

## Chapter 1

# General Introduction



## 1.1 Introduction

The measurement of motivation is a methodology used to assess resources that an animal values and, subsequently, to aid in determining its welfare. This method allows researchers to gain insight into the animal's own preferences for resources as animals are asked to 'pay a price' to access a resource (e.g. peck a lever or push through a door). Broom (1998) suggested that if an animal is willing to work hard to obtain a resource then it may be reasonable to conclude that its welfare is improved by accessing that resource. Although this may seem like a valid assumption, the relationship between high levels of motivation and welfare have not been investigated empirically. There are also very few data relating an animal's willingness to work to the degree to which it needs a specific resource (Patterson-Kane et al., 2011), or investigating how an animal estimates the costs and benefits of a resource (Spruijt et al., 2001). The measurement of animal welfare can be somewhat subjective and if we are to have confidence in the suite of measures used to assess welfare, and if motivation is to be one of them, then empirical evidence would be useful to better understand this measure and how it relates to welfare and welfare assessment.

Essentially, motivation is expressed by changes in behaviour (Toates, 1986) and is usually exhibited to allow an animal to access a resource or overcome a challenging environment. In this way, motivation can be viewed as a mechanism enabling behavioural changes and increases the likelihood that an animal can cope with and survive a changing environment (adaptive success) (Toates, 1986; Reeve, 2005). Changing motivation is likely to be an effective strategy if the challenge is short term in nature. If the challenge is longer term, then it is possible that motivation levels will decrease as changes in behaviour are no longer, or have not been, effective at returning an animal to a homeostatic state. At this point, other biological resources (e.g. physiological changes such as use of energy reserves), are likely to be employed (Moberg, 2000). When viewed within this framework, one question which arises is whether changes in motivational differences, and therefore adaptive processes and coping strategies, reflect changes in welfare.

Therefore, this thesis sets out to provide a better understanding of motivation by investigating the factors that influence it in a behavioural demand environment, using the feeding motivation of sheep as the experimental model. At a biological level, this thesis aims to test the regulation of motivation by internal and external factors. At a more practical level,

the need to understand the factors affecting the motivation of sheep to work for food may be of importance, since it could alter the value of motivation in animal welfare assessment.

The following literature review details the current measures used to assess adaptive success and welfare, as well as how the strength of motivation of an animal for a resource is used within this context. It then focuses on different motivational systems (e.g. motivation for social contact, motivation for food), and reviews the regulation of motivation of these systems to emphasise the complexity of underlying motivational mechanisms.

## Chapter 2

# Literature Review



## 2.1 Animal welfare

In 1964, Ruth Harrison's seminal work *'Animal Machines'* was published. It related to the reader the intensive farming conditions under which pigs, hens and veal calves were raised. These conditions had become the norm throughout the UK and much of the rest of the world. The public response to this book was powerful enough to prompt the British Government to direct an investigation into 'factory farming', which was chaired by Professor F.W.R Brambell (van de Weerd and Sandilands, 2008). The subsequent findings became known as *'The Brambell Report'* (1965), and outlined the ethical and biological principles upon which animal husbandry should be based. It found that all farm animals should, at the very least, have the freedom to perform a wide range of natural behaviours (Brambell, 1965). Over time, these ideas have evolved to become the 'Five Freedoms'. These freedoms include various provisions and state that an animal should be able to live free from hunger and thirst; free from discomfort; free from pain, injury and disease; free from fear and distress; and should have enough physical space to perform natural behaviours (Farm Animal Welfare Council, 1993).

Throughout the development of animal welfare science there has been two persistent and interconnected issues: 1) how welfare should be defined; and 2) which measures, or combination of measures, should be used to assess the welfare status of an animal (Mench, 2000). Broom (1986) has defined welfare as an animal's state as it attempts to cope with its environment. Webster (2005) noted that such definitions do not indicate whether the stimuli from the environment are internal, external or a combination of both, nor does it define what is good and bad welfare. Generally, however, welfare is viewed as a scientific judgement based on relatively objective measurements and an animal can be said to have poorer welfare if it is having difficulty coping, or failing to cope, and is experiencing increases in stress (Broom and Johnson, 1993; Moberg, 2000).

Welfare is generally measured on a continuum ranging from very good to very poor and it can be assessed with relative accuracy and will fluctuate during life (Broom, 1988). The amount of 'work' that is being done by an individual to cope with and adapt to environmental changes, and indicators of failure of these coping mechanisms, are used as measures of welfare state (Fraser and Broom, 1997). An issue that complicates the welfare continuum is that of deciding at what level of coping, or lack of coping, the welfare of the

animal becomes compromised. This appears to be rather straightforward at the extremes of the continuum, but becomes more difficult as the welfare of the animal moves away from these extremes. When this occurs, the placement of a threshold that identifies satisfactory from unsatisfactory welfare becomes difficult to define. For example, a ruminant animal that is under severe physiological stress (e.g. injury or disease), and is unable to forage successfully could be found to have reduced welfare on the continuum. It is unlikely that the same classification could be reached for an animal, if the stress was minor and the animal was adapting well and coping with the challenge. However, if the adaptive mechanism was costly or was required for long periods of time, this would also be likely to result in reduced welfare. Further research into understanding how various adaptive processes function and, specifically, how these processes underpin physiological, behavioural or immune function changes, may be useful, particularly, as these parameters are often used to assess welfare.

## **2.2 Current measures used to assess welfare**

Various measures are currently utilised to assess welfare. These include attempting to observe and measure behavioural and physiological changes within an animal, and to ascertain if these changes have any correlates with subjective experiences. Behavioural and physiological changes are appropriate to measure as the four major defence systems that respond to stressors are the behavioural response system, the autonomic nervous system, the neuroendocrinal system and the immune/biological system (Moberg, 2000; MacArthur Clark, 2008). These changes in behaviour and physiology are then used to indicate the welfare status of the individual, as well as provide information on the welfare of other animals in similar circumstances (Kirkden and Pajor, 2006b; 2006a).

Webster (2005) has acknowledged that although these methods have provided a diverse range of overlapping welfare indicators, there is still a certain amount of non-specificity due to some measures being observed in response to both aversive and pleasurable stimuli. Consequently, here, as in other aspects of welfare assessment, there is a strong argument for using a range of measures (i.e. behaviour, physiological, production and immune traits) when assessing welfare, as individually such measures may give conflicting and unreliable results (Terlouw et al., 1997).

Measures that are currently used to assess welfare are, in essence, assessing the level of the adaptive success of the animal to a challenge. This is regardless of whether the

challenge arises from adverse external stimulation or internal causal factors or both (Jensen and Toates, 1997). The following sections discuss measures that may be used to assess the welfare of an animal as it attempts to cope with a challenge. Where possible this will be illustrated using the model of a ruminant animal experiencing food deprivation, although both short and long term challenges to welfare will also be discussed.

### **2.2.1 Behavioural measures**

After an animal has perceived a stressor it may employ a variety of different coping mechanisms. Alterations in behaviour are generally the first adaptive response due to the biologically economical nature of this response (Moberg, 2000). In the example of the food restricted ruminant, which is presumably experiencing hunger, the animal may increase the time spent foraging in an attempt to compensate for the period of food restriction. This may well be at the expense of other activities (Houston and McNamara, 1989). It is also likely that a food restricted ruminant would alter both the rate of food intake and the time spent consuming specific food types (Villalba and Provenza, 1999b). Newman et al. (1994) reported that sheep fasted for 24h had significantly higher intake rates, achieved through increasing both bite mass and bite rate, as well as increased grazing time and alterations in food choices. However, if behavioural options, such as increasing intake rate, were not available and other behavioural responses were restricted or prevented from occurring, then alternative coping methods would need to be employed for the animal to regain and maintain a homeostatic state (Moberg, 2000).

Changes in behaviour can be used to assess both short term (as above) and longer term threats to an animal's welfare. These changes can be categorised as either normal or abnormal (Mench and Mason, 1997), although there can be considerable overlap between the two.

#### ***Normal behaviour***

The expression of normal behaviour is not as simple as it would first seem as most behavioural patterns are relatively variable and can be influenced by a variety of factors including internal stimulation, genetics and environmental cues (Špinka, 2006). Despite this, normal behaviour can be used in a variety of ways to gain information regarding the welfare of the animal. One method of assessing behaviour is the construction of an ethogram, or

catalogue of behaviours in a wild species, then comparing domestic and wild counterparts. This can demonstrate characteristics that should be typical of an animal in a more natural environment and, if these are expressed, may indicate better welfare. However, this can be complicated by a variety of issues such as genetic differences between populations that result in differences in behaviour (Dawkins, 1980), or whether a welfare compromise occurs when an animal has the capacity to perform specific behaviours but chooses not to (Hughes and Duncan, 1981).

Normal behaviours can also be used to indicate a disturbance, or stressor, and are frequently used as an assessment of pain (Dawkins, 2004; Weary et al., 2006). The frequency and intensity of specific behaviours, such as immobility, escape, avoidance and distress calls, can provide valid information about the welfare of an animal (Mench and Mason, 1997). For example, vocalisations are a common behaviour indicating a possible short term threat to welfare. Watts and Stookey (2000) point out that the vocalisations of cattle may allow a subjective commentary of the individual's internal state, and give insight into the welfare status of the animal, but this unlikely to be true for all species.

### ***Abnormal behaviour***

Stereotypies are an area of abnormal behaviour in which a large volume of work has been completed. It is generally believed that stereotypies result from a functional goal-orientated behaviour that is frustrated and then becomes fixed in a specific, repeated behavioural pattern (Nevison et al., 1999). This unvarying and repetitive behaviour (Mason, 1991) would appear to reflect a longer term animal welfare issue, although the association between stereotypies and other measures indicative of reduced welfare is inconsistent across studies. For example, when dairy cows were restrained, Redbo (1992) reported that oral stereotypies increased, while Korhonen et al. (2001) found that increasing the cage size, of a blue-farmed fox, assumedly improving welfare, actually increased the incidence of a locomotor stereotypy. Other authors have found no differences in physiological measures between animals that exhibited stereotypies and those that did not (Pell and McGreevy, 1999). In an attempt to clarify such anomalies, Mason and Latham (2004) reviewed stereotypical behaviour research and concluded that stereotypy was linked with good or neutral welfare as often as it was linked with poor welfare. The authors concluded that this occurred because situations that led to stereotypies tended to be linked with poorer welfare,

while individual stereotypy performance was often linked with improved welfare (Mason and Latham, 2004). In this way, individual stereotypies may actually aid in increasing the ability of an animal to cope with a challenging environment (Mason and Latham, 2004).

A variety of other abnormal behaviours can also be used to indicate compromised welfare, including misdirected behaviours. These are behaviours that begin as part of a normal repertoire and, for a variety of reasons, become misdirected. They can be focused on the animal itself and include self-mutilation and excessive grooming, focused on the environment surrounding the animal such as pica, hyperphagia and polydipsia, or focused on another animal and may include belly-nosing in pigs, inter-sucking in calves and feather pecking in poultry (Fraser and Broom, 1997).

The absence of specific behaviours during times of suffering or distress, or a failure of normal function, can also be used as indicators of reduced welfare. The loss of reproductive behaviours is one such example and there have been many investigations validating the theory that sexual behaviours, and the associated physiological mechanisms required for reproduction, are inhibited when an animal is in a state of reduced welfare and is failing to cope with environmental challenges (e.g. Rivier and Rivest, 1991; Ferin, 1999; Li et al., 2004; Boonstra, 2005; Fabre-Nys and Gelez, 2007).

Anomalous reactivity is another abnormal behaviour and may involve abnormally high or low levels of activity. It can occasionally be the result of a neurological issue, but is more frequently created by poor rearing or environments. Such behaviours may include prolonged inactivity, tonic immobility, unresponsiveness and hysteria (Fraser and Broom, 1997). A reduction or increase in these specific behaviours can be used, usually in conjunction with other welfare indicators, to determine the effectiveness of various management changes on welfare. Examples of this include assessing the use of environmental enrichment in pigs (Bolhuis et al., 2006; Docking et al., 2008) and the motivation of broilers to undertake locomotor movement (Bokkers et al., 2007).

### ***Using motivation behaviour to assess welfare***

Behavioural measures have often been used to gain a better understanding of how animals perceive specific situations. The interest in this area has been driven both by scientific research and societal and market drivers (Harper and Henson, 2001). In principle, motivation and preference testing allows some insight into what experiences and resources the animal values, rather than what researchers assume it might value. In an environment where various stimuli are present, an animal can indicate which objects or events it is highly motivated to obtain or avoid. This approach to assessing welfare assumes that an animal will work harder to achieve circumstances that meet its needs or wants, or to avoid those that cause stress, fear or pain (Clubb and Mason, 2002). Early research in this field proposed that it may be possible to ascertain if a chicken was distressed by being housed in a battery cage by training the animal to perform an operant task which allowed it into a second, more enhanced, type of cage. This effectively allowed the chickens to indicate their preference or “*vote with their feet*” (Dawkins, 1976, p.248). (These methodologies will be discussed in more detail in a later section).

#### ***2.2.2 Physiological measures***

When an animal is challenged by a stressor, the first physiological systems to respond are the sympatho-adrenal (SA) system and the hypothalamic-pituitary-adrenal (HPA) axis (Terlouw et al., 1997). The stimulation of the SA system readies the organism for ‘fight or flight’ (Cannon, 1932) and is aimed at immediately returning the individual to homeostasis (Englemann et al., 2004). Fully activated, the SA system can increase alertness, increase energy utilisation by cells, alter circulation, increase heart and respiratory rates and decrease the more non-essential systems, such as urine production and digestion (Martini, 2006).

Simultaneously, a longer term phase is activated involving the HPA axis (Herman et al., 2003). Activation of this system is indicated by the level of glucocorticoids (GCs) present in the body (Gordon, 2004). However, the HPA axis exerts its effects at a much slower rate than the SA system (Sapolsky et al., 2000). One main function of GCs is to stimulate hepatic gluconeogenesis, the generation of glucose in the liver from substrates such as amino acids and fatty acids (Purves et al., 2004). Glucocorticoids and their metabolites are present in various biological samples including plasma, saliva, urine and faeces (Gordon, 2004), and the levels of these can be assessed and used to reflect changes in welfare. This is one of the most

commonly used methods of assessing stress responses in a large variety of species, including baboons (Sapolsky, 1985), elephants (Ganswindt et al., 2005), bison (Mooring et al., 2006) rabbits (Cabezas et al., 2007), deer (Millspaugh and Washburn, 2003), dairy cows (Weiss et al., 2004) and mice (Touma et al., 2004). However, the use of GCs to indicate welfare status is not without issue. Increases in GCs have been reported in animals that are sexually aroused, playing or anticipating food, making the interpretation of the results difficult (Mason and Mendl, 1993; Dawkins, 2003). Furthermore, GCs are released episodically and fluctuate with an animal's circadian rhythm, genetics, external temperature, humidity and feed intake (Mormède et al., 2007).

Activation of the HPA system can also impact on immune function. There is a multilevel feedback inhibition of the HPA system that is critical for regulating the secretory pattern of the hormones involved (Handa et al., 1994), and GC levels in the blood act as a negative feedback signal to the hypothalamus (Purves et al., 2004). If the negative feedback loop is repeatedly activated, there is a loss of GC sensitive neurones in the brain (Gregory, 2004), which results in a decreased ability to halt GC production and can lead to suppression of various biological systems, particularly the immune system. Measures of immune function compromise have been well documented in the literature, as have links between GCs and a downregulation of the immune system (Minton and Blecha, 1990; Mori et al., 2001; Napolitano et al., 2003). This interaction allows parameters of immune function to be measured to identify levels of stress, particularly in response to environmental changes or husbandry procedures. Studies measuring red blood cell, leukocyte, neutrophil and lymphocyte percentages in animals have reported changes in levels of these parameters, reflecting possible welfare compromise (Gates et al., 1990; Minton and Blecha, 1990; Fell et al., 1991; Fell et al., 1999; Ernst et al., 2006).

There are a variety of metabolic and endocrine responses that are used as indicators of biological function in a food restricted animal, and changes in these responses are viewed as reflecting changes in welfare status. These responses are aimed, primarily, at maintaining a consistent internal environment, or homeostatic balance, and this often occurs through the mobilisation of energy reserves, particularly with longer term food restrictions. In ruminants, as in monogastrics, adipose tissue is the main storage site of body reserves so that when food supplies become restricted, body lipids are mobilised from adipocytes and used as energy to

maintain body function. The level of body fat mobilisation is dependent on the severity and duration of the food restriction (Chilliard, 2000), and the proportion of fat mobilisation can be estimated via body condition scoring and through ultrasound measurements of subcutaneous fat depth (Chilliard, 2000; Blache and Bickell, 2011). It is also possible to measure the metabolites, hormones and other products involved in the utilisation of body reserves such as free fatty acids (FFA's), which are released during the lypolysis of adipocytes, as well as ketone bodies, insulin and leptin (hormone produced in adipose tissue) (Bowden, 1971; Caldeira, 2007; Delavaud, 2007; Shimizu et al., 2007). These measures are then used to assess welfare and may, arguably, be more useful than measures of fat mobilisation as they can allow identification of welfare compromise and intervention to occur at an earlier stage (Kida, 2002).

### **2.2.3 Biological function and production measures**

Assessments of biological function can be undertaken using a variety of different methods with the main advantage being that most are easily quantifiable, and can be measured by changes in various production parameters. Alterations in biological function and associated production parameters, caused by either internal or external stressors, provide variables that can be used as indicators of reduced welfare. Measures of biological function in relation to extreme long term food deficits in ruminants include severe wasting (low body condition score, catabolism of body tissues, changes in body composition including decreases in fat, muscle and organ size), morbidity and death (e.g. Bush, 2006). There may also be a variety of production parameters that are affected by such alterations in function, although this is likely to be dependent on the species (e.g. decreased milk production, decreased muscle mass). Another measure of a biological disturbance is the assessment of reproductive success, which can easily be measured as the number of live young produced. External factors such as photoperiod and social sexual cues can impact on the reproductive success of a sheep (Blache and Bickell, 2011); however, nutrition also plays an important role. In Merino sheep, the plane of nutrition can impact the sperm production of the male and ovulation rate of the female as well as affect embryo and offspring survival (Robinson et al., 2006).

#### **2.2.4 Measures of disease and injury**

The level of disease, or susceptibility to disease, is another important aspect of the welfare of an animal. Although the connection between health and welfare would appear obvious, in that an animal that has a disease has compromised welfare when compared to one in good health, discovering relationships between the two can involve monitoring a broad range of indicators (Clubb and Mason, 2002). This can include correlating subjective feelings, such as discomfort, pain or distress, with disease or injury (Mellor et al., 2000). However, the most useful indicators for the assessment of pain and discomfort involve behavioural and endocrine measures (Flecknell and Roughan, 2004). Changes in behaviours, such as increased or decreased vocalisations, posture changes and alterations in locomotion (Mellor et al., 2000) are useful indicators. Clinical signs can also be used as indicators of possible reduced welfare. These include elevated body temperature, tissue damage, the presence of sepsis and infection, and the healing rates of wounds (Roger, 2008). Endocrine measures such as the levels of GCs are also useful as they represent the graded levels of acute stress, and can indicate whether the stress is elicited by pain or not (Mellor et al., 2000). However, the latency of the HPA response to an aversive stimulus suggests that acute stressors are better reflected in SA response measures (Lefcourt and Elsasser, 1995). Molony et al. (2002) attempted to validate the use of behavioural and endocrine changes in relation to the pain associated with tail docking and lamb castration. They compared these changes to the amount of tissue damage suffered and to tissue sensitivity and found that in over 80% of cases, the two approaches graded the pain to the same level (Molony et al., 2002).

#### **2.2.5 Affective state measures**

Recently, research into animal welfare has evolved to include the mental state, and emotions or affective state, of animals and there has been considerable effort spent developing measures that attempt to assess an animal's subjective experience or perception of a situation (Duncan, 2006; Matthews, 2008). The importance of focusing on the subjective experiences of animals was stated unequivocally by Dawkins (1990; p.1) when she wrote "*Let us not mince words. Animal welfare involves the subjective feelings of animals*".

The interest in this area has been driven by scientific research and by societal and consumer demand as consumers have been found to perceive food quality not only in terms of the overall nature of the end-product, but also in terms of the physical and mental welfare

of the production animal (Harper and Henson, 2001; Blokhuis, 2008; Oogjes, 2008). Consequently, more effort has been invested exploring the theory that the animal's perception of a situation is equally as important as its physical health. Put simply, this revised theory of animal welfare states that subjective experiences are key determinants for assessing the welfare of animals (Broom, 1991; Dawkins, 2008).

There have been several methodologies developed to assess the mental state of an animal and these include assessing the emotional or affective state. This generally refers to the changes in emotions (in conscious beings) that vary in terms of intensity and valence (Paul et al., 2005). Methods of measuring affective state include cognitive bias, attention bias and memory bias testing (Bush et al., 2006; Mendl et al., 2009).

There has also been much interest in the development of measuring positive welfare in animals as it has been argued that an animal's well-being is not simply related to the absence of negative welfare but also, and possibly predominately, to the presence of positive emotions (Fraser, 1995; Boissy et al., 2007). The premise behind this area of research is that if pleasure adds to the life of a human, why should it not be important for animals (Duncan, 2006)? Consequently, a variety of investigations have been conducted attempting to provide animals with positive experiences. Vinke et al. (2004) reported that American mink (*Mustela vison*) show positive anticipatory behaviour in response to food rewards, such as a general increase in locomotor activity and more time 'nose-poking' around the feeding area. Piglets have also shown increases in play behaviours (such as hopping, scampering, pawing and running) to an 'announced' enrichment (Dudink et al., 2006). Investigators have also researched the links between long term positive emotional states (moods) and animal temperament as it has been proposed that a predisposition to a positive emotional state is a suitable framework for further investigation into long-term positive emotions (e.g. Voisinet et al., 1997; Fell et al., 1999).

Unfortunately, how an animal perceives an experience cannot be directly assessed so indirect measures have been developed to allow further insight into an animal's perspective. The majority of these measures are operant tests and are based on the premise that animals can indicate, through their behaviour, experiences that they find either positively or negatively reinforcing (what they want or do not want) (Dawkins, 2008). This is particularly

useful when designing housing conditions and management strategies, with the premise being that an animal will work harder to access the resources or experiences that it wants or likes. However, there is not as yet, a direct empirical link between what an animal does or does not find reinforcing and its feelings or emotions, such as pleasure or suffering (Verbeek, 2010).

It should be noted that for the purposes of this thesis a distinct difference is drawn between assessing the value of experiences and resources from an animal's perspective (what it wants), and assessing an animal's affective or emotional state (how it feels). As mentioned, the development of affective state measurement is a growing area in the assessment of welfare, but a more in-depth discussion is outside the scope of this study (see reviews by Panksepp, 2005; Mendl et al., 2009).

When adaptations made by an animal are in response to a challenge, and the measures of the adaptation to such a challenge are used as welfare indicators, one underlying question which remains unanswered is; how do changes in the adaptive process reflect changes in the welfare of the animal? One possible way of better understanding the link between an individual's responses to challenges and its welfare is by understanding the regulatory mechanisms that influence the responses. Gaining a greater understanding of these mechanisms, with regard to motivation, is the main aim of this thesis and, consequently, the focus of the following sections.

### **2.3 Motivation and preference**

There are several differences between motivation and preference testing. A preference test ranks the decisions that an animal makes when it is given a choice between resources or stimuli. A motivation test quantifies the strength of motivation for a specific resource or experience, but does not necessarily allow an animal to make choices between different resources (Fraser and Matthews, 1997). A motivation test generally requires that an animal pay a cost to gain access to a resource or stimuli through an operant task (e.g. pressing a button or pushing through a weighted door). Kirkden and Pajor (2006a) have identified four distinct questions that preference and motivation tests are designed to answer:

- 1) whether the animal has a preference amongst alternative resources;
- 2) whether its preference, or strength of preference, is changed with variations in its environment;

- 3) whether an animal is motivated to obtain or avoid a resource and;
- 4) how strong the animal's motivation or preference is.

### **2.3.1 Preference testing**

Early preference testing involved attempting to solve relatively simple questions such as the type of flooring a hen preferred (Hughes and Black, 1973). Since then the method has been applied to study a large variety of environmental preferences including light intensity levels in turkeys and layer hens (Sherwin, 1998; Taylor et al., 2006; Zupan et al., 2007), dust bathing, bedding, foraging substrates (Fraser, 1985; Manninen et al., 2002; Jensen and Pedersen, 2006; de Jong et al., 2007) and other housing modifications such as floor heating (Pedersen et al., 2007).

Despite its widespread use, preference testing has been the subject of some controversy. Fraser and Matthews (1997) were critical of this type of testing and noted that three major issues needed to be considered. The first was ensuring that the question being asked had enough complexity to give valid results i.e. the choices given are not overly-simplistic and allowed for environmental factors that may alter a choice. The second issue involved enabling the most important features of an experiment to be replicated. This was to ensure that the results found were not overly influenced by specific experimental equipment, or the particular behaviour in which a preference was expressed. This was exemplified in the studies by Baxter (1991) and Phillips et al. (1992) who examined whether sows had a preference for a wide or a narrow farrowing stall. Baxter (1991) concluded that sows preferred the narrow stalls, while Phillips et al. (1992) determined the opposite. On closer inspection of the results, it became clear that the layout of the partitions and stalls were very different, indicating that more variables were influencing the results than stall width. The third issue involved taking into account prior experience on the effects of preferences. Shields et al. (2004) found that broilers had a preference for sand as a dust-bathing substrate (over wood shavings, rice hulls and paper), which appeared in the first week of life and did not alter over time. However, Nicol et al. (2001) found evidence that in laying hens, behaviours were generally flexible and influenced by the substrate the animals were housed on at the time of testing. In another example, Phillips et al. (1991) found that young sows showed a preference for partially enclosed farrowing crates when compared with open crates, but this preference largely disappeared with repeated exposure to the open crates.

### **2.3.2 Motivation testing**

#### ***Defining motivation***

Motivation has been defined in many different ways but the general consensus appears to be that motivation refers to a transitory state in which an individual is willing to alter its behaviour, often to work to access a reward or to escape from a punishment (Rolls, 2005). It is also referred to as a hypothesised internal event, which may not be directly observable, but is linked to an observable external event (Geen, 1995; Ferguson, 2000). For the purposes of this thesis motivation is defined as a process integrating a number of homeostatic mechanisms influencing behavioural change, for the purposes of increasing adaptive success and ultimately, survival (Toates, 1986; Ferguson, 2000; Reeve, 2005).

