for determining the welfare status of beef cattle in feedlots and a set of welfare indices for this model. This does not appear to have been attempted before for feedlot cattle, although, for some other species such as poultry and pigs, welfare assessment is well established (Dawkins 1977; van Putten 1989).

My review of the literature revealed that approaches to the assessment of animal welfare can be divided into three broad approaches: (1) examinations of the human-animal relationship, (2) assessment of the mental state of an animal and (3) assessment of the physical state of an animal. These approaches differ only in emphasis. The first approach argues that human beings make, and enforce, the decisions on an animal’s welfare. Therefore, it is important to examine the philosophical and ethical basis of different human attitudes to animals. I did not use this because with regard to feedlot cattle, there is a need for scientific contributions and evaluations to be brought into human discussions on animal welfare. However, any investigation into animal welfare has to be based on a clearly stated ethical framework. Therefore several moral theories were examined, and the theory of moral contractualism was chosen as an appropriate ethical basis. An important caveat was also made that there appears to be no perfect moral theory that covers all of the variations in human-animal relationships.

The second approach for assessing animal welfare employs techniques such as preference testing. The methodological difficulties of this approach have been widely discussed (Appleby and Hughes 1997). This approach was not followed, because of the problems associated with determining consciousness and assessing levels of consciousness in cattle, and because of an earlier finding of Grandin et al. (1994) which showed that the previous experience of cattle could confound preference testing results.
The third approach emphasises the physical measurement of stress. The underlying concept of this approach is that an animal which is in a significant state of distress, has poor welfare. There are challenges to this approach such as the definition of biological stress, the ambiguity of many indices with regard to non-stressful and stressful states, and the calibration and weighting of these indices (Broom and Johnson 1993).

I chose the third approach because I considered that an appropriate definition of stress could be determined, and a suitable experimental design could meet the previously mentioned challenges. The definition of stress chosen was Moberg’s pre-pathological state (Moberg 1987, 1996). This is a state whereby an animal’s homeostatic mechanisms, in attempting to cope with significant stressors, are overtaxed to the point where the animal is predisposed to metabolic declines and opportunistic infections. In a physiological context, it is identified by an elevated activity of the hypothalamus-pituitary-adrenal (HPA) axis, in conjunction with a depression of the immune system. One of the features of this definition is that it possesses a degree of non-specificity with regard to the types of stressors eliciting a stress response. Therefore, a range of different stressors can elicit a pre-pathological state, which is important from an experimental point of view.

A pre-pathological state is defined in physiological terms, but may well produce behavioural changes. If these could be identified, they would have potential as an aid to the field diagnosis of animals with poor welfare in commercial feedlot situations. There is also a possibility that these behaviours, if they existed, could be linked to cattle temperament. In this case, it could be possible to include them in future temperament screening procedures for cattle before they enter a feedlot. This would have the effect of improving the individual welfare of unsuitable animals (by directing them to pasture finishing rather than feedlot finishing) and raising the overall welfare level in the feedlot pens.
Having determined on an appropriate model and a suitable definition of stress, the strategy for the remainder of the study was to conduct three preliminary trials which examined certain behavioural aspects of feedlot cattle. These behaviours were then to be incorporated in the major trial of the study, the principal aim of which was to examine the physiological and behavioural responses of cattle under three different environments: pasture, "normal feedlot" and "stressed feedlot", with regard to pre-pathological states. Some aspects of the behaviour of feedlot cattle have been studied previously (e.g., Stricklin and Gonyou 1981; Stricklin 1983) but it appears that no thorough behavioural analyses have been performed. For example, at this stage there is no published ethogram of feedlot cattle from which certain behaviours could be selected.

7.2 Preliminary experimental work

The first trial investigated lying behaviour. At this stage, while there have been several studies on grazing cattle (e.g., Arnold and Dudzinski 1978; Kilgour and Dalton 1984) studies on feedlot cattle are uncommon, consisting of a series of publications by Czako (1975, 1977) which are mostly in Hungarian, elements of a postgraduate thesis (Gonyou 1980) and a study by Hicks et al. (1989) the conclusion of which was that feedlot steers in Oklahoma spend 54% of their time lying. Lying behaviour in feedlot cattle is of particular interest because of studies in other species, which show links between immune function and resting behaviour (e.g., Krueger and Karnovsky 1995; Moldovsky 1995; and Toth 1995). The results of this trial showed that there was a remarkably strong and consistent pattern of diurnal lying behaviour, which was completely unaffected by a range of different backgrounds and sources.