As noted by Reeve (2005) when motivation changes, behaviour also changes, therefore, the study of motivation involves investigating the processes that underlie the expression of a specific behaviour. Variations in the actions created by motivation can be caused by differing circumstances (momentary and situational differences), as well as inter-individual and inter-species differences (Ferguson, 2000), with memory and learning also playing a role. These factors have an intertwined relationship where motivation affects memory and learning, and memory and learning influence motivation (Karniol and Ross, 1996). Consequently, an animal that has already associated a particular behaviour with a reward may experience anticipation, or increased incentive or motivation, preceding the action that leads to the reward. All of the above factors indicate that motivation can only be assessed indirectly and with that, there will always be a degree of error associated with the interpretation.

#### ***The development of motivation testing***

The strength of the motivation in a motivation test can, theoretically, provide quantitative information regarding how important the experience or resource is to the animal (Rushen, 1990; Fraser and Matthews, 1997; Matthews, 2008). This type of testing has become increasingly popular and has been utilised for a wide range of situations and species from assessing the motivation of battery-caged hens for scratching and dust bathing substrates (Dawkins, 1983), to analysing the demand for food and social partners in cichlid fish (Galhardo et al., 2011). Dawkins (1983; 1990) was instrumental in the further refinement of this methodology for use in welfare science through the application of behavioural demand

theory. This is an economic concept derived from microeconomics and consumer demand theory (Lea, 1978), and applied to animal behaviour. This methodology requires that an animal ‘work’, or perform a specific operant activity, for access to a resource or experience. The ‘cost’ of accessing that resource is increased (e.g. an animal is asked to walk further and further for the same sized food reward), and the motivation to access the resource under increasing costs provides a quantifiable measure of the importance of that resource to the animal. The type of data that a behavioural demand experiment produces is often analysed using demand curve analysis where the demand curve relates the consumption of a resource to its price (Lea, 1978; Hursh et al., 1988). A typical demand curve for a food resource shows that consumption will decrease with increasing cost, assuming that food is only available by working for it.

### **2.3.3 Limitations of motivation testing**

As with the majority of welfare methodologies and measures, motivation testing and demand curve analysis has both critics and supporters who have discussed issues with methodology, validity and interpretation. A series of commentary articles (Mason et al., 1998a; 1998b; Matthews, 1998; Sherwin and Nicol, 1998) debated issues such as which measure, taken from the behavioural demand curve, most accurately assessed the strength of the motivation, whether animals should be able to control their own reward length and whether animals should be tested in open or closed economies. (A closed economy refers to a testing environment where an animal is placed in a facility for a longer period of time and only has access to the resource during the test. In comparison, in an open economy an animal is placed in a test facility for a shorter period of time with access to the same resource outside of the test environment.)

Another issue that has been raised is the difficulty of testing, usually social animals, in an isolated, artificial situation as this may impact the outcome (Jensen et al., 2004). Sherwin (2003) reported that mice allowed full social and physical contact with cage mates showed higher work rates than mice with restricted contact, although this was dependent on the particular resource they were working for. However, one issue that has not been thoroughly investigated is the validity of the link between the outcomes of a motivation test and the welfare of an animal. The relationship between these measurements and welfare is not well defined, with conclusions normally being based on the assumption that an animal’s welfare is

reduced if a ‘valued’ resource is not provided. According to Dawkins (1988), if an animal is highly motivated to obtain a resource or perform a behaviour and is unable to do so, it will suffer both physically and mentally, and have reduced welfare. Broom (1988) proposed a similar theory and stated that if an animal will work hard to obtain a resource, then it may be reasonable to conclude that its welfare would be improved by achieving that objective. However, this assumption is still a theoretical hypothesis rather than one which is based on empirical evidence.

#### **2.3.4 Motivation and adaption**

An animal’s willingness to pay a cost to access a resource suggests a link between an animal’s actions and its subjective feelings (Dawkins, 1990). However, when considering motivation further, it becomes more apparent that motivation may not be a measure of welfare status in itself; rather that it may be a coping strategy enabling an animal to adapt to an ever-changing environment. As noted by Reeve (2005), motivation benefits adaption and when motivation decreases, adaptive ability suffers. Rushen (1996) has suggested that a specific behaviour expressed by an animal may not be reflective of the individual’s emotional state, but rather is a method for solving a particular problem, i.e. an adaptive response. Koolhass et al. (1997) also noted that animals in their wild state have had to adapt to a dynamic and complex natural environment and to do so have developed a wide variety of defence mechanisms. As the observable expression of motivation is changing behaviour, it may be possible to conclude that motivation is one of the many defence mechanisms available to an animal. Within this framework it may be that decreases in motivation, rather than high levels of motivation, could indicate reduced welfare.

Verbeek et al. (2011) investigated the effects of food restriction and the perception of hunger in sheep and found that animals with a low body condition score had increased feeding motivation and appeared to ‘want’ food. However, as the authors noted, this alone did not provide an insight into the affective state of the animal, rather it indicated that it was capable of adapting to its situation and willing to work for food. It is possible that measures of affective state, combined with motivation testing could perhaps be a better indicator of the true mental well-being of an animal (Verbeek, 2010). However, before this type of testing is undertaken it may be necessary to better understand the factors influencing motivation.

Currently, the understanding of what motivates an animal to perform a specific behaviour and why it increases and then decreases or stops the behaviour, is missing from the literature. Without this, it is difficult to determine how the animal's effort, during testing, relates to the degree to which it needs the resource (Patterson-Kane et al., 2011). As noted by Spruijt et al. (2001), the ethologically orientated models that have investigated motivation from a welfare perspective (e.g. Jensen and Toates, 1993) have paid little attention to questions such as "How does an animal estimate the cost-benefit ratio of a specific behaviour prior to or during the activation of a motivational system?" and "How does an animal take its actual state into account enabling it to maximize the profit of ongoing behaviour?" Furthermore, the underlying neural mechanisms involved in integrating variables enabling animals to assess the value of resources are unclear (Minamimoto et al., 2012). It is also possible that the mechanisms that alter motivation vary with different resources and there are likely to be both similarities and differences when mechanisms that regulate motivation are compared between resources. For instance, energy balance and other metabolic and hormonal indicators are likely to be signal-specific to the motivation to feed, while a variety of resources may include the opioid reward system as a signal or regulator. When these factors, are more thoroughly understood, it may be possible to assess the impact that having or not having a resource has on the affective state and the welfare of the animal and how the factors influencing motivation can be manipulated. For example, if the motivation for food is determined by a combination of energetics and reward systems (e.g. changes in opioid levels) then these may be able to be manipulated and the impact on welfare measured through alternative assessments e.g. affective state analysis.

### ***2.3.5 Understanding motivational mechanisms for different resources***

A review of the literature indicates that most of the work defining and investigating motivation has occurred within the field of psychology with particular emphasis on ingestive behaviours (e.g. hunger and thirst). However, other work has also included non-ingestive motivational systems such as temperature control, social interactions and the self-administration of drugs (Toates, 1986; Rolls, 1999). Research into understanding motivational mechanisms includes mapping which brain structures are associated with specific motivational states, how these structures become activated, and what internal, environmental or daily events trigger the activation process (Reeve, 2005). The following section briefly discusses the mechanisms known to affect an ingestive and non-ingestive

motivational state to provide an overview of regulatory mechanisms influencing various behaviours, and to indicate the complexity and variation within such systems.

### ***Motivation for social contact***

Considerable research has been conducted on the effect of social contact and the importance of socialisation and social learning in non-human animals, particularly with respect to how the need for social contact alters other behaviours (Ferguson et al., 2002; Keller et al., 2011). Grazing livestock, such as sheep, are typically gregarious and motivated to remain in social groups, including when grazing. Group foraging enables animals to reduce the risks from predators (Molvar and Bowyer, 1994), and to learn about forage location and quality from other animals (Roguet et al., 1998). When placed in a situation where conflict between preferred grazing sites was tested against social contact it was found that an individual sheep would move to a preferred location provided its social group was near, but would not leave its group to reach a preferred site unless accompanied by several peers (Dumont and Boissy, 2000). Social interactions are activated through a complex system of both external and internal cues and the motivation for social contact is usually developed through early social experiences (Howery et al., 1998; Reeve, 2005), and maintained through visual, auditory, olfactory and tactile signals (Lynch et al., 1992).

The mechanics underlying motivational states are generally integrated interactions between the environment, environmental events, biochemical messengers and brain structures. The mechanics underlying changes in social behaviours are particularly complex due to the large variation in social behaviours expressed by human and non-human animals. There is evidence of a common social motivation ‘circuitry’ in mammals, and this is consistent throughout development (Nelson and Panksepp, 1998; Young, 2002). For example, the neuropeptides, oxytocin and vasopressin have been found to influence different forms of social interactions and behaviours including aggression (Ferguson et al., 2002; Ross and Young, 2009), while up- or down-regulation of vasopressin receptors have been found to impact on behaviours such as social dominance and defence, as well as social memory, social preference and social anxiety (Stevenson and Caldwell, 2012). Oxytocin and vasopressin, as well as catecholamines (norepinephrine), are also implicated in affiliative behaviours such as mother-infant bonding, play, sexual behaviours and the maintenance of group cohesiveness (Nelson and Panksepp, 1998). As most social behaviours have a reward component, it is

perhaps not surprising that endogenous opioid systems contribute to social attachments and regulate social responsivity (Nocjar and Panksepp, 2007).

Various studies have shown that opioids attenuate the effect of social separation (measured by isolation-induced vocalisations), and this was first reported in dogs (Panksepp et al., 1978) and domestic chicks (Panksepp et al., 1980). Early studies also showed that the levels of general social motivation and cohesion in rats decrease when animals are administered opioid receptor agonists (Panksepp et al., 1979). In contrast, opioid receptor antagonists (naloxone and naltrexone which inhibit endogenous opioid activity) can block the effect of opioids on social distress (Panksepp et al., 1980). Opioids are also released through social stimuli with play behaviour in rat pups resulting in a release of endogenous opioids, while antagonists can reduce the level of play (Niesink and Van Ree, 1989). Additionally, opioids have rewarding effects on social behaviour with Belluzzi and Stein (1977) theorising that a primary function of an endogenous opioid may be to signal a reward and thereby maintain specific advantageous types of social interactions. In this role, opioids reinforce learning (Kehoe and Blass, 1986; Nelson and Panksepp, 1998).

Although reproductive behaviour is a form of social interaction, it differs in both purpose and underlying mechanisms. During the expression of reproductive behaviours, vigilance is usually reduced increasing vulnerability to predators. Reproductive behaviours are timed to occur during the fertile phase of the females reproductive cycle and have therefore evolved to be under the control of reproductive hormones such as testosterone, oestradiol and progesterone (Bancroft, 1995; Blache and Bickell, 2011).

The above information indicates that the mechanisms that alter motivation for social interactions are complex and have a variety of regulatory systems. In the example of mother-infant bonding, factors such as thermoregulation, hunger, place-attachment mechanisms, visual, auditory and olfactory cues and underlying neural substrates and neurochemical systems all affect whether the animal exhibits social approach behaviours or separation and distress responses.

### **Motivation for drinking**

As with most motivations thirst, or the drive to seek fluid and drink it, is altered by a combination of external and internal factors. The intake of water is essential for survival and consequently, there are several integrated hormonal and neural mechanisms that have evolved to ensure that the state of thirst can be experienced, motivating animals to drink fluid when necessary (McKinley et al., 2004; Kyriazakis and Tolcamp, 2011). In humans, fluid intake has many causes including habit, customs, social interactions or rituals, disease, as an accompaniment to the ingestion of food, and physiological signals such as a dry mouth (McKinley et al., 2004). In ruminants, research on thirst has generally concentrated on the impacts of various, possibly detrimental, situations on an individual's fluid intake. For example, Jacob et al. (2006) and Jongman et al. (2008) investigated the effect of lairage on drinking behaviour, while Hogan et al. (2007) and Fisher et al. (2009) reviewed the effect of transport-associated water deprivation.

The mechanisms underlying the state of thirst have been found to be similar across species (cattle, sheep, rabbits, rats, mice) and the biological factor that exerts the most influence on the subjective experience of thirst is plasma osmolality (Denton et al., 1999). Alterations in serum osmolality can be induced by both intracellular and extracellular dehydration (Blair-West et al., 1994), and this has been found to stimulate fluid intake in all vertebrate species that show drinking behaviour (Fitzsimons, 1979; Fitzsimons, 1998). Increases in plasma osmolality of 1-2% have been found to increase the fluid intake of mammals; however, decreases in blood volume or blood pressure of 10% are also required (Denton et al., 1999). A reduction in the volume of extracellular fluid also initiates the motivation to drink but this is of lesser importance in the drinking motivation hierarchy (Hogan et al., 2007). Research has indicated that the motivational state of thirst is induced by signals from the hypothalamus and associated brain structures (Sewards and Sewards, 2000), and mediated by angiotensin II (a dipsogenic neuropeptide) through the renin-angiotensin-aldosterone axis (Hogan et al., 2007). This has been validated by many experiments such as that by Mungarndee et al. (2002) who found that hypothalamic neurons in sheep respond to the sight of water following intra-cerebroventricular (ICV) injection of hypertonic saline or angiotensin II. Stricker and Sved (2000) theorised that thirst is monitored by four excitatory signals and three inhibitory signals. The excitatory signals are increased plasma osmolality, decreased blood volume, increased gastric sodium load and increased circulating levels of

angiotensin II. The authors postulated that the three inhibitory signals are decreased osmolality detected by cerebral osmoreceptors, increased arterial blood pressure and increased gastric water load (Stricker and Sved, 2000).

The central opioid system has also been found to have stimulatory and inhibitory effects on the intake of water and sodium, although the effects are dependent on the dose of the opioid receptor agents administered, as well as the experimental methodologies used (Fitzsimons, 1998; De Castro e Silva et al., 2006). For example, Bodnar et al. (1995) administered naltrexone peripherally to rats and found a reduction in water intake of approximately 57% following 24h of prior water deprivation. The authors suggested that the mechanism through which opioids modulate water and saline intake may not work through a reward or palatability mechanism, but rather through the above mentioned fluid homeostasis mechanisms (Bodnar et al., 1995).

As with many investigations into a motivational state, operant tasks have been used to quantitatively measure the motivation of an animal for water, usually under conditions involving water restriction. Minamimoto et al. (2012) used this methodology to identify the internal variables that altered the motivation for drinking in rhesus monkeys subjected to a 22h water restriction. The authors reported that the osmolality level, used as a physiological indicator of the need for water, decreased as the total number of water rewards earned increased with a significant correlation found between the level of motivation and the osmolality level. They concluded that the motivational value of the water reward may be calculated with reference to the hydration level of the animal (Minamimoto et al., 2012). For further details on thirst see Fitzsimons (1998) and Sticker and Sved (2000).

The above section indicates that motivation for different resources is altered by a variety of mechanisms, although there are some similarities between systems. This suggests that it is important to understand the mechanisms influencing motivation before attempting to elucidate links between motivation and adaption, animal welfare and welfare assessment. The importance of resources that elicit low to intermediate levels of motivation can be somewhat ambiguous in meaning (Patterson-Kane et al., 2011), so when attempting to gain an understanding of the factors that influence motivation in a demand test, it was decided that using a resource with a known high value, such as food, would be a suitable starting point.

## 2.4 Main factors influencing feeding motivation

Food is clearly a biological need and a variety of physiological, hormonal and neural pathways are involved in regulating consumption in a complex and integrated manner so that intake meets an animal's nutritional and energetic requirements. Single-factor theories have been proposed to account for levels of energy intake or storage (e.g. stomach distension, body fat stores and blood glucose concentration), but these have not fully explained how food intake and feeding motivation are controlled under all circumstances (Forbes, 2007). It seems likely that a complex system involving taste, palatability and orosensory signals, as well as post-ingestional feedback from chemo-, mechano- and osmoreceptors, and various hormones and metabolites is integrated in the central nervous system (CNS). It is probable that feedback from hedonic reward systems and information previously learnt about the consequences of feeding also play a role.

It should be noted that the following is not an in-depth review of the individual regulators of feed intake (e.g. ghrelin, leptin, glucose, the role of adipose tissue and fatty acids, taste and palatability). Forbes (2007) provides a general review of voluntary food intake and Forbes (1996; 2003) and Roche et al. (2008) review the regulation of food intake in ruminants. Rather, the aim of the following section is to present an overview of the main factors that may influence feeding motivation. This includes the factors that are likely to be important for an animal when it assesses the value of a food reward in a behavioural demand test environment.

A theoretical model (Figure 2.1) is proposed to explain the role of the underlying determinants of feeding motivation and the integration of other regulatory systems, as well as to identify areas of research relevant to this thesis. While individual determinants have been proposed as the factors through which motivation for food is altered, it is unlikely that they act as individual systems. It is more probable that there is an integration and overlap of factors that influence the motivation of an animal and these changes in motivational states are measurable through behavioural alterations.

### 2.4.1 Energy balance

For the purposes of this thesis energy balance is defined as the difference between the energy consumed and energy expended within a specific period of time. Due to the design of

the experiments in this thesis, no accounting of energy reserves or prior energy intake has been accounted for. The impact of energy balance (as defined above) on feeding motivation has been investigated to some degree. Dumont et al. (1998) investigated how ewes made choices between varying amounts of a good quality hay (2, 4, 8, 16 or 32g) that was available at differing distances (either 23 or 46m from the start position), with poorer quality hay that was freely available. The authors found that the quantity of reward affected the ewes motivation levels, with increases in the intake of poorer quality hay occurring as the amount of high quality hay was decreased. This follows previously identified patterns of food intake which indicate that selection is determined by the quality and taste of food, as well as the costs involved in accessing food (Pyke, 1984; Ginane et al., 2002). The costs alter the intake of food according to optimal foraging theory which predicts that animals will make food choices to maximize the net energy gain per unit of feeding time (MacArthur and Pianka, 1966; Pyke, 1984; Stephens and Krebs, 1986). Similar patterns of foraging, following an energy gain maximisation hypothesis, have been found in cattle when the strength of motivation for food was assessed (Ginane et al., 2002). This pattern was also reported by Schütz et al. (2006) who investigated the impact of feed deprivation on the willingness of dairy cows to walk for access to a small amount of silage. They found that both lactating and non-lactating dairy cows did not walk distances further than 100m. The authors had calculated that at this distance the energy expended in walking and maintenance would be equal to that of the energy in the food reward (Schütz et al., 2006). This contrasts with recent work done by Verbeek et al. (2011) who reported that ewes continued to work for access to a small (approximately 5g) food reward, although this meant they spent more energy on walking and maintenance than was gained from the food.

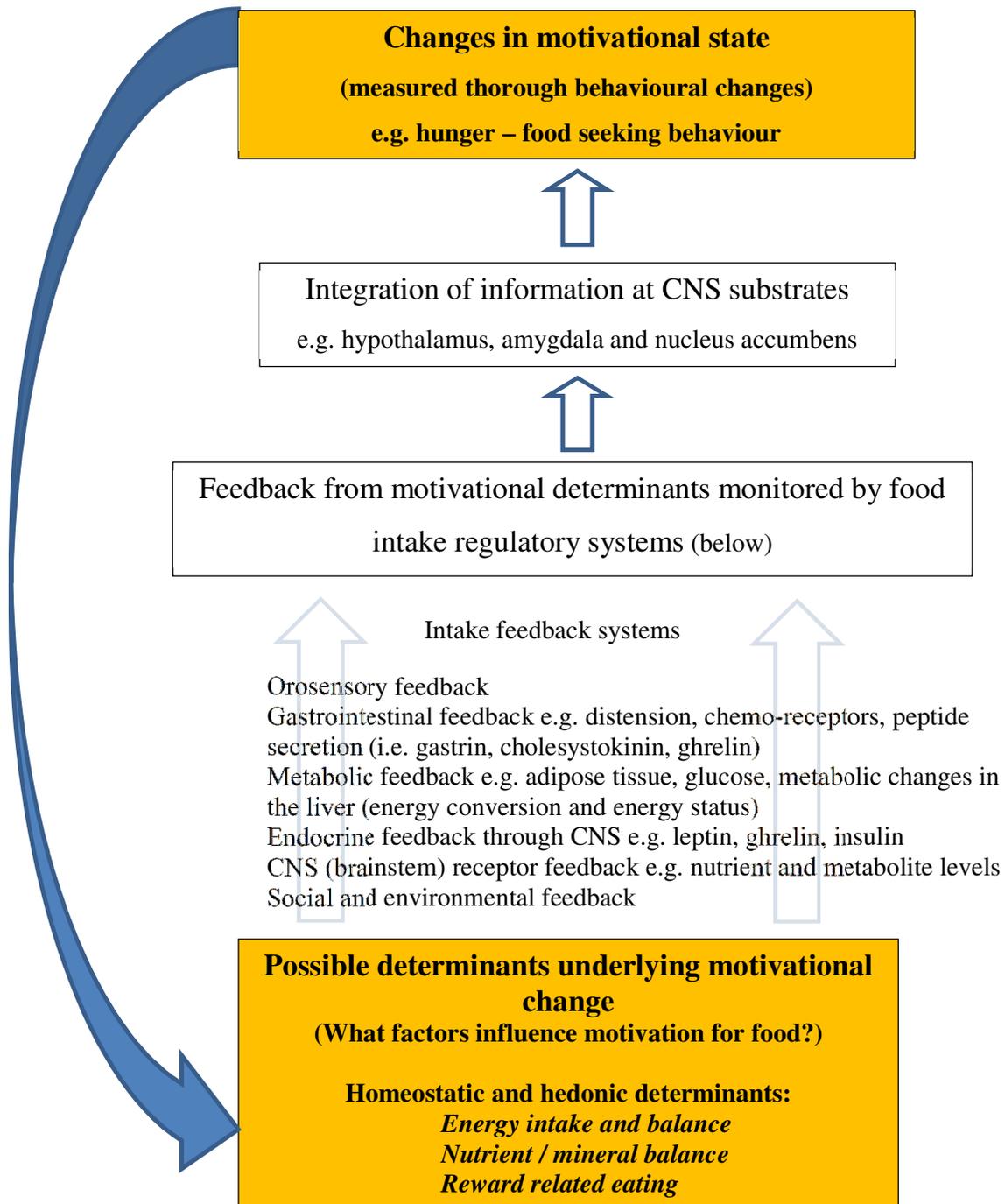


Figure 2.1 Proposed model outlining the main factors underlying changes in the motivation for food in ruminant animals. Sections highlighted in orange are the focus of this thesis.

There is considerable literature available, both in ruminants and monogastrics, detailing the physiological changes of energy homeostasis and how physiological, hormonal and neural pathways interact. For example, in ruminants the role of rumen distension and the restrictive capacity of the digestive tract has been investigated and reviewed (Forbes and Barrio, 1992; Allen, 1996; Forbes, 2007), as has the regulation of intake through feedback from volatile fatty acids (VFA's) (Mbanya et al., 1993; Aluwong et al., 2010). Additionally, the role of hormones and peptides such as leptin (hormone produced in the adipose tissue) and ghrelin (a 28 amino acid orexigenic peptide produced mainly in the stomach) (Gil-Campos et al., 2006) have been researched as well as the combination of the above factors and the integration of the brain-gut axis (Konturek et al., 2004). More recently, work has concentrated on the role of the CNS and various brain substrates implicated in the regulation of food intake (Schwartz, 2000; Figlewicz et al., 2007; Figlewicz and Sipols, 2010; Harrold et al., 2012). Work has also been undertaken in ruminants with regard to the efficiency with which animals are able to convert food energy to animal products (Stubbs and Tolkamp, 2006). While all of this research has an impact on understanding the regulation and the factors involved in energy homeostasis, there are little data available on how energy balance, either deficit or surplus, affects the motivation of a ruminant animal to seek and eat food, particularly when under behavioural demand test conditions. However, recent publications have shown that work has begun in this area with Schütz et al. (2006), exploring the impact of food restriction on feeding motivation in dairy cows, and Verbeek et al. (2011; 2012a) investigating the impacts of food restriction and body condition score changes on the motivation of ewes for food.

#### **2.4.2 Nutrient and mineral balance**

Ruminants sample food and eat a widely varied diet in order to fulfill their nutritional and mineral needs (Forbes, 2003). As mentioned above, the ability to determine which foods are appropriate for nutrient or mineral (or macronutrients) balance is thought to be primarily experiential in origin as well as involving orosensory (taste, smell, texture, appearance) feedback. However, exactly how this variation in food choice is regulated is not well understood (Tolkamp and Kyriazakis, 1997). Palatability is not simply the preference, acceptability or hedonic value of the food in question, but takes into account previous experience with the food and metabolic status of the animal (Forbes, 2007; Ginane et al., 2011). Ruminants generally develop preferences for feed that will rapidly provide a high level of satiety. Therefore, palatability integrates the nutritive value of the food as well as the

sensory response of the animal (Baumont, 1996). This occurs through neurally mediated interactions between the senses and the viscera enabling animals to gain feedback with regard to the consequences of the ingested food (Provenza, 1995). Taste is viewed as important as it is the last sense that can inform an animal about the food it is ingesting before it is swallowed. This enables valuable information to be gained regarding the ingestion of suitable foods and the rejection of unsuitable ones (Bell, 1984).

Ingesting food that induces physical discomfort, such as malaise or illness, can be caused by toxins and by either a deficit or excess of nutrients. This occurs through feedback from portal and jugular blood metabolites and may enable the animal to sense the effects of the ingestion within 15 minutes (De Jong, 1981). Consequently, an aversion to these foods develops. Aversion occurs through an excess of by-products (e.g. VFAs, lactate and propionate) caused by microbial fermentation (Provenza, 1995). Some of this 'nutritional wisdom' about what foods to avoid or consume can be acquired from previous experience or from conspecifics (Mirza and Provenza, 1994).

Requirements for protein can also affect feeding behaviour with dairy cows able to choose a diet with consistent protein levels when provided with a low protein or high protein feed (Tolkamp and Kyriazakis, 1997; Tolkamp et al., 1998). Lambs can also acquire aversions to a food when previous intake has resulted in an imbalance in essential amino acids (Provenza, 1995). In this circumstance, the nutrient deficient diet can cause the animal to increase the diversity of intake and seek foods that rectify the deficit (Provenza, 1995), thereby altering the motivation for specific food.

There are also feedback links between the orosensory signals and nutrient values of food. When lambs were provided with either of two flavours, orange or grape, paired with either saccharin or glucose they demonstrated a preference (96% to 4%) for the flavour paired with glucose (Burrill and Provenza, 1992). As the lambs did not exhibit any preference for either glucose or saccharin in short term tests, the authors concluded that the pairing, and subsequent activation of the orosensory feedback signals, reflected the preference for the nutritive properties of the solution (Burrill and Provenza, 1992). Furthermore, nutrients that are required in the largest amounts should have the greatest influence on motivational state. Scott and Provenza (2000) showed that when lambs were fed either a low protein/high energy

or low energy/high protein diet they foraged on food that contained the highest concentration of the macronutrient lacking in their basal diet. Therefore, while energy is of obvious importance, other nutritional needs can take precedence at times (Kyriazakis and Oldham, 1993; Webster, 1993; Provenza, 1995).

### **2.4.3 Influence of hedonic reward system**

There are various aspects of feeding behaviour, such as anticipation of food and motivation for food that are regulated by both homeostatic and hedonic systems. While the homeostatic system is regulated by the factors discussed above (e.g. stomach distension, blood metabolites), the hedonic system is modulated by dopaminergic and opioid brain systems (Barbano and Cador, 2006). Although there is currently no consensus about the specific roles of the dopaminergic and opioid systems, it seems likely that dopamine impacts on the anticipatory activity related to motivationally relevant stimuli (Barbano and Cador, 2007) or more specifically, the motivational tendency or the effort required for a reward (Salamone et al., 1994; Salamone and Correa, 2009). In contrast, opioids are involved in aspects of motivation driven by food palatability (Hanlon et al., 2004; Taha, 2010), although it is now thought possible that the hedonic reward system can increase the pleasure associated with eating any type of food whether it is palatable, bland or even aversive (Olszewski et al., 2011). Opioid reward systems have been found to influence feed intake regulatory systems and mediate food motivation predominately through the CNS, although peripheral effects on taste and gastrointestinal function may also be important (Levine, 1985). For example, support for the hypothesis that changes in food intake caused by orosensory signals are, at least, partially mediated by the feedback from the opioid system arises from the experiments where opioids had strong effects on sham-fed animals when post-ingestive feedback had been minimised (Kirkham and Cooper, 1988; Leventhal et al., 1995).

As previously mentioned, opioids also appear to increase the consumption of foods that are particularly palatable or energy dense (Taha, 2010), and in this way are one of the underlying factors that can affect the intake of food, even after energetic and nutritional requirements have been met. Early studies concentrated on sweet tastants and it was found that general opioid receptor antagonists (e.g. naloxone) could significantly decrease the intake of sucrose in rats (Levine et al., 1982), and much work has since been undertaken in this area (e.g. Kirkham and Blundell, 1986; Salamone et al., 1994; Naleid et al., 2007). In

comparison, little work has been undertaken in ruminant species with regard to the role that opioids play in altering feeding motivation. However, Alvai et al. (1991; 1993) investigated the role of naloxone (antagonist) on fasting sheep and on lean and obese sheep. The authors found that naloxone was able to suppress the appetite in sheep with the impact being inversely related to the duration of fasting, and that the obese sheep had an enhanced responsiveness to the intake-inhibitory effects of naloxone. Baile et al. (1981; 1987) have also investigated opioid mechanisms in sheep but concentrated on studying the specific brain substrates, peripheral systems and receptor types involved. Other work involving opioid systems and sheep has been undertaken but has focused on additional factors such as investigating the role of opioids on rumen motility (Maas and Leek, 1985; Maas et al., 1986).

Despite the large volume of work that has been undertaken, particularly in rodent models, at this point the exact role of opioid feedback in macronutrient choice is unclear. In rodents, studies have found that administration of an opioid agonist increased consumption of a preferred food choice (Naleid et al., 2007), irrespective of macronutrient content (Glass et al., 1996) and, conversely, increased the intake of fat (Marks-Kaufman and Kanarek, 1980). When motivation for food is assessed in a behavioural demand environment the components of the reward can be manipulated and the effects on the motivational state measured. This may allow insight into the mechanisms that are likely to link opioids, orosensory feedback signals, macronutrient choice, and palatability.

## **2.5 Conclusion**

As mentioned, the measurement of motivational change is a method used to assess resources that an animal values and, subsequently, used to aid in making conclusions regarding an animal's welfare status. However, as indicated by this review, little research has been reported on understanding the factors that influence motivation in a behavioural demand situation (Spruijt et al., 2001). While it is important to understand how situations and resources are perceived by the animal (Erhard, 2003), it is also important to better understand how the animal's experiences and motivations relate to its welfare if we are to continue to use motivational testing for the purposes of improving the well-being of an animal. As previously mentioned, an animal's willingness to pay a cost to access a resource suggests a link between its actions and subjective feelings (Dawkins, 1990). However, when motivation is viewed in terms of a behaviour that confers an adaptive advantage, it becomes more difficult to

extrapolate direct links between motivation for a resource and the impact of this motivation on the resultant welfare. One possible way to better identify the relationship between changes in motivation, adaption, welfare and welfare assessment may be to investigate the mechanisms that underlie and influence motivation. Therefore, this thesis attempts to explore this relationship by gaining a better understanding of the factors that influence motivation for food in a behavioural demand environment.

## **2.6 Scope of thesis**

The aim of this thesis was to investigate the underlying factors, homeostatic balance and hedonic reward, which influence an animal's motivation for food. Additionally, this thesis focuses on understanding how these factors interact with feeding motivation in a ruminant species (sheep) when assessed in the context of a behavioural demand test. This was undertaken with the overall aim of gaining more insight into possible links between the level of motivation, adaption and possible implications this may have for welfare assessment. This was done through a series of experiments examining:

1. The most appropriate parameters and models to use to assess data produced in the behavioural demand testing of a ruminant for food (Chapter 3);
2. The associations between level of motivation and energy balance following short term food deprivation conditions (Chapter 3);
3. How motivational levels are impacted by food rewards of differing energy densities (Chapter 4);
4. The role of the opioid reward system on motivational levels for foods of differing energy densities (Chapter 5).

## Chapter 3

# Assessing the impact of food deprivation on motivation for food using different behavioural demand analysis methods



### 3.1 Abstract

A number of quantitative measures have been used to assess the strength of motivation of animals for a reward. Behavioural demand analysis has a theoretical advantage over more traditional operant conditioning measures in that it quantifies an animal's changing motivation in relation to the resource consumption and constraint. Nonetheless, much debate exists regarding the most useful model with which to appropriately scale the reinforcing strength of a reward or resource. The first section of this experiment (part 1) compared measures of behaviour (maximum price paid and total number of rewards consumed), with two behavioural demand models; the linear-elasticity model ( $P_{\max}$  and  $O_{\max}$  measures), and the exponential demand model (alpha value) for utility. These types of behavioural demand analysis can be used to assess the resources and rewards that an animal values and, subsequently, to aid in determining its welfare. However, the relationship between animal motivation and welfare is not necessarily well defined. One way to better understand this relationship is by investigating the factors that influence motivation in a demand test. Therefore, part 2 of this experiment aimed to test the hypothesis that a short term food deprivation, and subsequent alteration to energy balance, would increase the motivation of sheep to work for food. To assess motivation for food, 8 Merino wethers were required to walk an increasing distance for a 4g food reward following either a 14h food deprivation or no deprivation (control), in a cross-over design where each animal was used as its own control and exposed to all treatments. The distance (cost) that the sheep walked was increased progressively on a log scale (1.5 - 105m). The results showed that all animals reduced the number of food rewards obtained as the cost (distance walked) increased ( $P < 0.001$ ), and differences between the treatments tended towards or reached significance at the shorter costs (1.5m,  $P = 0.08$ ; 6.1m,  $P = 0.09$ ; 12.3m,  $P < 0.001$ ). The exponential demand model indicated that under the deprivation treatment, food had a significantly lower alpha measure, equating to a higher essential (rewarding) value, ( $P = 0.017$ ). There were no significant treatment differences when assessing the data using the linear-elasticity model. These results indicate that the exponential demand model may be more sensitive to measuring small changes in the value of a resource when compared to the linear-elasticity parameters or to more quantitative measures, such as the number of rewards consumed. The energy balance differences between treatments at the 1.5m cost were found to be significant ( $P < 0.05$ ), and there was a tendency for this trend to continue at the 6.1 and 12.3m costs ( $P = 0.057$ ,  $P = 0.058$ , respectively), with the deprived sheep working harder for food rewards at these costs than the control animals.

The cost at zero energy balance (energy intake = energy expenditure) and the cost at  $P_{\max}$  were similar (between 30 and 39m,  $P>0.05$ ) for both the deprived and the control treatments, although the sheep did not stop walking at these distances. This indicates that sheep showed a reduced motivation for food at the point of zero energy balance, but continued walking beyond this estimate. These results suggest that in a demand test, energy intake and expenditure may aid in determining motivation for food. However, it is likely other feedback factors are also involved in this process, but further investigation will be necessary before any conclusions can be made.

## 3.2 Introduction

The use of motivational testing has become a popular method in animal welfare science (Dawkins, 1983; 1990) for measuring an animal's preference for a range of resources and rewards. It is derived from theories in microeconomics and other economic concepts which have been used in experimental psychology (Hursh, 1984; Hursh et al., 1988; Jensen and Pedersen, 2008). A typical test involves assessing the strength of an animal's motivation to obtain a specific resource as the 'cost' (e.g. distance walked or the number of lever presses) of obtaining that resource increases. This approach is based on the observation that animals will work to gain access to resources, with the strength of the motivation to work providing quantitative information on how important the experience or resource is to the animal.

There are a variety of methods which can be used to assess the strength of an animal's motivation for a resource. The simplest of these is to measure the consumption of the resource by an animal undergoing an operant test (e.g. number of rewards consumed), or to assess the maximum amount of effort an animal will expend to gain access to a resource, referred to as the maximum price paid or reservation price (e.g. Kirkden et al., 2003; Hovland et al., 2006). Demand functions are a more complex method of analysis and describe to what extent the change in the price of one resource affects the consumption of that resource, and there is a range of estimates that can be obtained from this function (Jensen and Pedersen, 2008). In more theoretical terms, this methodology is used as a means of scaling the strength of a resource, i.e. how well the resource or reward reinforces the specified operant behaviour. Learning theorists have spent many years attempting to reach agreement on the most appropriate method to rank the strength of different resources (Christensen et al., 2008a) with, as yet, no real consensus (Ng, 1990; Matthews, 1998; Kirkden et al., 2003; Jensen and Pedersen, 2008).

When using the linear-elasticity model, one of the measures that can be obtained from a demand function and used to assess motivational strength is the elasticity of demand (price elasticity). This assesses the slope of the demand function (changes in consumption relative to changes in price) in log-log coordinates (Dawkins, 1988). Log-log coordinates are used as this converts absolute changes into proportional changes and standardizes the units so that they are independent of the unit of original measurement. However, this measure may not be

valid under all circumstances. As noted by Kirkden et al. (2003), elasticity of demand is only valid when the elasticity is constant across a range of costs, or the demand curve is a true straight line on log-log coordinates. This is often not the case (e.g. Hursh and Silberberg, 2008) as demand for a resource is usually defended at low costs, but motivation for the resource decreases as the costs increase. The use of this analysis has been the topic of some debate among welfare scientists due to a lack of agreement over which parameter of the demand curve best describes the strength of motivation (Kirkden and Pajor, 2006a).

Measures derived from the linear-elasticity model and used to assess changes in motivation include  $P_{\max}$  and  $O_{\max}$ .  $P_{\max}$  is the cost at which maximum responding occurs or the point where the slope of the behavioural demand curve is equal to -1 and moves from inelastic to elastic, while  $O_{\max}$  is the maximum work done at  $P_{\max}$  ( $O_{\max} = P_{\max} \times$  number of trips at  $P_{\max}$ ) (Hursh and Winger, 1995; Matthews, 2008).  $P_{\max}$  has been described as an accurate method by which to compare elasticity across demand curves that have continuously varying slopes, or where elasticity varies with cost (Hursh and Winger, 1995), and has been utilised in a variety of studies (Foltin, 1991; Bokkers and Koene, 2002; Kirkden and Pajor, 2006b; Galhardo et al., 2011).  $O_{\max}$  has been proposed as the best measure to quantify the subjective experiences of animals (Madden et al., 2007a; Matthews, 2008), and has recently been used in an attempt to quantify differences in hunger in sheep (Verbeek et al., 2011; 2012a).

Another methodology that has been proposed and has undergone preliminary testing is that of the exponential model of demand (Christensen et al., 2008a; Hursh and Silberberg, 2008). This model is argued to be more appropriate than the more frequently used linear-elasticity model, as it is capable of indexing the rate of decline of a demand curve rather than measuring it as two parameters,  $a$  and  $b$  (where  $a$  is the acceleration in slope as price increases, and  $b$  is the initial level of the curve at minimal cost) (Hursh et al., 1988). The derived value is referred to as alpha or the 'essential value' (Hursh and Silberberg, 2008). Hursh and Silberberg (2008) propose that alpha measures the reinforcing strength of a reward or the degree to which the reward is capable of maintaining behaviour independent of the reward size or potency. The value of alpha is inversely related to the value of the reward so that resources which generate a steeply declining demand curve have a higher alpha value, and therefore a lower reward value, when compared to rewards which generate demand

curves which drop slowly with increases in cost (Christensen et al., 2008a). To date, this method has not been applied in motivation studies involving livestock, but has been successfully utilised in rodents to assess the value of cocaine when compared to food (Christensen et al., 2008a).

Other authors, however, have argued that the maximum price paid is a more reliable measure of quantifying animal motivation and resource rank (Kirkden and Pajor, 2006a). The maximum price paid (MPP, also referred to as reservation price) is the highest price that an animal is prepared to pay to gain access to a resource (Hursh and Silberberg, 2008), and MPP has been widely utilised in studies of animal preference and motivation (Robinson et al., 1995; Mason et al., 2001; Patterson-Kane et al., 2002; Hovland et al., 2006). Proponents of this measure have argued that while demand curves may be able to assess the price that an animal will pay, they do not necessarily measure the benefits of a resource and that MPP allows the assessment of discrete, non-divisible resources (e.g. the demand for social contact or nest boxes) which are goods unsuited to demand curve methodologies (Cooper and Mason, 2001). However, this methodology has been criticised because the criterion for determining an animal reaching the MPP is often arbitrary and varies between testing situations and resources, and that the data may be confounded by the rate of access and rate of work (for a full review see commentaries by Mason et al., 1998b; Matthews, 1998; Sherwin and Nicol, 1998).

It has been proposed that if an animal will work hard to obtain a resource (e.g. push through increasingly heavier doors, jump progressively higher barriers or walk further distances), then it may be reasonable to conclude that its welfare is improved by achieving that objective (Broom, 1988). However, the relationship between animal motivation, welfare and welfare assessment is not well defined, neither does it appear to have been investigated empirically. It is also difficult to determine how the animal's effort relates to the degree to which it needs the resource (Patterson-Kane et al., 2011). As noted by Spruijt et al. (2001) the ethologically orientated models that have investigated motivation from a welfare perspective (e.g. Jensen and Toates, 1993) have paid little attention to questions such as "How does an animal estimate the cost-benefit ratio of a specific behaviour prior to or during the activation of a motivational system?" and "How does the animal take its actual state into account?". One possible way to gain insight into these questions is by investigating the underlying

determinants that influence motivation. As the importance of resources which elicit low to intermediate levels of a response in an animal can be somewhat ambiguous in meaning, (Patterson-Kane et al., 2011) it was decided that a resource should be used with a known high value. Food is one such resource.

Food is clearly a biological need and a variety of mechanisms and physiological, hormonal and neural pathways are involved in its regulation in a complex and integrated manner. This is designed to allow food intake to match energy output and to ensure that the animal's nutritional and energetic requirements are met. As noted by Forbes (2007), the phrase 'animals eat for calories' is not true under all circumstances, particularly when referring to ruminants, neither is the prediction that animals will eat to meet their energy requirements unless prevented by a limiting factor (e.g. Conrad et al., 1964; Forbes, 2003). However, the role of caloric intake, or energy balance, could be considered a good starting point when investigating factors that may influence the motivation of a ruminant for food in a demand test.

Food restriction has successfully been used to determine an animal's motivation to work for food. Jackson et al. (1999) exposed sheep to different food restriction levels ranging from 0 to 24h and tested the resultant feeding motivation using operant tasks. The authors reported that when sheep were required to walk a race and push through a door, there was an increase in feeding motivation following a food restriction of as little as 6h. More recently, Verbeek et al. (2011) have shown that when ewes were required to walk varying distances, or 'costs', following exposure to a food restriction of 24h, restricted ewes displayed significantly higher motivation for access to food.

The impact of energy balance on the motivation of animals for food has also been studied to some degree. Dumont et al. (1998) investigated how ewes made choices between varying amounts of a good quality hay, which was available at differing distances, with poorer quality hay that was freely available. The authors reported that the quantity of reward affected feeding motivation as the poorer hay was preferred as the reward amount of the high quality hay decreased. This follows previously identified patterns of food intake which indicate that selection is determined by preferences in both the quality and taste of food as well as the costs involved in accessing food (Pyke, 1984; Ginane et al., 2002). These costs

alter the intake of food according to the optimal foraging theory which predicts that animals will make food choices to maximize the net energy gain per unit of feeding time (MacArthur and Pianka, 1966; Pyke, 1984; Stephens and Krebs, 1986). Similar patterns of foraging, following an energy gain maximisation hypothesis, have been found in cattle when the strength of motivation for food was assessed (Ginane et al., 2002; Schütz et al., 2006).

Another issue with behavioural demand testing is the test length. Broom (1988) noted that many experiments need to be a considerable length as specific activities may only be carried out at certain times of the day over long time periods. A proven protocol for testing food motivation in ruminants allows unrestricted access to food over 23h (Verbeek et al., 2011), meaning that only one animal can be tested at each access price in each set of apparatus per day. Consequently, each experiment can be a considerable length depending on the number of treatments and animals per treatment. Ideally, it would be useful if several animals could be tested per day.

Given the above mentioned debate surrounding the use of particular demand function measures, the first aim of this experiment (part 1) was to compare quantitative measures (MPP and rewards consumed) with two demand models (the linear-elasticity and exponential demand) and the various measures derived from these models ( $P_{\max}$ ,  $O_{\max}$  and alpha). This was done by quantifying the effect deprivation of an important resource (energy) had on these measures of motivational strength.

The present study also aimed to test the hypothesis that creating an energy deficit by depriving sheep of food for 14h would increase their motivation to work for food (part 2). A secondary aim of this part of the experiment was to determine whether shorter periods of testing could differentiate food motivation levels between animals exposed 0h and 14h food deprivation.

### **3.3 Materials and methods**

This study was approved by the CSIRO's Animal Ethics Committee and conformed to the standards in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Animal Research Authority 09/10). All animals were closely monitored during the experiment and body weight and body condition were monitored weekly. The

sheep did not show any signs of health problems, illness or injury at any time, and did not lose body condition or body weight during the trial.

The testing was undertaken at the CSIRO in Armidale, NSW at the Chiswick FD McMaster Laboratory. Chiswick is located at the southern end of the Northern Tablelands in the New England region of New South Wales, 16km south of Armidale. Chiswick has an annual rainfall of 866mm and is situated 1,046m above sea level at 30°south, 151° east. This region has a temperate climate with significant daily and seasonal changes in temperature. The mean temperature range is 12° - 24°C during summer and 0° - 11°C during the winter months.

### **3.3.1 Animals and housing**

Twenty, 2-year-old Merino wethers weighing  $45 \pm 3.4$ kg with a mean body condition score of 3.1 ( $\pm 0.04$ ) were used for this experiment. All sheep in the experiment (and other experiments described in this thesis) were body conditioned scored using a 5-point system where a score 1 corresponded to an underweight animal where the scorer could feel sharp spinous and transverse processes and shallow eye muscles, through to a score 5 that corresponded with an overweight animal or an inability by the scorer to manually feel the spinous and transverse processes which was accompanied by a thick fat covering over the eye muscles (Russel, 1984).

During testing the sheep were maintained in 3 outdoor pens (16m x 12m) and moved freely between the pens. On non-test days, all sheep were fed 110% of a theoretical maintenance ration (AFRC, 1995; Primary Industries Standing Committee, 2007), which consisted of a commercially manufactured (Ridleys, Tamworth, NSW) concentrate lucerne pellet (AH or Animal House pellets) (ME=11.0 MJ/kgDM; CP=21.4%) and oaten chaff (ME=8.4MJ/kgDM and CP=4.9%) in a 9:1 ratio. Feeding occurred twice daily at approximately 8:00am and 4:30pm during both training and test periods. During test periods sheep had access to the AH lucerne pellets only. Water was freely available at all times.

### **3.3.2 Facility and equipment**

The facility was designed so that an animal's willingness and motivation to walk for a food reward could be assessed. A walking test was chosen as it has been found to more a

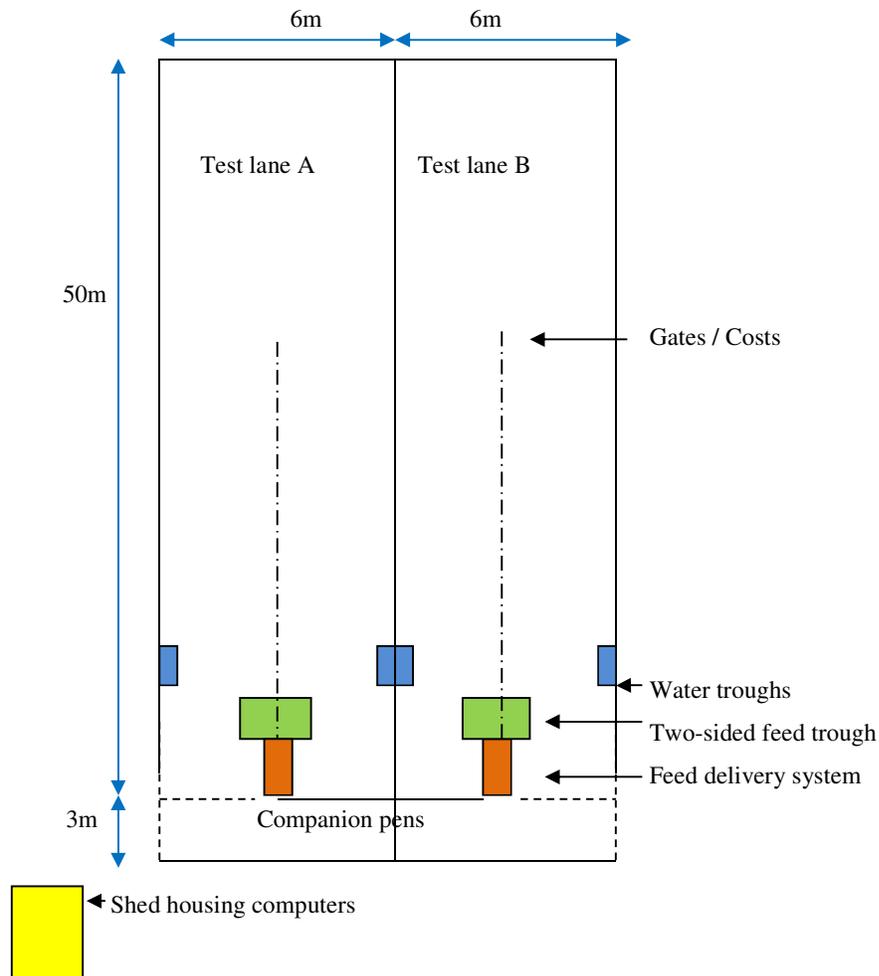
naturalistic activity for a ruminant as it is associated with foraging behaviour (Jackson et al., 1999). The animal's motivation for food was tested by investigating the number of food rewards that were consumed as the distance walked (cost) to access the reward increased. Two identical laneways (6m x 50m) were used to assess the demand of the sheep for food (Figure 3.1). The dividing fence between the two lanes was approximately 1.8m high and was covered with a double thickness of shade cloth so that the sheep could not see each other or be influenced by the other animal's behaviour. A purpose built feeder with 2 access bays was located at one end of each laneway. A feed hopper and motor were located above the feed trough on the outside of the laneway and polyvinyl chloride pipes ran from the base of the hopper to each side of the feed trough, allowing gravity assisted delivery of the pellets. An animal's presence at one bay was detected by infrared beams (Housewatch IR-1200L-12m Reflector Beam, Australia), and activated feed delivery to the opposite bay. Access to that feed could be gained when the animal moved from one side of the feeder to the other. Continued access to food required the animal to cycle back and forth between bays.

The control system (Dell Latitude E3400 laptop (Dell, Australia), National Instruments multi input / output card (National Instruments, Australia) plugged into the PCI bus of the computer and an interface box containing surge protection devices and relays), and associated control software (UNE, Australia), monitored the infrared beams and when the appropriate beam was broken moved a butterfly valve and activated the motor allowing feed to be dispensed into the correct feeding pipe. The amount of feed delivered each time was measured using a mild steel cylindrical measuring cup. The cups were 77mm in length with a diameter of 33mm. Halfway along the length of the cup was a hollow that held approximately 4g of pellets. Holes were drilled into one end of the cup and prongs, which were turned by the motor, fitted into these holes. The motor included a gearbox and switching mechanism so that when it was activated, via a voltage pulse, one full revolution of the feed measuring cup allowed both collection and delivery of the pellets. The battery used to deliver the voltage pulse was a 600AMP Marine Deep Cycle battery (Autocraft, Australia), and via this system pellets were delivered to alternating bays of the feed trough.

The feeders were calibrated weekly by taking 100 samples from each of the 4 feeding troughs to determine the mean reward delivered from each set of feeding apparatus ( $3.8 \pm 0.1\text{g}$ ). A reliability test over 24h was conducted before the start of the experiment to ensure

that the machinery would operate under continuous conditions. This was achieved by attaching the mechanisms from analogue clocks to both of the feeder walls in such a position that the second hands would break the infrared beams, on alternating sides of the feeders, and activate the feed delivery system every 30 seconds.