The second trial investigated agonistic and affiliative behaviours. These social behaviours have been widely studied (as reported earlier in the literature review) but not particularly in the feedlot situation except for studies of social dominance by Stricklin (1983) and the work of Fell and Clarke (1993) which recorded the incidence of agonistic and affiliative behaviours in a number of Australian commercial feedlots. They are highly distinctive behaviours in the pasture and feedlot environment, are
easily observed by feedlot staff and could be good candidates as indicators of hierarchical status and cattle temperament, although a possible qualifier is that this may be only in the first few months of feedlot life. Anecdotally, they tend to become reduced over time, perhaps because a hierarchy has been formed and is maintained by threats rather than physical interactions. The results of this trial showed that agonistic and affiliative encounters occurred significantly in the first three weeks of feedlot life, and with regard to agonistic interactions, there is clear structure of winners and losers plus a major emphasis on the head push as an agonistic behaviour. They appeared to be worthwhile to study further as possible correlates to pre-pathological states.

A third trial developed the methodology for determining preferences for grid space occupation and the frequency and durations of nearest neighbour relationships. This was performed as a preliminary step for investigating the hypothesis that feedlot cattle have preferences for certain locations in a feedlot and for being with certain other cattle. Again, there has been very little research on these behaviours (Stricklin 1979; Fraser 1980). The result of this trial showed that there were significant differences (which indicate preferences) in grid square occupation and nearest neighbours, although the interpretation of these two findings was limited by the use of a relatively short observation period.

7.3 Major experimental trial
The principal aim of this trial was the evaluation of the physiological and behavioural responses, with regard to pre-pathological states, of three matched groups of cattle placed under three different environments or treatments. These were pasture, normal feedlot, and stressed feedlot. The stressed feedlot group differed from the normal feedlot group in that there was a high stocking density (6 square metres per head) and the substrate of the pen was kept continually wet and muddy, the idea being to create a significant stressor by affecting lying behaviour (through the wet substrate) and increasing social interaction and competition for resources. These stressors were also designed to be representative of a poorly managed feedlot under prolonged wet conditions. The principle behind this experimental design was to have the pasture
group as a control against which the responses of the feedlot groups could be measured, although this could not be considered in terms of between-group behaviour because obviously behaviours will change so that an animal can accommodate different environments.

The results showed that there was a significant increase in relative adrenal mass and adrenal cortical volume in the feedlot groups compared to the pasture group, and out of 20 immune variables, only two: serum IgA, and the gamma delta T-lymphocyte WC1, had lower levels in the feedlot groups compared to the pasture group. They were the only variables supportive of pre-pathological states occurring in the trial. The differences in measurements of the adrenal measurements between treatments were quite clear with regard to pre-pathological states, but the two immune variables alone are not convincing proof of depressed immune function. Altered levels of serum IgA have been linked to pathological states in other species such as dogs (e.g., Campbell 1991). There is little known about the gamma delta T-lymphocyte WC1 at this stage. WC for example, refers to Workshop Classification (Colditz, pers comm). Morrow Tesch et al. (1996) considered that these lymphocytes may be important in cattle because they are proportionally higher in ruminants than in all other species except pigs and, being mainly found in epithelial tissue, they may function as a primary line of defence at skin surfaces. The results of Morrow-Tesch’s investigations between WC1 lymphocytes and heat stress were inconclusive.

In summary, it was concluded that the two immune variables were not sufficiently representative of the immune system to be able to conclude that it was depressed. Therefore, it could not be concluded that cattle exhibited pre-pathological states in this experiment.

Some previous work in other species has provided similar results. Klein et al. (1992) for instance, found in an experiment on chronic stress in Wistar rats, that there was a prolonged elevation of HPA activity, but no significant changes in a range of immune
functions. In the literature review, other studies were also detailed which gave inconsistent results regarding stress and immunity (e.g. Ursin 1994; Booth 1996).