The distance the sheep walked to access the bays of the feeder was increased by a series of panels placed down the middle of the facility at log specific intervals. Each event (breaking of the beam and consumption of food) was time and date stamped and recorded in a text file.



**Figure 3.1** A schematic representation of the behavioural demand facility. Two sheep were tested simultaneously on one day and two companion sheep were placed in each of the companion pens.

### **3.3.3 Habituation and behavioural demand training**

Sheep were initially habituated to lucerne pellets whilst in a paddock. This was conducted 3 or 4 times weekly for 8 weeks and 6kg of lucerne pellets were provided to the group on each visit. This process also habituated the animals to human contact. On the first three days of training in the behavioural demand (BD) facility a group of 10 sheep were moved into each of the lanes. The trainer then placed a small amount of pellets (50g) into both sides of the feeder and the sheep were left in the facility for 1h to familiarise themselves. Over the next 9 days sheep were trained under the same conditions, but the number of sheep per group was gradually decreased to individual animals. The feeder was primed with fewer pellets (10g) and the feeding machinery was activated. At this point, sheep were observed for general behaviour, including fearfulness in the facility and willingness to continuously feed out of both sides of the feeder. Eight animals were selected for the study based on these observations. Each individual animal was then trained at varying distances (1.5m, 10m, 20m, and 40m) and reward sizes were decreased from 10g to 5g. The sheep were classified as trained when they successfully activated the feeder in a continuous manner 10 times. The remaining animals were used as companions during test sessions.

### **3.3.4 Experimental design**

A cross-over design, where each animal was its own control, was employed to investigate the changes of the deprivation on motivation and the 8 trial Merino wethers were randomly allocated to 2 groups (n=4) and assigned to a first treatment, either no food deprivation (control animals) or 14h food deprivation, and to a specific BD lane. Nine costs were used in ascending order (see below), and each pair of sheep were exposed to the same cost at the same time but differed in level of feed deprivation i.e. on day 1 both sheep were given a 1.5m cost, with one animal being exposed to the control treatment and the other the 14h food deprivation treatment. One pair of animals were tested every day, for a 23h period on each occasion, for a 36 day period with each pair having a 3-day break between tests. The first 8 days of each 36 day period (C1 and C2) have are shown below (Table 3.1). The pattern shown continued for all 9 costs and ran for 2 x 36 day cycles.

A 14h deprivation period was chosen for the food deprivation treatment as it was thought to be a sufficient compromise between restricting the sheep so that an effect on their motivation for food could be seen and not restricting so greatly that a demand for food would

continue past the space limitations (105m) of the facility. It should also be noted that Jackson et al. (1999) found increases in food intake in sheep deprived of food for as little as 6h.

**Table 3.1** The first 8 days of each 36 day cycle (cycle 1 or C1 and cycle 2 or C2) shown for each pair of sheep. The grey blocks indicate the control treatment and the green blocks indicate the 14h food deprivation treatment.

<b>C1</b>	<b>Lane A</b>	<b>Lane B</b>	<b>Cost</b>
Day	Sheep No.	Sheep No.	
1	1	2	1.5m
2	3	4	1.5m
3	5	6	1.5m
4	7	8	1.5m
5	1	2	6.1m
6	3	4	6.1m
7	5	6	6.1m
8	7	8	6.1m

<b>C2</b>	<b>Lane A</b>	<b>Lane B</b>	<b>Cost</b>
Day	Sheep No.	Sheep No.	
1	1	2	1.5m
2	3	4	1.5m
3	5	6	1.5m
4	7	8	1.5m
5	1	2	6.1m
6	3	4	6.1m
7	5	6	6.1m
8	7	8	6.1m

To be able to compare different demand curve parameters the underpinning methodologies had to be revised as the  $P_{\max}$  measures require an assessment of the change in curve at the point where the slope is equal to -1.0, while MPP requires extinction of the specified behaviour. Generally, the  $P_{\max}$  approach uses a series of pre-determined costs given to the animals in a random order to produce a curve from which the  $P_{\max}$  can be obtained. This methodology also allows for recovery days between tests. The MPP method also has pre-determined costs, but generally presents these in ascending order and can impose these as either a series of costs in one session or as one cost per session. In the present experiment

these methods were combined by calculating two sets of log costs, one which spanned 0–50m (used to calculate  $P_{\max}$ ) and the other 50–105m (used to reach MPP). As sheep can walk an average of 10km/day (Squires and Wilson, 1971; Squires, 1976), the final cost for the MPP parameter was chosen at 105m, meaning that the sheep would need to complete almost 100 events to reach 10km. Little data are currently available regarding how much work a ruminant will do when asked to pay a maximum price for a resource so the distance for the final cost was chosen as an estimate of the possible work which could be undertaken. The distance of 105m was also the longest cost available within the structural limits of the facility.

The costs were presented to all of the sheep in ascending order with a recovery period of 3 days between tests to ensure that the appropriate level of feed deprivation could be maintained, and that there was no carry-over effect from having been in the testing facility on the previous day. The final costs used were 1.5, 6.1, 12.3, 24.8, 50.0, 60.2, 72.5, 87.2 and 105m. To obtain the final cost of 105m, panels were placed from 60m onwards (i.e. 30m from the feeder) at right angles to the direction that the sheep were travelling. This ensured that the sheep moved through the latter section of the facility by walking in a zig-zag pattern and this allowed a cost of 105m within a facility with a length of 50m. The termination point for the MPP was defined as an animal consuming zero rewards during the 23h test period.

Under grazing conditions Merino sheep walk an average of 10km per day (between 3 and 17km per day) (Squires and Wilson, 1971; Squires, 1976; Brand, 2000). Taking into account the energy requirements of a 45kg wether for; theoretical maintenance (8.2 MJ per day), walking 10km on horizontal terrain (1.2MJ per day), standing for 12h (0.2MJ per day) and 12 positional changes (0.1MJ per day) (Figures taken from SCA, 1990; AFRC, 1995; Primary Industries Standing Committee, 2007), the sheep had a total energy requirement of approximately 9.7MJ to recoup the energy spent on maintenance and activity over the test period. The number of events required at each cost to walk 10km was then calculated, as well as the amount of energy required per rewarded event to achieve an intake of 9.7MJ. For example, at the 50m cost, 200 events would be needed to reach 10km. A theoretical maintenance requirement of 9.7MJ then needs 0.048MJ per event to allow the animal to attain maintenance. Given this, we hypothesised that the sheep would reach a state of zero energy balance between the 24.8 and 50m costs and may stop walking at or around this point.

### 3.3.5 Behavioural demand testing

Two repetitions of the experiment were run for 36 days each from the 24<sup>th</sup> of November, 2009, to the 1<sup>st</sup> of January 2010 (C1) and from the 7<sup>th</sup> of January, 2010, through to the 11<sup>th</sup> of February, 2010 (C2). On the morning preceding a motivation test 2 test sheep were placed in individual pens for a 24h period prior to testing. Both sheep were fed pellets and chaff at 8:00am and 4:30pm. At 7:00am on the morning of the test the control group animals were provided with their usual ration of AH pellets (minus chaff) and were allowed 30 minutes in which to consume the pellets (residual was measured and all sheep ate 100% of the feed provided to them). The restricted treatment animals were not fed prior to the motivation testing. At 7:30am both sheep were moved into their designated lanes and companion animals were moved into the pens at the front of each laneway. Sheep had *ad libitum* access to the AH pellets during the testing period. At approximately 7:15am the day following the motivation test the 2 test sheep were removed from the facility and the feeder mechanisms were checked to ensure they were functioning correctly. The pellets remaining in the hopper were then removed, weighed and recorded. The feed trough was also checked for residual pellets; however, the sheep consumed 100% of all food earned. The number of rewards consumed, date and time stamp information was downloaded at this time. Nine costs were used and costs were altered every fourth day. Water was freely available at all times.

Twenty-four hour video footage was also recorded of the first 15m of the lane, including the feeder. Samsung Day/Night Colour (SSCB2013P Colour Camera, Samsung, Homebush, Australia) cameras were mounted in all-weather housing and footage was recorded using PaCom (Castle Hill, Australia) software. To ensure the sheep could be viewed at night, a 240 watt spotlight was mounted below the camera in each laneway and turned on, via a digital plug-in timer (HPM Industries Ltd., Auburn, Australia), at 7:00pm and off at 7:00am.

### 3.3.6 Statistical analysis

The number of rewards consumed by each sheep at each cost were analysed using Genstat 14 (VSN International Ltd., Hemel Hempstead, U.K.). The effects of cycle (cross-over), treatment (level of deprivation), cost (distance walked), order of treatments (deprivation then control or vice versa), and the interaction between these on the number of rewards consumed (motivation) were tested using analysis of variance (ANOVA). Cycle,

treatment, cost and treatment order were fitted as fixed effects with the number of rewards consumed fitted as the dependant variable. The individual sheep was fitted as the random variable and the number of rewards consumed was transformed (square root) to improve normality of distribution. Data and residuals were also tested for heteroscedasticity. It should be noted that a repeated measures analysis was not used as this assumes that the two measures are correlated i.e. that one measure influences the next (Petrie and Watson, 1999). The underlying assumption for the current (and the following experiments described in this thesis) is that the measures are independent of each other. The 3 day break between each test ensures that the animals are not affected by previous deprivation levels and no evidence of serial correlation was found.

The linear-elasticity and the exponential demand models were also fitted using GenStat 14. The number of rewards was tested for normality and heteroscedasticity was normalised via square root transformations. To obtain the  $P_{\max}$  (cost at which maximum responding occurred) and  $O_{\max}$  (maximum work done) values, a residual maximum likelihood (REML) analysis was used to estimate the treatment by cost means. These data were then log transformed and fitted to the generic demand function (Hursh and Winger, 1995):

$$\ln(Q) = \ln(L) + b [\ln(P)] - a(P),$$

where  $Q$  is the measured resource consumption,  $P$  is the price for a unit of the resource,  $L$  is the initial level of the curve at minimal price,  $b$  is the corresponding slope of the demand curve at minimal price and  $a$  is the acceleration, or increase in slope, with increases in price. Once  $P_{\max}$  values were obtained  $O_{\max}$  was calculated as:

$$O_{\max} = P_{\max} \times NR_{P_{\max}},$$

where  $NR_{P_{\max}}$  is the number of rewards obtained by the animal at  $P_{\max}$ .

The exponential model of demand was calculated firstly by normalising the data. Following this the normalised data were fitted to the exponential equation:

$$\log Q = \log Q_0 + k (e^{-\alpha \cdot Q_0 \cdot C} - 1),$$

where  $Q$ ,  $Q_0$  and  $e$  represent units of consumption, units of consumption at the lowest price on the demand curve, and the base of the natural logarithm, respectively. The value of  $k$  depends on the resource being evaluated (generally a value between 1 – 4 log units) and was 3.5 in the current study. The  $C$  value is defined as the cost (e.g. a fixed ratio schedule of 5 has a  $C$  value of 5) and alpha is the free parameter which is adjusted to allow minimisation of the

differences between the modelling predictions and each demand curve (Christensen et al., 2008a).

Energy balance (EB) estimates were calculated as  $EB = EC - (TM + AE)$  where energy consumed (EC) was the number of food rewards earned at 41.8kJ per reward (as provided by analysis of the feed;  $3.8 \pm 0.1g$ ) within the 23h test period, and energy expended was comprised of a theoretical maintenance (TM) requirement figure and an estimate of the energy expended on activity (AE). No accounting of prior energy intake or energy restriction was made (e.g. Schütz et al., 2006). TM was calculated as 8.2MJ for a 45kg wether and the activity portion of the calculation involved actual distance walked to access feed rewards as well as estimates of energy used for standing and positional changes. Therefore, estimates of energy expenditure were calculated as: Energy Expenditure (kJ per day) = [horizontal movement (2.6J per kg body weight divided by metres walked) + standing 12h (5kJ per kg body weight per day) + 12 positional changes (3.1kJ per kg body weight)], (AFRC, 1995; Primary Industries Standing Committee, 2007). Estimates of the amount of time spent standing and number of positional changes were kept constant so that the energy utilised for walking to access rewards could be calculated, meaning that the final calculation was:

Energy Expenditure (AE) (kJ/day) =  $[(2.6 \times \text{metres walked}) + 5.0 + 3.1] \times BW$  (AFRC, 1995).

To estimate the cost at the point of zero energy balance (y intercept or point where sheep changed from a state of positive energy balance to negative energy balance), 2 points about the line (last point above the x axis and first point below x axis) were taken and a straight line was approximated between these points. The straight line equation of  $y = a + bx$  was then solved so that y equalled zero. Other analysis was also undertaken to ascertain the value at which y was equal to zero. For example, the non-linear least squares method was used to fit various equations to the data and Solver (Excel) was used to estimate the specific cost at which the energy balance was equal to zero. However, due to the lack of costs tested around the point of zero energy balance it was not possible to determine which equation best modelled the behaviour of the curve at this point, therefore, a straight line was deemed to be the best approximation. However, this meant that there were no error values at the point of zero energy balance as the data was taken from energy intake at two tested costs.

Significance levels between  $P_{\max}$  and zero energy balance costs were tested with a two-tailed  $t$ -test (GenStat 14). Cumulative work rate (a cumulative measure of the work done over the 23h test in 3h test periods e.g. work done in periods 0-3h, 0-6h) and interval work rate (work done over specific time intervals e.g. work done in 0-3h, 3-6h) treatment differences were assessed with Chi-squared tests. All data are presented as actual mean  $\pm$  standard error of the mean (SEM), unless otherwise stated. The simplest statistically significant model has been presented here (and throughout the remainder of this thesis).

### **3.4 Results**

The number of rewards consumed declined as the cost increased ( $P < 0.001$ ). However, there were no significant treatment differences in the total distances walked, over time (between C1 and C2), or associated with the order in which the treatments were allocated (control then deprivation or vice versa). Analysis of the mean number of rewards consumed showed that differences between the treatments at individual costs did reach significance at 12.3m ( $P < 0.001$ ), and trended towards significance at the 1.5m and 6.1m costs ( $P = 0.08$  and  $P = 0.09$ , respectively; Figure 3.2). However, overall there were no significant treatment differences between the mean amount of rewards consumed ( $P > 0.05$ ) and no significant interactions.

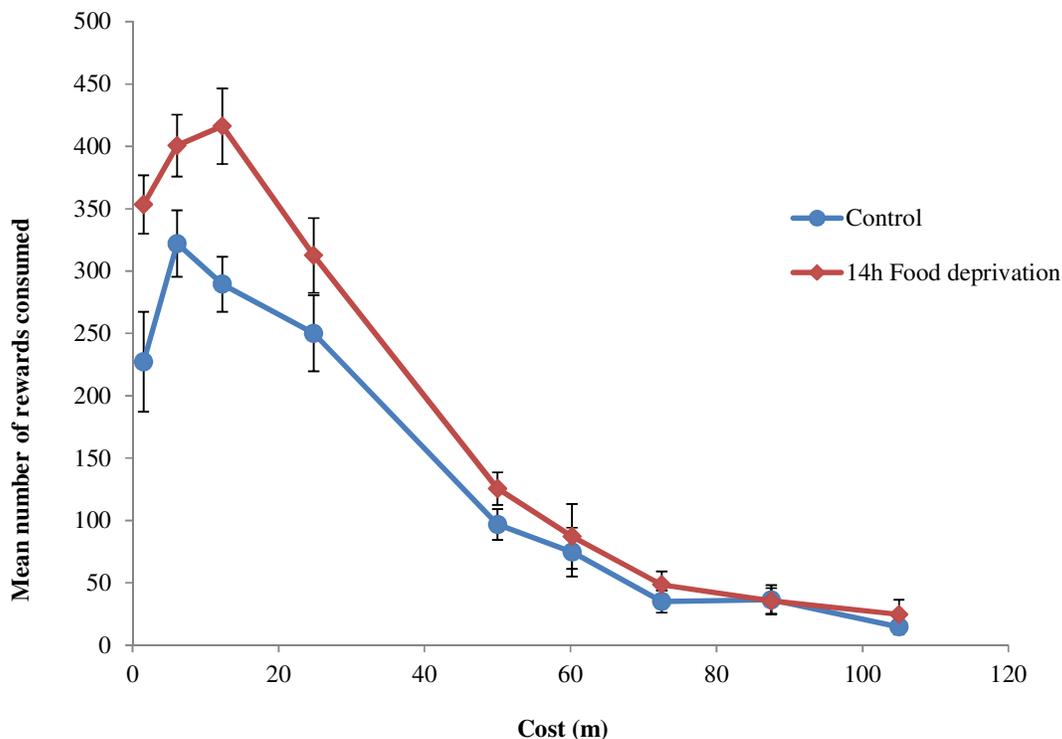


Figure 3.2 Actual mean number of rewards consumed ( $\pm$ SEM) for both the control (blue circle) and 14h food deprivation (red diamond) treatments at all cost levels ( $n=8$ ).

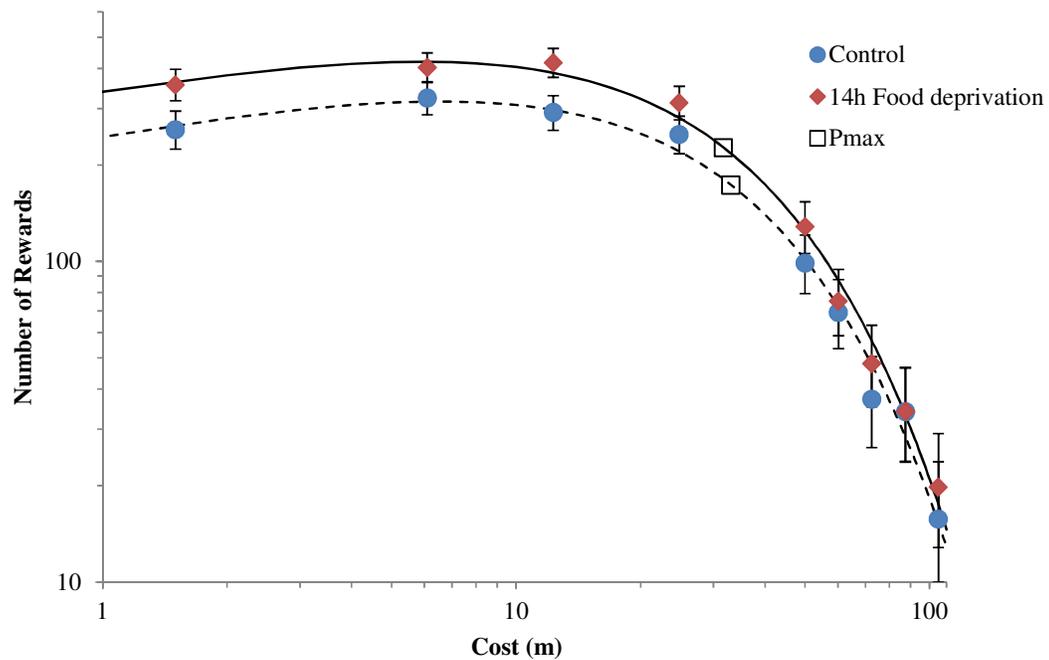
### Part 1 Comparing behavioural demand analysis methods

There were no significant differences ( $P>0.05$ ) between treatments found by analysing the data with linear-elasticity modelling. The mean  $P_{\max}$  of the control and food deprived treatment groups was  $29.8 \pm 2.9\text{m}$  and  $29.9 \pm 3.1\text{m}$  (Figure 3.3), respectively. The  $O_{\max}$  was also similar being  $6,005 \pm 905\text{m}$  for the control and  $7,812 \pm 1,090\text{m}$  for the food deprived treatments ( $P=0.35$ ).

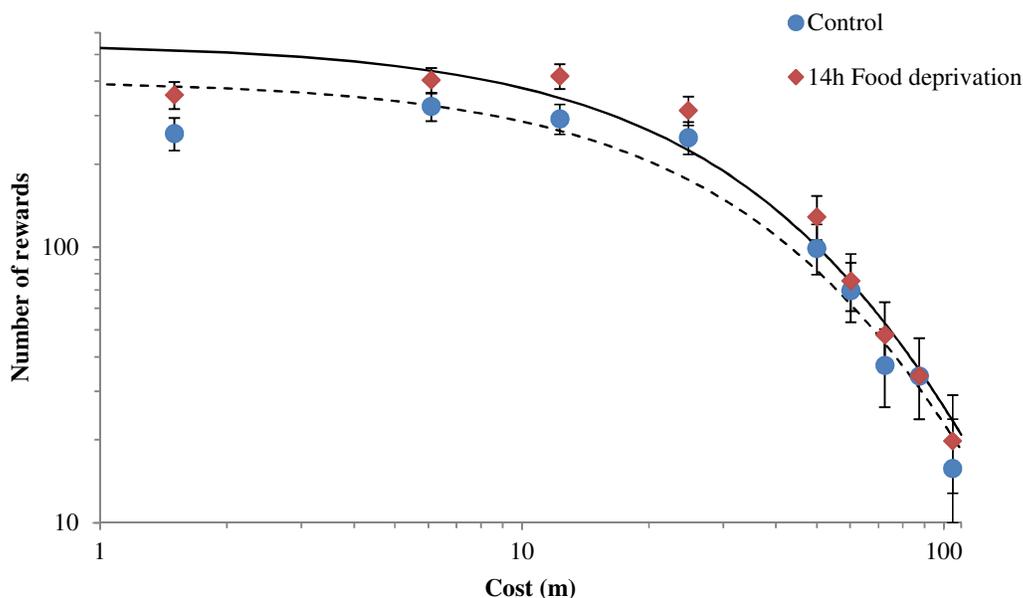
The maximum price paid (MPP) could not be determined as only 25% of the sheep actually reached this point (stopped walking for food). However, assuming linear increases, the extrapolated MPP was estimated at  $153 \pm 18\text{m}$  and  $211 \pm 33\text{m}$  for the control and food deprived treatments, respectively ( $P>0.1$ ). The exponential demand model revealed significant treatment differences (Figure 3.4), with the alpha value (reinforcing strength of a reward) being lower in the food deprived treatment than the control treatment ( $P<0.05$ ; Table 3.2). (A lower alpha value indicates a higher essential, or reinforcing, value). This model also showed significant differences ( $P<0.05$ ) in the strength of the reward between the cycles indicating an interaction between treatment and time; however, no other interactions were shown.

**Table 3.2 Summary data of the linear-elasticity and exponential demand models given as mean  $\pm$  SEM.**

		Treatment		P value
		Control	Restricted	
Linear-elasticity model	Mean $P_{\max}$ (m)	29.8 $\pm$ 2.9	29.9 $\pm$ 3.1	0.98
	Mean $O_{\max}$ (m)	6,005 $\pm$ 905	7,812 $\pm$ 1,090	0.35
Exponential demand model	Alpha value ( $\alpha$ )	1.28E-05 $\pm$ 1.37E-06	1.03E-05 $\pm$ 1.35E-06	0.02



**Figure 3.3 Linear-elasticity demand curve with the back transformed mean number of rewards shown as a function of cost on a log-log scale for the control (blue circle, dotted line) and 14h food deprivation (red diamond, solid line) treatments.  $P_{\max}$  estimates are represented by open boxes ( $n=8$ ).**



**Figure 3.4** Exponential demand curve with the back transformed mean number of rewards shown as a function of cost on a log-log scale for the control (blue circle, dotted line) and 14h food deprivation (red diamond, solid line) treatments ( $n=8$ ).

### **Part 2** Assessing the impact of energy balance on motivation for food

The energy balance data (Figure 3.5) indicated that all sheep, in both treatments, initially sustained a state of positive energy balance (energy gain) but were in negative energy balance (energy deficit) at the higher costs (24.8m and above). The energy balance differences between treatments at the 1.5m cost was found to be significant ( $P<0.05$ ) and there was a tendency towards significance at the 6.1 and 12.3m costs ( $P=0.057$ ,  $P=0.058$ , respectively), with the food deprived sheep maintaining a bigger energy surplus at these costs. No significant differences were found between the individual cost levels at any of the longer costs (above 40m). The estimated cost at zero energy balance (point at which sheep moved from a positive to a negative energy balance, or where energy consumed equalled energy expended) was  $31.1 \pm 2.9\text{m}$  for the control treatment and  $38.5 \pm 3.4\text{m}$  for the food deprivation treatment (Figure 3.5).

The average distance walked by a grazing sheep over a 24h period is approximately 10km (Squires, 1976). Calculating the energy required for maintenance and activity, and given the amount of energy available per reward, it was estimated that the sheep would reach

a point of zero energy balance between the costs of 24.8 and 50m. As the mean distance walked was less than 10km;  $4,114 \pm 799\text{m}$  for the food deprived treatment and  $3,247 \pm 658\text{m}$  for the control treatment, these estimates were re-analysed. Using 4,000m as the average walked in one test period and the energy available to the sheep on a 'per reward' basis, the new estimates indicated that the sheep would reach a point of zero energy balance between the 12.3 and 24.8m costs.

Mean costs at  $P_{\text{max}}$  (cost of maximum responding) and at zero energy balance (where energy expenditure equalled energy consumed) were calculated and ANOVAs were performed on the resultant data to test whether the costs at  $P_{\text{max}}$  and at zero energy balance differed between treatments (Table 3.3). This analysis indicated that the cost at which  $P_{\text{max}}$  occurred was not significantly different to the cost at zero energy balance for the control and food deprivation treatments ( $P > 0.1$ ;  $P > 0.5$ , respectively).

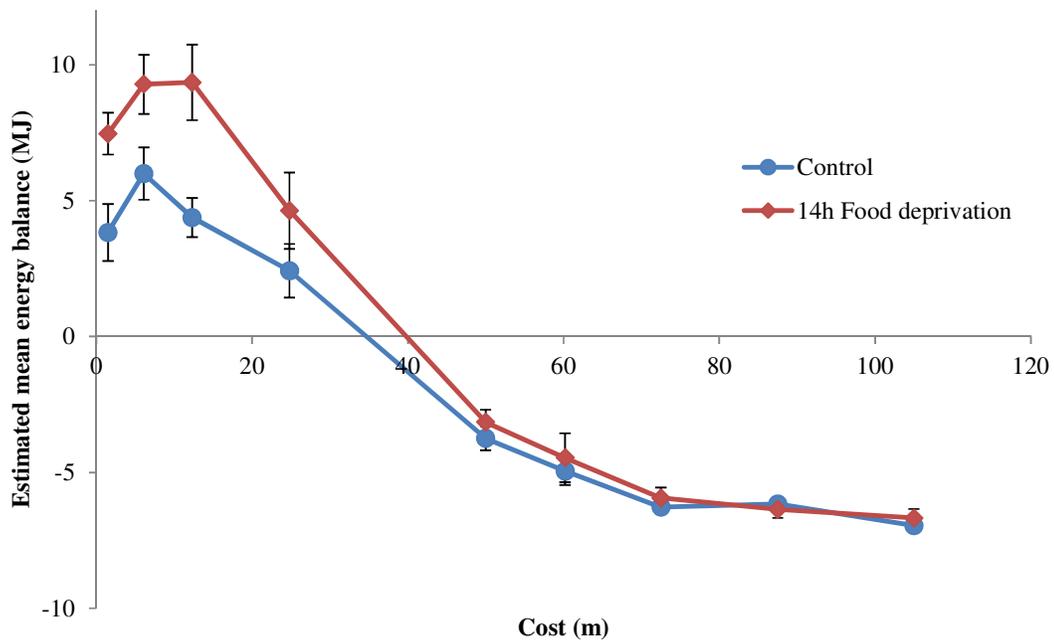


Figure 3.5 Estimated mean energy balance ( $\pm$  SEM) at each cost level for control (blue, circle) and 14h food deprivation (red, diamond) treatments ( $n=8$ ).

**Table 3.3 Differences between the cost estimates at  $P_{\max}$  and at zero energy balance for control and 14h deprivation treatments ( $P>0.05$  for all comparisons).**

	Control	14h deprivation
Cost at $P_{\max}$ (m)	29.8	29.9
SEM	2.9	4.3
Cost at zero energy balance (m)	31.1	38.5
SEM	2.9	3.4

A *post hoc* assessment of the cumulative work rates (number of rewards in time period divided by the number of rewards in total period) of the sheep during testing indicated that in the 0-3h period the control treatment animals completed  $53 \pm 2.2\%$  of the total work done in the 23h test period, while the deprivation treatment animals completed  $74.4 \pm 2.4\%$  (Figure 3.6). After 9h, the control treatment had completed  $69 \pm 2.7\%$  compared to  $87.5 \pm 2.1\%$  for deprivation treatment. The differences between the work rates of the treatment groups remained significant until 12h ( $P<0.01$ ) when compared with a Chi-squared test following a square root transformation. To further investigate the eating patterns and intake rates within the test period an analysis of the work rate in 3h intervals, accounting for cost levels, was undertaken (Figure 3.7). This showed that both the control and the deprivation treatment animals exhibited similar patterns of work rates irrespective of the costs involved. For example, the mean amount of work done in the control treatment across all costs in the 0-3h time period was  $12.7 \pm 3.4\%$ , compared to  $17.1 \pm 4.4\%$  for the deprivation treatment ( $P>0.1$ ).

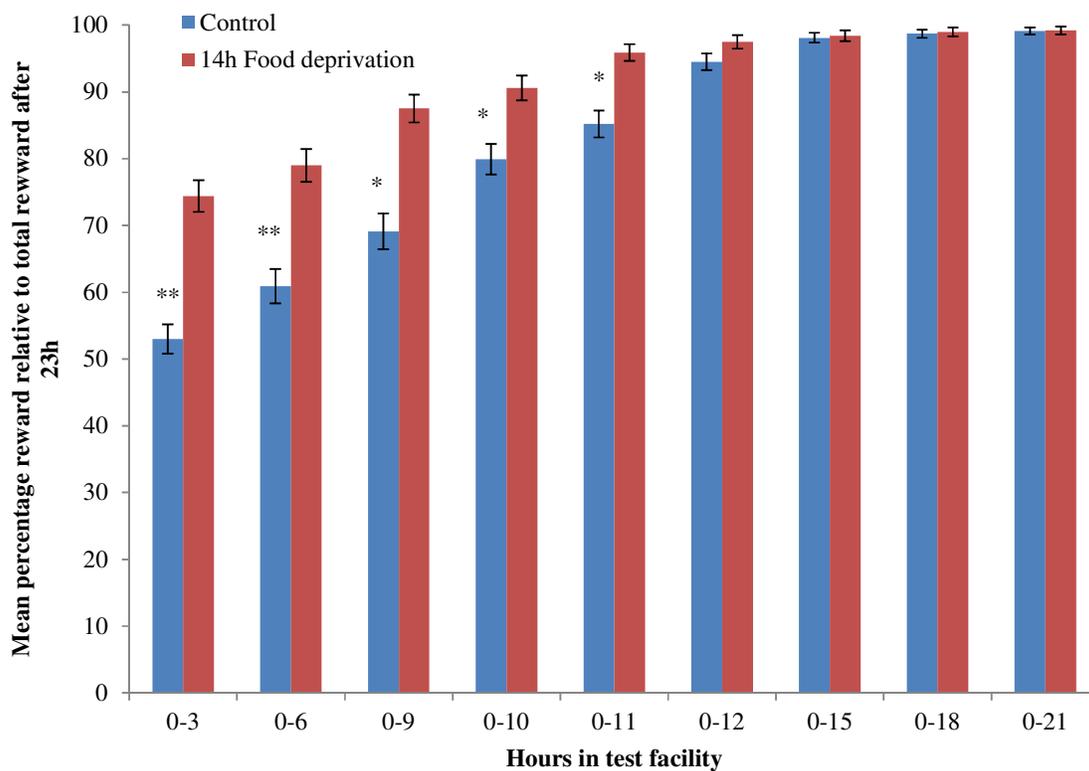
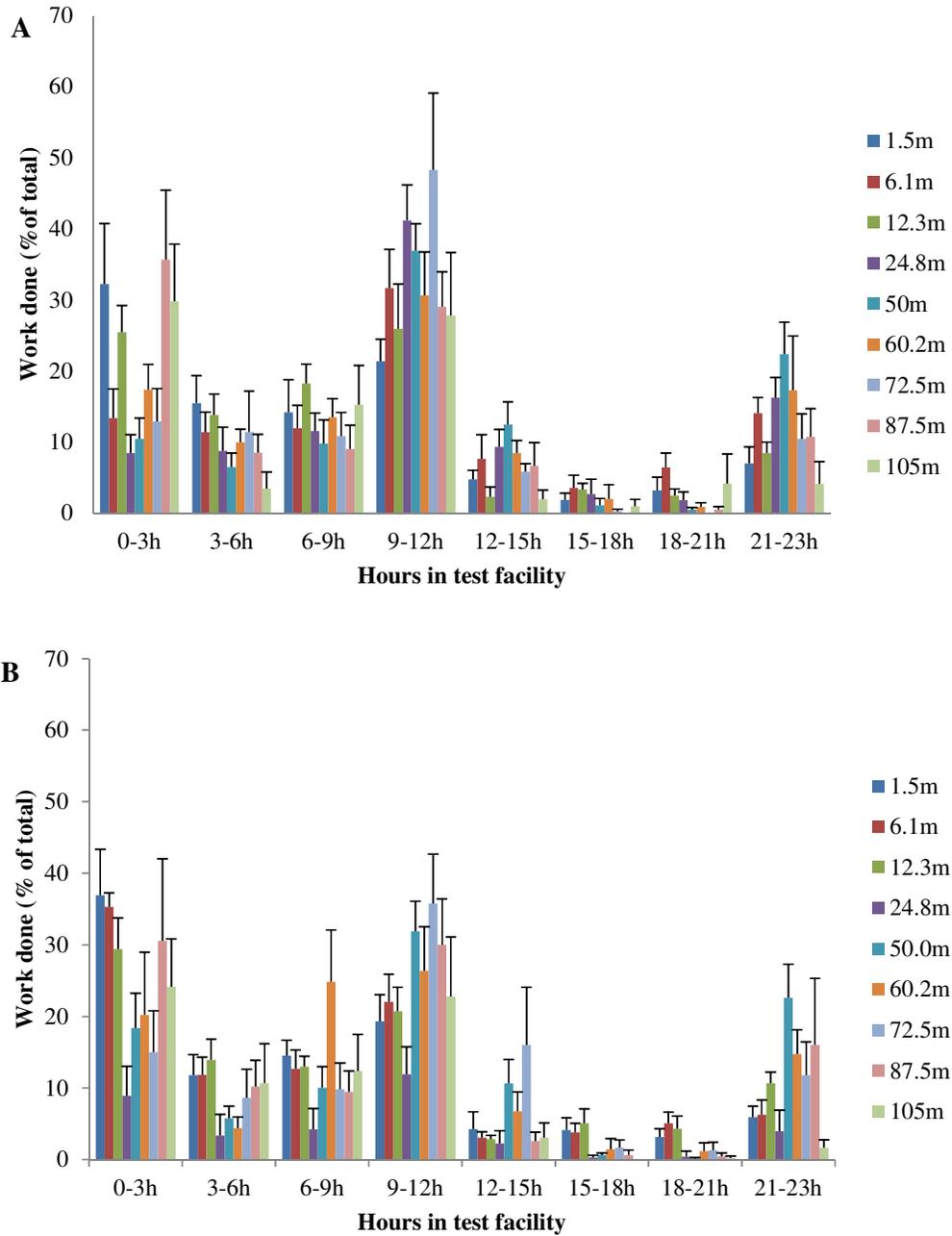


Figure 3.6 Mean cumulative percentage reward ( $\pm$  SEM) relative to total reward for control (blue) and 14h deprivation (red) treatments ( $n=8$ ). Significance levels are as indicated \*  $P<0.01$ , \*\* $P<0.005$ .



**Figure 3.7** Mean interval work rates (+SEM) given as a percent of the work completed over the total test period for the control (A) and 14h food deprivation (B) treatments ( $n=8$ ).

### 3.5 Discussion

#### **Part 1      *Comparing behavioural demand analysis methods***

The aim of this part of the experiment was to compare two models used to analyse behavioural demand curves; linear-elasticity and exponential demand, and within this to compare different measures to ascertain if one model or measure could more accurately identify differences in the changes in motivation of sheep for food. No significant treatment differences between the  $P_{\max}$  or  $O_{\max}$  parameters were observed. Similarly, non-significant differences in  $P_{\max}$  were also described by Verbeek et al. (2011; 2012a). However, these authors reported that  $O_{\max}$  was almost twice as large in restricted compared to control sheep. The two studies differed in the amount of time allowed for food restriction and it is possible that the 14h deprivation in the current study was not sufficient to cause an increase in feeding motivation, similar to that shown in the Verbeek et al. study.

In contrast to the linear-elasticity model, significant treatment differences were observed when the data was analysed with the exponential model. These results indicate that the deprived animals found the food to have a greater essential value than the control animals. This suggests that the exponential demand model may be more sensitive to small changes in willingness to work, and perhaps more accurately measures the reinforcing strength of a resource or reward. As this method analyses data by scaling the rate of decline of the curve and provides a value that is independent of the reward size or type (Christensen et al., 2008a; Christensen et al., 2008b; Hursh and Silberberg, 2008), it is possible that this type of analysis may be particularly useful in an animal welfare context as it would allow two different but competing resources to be compared.

The results also indicated that cost was the only factor that, overall, had a significant impact on feeding motivation while the number of rewards consumed did not differ between treatments. Verbeek et al. (2011) reported similar findings in regard to cost. However, in contrast to the current study, these authors reported an overall significant difference between control and feed restricted treatment groups with the feed restricted groups consuming more food rewards than the control group. The results of the current study did show trends following the same pattern but only at the shorter costs (less than 25m). Verbeek et al. (2011) also reported that sheep on a 24h food restriction treatment walked significantly further than

animals on a maintenance control treatment. Again, this disparity is probably associated with the difference in the duration of food restriction.

In this study, MPP could not be measured for 75% of the sheep as only 25% of the animals stopped walking for access to food. There is a lack of feeding motivation studies using MPP in ruminants, as to date, most have focused on monogastrics. For example, Bokkers et al. (2004) assessed the demand of broiler poultry for food under varying motivation schedules, Cooper and Mason (2000) and Mason et al. (2001) assessed the willingness of American mink (*Mustela vison*) to pay for access to various resources (water bath, novel object, raised platform) and Kirkden and Pajor (2006b) quantified the motivation of gestating sows for group housing. These studies were able to use the MPP parameter to estimate the strength of the animal's motivation for specific resources; however, the estimate of MPP in relation to feeding motivation may be more problematic in ruminants given the constant supply of energy, which is retained in the rumen.

The estimate of MPP in this type of motivation study also raises some practical issues. Our experiment relied on a natural operant task (walking) to assess motivation for food and this was problematic as sheep simply did not stop walking. The extrapolated data indicates that to reach the MPP for all sheep the facility would have had to be extended a further 70m and the experiment would have taken considerably longer to complete. However, using the same operant task we were able to ascertain values for the other measures and this indicates that practical constraints should be considered when deciding on which parameters to use to assess the motivation of a natural behaviour. When assessing feeding motivation in ruminants, it may be necessary to consider trade-offs between measures that assess the desired trait with financial, labour, time and infrastructure constraints. It should be noted that the 14h deprivation period was chosen in the current experiment as it was thought to be a sufficient compromise between restricting the sheep so that an effect on their motivation for food could be seen, and not restricting the animal so greatly that demand for food would continue past the space limitations (105m) of the facility.

## **Part 2      *Assessing the impact of energy balance on motivation for food***

This section of the study aimed to test the hypothesis that an energy deficit, caused by a 14h period of food deprivation, would increase the motivation of sheep to work for food. At

the shorter costs (1.5-12.3m) the results from our study showed trends that were consistent with this hypothesis. These results also agree with data from other studies. For example, Dumont et al. reported that ewes were more reluctant to walk for access to a good quality hay in a longer walking system (46m), when compared to a shorter walking system (23m). The authors also noted that the sheep changed preferences from the good quality hay to the freely available poorer quality hay as the reward size dropped from 32g to 2g.

It is possible that the behaviour patterns seen in the current experiment may be partially explained by optimal foraging theory. This theory hypothesises that the cost associated with energy gain will alter the feeding choices that an animal makes as the animal attempts to maximise the net energy gain per unit of feeding time (Pyke, 1984). The main cost to a grazing animal is that of walking (Osuji, 1974), and the results from the current experiment indicate that at the shorter costs an energy gain maximisation framework could be a factor in determining the willingness of sheep to work for food. To some extent, the sheep appeared to be able to optimise their foraging behaviour under test conditions by increasing their work rate at shorter costs to gain more food rewards. However, as discussed below, the behaviour shown at longer costs (greater than 25m) indicates that the animals were displaying suboptimal foraging patterns, so it is unlikely that foraging theory can be used to fully explain motivation for food.

We considered the possibility that the sheep may stop walking at or around a point of zero energy balance as calculating the estimated theoretical energy changes showed that at costs of greater than 50m, the energy expenditure of accessing food would outweigh the energy gain. This seemed to be a reasonable assumption as, from the perspective of foraging theory, an animal's food selection and the amount of food consumed are essentially determined by metabolic needs (Schütz et al., 2006), and obtaining sufficient energy is one of the main motivations underlying food intake (Toates, 1986). However, the animals did not stop walking at costs above 50m, and only 25% of the experimental sheep stopped walking at the highest cost (105m). This indicates that the sheep continued to work for food even though they were in an energy deficit. Similar findings have been observed in both sheep (Verbeek et al., 2011) and sows (Hutson, 1991). However, it should be noted that the energy balance calculations for this experiment are estimates and include only the energy consumed and expended within the 23h test period. Further studies will be needed to verify this finding and

it may be useful to explore different definitions of energy balance that include energy reserves such as the use of glycogen from the liver and muscle, and fat reserves.

Accounting only for the energy consumption and expenditure occurring within a test period appears to be the usual methodology when using optimal foraging theories (e.g. Ginane et al., 2002). Even so, our results are in direct contrast with a study undertaken in lactating and non-lactating dairy cows (Schütz et al., 2006). This study provided the dairy cows with access to a small food reward (approximately 30g) which could be continually accessed by walking 100m in a laneway. The reward size was calculated so that if the cow walked less than 100m it would be in a positive energy state while walking further than 100m would give a state of energy loss. The authors reported that both the lactating and non-lactating cows seldom walked further than 100m. This indicated that they were choosing not to spend more on energy than was available from the reward and therefore, were behaving in accordance with optimal foraging theories (Schütz et al., 2006).

There are several possible explanations that might provide a reason for the continued negative energy balance of the sheep in our experiment. One is that the sheep were likely to have a limited time budget to spend on foraging and eating and there may have been a trade-off between the walking involved for food acquisition and other activities, such as resting (Houston and McNamara, 1989). Rest has been found to be very important to ruminants (Metz, 1985; Jensen et al., 2005) and penned sheep can rest for up to 15h per day (Bøe et al., 2006; Bøe and Andersen, 2010). It may be that the sheep in this experiment spent the maximum amount of time available to them in the test situation on acquiring food; however, this was not enough to allow them to remain in a state of continued energy surplus. The cumulative work rate data collected in the experiment showed the sheep performed 95% of the work they would do over the 23h period in the first 12h. This pattern remained the same regardless of the cost involved. As there were still 11h remaining in each test period, there was time for the animals to undertake more work to acquire food. However, it is possible that in this situation a trade-off may have occurred between obtaining food and other necessary activities. Video footage taken over the duration of the experiment was unable to provide further insight into activities in the 11h 'reduced work' period. This is because only the first 15m of the lanes were in camera range and sheep were found to be outside the field of view of the camera for long periods of time. The sheep may have been able to optimise their time

spent foraging if they were able to adjust the intake rate at which the food acquisition occurred (Houston and McNamara, 1989; Newman et al., 1994). However, as the reward size was kept constant, the sheep were only able to exert control over the intake rate by increasing the time spent walking for food, or by increasing the walking speed. As mentioned above, the sheep may not have been able to increase the time spent walking for food due to the time constraints of other activities and approach speed to the feeding station was not measured in our experiment. However, latency to feed (as measured by time taken to approach the feeder) has been shown to increase in sheep when costs become more time consuming (Dumont et al., 1998; Verbeek et al., 2011), and this may also have occurred in our experiment.

Another possible interpretation for the continued negative energy balance is related to the methodology of our experiment. Schütz et al. (2006) reported that the dairy cows in their experiment seldom spent more energy on costs than was available through the food reward. However, these animals appear to have been tested under an open economy. A typical open economy using food involves a short experimental session. This is either followed or preceded by the opportunity for the animal to consume enough food so that a given percentage of its body weight can be maintained. This is in contrast to a closed economy where the animal has the opportunity to work to access food for a longer period of time, usually throughout the day (Houston and McNamara, 1989), and is not supplemented to maintain body weight. The dairy cows that Schütz et al. (2006) used were taken off pasture and tested during a 4h period in the afternoon. This contrasts with our experiment where food could only be accessed during the test period. It may be that when food deprivation and the subjective experience of hunger are involved, the animals in a closed economy maintain a higher work rate than those in a shorter duration, supplemented test. Furthermore, it is possible that the foraging behaviour and associated motivation for food under short test durations (e.g. minutes to hours) is different to feeding motivation when diurnal variation is accounted for. Due to diurnal variation in feeding, short tests may not cover periods when feeding motivation is high, and thus, animals may not achieve satiation during these tests.

Another methodological reason for the sustained negative energy balance may be that the data collected provided a false estimation of the energy surplus and expenditure of the experimental animals. Not accounting for the energy gained or expended during the days prior to the test, or the animal's actual energy reserves may have influenced the results. It is

possible that if we had continued the test further than the 23h period, the sheep may have eaten enough in the longer term to maintain, or re-enter, a positive energy balance. This is an area worthy of further investigation.

A final reason that may partly explain the sustained energy deficit during this type of test situation is that the actual process of food consumption may elicit a pleasurable reward, or hedonic response (Ginane et al., 2002). The opioid and dopaminergic systems have been found to play an important role in feeding and the pleasurable reward associated with food intake (Cleary et al., 1996). Much of the literature regarding the action of the opioid and mesolimbic dopaminergic systems relates to addiction and obesity and has been carried out predominantly in rodent species (e.g. Schaefer et al., 1994; Naleid et al., 2007). This body of literature suggests that strong relationships exist between opioids and changes in food intake (Koch et al., 1995; Glass et al., 2000; Barbano and Cador, 2006; Barbano et al., 2009). Despite this, Berthoud (2007) has noted that investigations relating to the links between cognitive and emotional neural processes and energy balance have received little attention. The author hypothesised that information from the cognitive and rewarding brain systems may, at times, override homeostatic regulation of food intake, and it may be that this was occurring in our experiment.

The results from this experiment also indicated that the costs at  $P_{max}$  and at zero energy balance were similar and were between 30 and 39m, irrespective of the treatment. This suggests that the work output of the sheep began to decline around the point of zero energy balance. These findings may point to the presence of metabolic feedback, possibly with feedback cues derived from nutritional parameters, influencing the motivation to work for food. The presence of a relatively fast acting metabolic feedback system is perhaps harder to reconcile in ruminants when compared to monogastrics due to the constant supply of energy available from the gut. Furthermore, it is difficult to precisely identify which signals or regulators may be involved. Additional investigation will be necessary to determine if this effect is real and to provide greater insight into what mechanisms may be underpinning the change in feeding motivation.

We also aimed to investigate whether a shorter period of testing would be able to differentiate between levels of feeding motivation in animals exposed to different food

restrictions. We found that the differences between the treatments were significant when assessed within the first 11h; however, as mentioned above, no feed deprivation treatment differences were found over the 23h period. This indicates that the work done in a behavioural demand test of 12h may provide a good representation of the total work done as well as the treatment differences present after 23h. However, the duration of the test appears to influence whether significant treatment differences can be detected and this should be considered in future work. The pattern of intake also appears to follow a circadian cycle, and this may be as expected as the sheep has access to the food rewards for a 23h period. However, this was a retrospective analysis so further investigation into the intake cycles was not possible.

In conclusion the  $P_{\max}$ ,  $O_{\max}$  and MPP measures and number of rewards consumed did not reflect significant treatment differences in feeding motivation. In the case of  $P_{\max}$  and  $O_{\max}$  this may have been due to issues such as the length of the food deprivation and the suitability of the measures to assess changes in feeding motivation. The use of MPP was also not particularly useful in this study. This was due the sheep continuing to access food rewards even at the longest cost of 105m. This is an issue that may arise when requiring animals to perform a natural behaviour as an operant task, particularly when combined with practical and physical constraints of the experiment. Given this, it is not possible to make a definitive comment on the usefulness of these measures without further data. However, this investigation has indicated that the exponential demand model appears to be more sensitive to detecting small changes in a resource's value than either the linear-elasticity measures or the quantitative measures (MPP and number of rewards consumed). Therefore, this method of analysis may potentially be useful for future animal welfare studies as it provides a seemingly sensitive value that is independent of reward size or type.

Overall, the willingness of the sheep to walk to access food decreased with increasing costs. The feed deprivation treatments showed significance, or patterns trending towards significance, at the shortest costs (1.5, 6.1, 12.3m) only, rather than showing overall differences as was hypothesised. This is likely due to the short (14h) length of the feed deprivation treatment. However, the sheep continued to walk when in an energy deficit. There are several possible interpretations of this behaviour and further work will be needed to ascertain which, if any, of our hypotheses are correct. This experiment also found that sheep

decreased the work done at the estimated point of zero energy balance which may indicate the presence of a metabolic feedback system influencing motivation to work for food. This also needs further validation before conclusions can be made with regard to the possible mechanisms that may be determining this change and any implications this may have on an animal's welfare or on the assessment of welfare. It also appears that significant treatment differences can be ascertained when shortening the test length from 23h to 12h. However, it seems unlikely that this will result in faster testing protocols as sheep still need to be placed in the test facility early in the morning to ensure daily feeding cycles are accounted for. Nonetheless, overall significance levels differed across time so the test length in the design of future experiments would need to be given careful consideration.

## Chapter 4

# The influence of energy density on feeding motivation



## 4.1 Abstract

Intake of food by ruminants is controlled by a wide range of internal and external variables and the requirement for energy is one of the most important of these determinants. Therefore, gaining a better understanding of the role of energy in determining an animal's motivation for food is likely to be important when attempting to understand factors which influence feeding motivation. This experiment aimed to test the hypothesis that alterations to the energy density of a food reward would alter the motivation of sheep to access that reward. Behavioural demand analysis of the data was used to assess differences in the strength of motivation. Ten mature Merino wethers were tested to see how many times in a 20h period they would walk a predetermined distance (cost) to access a 4g food reward. In each test run the sheep were offered either a low energy (LE) pellet (48kJ/4g reward) or a high energy pellet (61kJ/4g reward) and food type was tested for both treatments at 5 out of 7 of the following costs (1.5, 10, 20, 30, 45, 60, 75m). We found that, overall, energy density did not affect the distance walked ( $P>0.1$ ) and there was no cost by energy density interaction ( $P>0.1$ ), although all animals reduced the number of rewards consumed as the cost increased ( $P<0.001$ ). Further analysis showed that at the lowest cost of 1.5m, sheep exposed to the LE treatment worked harder and consumed more rewards than sheep given the HE alternative ( $P=0.02$ ). Demand functions fitted to the data demonstrated that the cost at which maximum responding occurred ( $P_{\max}$ ), the maximum output at that cost ( $O_{\max}$ ) and the alpha value (reinforcing strength of a reward), were not sensitive to changes in motivation. The energy balance data (the difference between energy consumed and energy expended) showed that the sheep were willing to sustain an energy deficit to gain additional access to food, and this was not dependent on treatment ( $P>0.1$ ). In conclusion, we found a trend indicating that sheep were able to differentiate between food of varying energy densities at the 1.5m cost in the behavioural demand environment. However, they were not willing to maintain a high level of responding for one food type over another as costs increased. This may be due to both feed types providing adequate energy to meet daily intake requirements. That sheep were willing to continue walking when in a state of energy deficit helps to validate previous results, although the actual mechanisms or inputs providing feedback to the sheep, allowing them to alter their behaviour, still require further investigation.

## 4.2 Introduction

The intake of food is controlled in an integrated manner and involves energy-sensing mechanisms as well as bulk-sensing mechanisms, nutrient levels, disease, environmental conditions, and social pressures and influences. This integration allows food intake to match energy output so that an animal's nutritional and energetic requirements can be met. Although the phrase 'animals eat for calories' is not true in ruminants under all circumstances (Forbes, 2007), energy intake is proposed to be one of the major determinants of food control (Forbes, 2003). Therefore, gaining a better understanding of the role of energy in determining an animal's motivation for food is likely to be important when attempting to understand factors that influence feeding motivation in a behavioural demand test.