This study can be seen as a foundation work with regard to the welfare of feedlot cattle. The main experiment was designed so that confounding influences such as exposure to strange pathogens and different effects of backgrounds from unmatched cattle were not introduced (it is likely that the mixing of unfamiliar cattle introduces new and different pathogens). These constraints were necessary to preserve the integrity of the experimental design. However, future work could introduce the following elements to the design. Firstly, the immune system of all animals could be challenged with a mild antigen stimulus (perhaps with a vaccination process) midway through the treatment regimes. A strong antigen challenge is probably not desirable because it could precipitate a rapid transition from normal to pre-pathological to pathological states. However, a mild challenge would have the effect of focussing the immune system on a pathogen challenge and thus identifying animals of lower immune competence. Secondly, perhaps an extra two samplings could be conducted by extending the trial. This would allow for a clearer interpretation of trends as well as increasing the effect of the treatments. Thirdly, 3-4 samplings of all animals at 14-day intervals before the start of the treatments would clearly establish the baselines of the tested immune variables.

The experimental protocol developed for this study is applicable to welfare studies of many domestic animal species. For example an examination of the time course of pre-pathological states could be worthwhile, are they of relatively short duration, or are they long term conditions? It has been shown that some immune variables have altered levels some days before the occurrence of clinical symptoms (Amadori et al. 1997).

7.4 Conclusions

This study has developed a model for the assessment of welfare and identified measurements that may be used to develop a physiological index of welfare for the
intensive beef industry (relative adrenal weight at the time of slaughter, adrenal index, and the immune indices, serum IgA and the gamma delta T-lymphocyte WC1). These physiological measures are scientifically credible that is, there is adequate evidence of their role in welfare biology, and they are reasonably understandable to the wider community. These are two important criteria for an index of welfare.

It was not possible to link the behavioural data to pre-pathological animals and thus investigate behavioural correlates. Nonetheless, this study opened up an important area of behavioural research in feedlot and pasture cattle and has provided a large body of behavioural data which after a complete analysis will facilitate a better understanding of cattle behaviour. This in turn will assist in the management of feedlot cattle.

Major findings from the behavioural analyses were as follows:

1. It was hypothesised that a wet substrate and double stocking density in the stressed feedlot would reduce lying behaviour and thus act as a stressor for the stressed feedlot animals. The time budgets of the three groups (see page 147) showed that this was not the case. An analysis of the circadian pattern of lying (page 148) shows that the stressed groups were compensating for their wet conditions by having a higher lying activity than the other groups during the day, and lower lying activity during the night. Therefore lying behaviour was disrupted but not reduced. These results indicate a very strong motivation for lying. A finding that agrees with the work of Ruckebusch (1972) and Metz (1985) on cattle in pasture conditions.

2. Cattle have preferences for certain areas of a feedlot. These preferences are different for activities of lying, standing, and feeding.

3. Cattle spend far more time on their own than with animals less than one steer's length away from them. This indicates that the use of the length of a steer as a meter for determining nearest neighbour relationships should be revised.
4. The pasture group had the lowest percentage of lying time of the three groups. This was consistent for both trials. The reason for this was related to the higher percentage of feeding time they had compared to the feedlot animals. This also reduced their percentage of standing time.

5. There was a marked difference in the rates of agonistic behaviours between the first and second trials. This demonstrates that different animal backgrounds contribute strongly to these behaviours.

6. With regard to time spent feeding, the pasture animals showed little difference between the two trials (see Figure 6.43, page 147), yet the grass cover was much higher and more dense in the second trial. This indicates that when there is adequate grass cover, cattle eat for as long as when there is low cover. This could be attributed to them being more selective in their feeding when there is abundant grass cover.

This study has addressed some of the ethical issues surrounding human-animal relationships. It has determined on an ethical framework as one basis for research in this field and a model for assessing the welfare of beef cattle in feedlots. It has demonstrated the resilience and adaptiveness of cattle to wet and crowded conditions, yet has also shown that feedlot conditions do have an effect on cattle physiology in terms of stress and that they are making larger responses in coping than pasture cattle. This finding has implications in feedlot management in that if other stressors such as a high pathogen challenge and/or social mixing of unfamiliar cattle are introduced then there is a high potential for not only pre-pathological but pathological states to occur.
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