Ruminants have the ability to differentiate between food containing various macronutrients (protein, fats and carbohydrates) and when offered complementary diets have routinely been found to ingest a diet with a constant energy-to-protein ratio (Provenza et al., 1996; Wang and Provenza, 1996; Villalba and Provenza, 1999a; Scott and Provenza, 2000). It may be useful to use this ability to further investigate the role of caloric intake in determining motivation for food; specifically to investigate the impact energy density has on the feeding motivation of sheep. Several studies have been reported in livestock species assessing changes in the level of motivation for various dietary components using behavioural demand techniques (Robert et al., 1997). For example, Ramonet et al. (2000) used an operant task (pressing a switch for access to food) to measure the feeding motivation of pregnant sows where three food types with similar metabolisable energy (ME), but differing fibre content, were accessible. Studies investigating the differences in feed types in ruminants have also been undertaken by Dumont and Petit (1995) and Dumont et al. (1998). This group investigated how ewes made choices between different amounts of good quality hay, which was available after walking a specified distance, with poorer quality hay, which was freely available. The authors found that the quantity of reward affected the motivation levels as the ewes changed from the high quality feed to the poorer quality when the distance to the high quality feed increased (Dumont et al., 1998). The same experimental methodology was used in a cattle study with similar results (Ginane et al., 2002), indicating that ruminants can recognise differences between foods in a behavioural demand setting and, consequently, alter behaviour as their motivation changes. However, these experiments used operant

techniques to test the motivation of the ruminant for an overall food type, good versus poor quality, rather than one specific component or macronutrient contained within the food.

The overall palatability of food may also play a role in ruminant preference, and corresponding motivation, for one food over another. However, palatability in ruminants does not simply take into account the taste, smell, texture or appearance of the food, but also the animal's previous experience with the food and current metabolic status (Ginane et al., 2011). Ruminants have generally been found to develop preferences for feeds that rapidly provide high levels of energy and satiety. Therefore, it has been concluded that palatability integrates the energetic and nutritive value of the food as well as the sensory response of the animals (Baumont, 1996).

It is possible that of the amount of work required to gain a food reward and the size of the food reward may confound the measure of motivational strength. However, it is also possible this problem may be avoided by assessing the unit price of the reward. In economic terms the response requirement and reward magnitude interact to determine the consumption of a specific resource, with the interaction quantified as the unit price (Hursh, 1980). In other words, the ratio of costs over benefits, or operant response over reward size, is defined as the unit price (Greenwald and Hursh, 2006). One of the predictions made by economic theory in regards to unit price is that total consumption and total work done should be determined by the value of the unit price, independent of its cost and benefit components (Madden et al., 2000). For example, if three pellets of food were delivered after 30 operant responses and 5 pellets of food were delivered after 50 responses the unit price (10) would be the same and test subjects would work equally hard to obtain the reward in both cases. It is also noted that if given a choice between unequal unit prices, such as 30 responses for 3 pellets (unit price of 10) and 60 responses for 3 pellets (unit price of 20), the lower unit price should be preferred. The use of unit price has not previously been associated with many investigations involving motivation in animals, particularly within an animal welfare context, as the majority of work has been undertaken on drug addiction in humans (e.g. Bickel et al., 1991; Greenwald and Hursh, 2006). Therefore, this experiment provided an opportunity to assess the use of unit price in measuring the motivational strength of a ruminant for food.

Consequently, the purpose of the present experiment was to further investigate the role of energy balance in feeding motivation by determining if altering the energetic profile of a food reward could alter the motivation to work for that reward. The experiment aimed to test the hypothesis that sheep would be able to recognise the differences in energy levels within the two types of food and would work harder for access to the food that was higher in energy. The number of rewards consumed and measures derived from a demand function curve, as well as unit price, were used to assess differences in motivational strength.

### 4.3 Materials and methods

The materials and methods used in this chapter are similar to those more fully described in Chapter 3. To avoid repetition, only the differences in the material and methods between the previous experiment (Chapter 3) and the current experiment (Chapter 4) have been described.

This study was approved by the CSIRO's Animal Ethics Committee and conformed to the standards in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Animal Research Authority Number 10/19).

#### 4.3.1 Animals and housing

Twenty, mature Merino wethers were divided into a group of 10 experimental animals and a group of 10 companion animals. (An additional 2 animals were added to the experimental group to improve the validity of the statistical analysis.) The mean weight of the 10 experimental sheep was  $54.0 \pm 1.7$ kg with a body condition score of  $3.3 \pm 0.06$  (scale of 1-5). During testing all sheep were maintained in three outdoor pens (16 x 12m) and moved freely between the pens. Before beginning the experiment it was determined that the sheep were able to maintain both their weight and body condition on a diet of 100% of theoretical maintenance for mature (non-growing) animals (AFRC, 1995; Primary Industries Standing Committee, 2007), and so were provided with this ration on non-test days. On these days all sheep were fed a basal diet of a lucerne-based total ration pellet (referred to as Animal House (AH) pellets; Table 4.1) and lucerne chaff (ME= 10.4MJ/kgDM, DM =83.6%, CP =23.6% of DM) in an 80:20 ratio. Feeding occurred once daily at approximately 8:00am for non-test animals. On test days experimental sheep were provided with *ad libitum* access to either the

‘low’ energy ration (LE) or the ‘high’ energy ration (HE; Table 4.1) within the test facility for a 20h period.

For a period of 1 month during the training process sheep were slowly introduced to the HE and the LE food types. These pellets were mixed in equal amounts with the AH pellets. Individual animal observations of the consumption of the HE and LE feeds were also undertaken to ensure that each sheep was consuming both the LE and the HE feed.

The AH pellets were commercially manufactured (Ridleys, Tamworth, NSW), while the LE and HE pellets were made at the University of New England feed mill. All pellets were 4mm in diameter and approximately 7-10mm in length. Unfortunately, there was significant difficulty in manufacturing the low energy pellets. This was due to the lack of machinery available to produce a pellet that was high in fibre and low in energy and with all other nutrients kept as constant as possible. Keeping macronutrient levels (e.g. protein) of the two feed types as similar as possible was important to avoid introducing confounding variables that may have affected the palatability of the food. Various ingredients (e.g. cottonseed hulls), formulations and pellet sizes were tested prior to the final LE pellet being produced. Due to these manufacturing difficulties the final metabolisable energy (ME) value of the LE pellet was higher than originally intended (Table 4.1).

**Table 4.1. Summary and analysis of the feed types used during the experiment.**

<b>Feed Type</b>	<b>Animal House (AH) pellets</b>	<b>Low energy (LE) pellets</b>	<b>High energy (HE) pellets</b>
Ingredients	Lucerne	Lucerne pellets	Lucerne pellets
	Wheat	Copra meal	Wheat
	Pollard	Hardwood sawdust	Vegetable Oil
	Bran		Urea
	Salt		
	Ammonium chloride		
Metabolisable energy (MJ/kg DM)	12.2	12.2	15.3
Dry Matter (%)	89.7	90.3	91.1
Crude Protein (%)	21.5	22.3	25
Crude Fat (%)	2.5	5.2	9.8
Acid detergent fibre (%)	16.4	29.4	8
Neutral detergent fibre (%)	30	44	18

#### **4.3.2 Facility and equipment**

The test facility has been described previously in Chapter 3.

#### **4.3.3 Habituation and behavioural demand training**

The sheep used in the current experiment were the same animals used in the experiment described in Chapters 3, so the habituation process was not required and the training schedule followed was the same as was described in Chapter 3. During the period between experiments the sheep were grazed on pastures and were fed AH pellets twice weekly.

#### **4.3.4 Experimental design**

An incomplete cost design was used whereby each of the 10 trial sheep were exposed to 5 costs, in random order, out of the 7 costs (distances) used, for each treatment. An incomplete cost design was used as an attempt to reduce the length of the experiment (when compared to the experiment described in Chapter 3), while not compromising the statistical validity of the experiment. The experiment described in Chapter 3 used 9 costs and, while this provided a large data set, it was found to be taxing in terms of the time and labour required to successfully complete the trial. Other behavioural demand experiments have successfully used 5 costs (Verbeek et al., 2011; 2012a), so a cost allocation was designed that would provide a similar quality of information, using fewer costs.

The costs used were 1.5, 10, 20, 30, 45, 60 and 75m. Each sheep was allocated a test run at the costs of 1.5, 30 and 75m for the HE and the LE treatments. Sheep were then randomly allocated to 2 of the 3 remaining costs (10, 20 and 45m), and so completed a total of 5 costs for the HE treatment and 5 costs for the LE treatment (10 test runs in total per sheep). The order of both the treatments and costs were randomly allocated. A recovery period of 4 days was maintained between tests to ensure that there was no carry-over effect from having been in the testing facility on the previous day.

A mean reward size of  $4.1 \pm 0.08\text{g}$  was given to the sheep at each rewarded event (a sheep successfully activating the feeding machinery and eating the amount of food provided). Following analysis of the feeds it was calculated that 4.1g of food equated to 61kJ per reward for the HE, and 48kJ per reward for the LE feeds (Primary Industries Standing Committee,

2007). These data was then utilised to estimate the point at which the animal would reach a state of zero energy balance (where energy consumed equals energy expended; see below).

#### **4.3.5 Behavioural demand testing**

The experiment was run from the 6<sup>th</sup> of May to the 3<sup>rd</sup> of July, 2011. On the morning preceding a motivation test, 2 experimental sheep were placed in individual pens (companions were always in visual and auditory contact) and remained in these pens for a 24h period prior to behavioural demand (BD) testing. During this time in individual pens, both sheep were given feeds comprising of AH pellets and chaff at 100% of theoretical maintenance at 7:00am. Residuals were measured and all sheep consumed 100% of the feed given.

At 6.00am on the morning of a test, both sheep were moved into their designated lanes and companion animals were moved into adjoining pens. At approximately 5:00am the day following the motivation test, the 2 test sheep were removed from the lanes and the feeder mechanisms were checked to ensure they were functioning correctly. The pellets remaining in the hopper were then removed and weighed. The feed trough was also checked for residual (food that had been earned but not consumed) and this was always empty. Cost was altered daily.

At the time of experimentation, the cold night time temperature caused moisture to freeze on the infrared beam reflectors. As a result the trial was stopped at the earliest time that the equipment became non-functional (2:00am), and the data recorded after this time was excluded from the analysis.

#### **4.3.6 Preference testing**

In an attempt to better understand the impact of the energy level treatment on the palatability of the food and consumption choices made by the sheep in the behavioural demand test, a series of individual animal preference tests were undertaken following the experiment. Preference testing was conducted on the 7<sup>th</sup> and 8<sup>th</sup> of July 2011, days 4 and 5 after completion of the experiment, and again on the 7<sup>th</sup> and 8<sup>th</sup> of September 2011, which was days 62 and 63 after the completion of the experiment. During the 3 days leading up to each preference test, sheep were maintained on pasture and were supplemented with a pellet

mixture comprised of equal amounts of the LE and HE food types. All 10 experimental sheep underwent 2 preference tests; the first in July and the second in September. The first preference test was designed to assess a base preference following testing and the second was to assess changes in preferences over time.

To test sheep preference for the two types of pellet, 1kg each of LE and HE pellets, were placed in separate feeders in individual pens. All sheep had previously been exposed to both the feeders and the pens. Both types of pellets were presented to the sheep in identical feeders that were placed 50cm apart. Half of the sheep were allocated HE feed placed in the right side feeder while the remaining sheep were allocated HE feed on the left side, and this was alternated for each sheep between test 1 and test 2. Sheep were then randomly allocated to testing order and test pen. The sheep were placed individually in the pens for 1h between 7:00am and 10:00am on the day of testing and given free choice of both feed types (at zero cost). Water was available at all times and companions were in constant visual and auditory contact. On completion of each test the sheep were removed from the test pen and the remaining feed was weighed. No sheep consumed all the food available.

#### **4.3.7 Statistical analysis**

The effects of energy treatment (pellet type), cost (distance walked), and the interaction between these on the number of rewards consumed (motivation) were tested using the residual maximum likelihood (REML) procedure in GenStat 14 (VSN International Ltd., Hemel Hempstead, U.K.). Treatment and cost were fitted as fixed effects as was run order (chronological order in which the tests were conducted) and lane. The individual sheep were fitted as the random effect with the number of rewards received fitted as the dependant variable. Number of rewards earned was transformed (square root) to improve normality of distribution. The data were then analysed by a REML procedure. Residuals were tested for heteroscedasticity and no evidence of serial correlation was found within these REML residuals.

The derivation of the  $P_{\max}$  (cost at which maximum responding occurs),  $O_{\max}$  (maximum work done) and alpha values (reinforcing strength of a reward) have previously been described in Chapter 3. The calculation of the energy balance estimates has also previously been explained in Chapter 3; however, in the current experiment 2 sets of

calculations were undertaken to estimate energy balance (HE food at 61kJ per reward and the LE food at 48kJ per reward), and theoretical maintenance was increased from 8.2MJ to 9.5MJ to account for the increase in weight of the sheep. The calculation of the cost at which zero energy balance occurred has also previously been described in Chapter 3.

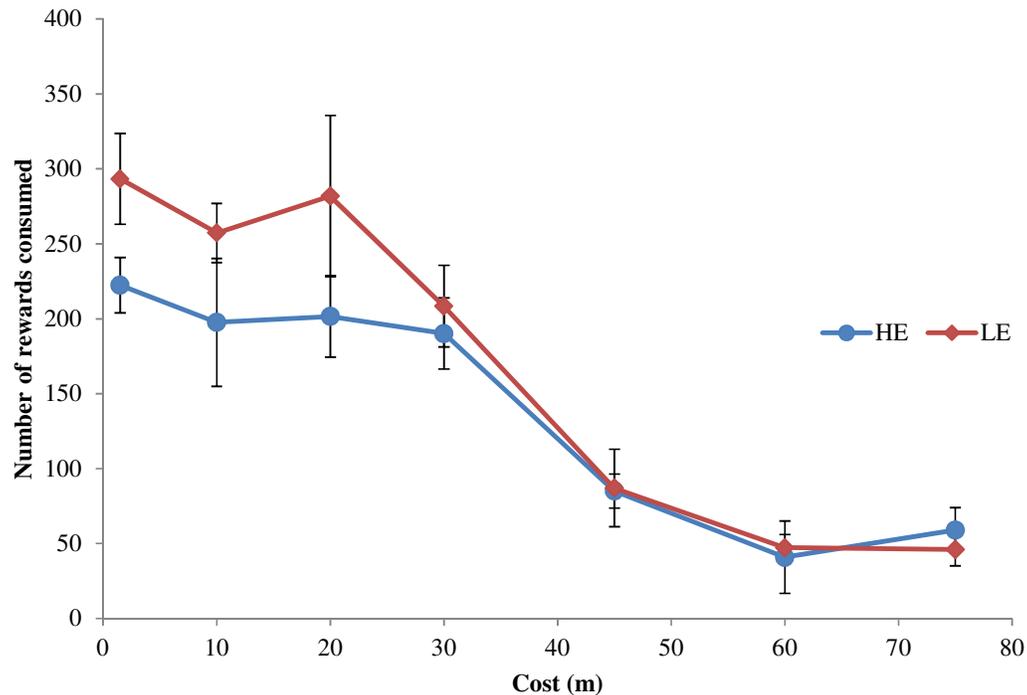
Significance levels between differences at  $P_{\max}$  and zero energy balance estimates were tested with a two-tailed  $t$ -test (GenStat 14). Cumulative work rate (a cumulative measure of the work done over the 20h test in 3h test periods e.g. work done in periods 0-3h, 0-6h) treatment differences were transformed (square root) and the differences between the treatments for each period were assessed with Chi-square tests. Interval work rates (work done over specific time intervals e.g. work done in 0-3h, 3-6h) were assessed using the REML procedure with treatment and cost as the fixed effects and animal as the random term. Unit price was calculated as the distance walked divided by the MJ consumed (m/MJ) and treatment differences were analysed using the REML procedure with cost and treatment as the fixed effect and sheep as the random effect. Additionally, an analysis of variance (ANOVA) procedure was used to check the unit price data produced from the REML analysis to ascertain if differences in the statistical procedures would alter the outcomes.

To analyse the preferences of the sheep, the raw data (amount (g) eaten per test) were transformed to a percentage ratio of HE:LE eaten in each test. Differences between the mean percentage ratios were then analysed using the two sample  $t$ -test procedure (GenStat 14). All data are presented as actual mean  $\pm$  standard error of the mean (SEM) unless otherwise stated.

#### 4.4 Results

All 10 sheep completed their 5 test runs for each treatment successfully and the average reward size was  $4.1 \pm 0.08\text{g}$  for both pellet types across the test runs. Mean rewards accessed across all costs for the LE treatment was  $177 \pm 17$  with a minimum of 4 rewards (at the 75m cost) and a maximum of 488 (at the 1.5m cost). For the HE treatment mean rewards accessed across all costs were  $147 \pm 13$  with a minimum of 2 rewards at the 75m cost and a maximum of 332 at the 1.5m cost.

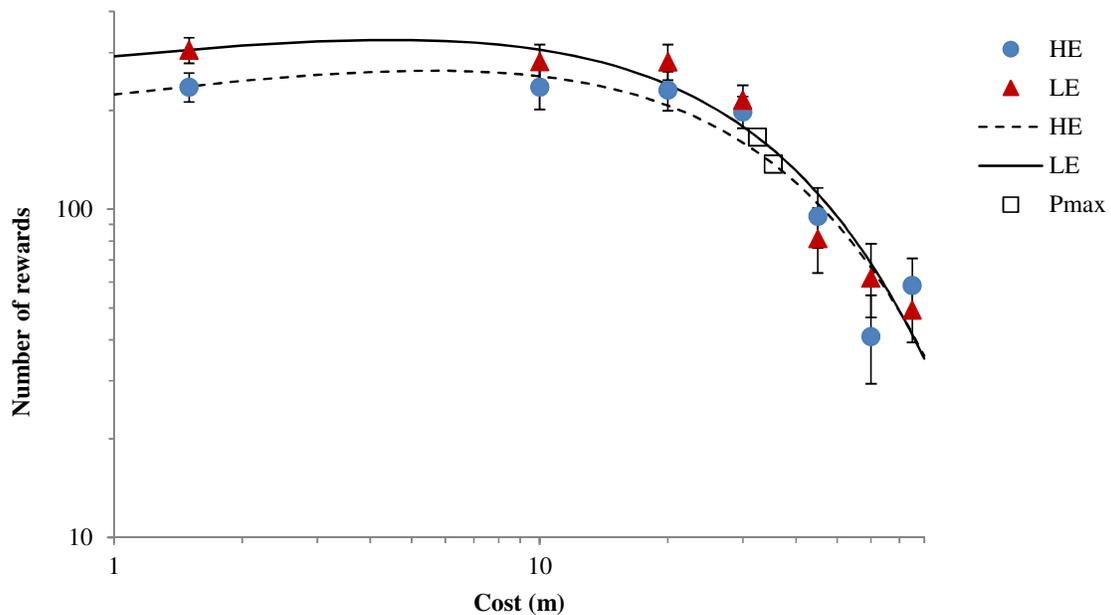
The cost had a significant effect on the number of rewards consumed with all sheep decreasing the rewards consumed as the cost increased ( $P<0.001$ ), although there were no significant effects of energy density on the total number of rewards earned or the total distance walked or any energy density by cost interaction. However, single cost contrasts showed a significant treatment difference at the 1.5m cost ( $P<0.05$ ) with sheep exposed to the LE treatment collecting a higher number of rewards at this cost compared to the HE treatment (Figure 4.1). A similar trend, although not significant, was also apparent at the 10 and 20m costs ( $P<0.1$ ). No other significant pair differences between treatments at different costs were found, nor was there any impact of the chronological order in which the tests were conducted, or a significant effect of the test lane used ( $P>0.05$ ).



**Figure 4.1** Mean number of rewards earned ( $\pm$  SEM) for each cost level at the low energy (LE, red diamond) and the high energy (HE, blue circle) treatments ( $n=10$ ).

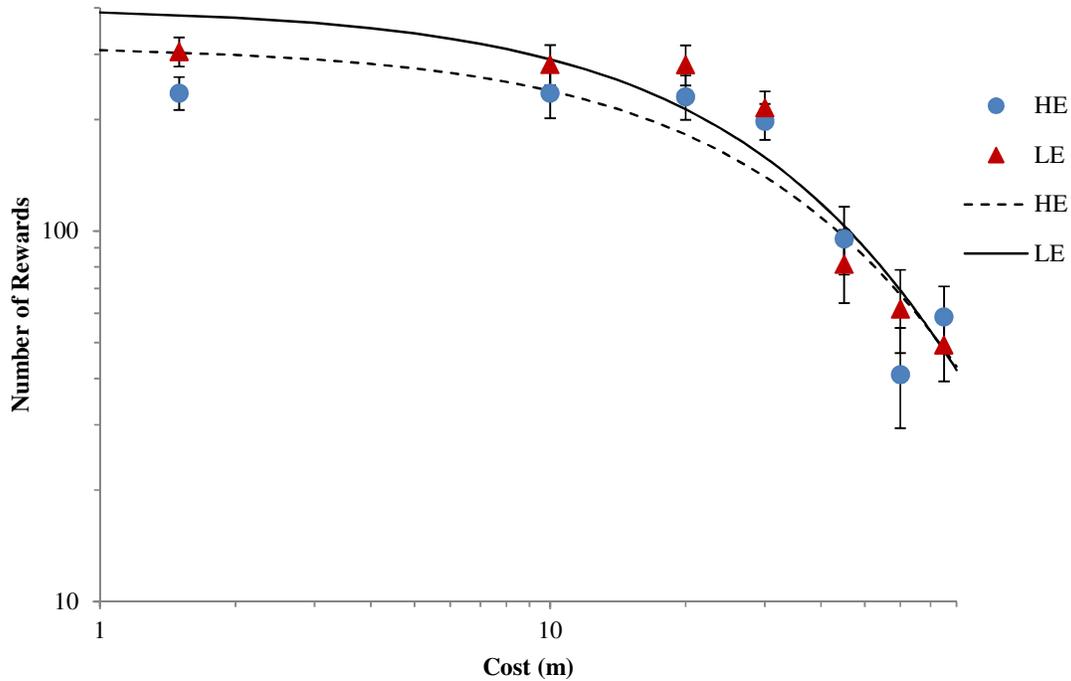
The  $P_{\max}$  values were  $41.4 \pm 6.5$ m for the HE treatment and  $35.0 \pm 4.3$ m for the LE treatment (Figure 4.2). The mean  $O_{\max}$  value, or total work output at  $P_{\max}$ , was  $5,370.5 \pm 525.1$ m and  $5,567.2 \pm 493.7$ m for the HE and LE treatments, respectively. The treatment

differences between the  $P_{\max}$  and  $O_{\max}$  values were not significant. However, when one outlier was excluded from the data set, due to being greater than 3 standard deviations away from the mean (Petrie and Watson, 1999), there was a significant treatment difference between the  $O_{\max}$  values showing that sheep exhibited higher feeding motivation when exposed to the LE treatment ( $P=0.04$ ). The alpha value, derived from the exponential model of demand, was not significant between LE and HE treatments (Figure 4.3).



**Figure 4.2** Linear-elasticity demand curve showing the mean number of back transformed rewards as a function of cost on a log-log scale for the high energy (HE, blue circle, dotted line) and low energy (LE, red diamond, solid line) treatments ( $n=10$ ). The differences in  $P_{\max}$  between the LE and HE treatments are represented by open boxes.

Analysis of unit price differences (calculated as the meters walked in a test run divided by the MJ consumed within the run, or m/MJ) revealed that there was no effect of treatment ( $P>0.1$ ) or a treatment by cost interaction ( $P>0.1$ ), although there was an overall effect of cost on unit price ( $P<0.001$ ). The variation between costs ranged from a mean unit price of  $0.12 \pm 0.008$  at the 1.5m cost to  $89.97 \pm 33.52$  at the 75m cost.



**Figure 4.3** Exponential demand curve showing the back transformed mean number of rewards as a function of cost on a log-log scale for the high energy ( HE, blue circle, dotted line) and low energy (LE, red diamond, solid line) treatments ( $n=10$ ).

Analysis of the energy balance data indicated that all sheep in both treatments sustained a state of positive energy balance at costs of less than approximately 30m, but did not continue this level of consumption and were in negative energy balance at the longer costs (Figure 4.4). The estimated cost at zero energy balance (point at which sheep moved from a positive to a negative energy balance) was  $33.3 \pm 3.3\text{m}$  for the HE treatment and  $31.3 \pm 3.0\text{m}$  for the LE treatment. There was no significant difference between the treatments at the point of zero energy balance ( $P>0.1$ ), and there were also no significant treatment differences between energy balance estimates ( $P>0.1$ ) at any of the individual costs.

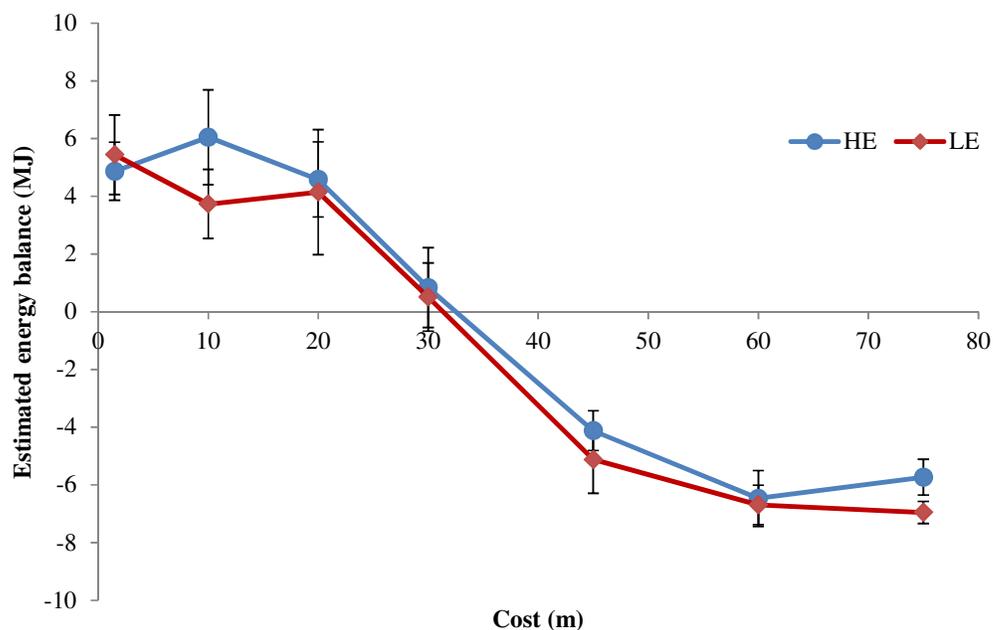


Figure 4.4 Estimated mean energy balance ( $\pm$  SEM) calculated for low energy (LE, red diamond) and high energy (HE, blue circle) treatments ( $n=10$ ).

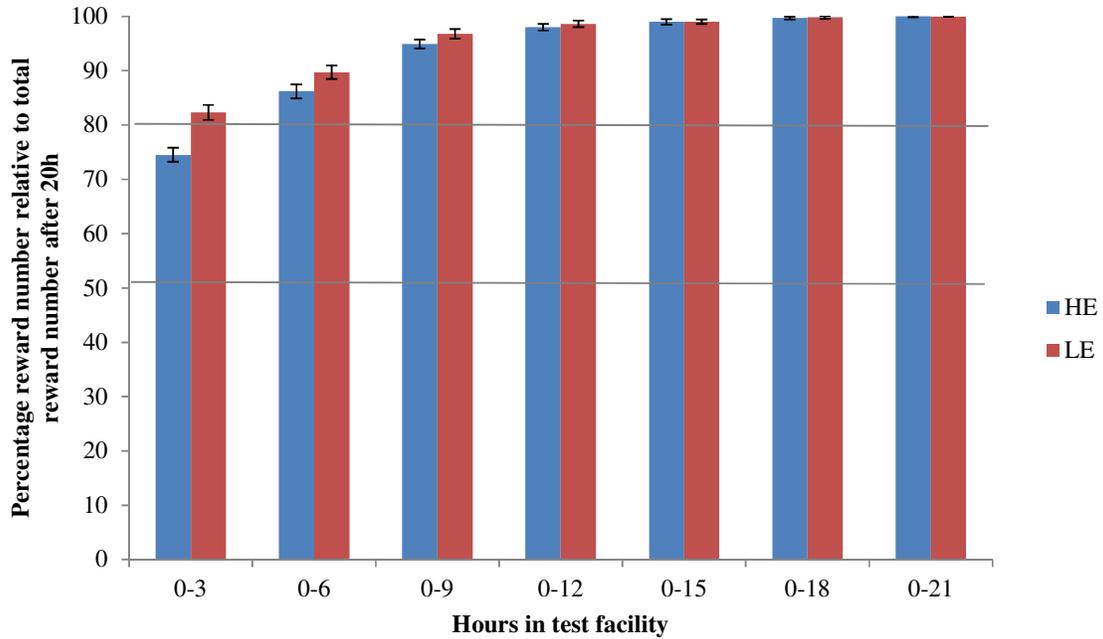
The estimated cost at  $P_{\max}$  was similar to the cost at zero energy balance (Table 4.2). There were no significant treatment differences between the  $P_{\max}$  costs and zero energy balance estimates for the HE treatment ( $P>0.1$ ), or for the  $P_{\max}$  costs and zero energy balance estimates for the LE treatment ( $P>0.1$ ).

Table 4.2 Differences between costs at which  $P_{\max}$  and the estimated zero energy balance occurred ( $P>0.1$  for all comparisons).

	Energy treatment	
	High energy (HE)	Low energy (LE)
Cost at $P_{\max}$ (m)	41.5	35.0
SEM	6.5	4.3
Estimated cost at zero energy balance (m)	33.3	31.3
SEM	3.3	3.0

Although not a specific aim of this experiment *post hoc* analysis of the eating patterns and intake rates of the sheep within the test period was undertaken. The cumulative work rate analysis and the interval work rate analysis was done to better understand how sheep allocated their activity budgets and to ascertain how using only the initial short portion of the 20h test (e.g. the first 6-12h) would impact the results. Specifically, the aim of the analysis was to better understand if an animal would do the majority (defined arbitrarily as 80%) of work it would do in a 20h test in the first 6h (cumulative work rate data) and to see if diurnal intake patterns (interval work rate data) varied with cost. This was then compared to similar data described in Chapter 3.

The cumulative work rate data (number of rewards in time period divided by the number of rewards in 20h period; Figure 4.5) indicated that within the first 3h the sheep exposed to the LE treatment completed  $82.3 \pm 1.3\%$  of the total work they would complete within the 20h test period, while the HE sheep completed  $74.5 \pm 1.4\%$ . Eighty percent was chosen as an arbitrary value to indicate the time point at which sheep had completed the majority of work done and this was reached by 6h in both treatments (LE  $89.7 \pm 1.3\%$ , HE  $86.2 \pm 1.2\%$ ). There were no significant differences at any time interval between the two treatments ( $P>0.1$ ).



**Figure 4.5** Mean cumulative percentage reward number relative to total reward number after 20h for both the high energy (HE) and low energy (LE) feeds. Horizontal lines highlight intervals at which 50% and 80% of intake was achieved ( $n=10$ ).

The interval work rate data showed that both the HE and the LE (Figure 4.6) treatments exhibited similar patterns of work done, irrespective of the costs involved. The trends of work done within each 3h time interval was then analysed for each treatment and there were no significant differences found between any of the intervals.

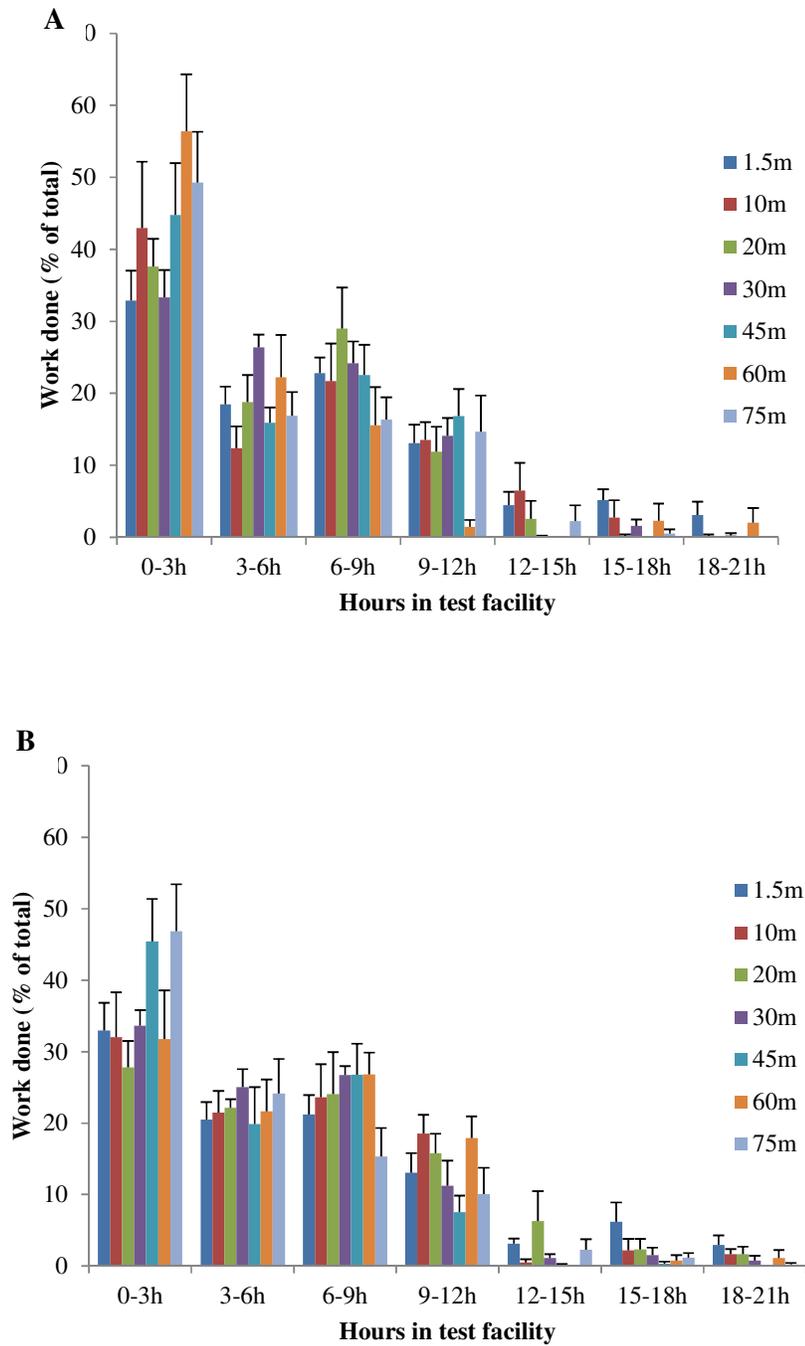


Figure 4.6 Mean interval work rates (+ SEM) given as a percent of the work completed over the 20h test period for the high energy (HE; A) and low energy (LE; B) treatments ( $n=10$ ).

The results of the preference testing showed no treatment difference in preferences ( $P>0.05$ ), and individual preferences appeared to remain relatively constant (Table 4.3).

**Table 4.3 Individual food preferences of sheep in a free-choice test. Tests were conducted on days 4 and 5 (test 1) and days 62 and 63 (test 2) after completion of the behavioural demand testing. Intake is given as a percent of the total intake for the high energy (HE) and low energy (LE) food types.**

Sheep No.	Test 1			Test 2			Overall
	High energy (HE) %	Low energy (LE) %	Preference	High energy (HE) %	Low energy (LE) %	Preference	Preference change
15	31.1	68.9	LE	13.6	86.4	LE	LE - LE
17	66.5	33.5	HE	60.6	39.4	HE	HE - HE
19	73.0	27.0	HE	57.7	42.3	HE	HE - HE
20	91.9	8.1	HE	87.4	12.6	HE	HE - HE
21	32.7	67.3	LE	58.7	41.3	HE	LE - HE*
22	14.1	85.9	LE	41.6	58.4	LE	LE - LE
25	27.0	73.0	LE	54.7	45.3	HE	LE - HE*
28	79.0	21.0	HE	22.1	77.9	LE	HE - LE*
29	97.5	2.5	HE	60.9	39.1	HE	HE - HE
30	48.7	51.3	LE	32.2	67.8	LE	LE - LE

\* denotes change in preference from test 1 to test 2

## 4.5 Discussion

The aim of this study was to further investigate the role of energy intake and energy balance in a ruminant's motivation for food in a behavioural demand test. This was undertaken by determining if the different energy densities (high energy versus low energy) of a food reward altered feeding motivation.

Overall, the results indicated that there were no significant differences between the two energy density treatments. However, the cost (distance walked) did influence the number of rewards consumed as these declined, with increasing costs. This finding agrees with other studies (Verbeek et al., 2011; 2012a) that have used a similar methodology and this finding does not appear to be dependent on species (monogastric or ruminant) or reward type. For example, Asher et al. (2009) investigated the willingness of captive starlings to push through a weighted door to access either a tray of turf or protective foliage cover and found that the cost (weight of the door) was the most important determinant of demand.

This finding was also confirmed in the unit price analysis in the present study where a direct relationship between cost and unit price was observed. One of the predictions made by economic theory in regards to unit price is that total consumption and total work done should be determined by the value of the unit price, independent of its cost and benefit components (Madden et al., 2000). It is also noted that if given a choice between unequal unit prices, such as 30 responses for 3 pellets (unit price of 10) and 60 responses for 3 pellets (unit price of 20) the lower unit price should be preferred over the higher one (Madden et al., 2000). If the behaviour favoured differs between alternatives then it can be concluded that the relative contributions of the costs and benefits to the unit price are not equal (Madden et al., 2000). In the current experiment, the unit price increased as the cost increased, indicating that the relative contributions of the costs and benefits to the unit price were not equal and subsequently, the lower unit price (walking to access food when the cost was short) was preferred to the higher unit price (walking to access food as the cost increased).

Specifically, this experiment aimed to test the hypothesis that sheep would be able to recognise differences between foods with differing energy densities. We also hypothesised that the sheep would work harder for access to food with the higher energy (HE) density as ruminants have been found to develop preferences for feed that rapidly provides them with high levels of satiety (Baumont, 1996). Our hypotheses were not confirmed as there were no overall differences between the LE and HE treatments in the behavioural demand environment or in the free-choice preference test. However, a significant treatment difference was present when analysing the data at the 1.5m cost level, with the sheep showing higher feeding motivation for the LE than the HE treatment. There was also a tendency for this treatment effect to be present at the 10 and 20m costs. One explanation for this trend could be that sheep were willing to work harder for access to the LE food as more of this would be needed to meet the levels necessary for metabolic and nutritional requirements. However, this would seem to be dependent on the effort required as the lack of treatment differences shown at higher costs may indicate that differentiation between energy levels was only worth the effort at lower costs.

The final HE formulation produced food of 15.3MJ and LE food of 12.2MJ which were essentially 'high and higher' levels of energy as opposed to truly 'low' and 'high' values, although the energy difference between the feeds was approximately 20%.

Nevertheless, if feeds could have been produced with the same approximate difference but with lower MJ values (e.g. 9.3 and 12.3MJ), animals may have shown a more pronounced increase in work rate at lower costs and, possibly, maintained an increased work rate at higher costs. The production of pellets with a low energy density and high fibre content proved challenging, as it was difficult to achieve pellet integrity keeping other factors as similar as possible.

One factor that was not able to be kept similar between the HE and LE pellets was the fat content. As indicated in Table 4.1 the LE pellets had a crude fat content of 5.2% compared with 9.8% for the HE pellet. As previously discussed the existing literature on palatability and preference indicates that a complex range of variables alter the food choices of ruminants, and preference depends on how these variables (e.g. smell, taste) align with post ingestive energy feedback (Baumont, 1996). While it is possible that the differing levels of crude fat could have influenced the preference for one feed type over the other, it is difficult to make any valid conclusions as, to our knowledge, there is no evidence in the literature that the levels of fat in the pellets used in this experiment influences palatability, feed choice and feed intake of ruminants. As preference generally develops for feeds that provide more energy (Provenza, 1995), it would seem likely that if the fat levels had impacted the preference then the HE pellets would have been the preferred food type and this was not the pattern seen in the current experiment. Although there is a lack of literature on ruminant preference for fat and/or energy, there is a large body of work on the preferences of fat and energy in rodent species (Marks-Kaufman, 1982; Drewnowski et al., 1995; Berridge, 2009; Taha, 2010). This area will be further discussed in relation to the opioid reward system and feed preferences in Chapter 5.

Previous investigations undertaken to assess the impact of energy balance estimates on feeding motivation have found that animals will sustain an energy deficit to gain additional food (Hutson, 1991; Verbeek et al., 2011), and this finding was confirmed in the current experiment. Potential reasons for an animal's continued willingness to work for food once it enters an energy deficit have been fully discussed (Chapter 3) and various theories to explain this behaviour were proposed. However, due to the repetition of the results it may now be possible to discount some of these explanations. For example, one explanation proposed was that ruminants have a limited time budget to spend on the acquisition of food,

and animals in this experiment may have had to consider a trade-off between time spent working for food and other important activities, such as rest (Houston and McNamara, 1989). However, the cumulative work rate data suggests that the animals were able to complete the majority of their reward consumption (80%) in the first 6h. Further analysis (interval work rates) indicated that this level of work done was relatively constant, regardless of cost. This suggests that sufficient time was available for other activities as well as for the further acquisition of food. Although the methodologies, with regard treatment, differed between this experiment and the experiment described in Chapter 3 (food deprivation in Chapter 3 and different energy densities in the current experiment), the cumulative and interval work rate data are similar between experiments, suggesting that these patterns can be validated and that the trade-off theory may not be appropriate in this situation.

As the methodology for the current experiment, with regard to prior energy balance, was similar to that of the previous experiment (Chapter 3), no further information could be gained regarding the impact of long term energy reserves on energy balance. As previously mentioned, estimates of long term energy reserves, or providing *ad libitum* food, in the days prior to the experiment and longer testing periods may provide more insight into this. Due to time constraints this was not feasible for the current experiment.

Neither the  $P_{\max}$ ,  $O_{\max}$  or alpha values, derived from the linear elasticity model and exponential model of demand, respectively, were found to differ for the two treatments. The finding that  $P_{\max}$  did not differ between treatments supports other recent findings (Verbeek et al., 2011; 2012a). One explanation for this is that  $P_{\max}$  does not account for differences in actual levels of food consumption or response output and therefore  $O_{\max}$  has been proposed as a more useful measure of motivation in a variety of studies (Hursh and Winger, 1995; Madden et al., 2007a; 2007b). However, treatment differences were not observed when analysed with the  $O_{\max}$  or the alpha value, although  $O_{\max}$  was significant when outliers were removed. As previously mentioned the unit price analysis supported the findings of the effect of cost on feeding motivation. This may suggest that in this experiment, the measure of unit price was a useful, but perhaps not necessary, addition to the suite of measures used to assess changes in motivation.

The costs at which zero energy balance and  $P_{\max}$  occurred were similar and were between 31 and 41m, irrespective of treatment. This suggests that the work output of the sheep began to decline around the point of zero energy balance and is similar to earlier results (Chapter 3). Although these findings indicate that this effect is repeatable, it remains difficult to theorise which metabolic feedback system or nutritional parameters may be influencing feeding motivation. This is particularly so in a ruminant where there is a constant supply of energy available from the gut. Additional investigation will be necessary to provide greater insight into what mechanisms may be providing feedback to the sheep allowing it to alter its behaviour so quickly. Due to the speed with which the feedback appears to be integrated and behavioural changes initiated, a feasible starting point for further research could involve short term hunger and satiety patterns and may involve investigating what impact factors such as ghrelin, an amino acid orexigenic peptide produced mainly in the stomach, insulin and leptin might have on  $P_{\max}$  and zero energy balance estimates (Williams and Morbarhan, 2003; Gil-Campos et al., 2006; Figlewicz and Sipols, 2010).

In conclusion, there was a trend suggesting that sheep may have been able to differentiate between the two feed types of varying energy densities in the behavioural demand environment, and were willing to work slightly harder for the lower energy treatment at the 1.5m cost. This, combined with the relatively high levels of energy density for both feed types, suggests that the sheep may be reluctant to maintain higher levels of feeding motivation as the cost increases, if both feed types supply sufficient energy to meet daily requirements. In this experiment sheep were willing to sustain an energy deficit to gain additional food and decreased the work done at the estimated point of zero energy balance. This finding supports results from a previous experiment (Chapter 3) with regard to energy balance, and indicates that this effect is repeatable. However, the current experiment was not able to provide further insight into what type of feedback system may be influencing feeding motivation. There are still several potential interpretations for this behaviour and further work will be needed before conclusions can be reached with regard to possible mechanisms which may be underlying these behavioural changes.

## Chapter 5

# The influence of the opioid reward system on feeding motivation



## 5.1 Abstract

Research conducted since the early 1970's has shown that the endogenous opioid system is involved in the elicitation and/or control of food intake. Gaining a better understanding of the role of the opioid reward system in determining an animal's motivation for food is likely to be important when attempting to understand factors that alter the motivation for food in a behavioural demand test. This study aimed to test the hypothesis that down regulation of the opioid system, through administration of the  $\mu$ -opioid antagonist, naltrexone hydrochloride (NTX), would decrease the willingness of sheep to walk for food in a demand test. It was also hypothesised that this decrease could be modulated by the energy density of the food through either; 1) underlying preferences of one food over the other, or 2) by the energy and fat content of the diets. Ten sheep were trained in a 50m U shaped laneway to access a double-sided feeder and gained a reward with each access event. NTX was administered at 1mg/kg intramuscularly and sheep were tested to see how many times in a 20h period they would walk a specific distance for a 4g reward of either high energy (HE), or low energy (LE) food. Each sheep was exposed to 4 of 6 distances (1.5, 10, 25, 40, 55 and 75m) in a random order at each of the four treatment levels (HE, HE+NTX, LE, LE+NTX). A free choice preference test was also conducted before and after the behavioural demand experiments to determine preferences of the two food types. The number of food rewards earned decreased as the distance walked increased in all sheep, for all treatments ( $P < 0.001$ ). Following administration of NTX sheep earned fewer rewards in the first 6h compared to test runs without the drug ( $P = 0.010$ ). Within this time period there was also a cost by food interaction, with sheep more motivated for the LE than HE treatment at the 1.5 and 10m costs ( $P = 0.013$ ), and a cost by drug interaction, which indicated that at shorter costs sheep administered with NTX exhibited lower levels of feeding motivation ( $P = 0.004$ ). The sheep were also more motivated to access the LE compared to the HE food over the 20h period ( $P < 0.001$ ). Although overall preference testing indicated that in the free-choice test sheep preferred the HE to LE treatment ( $P = 0.039$ ), there were no clear preferences determined during baseline testing, (the preference test that occurred before behavioural demand testing). There was no significant association between the preference for food in the free choice test and the food consumed in the behavioural demand facility. These results indicate that the opioid reward system may influence the motivation of sheep to work for food in a behavioural demand test, and suggest that sheep worked harder to access the LE food at short costs, possibly, to maintain adequate energy intake.

## 5.2 Introduction

The regulation of feeding behaviour and motivation is dependent on many variables including the physiological and nutritional state of the animal (level of food restriction and hunger, overall health, age, and pregnancy) as well as characteristics of the food (macronutrient composition, energy density, palatability), social environment, learning and memory (Olszewski et al., 2011). However, there are other factors that also impact food intake and the opioid reward system has been proposed as one such mechanism (Bodnar, 2004). It is generally accepted that opioid receptors, such as the receptor subtype  $\mu$ , regulate feeding behaviour through modulating the hedonic or pleasurable aspects of food and food intake (Barbano et al., 2009), and that opioids are particularly involved in the regulation of intake of palatable foods, at least in humans and rodents (Olszewski et al., 2011).

Extensive research has been done investigating the impact of opioids on food intake and feeding motivation, particularly in rodents (Barbano and Cador, 2006; 2007; Barbano et al., 2009; Fulton, 2010), using both free feeding and operant task methodologies. The mechanism by which opioids impact feeding motivation is continually under review and current theories suggest that opioids may influence feed intake through regulation of the orosensory reward system, patterns of meal intake or the quality and energy of the food ingested (Obese, 2007). However, based on the extensive distribution of opioid receptors in the central nervous system, it is possible that opioids impact multiple feed intake systems. For example, Glass et al. (1999) has suggested that hindbrain opioids may be involved in metabolic and sensory features of food intake, and that the amygdala and the hypothalamus influence emotions and energy requirements, respectively. Generally, acute administration of morphine, an opioid receptor agonist, causes increases in food intake and weight gain in different species, such as rats (Levine, 1988) and sheep (Obese, 2007). In contrast, administration of opioid receptor antagonists (e.g. naloxone and naltrexone) decrease the intake of both caloric food (Marks-Kaufman et al., 1984; Naleid et al., 2007) and non-caloric fluids, such as saccharin (Beczowska et al., 1993). However, the impact of opioids on ingestive behaviour varies depending on the type of opioid, the nutritional state of the animals prior to drug administration (Alavi et al., 1993), the route of administration (Glass et al., 2000), the type of diet presented, and previous preferences of the animal towards the food types (Rockwood and Reid, 1982; Naleid et al., 2007; Taha, 2010).

The role of previous or baseline preferences has become important in better understanding opioid signalling with regard to macronutrient preference. Taha (2010) identified that there are two principle theories that have been proposed to explain macronutrient (e.g. fat, carbohydrate, protein) preferences: 1) that opioids increase the consumption of preferred foods; independent of macronutrient content (e.g. Gosnell et al., 1990); or 2) that opioids increase the intake of foods high in fat as well as fat itself (e.g. Marks-Kaufman and Kanarek, 1980; Marks-Kaufman, 1982; Marks-Kaufman et al., 1984). However, these alternative theories are somewhat confounded, particularly in rats, as fat is highly palatable and is often preferred to other sources of energy (Taha, 2010). This has been overcome to some extent by characterising baseline preferences for two alternatives and then testing the effects of opioids on the consumption of the preferred food. For instance, Glass et al. (1996) reported that naltrexone significantly reduced the intake of carbohydrates in carbohydrate-preferring rats and of fats in fat-preferring rats and similar patterns of intake were found after a 24h food restriction. In contrast, most studies show little difference in protein intake caused by opioid administration (Marks-Kaufman and Kanarek, 1980; Taha, 2010).

The pharmacological use of opioid administration to better understand the role of opioids in ruminant food intake has been explored to some extent. The results suggest that the system generally functions in a similar manner to monogastrics, although particular aspects (e.g. brain locations and functions) may be species-specific (Baile et al., 1987). Research has shown that syndyphalin-33 (SD-33) a  $\mu$ -opioid receptor ligand, increases feed intake in sheep (Obese, 2007). This is supported by earlier work which reported that intracerebroventricular administration of opioid receptor agonists to sheep increased food intake, while peripheral administration of an antagonist had the opposite effect (Baile et al., 1981). Furthermore, the administration of an opioid receptor antagonist to obese sheep caused a significantly greater reduction in food intake when compared to lean sheep (Alavi et al., 1993), and the reduction in intake was inversely related to the food deprivation level of the sheep (Alavi et al., 1991).

The role of endogenous opioids in ruminant physiology has also been investigated in relation to maternal behaviour (Caba et al., 1995), rumen motility (Maas and Leek, 1985; Maas et al., 1986) and immune function (Gates et al., 1990). However, the use of a pharmacological approach to better understand indicators of animal welfare is relatively new.

Although the role of opioids in stress responses in dairy cows (Rushen et al., 1999) and affective state in sheep has been reported (Verbeek et al., 2012b), few studies have investigated the role of opioids in the feeding motivation of a ruminant when tested using operant conditions.

The objective of the present study was to investigate the impact of an opioid receptor antagonist on feeding motivation in sheep. The number of rewards consumed and measures derived from a demand curve were used to assess the changes in motivation. A second objective was to assess if opioid signalling changed the level of motivation for a preferred food, when measured against baseline preferences, or changed the motivation for the food that was highest in energy and fat. We hypothesised that the opioid antagonist would decrease the willingness of the sheep to work for food for a short period, and that intake of the high energy diet would decrease more following drug administration compared to the intake of the low energy diet, given that diets that rapidly provide high levels of satiety and are highly digestible are generally preferred. (Baumont, 1996).

### **5.3 Materials and methods**

The materials and methods used in this chapter are similar to those more fully described in Chapter 3. To avoid repetition, only the differences in the material and methods between the previous experiments (Chapter 3 and 4) and the current experiment (Chapter 5) have been described.

The study was approved by the CSIRO's Animal Ethics Committee and conformed to the standards in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Animal Research Authority Number 11/22).

#### **5.3.1 Dose response study**

This experiment was divided into two phases: 1) a dose response study to investigate the suitability and impact of the chosen opioid antagonist, naltrexone hydrochloride (NTX), on the general behaviour and motivation of sheep; and 2) a behavioural demand study to investigate the impact of NTX on the feeding motivation of sheep. An antagonist was used (as opposed to an agonist) as literature existed on approximate dose amounts that may be suitable for use in ruminants when assessing the effect of regulation of the opioid reward

system on feed intake (Alavi et al., 1993; 1994). This was used as a starting point for the dose response trial.

The dose response study involved testing 4 cohorts (n=8) of Merino wethers (32 sheep in total) with each cohort randomly allocated a different drug treatment level. Treatments comprised of a control group (sham injected) and differing NTX dose rates of 0.1, 0.5 and 1.0mg/kg injected intramuscularly into the gluteus muscle. Sheep were individually housed in a partially enclosed facility, fed a complete, concentrate diet (Animal House or AH pellets) and water was available at all times. Feed intake over 24h was measured prior to and following NTX administration to assess the impact of dose rate on feeding motivation as well as the recovery rate of feed intake over time. An approach/avoidance conflict motivation test was also used to investigate behavioural and decision making changes that occurred following administration of NTX. The analysis of the results indicated that a dose of 1.0mg/kg would be appropriate for use in the behavioural demand testing (see Appendix 1 for further information).

### ***Behavioural demand study***

Twenty, mature Merino wethers were divided into a group of 10 experimental animals and a group of 10 companion animals. The mean weight of the 10 experimental wethers was  $58.1 \pm 1.8$ kg with a body condition score of  $3.3 \pm 0.09$  (scored from 1-5). On non-test days all sheep were group fed a basal diet of a lucerne-based total ration pellet (referred to as Animal House (AH) pellets) (Table 5.1) and oaten chaff (ME= 8.9MJ/kgDM, DM =87.5%, CP =10.9% of DM) in an 80:20 ratio. Feeding occurred once daily at approximately 8:00am for non-test animals. On test days experimental sheep were provided with *ad libitum* access to either the 'low' energy ration (LE) or the 'high' energy ration (HE) (Table 5.1.) within the test facility. In the weeks prior to testing, sheep were familiarised to all feed types as their AH ration was supplemented with equal amounts of the HE and LE feed types. Individual animal intake tests were also done to ensure that all animals were eating all types of pellets. Water was freely available at all times.

**Table 5.1 Summary of the pellet type, ingredients and composition**

<b>Feed Type</b>	<b>Animal House (AH) pellets</b>	<b>Low energy (LE) pellets</b>	<b>High energy (HE) pellets</b>
Ingredients	Lucerne Wheat Pollard Bran Salt Ammonium chloride	Lucerne pellets Copra meal Hardwood sawdust	Lucerne pellets Wheat Vegetable Oil Urea
Metabolisable energy (MJ/kg DM)	12.2	12.2	15.3
Dry Matter (%)	89.7	90.3	90.8
Crude Protein (%)	21.5	22.3	23.1
Crude Fat (%)	2.5	5.2	10.0
Acid detergent fibre (%)	16.4	29.4	8
Neutral detergent fibre (%)	30	44	19

### **5.3.2 Facility and equipment**

The test facility has been described previously in Chapter 3.

### **5.3.3 Habituation and behavioural demand training**

The sheep used in the current experiment were the same animals used in the experiments described previously in Chapters 3 and 4 so the habituation process was not required and the training schedule followed was the same as was described in Chapter 3. During the period between experiment described in Chapter 4 and the current experiment the sheep were grazed on pastures and were fed AH pellets twice weekly. The frequent handling of the sheep over the current (Chapter 5) and previous (Chapters 3 and 4) test periods meant that no habituation or training and very little restraint was necessary to safely administer the NTX.

### **5.3.4 Experimental design**

An incomplete 6 cost design was used whereby each sheep was exposed to 4 random costs, out of the 6 costs (distances) used, at each of the 4 treatment levels (16 test runs in total per sheep). Treatment levels were low energy (LE), high energy (HE), LE with naltrexone (LE +NTX) and high energy with naltrexone (HE+NTX). Cost and food type were randomly assigned while NTX was pseudo randomised to ensure that administration of the drug occurred only on alternating test runs e.g. test 1 was with NTX administration, test 2 was without. The injection site was also altered between left and right sides of the animal to

reduce the impact of repeated intra-muscular injections. The costs used were 1.5, 10, 25, 40, 55 and 75m. A recovery period of 4 days was maintained between tests to ensure that there was no carry-over effect from having been in the testing facility on the previous day.

A mean reward size of  $4.1 \pm 0.08\text{g}$  was given to the sheep at each event. Following analysis of the feed it was calculated that for the HE feed type this equated to 65kJ per reward and 48kJ per reward for the LE feed type (SCA, 1990; Primary Industries Standing Committee, 2007).

### **5.3.5 Behavioural demand testing**

The experiment was run from the 28<sup>th</sup> of February to the 18<sup>th</sup> of May 2012. The same testing methodology described in Chapter 4 was used for this experiment. The sheep were administered the NTX immediately prior to entering the behavioural demand facility at 6:00am on each test morning. To administer the drug the test sheep and companion sheep were moved to a drafting race and the test sheep were restrained and injected as they moved through the draft.

### **5.3.6 Preference testing**

Preference testing was undertaken prior to and following the experiment and the methodology has previously been described in Chapter 4. However, for this experiment testing occurred prior to and following the behavioural demand experiment so that both a baseline preference could be determined and to ascertain if any changes in preference occurred over the course of the experiment. Testing was conducted on the 24<sup>th</sup> and 25<sup>th</sup> of February 2012 (3 and 4 days prior to the behavioural demand testing), and again on the 28<sup>th</sup> and 29<sup>th</sup> of May 2012 (10 and 11 days after the conclusion of the behavioural demand testing).

### **5.3.7 Statistical analysis**

The effects of food energy density (LE or HE), administration of NTX, cost (distance walked) and the interaction between these factors on the number of rewards consumed were tested using the REML procedure in GenStat 15 (VSN International Ltd., Hemel Hempstead, U.K.). Opioid antagonist treatment, food type and cost were fitted as fixed effects, as was run order (chronological order in which the tests were conducted) and lane. The animal was fitted

as the random effect with the number of rewards consumed fitted as the dependant variable. The number of rewards was transformed (square root) to improve normality of distribution. Residuals were tested for heteroskedasticity and no evidence of serial correlation was found within the REML residuals.

Analysis of cumulative data of number of rewards consumed at 0-6h was performed to ascertain the impact of NTX on feeding motivation, as it was within this time period that the drug was most likely to be acting (Appendix 1). Previous experiments (Chapters 3 and 4) also indicated that a large portion (80%) of the work done over a 20h period could be completed in the first 6h, ensuring that sufficient work could be done to enable valid and reliable statistical analysis to be undertaken. Cumulative analysis, based on the 0-20h data, was also performed, as was similar analyses over specific test periods; 6-12h and 12-20h. Analysis of the latter two periods was undertaken to examine the temporal effects of the NTX treatment on feeding motivation. Cost, food type and NTX treatment were fitted as fixed effects with animal fitted as the random effect. The impact of these effects on motivation (number of rewards consumed) and interactions between these effects were tested using a REML analysis. All *post hoc* contrast analyses were Bonferroni corrected.

The results from the dose response study (Appendix 1) showed that there was a pattern of compensatory feeding occurring 8-12h after NTX administration. To examine this further in the current study, separate contrast analyses of the food by cost by drug interactions for the 6-12h and 12-20h periods, post NTX administration, were undertaken using the same REML analysis as mentioned above.

The derivation of the  $P_{max}$  (cost at which maximum responding occurs),  $O_{max}$  (maximum work done at  $P_{max}$ ) and alpha values (reinforcing strength of a reward) have previously been described in Chapter 3. The calculation of the energy balance estimates have also previously been explained in Chapter 3. Noteworthy differences with these estimates are that 2 sets of calculations were undertaken to estimate energy balance (HE food at 65kJ per reward and the LE food at 48kJ per reward), and that the calculation of theoretical maintenance was increased from 9.5MJ (Chapter 4) to 9.9MJ (this experiment) to account for the increase in weight of the sheep. The estimation of the cost at which zero energy balance occurred has also been previously described in Chapter 3; however, normalisation of the

resultant data was improved through a square root transformation and treatment differences between the points of zero energy balance were analysed by REML procedure with treatment as a fixed effect and sheep as a random effect. The significance of the difference between  $P_{\max}$  and the cost at zero energy balance estimates were tested using a two-tailed *t*-test (GenStat 15).

To analyse the preferences for the LE or HE food type of the sheep the raw data (amount (g) eaten per test) were transformed to a percentage ratio of HE:LE consumed in each preference test. Differences between tests were then analysed using the two sample *t*-test procedure (GenStat 15). To compare motivation for the different food types between the behavioural demand test and the free-choice base preferences (preferences prior to behavioural demand testing), the total rewards collected for each treatment (all costs) were collated and percentage ratios produced (i.e. ratio of HE:LE rewards collected with and without NTX administration). Linear regression (GenStat15) was then used where the relationship between the HE:LE ratio from the base preferences and the HE:LE ratio from the behavioural demand test was examined. All data are presented as actual mean  $\pm$  standard error of the mean (SEM) unless otherwise stated.

## 5.4 Results

All 10 sheep successfully completed 4 test runs for each treatment. The number of food rewards across all costs for the 20h data ranged from a minimum of 11 at the 70m cost for the LE treatment to a maximum of 500 at the 1.5m cost for the LE+NTX treatment. The distance walked in the 20h test period ranged from a maximum of 169.5 at the 55m cost to 14,025m at the 25m cost.

REML analysis of the cumulative 0-6h data indicated that overall, the number of rewarded events decreased as the distance the sheep were required to walk increased ( $P < 0.001$ ). Overall analysis also showed that when the sheep were administered NTX they earned fewer rewards compared to test runs without the drug ( $P = 0.010$ ), and tended to show higher motivation for the LE compared to the HE treatment ( $P = 0.063$ ). Further analysis of this time period revealed that there was a cost by food interaction (Figure 5.1), demonstrating that at the shorter costs (1.5 and 10m) the sheep were more motivated for the LE compared to the HE treatment ( $P = 0.013$ ). There was also a cost by drug interaction (Figure 5.2) which

showed that at the 1.5 and 10m costs sheep administered NTX exhibited a lower level of feeding motivation ( $P=0.004$ ).

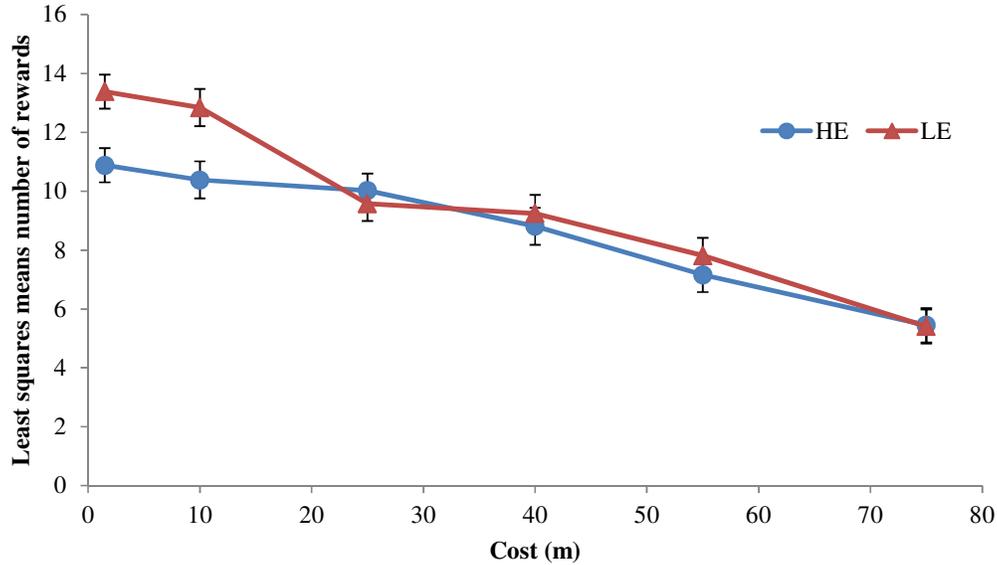


Figure 5.1 Least squares means number of rewards ( $\pm$  SEM) for the 0-6h test period for the high energy (HE, blue circle) and low energy (LE, red triangle) treatments ( $n=10$ ).

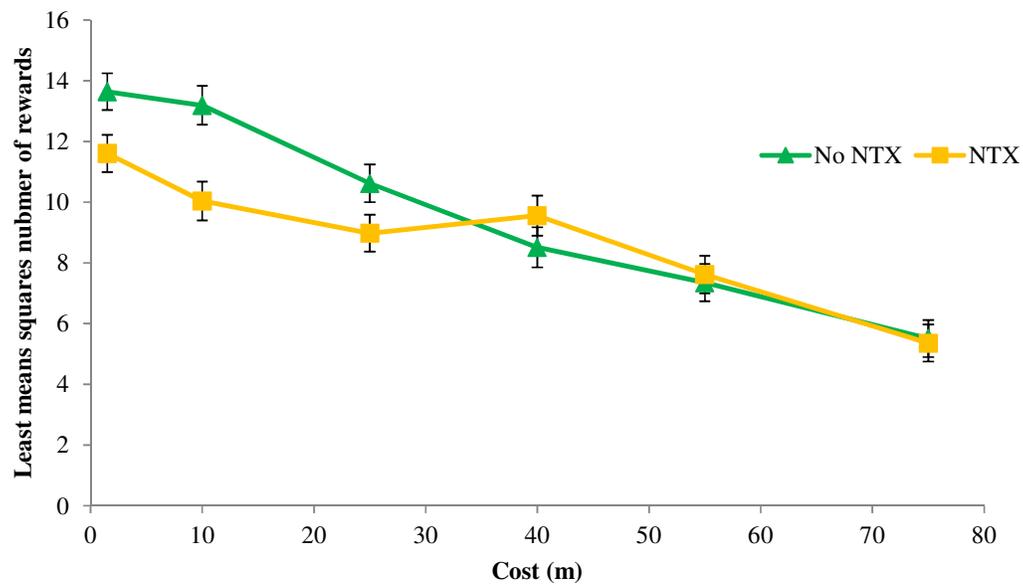
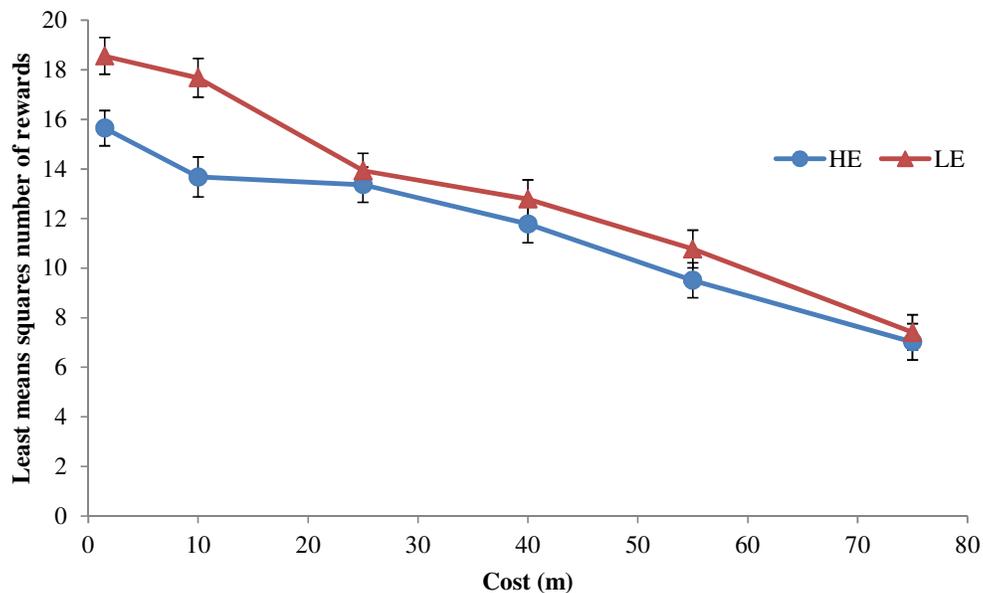


Figure 5.2 Least squares means number of rewards ( $\pm$  SEM) for the 0-6h test period for treatments with naltrexone (NTX, yellow square) and without naltrexone administration (No NTX, green triangle) ( $n=10$ ).

Analysis of the 0-20h test period data showed that overall, the number of rewards earned decreased as the distance walked increased ( $P<0.001$ ), and the sheep worked significantly harder to access the LE compared to the HE food type ( $P<0.025$ ). There was no overall effect of NTX administration ( $P=0.7$ ) on feeding motivation, nor was there an effect of run order (chronological order in which the tests were administered;  $P=0.2$ ). However, there was a cost by food interaction (Figure 5.3), which was similar to the 6h data in that it indicated that at the two lowest costs (1.5 and 10m) feeding motivation was significantly higher for the LE than the HE treatment ( $P=0.046$ ).

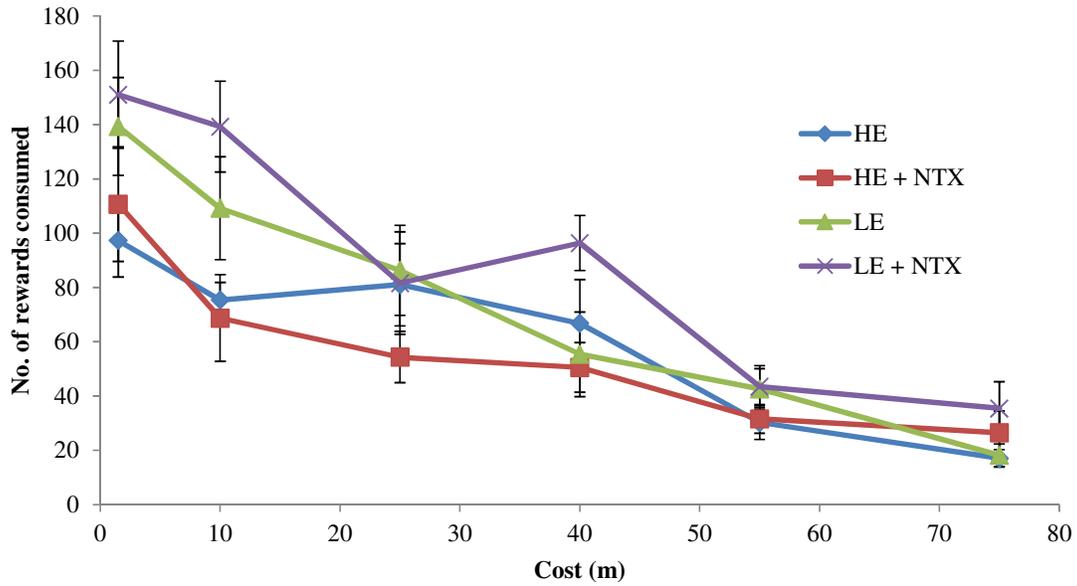


**Figure 5.3** Least squares means number of rewards ( $\pm$  SEM) for the complete (20h) test period for the high energy (HE, blue circle) and low energy (LE, red triangle) treatments ( $n=10$ ).

The results from the dose response study (Appendix 1) showed that there was a pattern of compensatory feeding occurring 8-12h after NTX administration. To examine this further in the current study, separate contrast analyses of the food by cost by drug interactions for the 6-12h and 12-20h periods, post NTX administration, were undertaken.

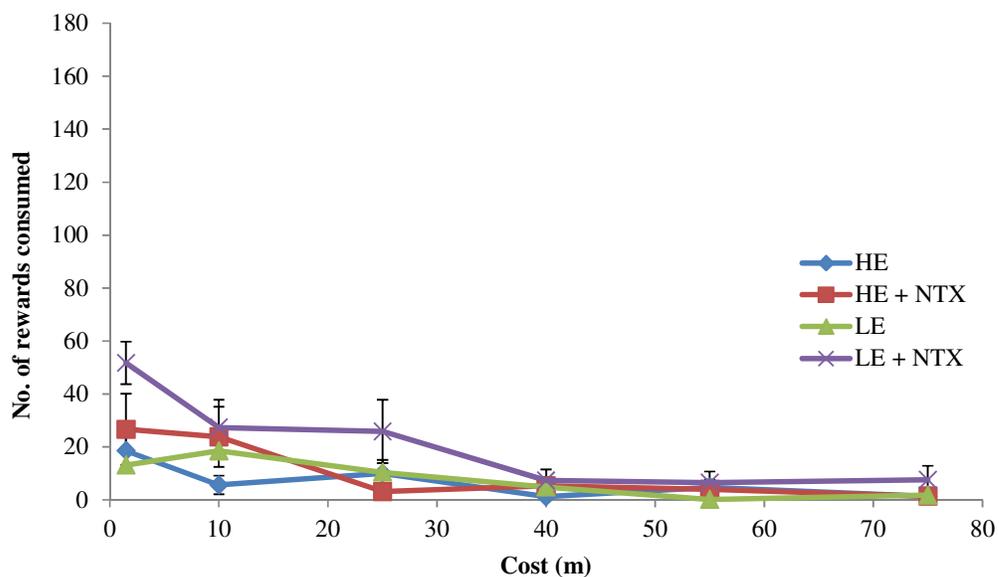
The overall analysis of the 6-12h period showed that the cost the sheep were exposed to had a significant effect ( $P<0.001$ ) with the sheep decreasing the number of feed rewards consumed as the distance increased (Figure 5.4). The sheep continued to work harder for

access to the LE compared to the HE treatment ( $P=0.008$ ) and there were no significant differences between NTX treatments in the 6-12h period, although administration of NTX tended to increase the number of LE rewards earned at the 10 and 40m costs (10m,  $P=0.061$ ; 40m,  $P=0.055$ ).



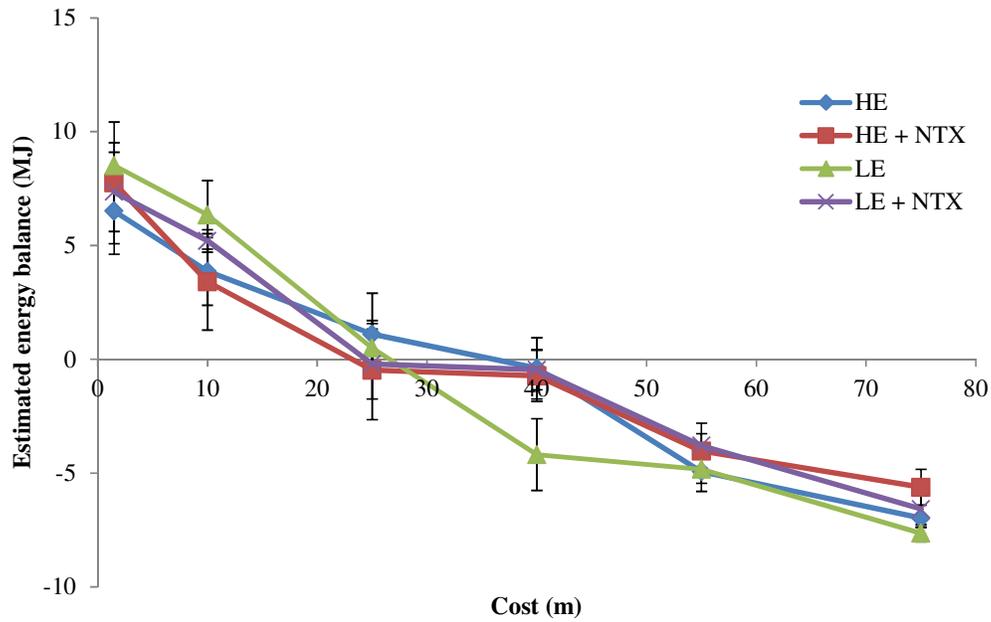
**Figure 5.4** Mean numbers of rewards consumed ( $\pm$ SEM) between 6 and 12h of the 20h test period for the high energy feed (HE), low energy feed (LE), high energy feed with naltrexone (HE+NTX) and low energy feed with naltrexone (LE+NTX) treatments ( $n=10$ ).

Analysis of the number of rewards consumed in the final time period (12-20h) showed similar results in terms of distance walked as the number of rewards decreased with increasing cost ( $P<0.001$ ). There were no differences found in any of the individual cost contrast analyses except at the 1.5m cost (Figure 5.5). At this cost the sheep consumed a higher number of rewards following NTX administration (contrasting LE with LE+NTX;  $P=0.009$ ).



**Figure 5.5** Mean numbers of rewards consumed between 12 and 20h of the 20h test period for the high energy feed (HE), low energy feed (LE), high energy feed with naltrexone (HE+NTX) and low energy feed with naltrexone (LE+NTX) treatments ( $n=10$ ).

Analysis of the theoretical energy balance data showed that all sheep in all treatments sustained a state of positive energy balance at lower costs, but were in negative energy balance at costs of 40m and above (Figure 5.6). The estimated cost at zero energy balance (point at which sheep moved from a positive to a negative energy balance) was  $35.5 \pm 3.9$ m and  $28.4 \pm 3.3$ m for the HE and LE treatments, respectively. Theoretical zero energy balance for treatments with NTX was  $29.4 \pm 4.4$ m and  $29.8 \pm 4.6$ m for the HE+NTX and LE+NTX treatments, respectively. There were no overall significant differences between the estimated cost at zero energy balance for any of the treatments. There were also no significant treatment differences between energy balance estimates at any individual costs.



**Figure 5.6** Estimated mean theoretical energy balance ( $\pm$  SEM) calculated for each cost level for the high energy (HE), low energy (LE), high energy with naltrexone (HE+NTX) and low energy with naltrexone (LE+NTX) treatments ( $n=10$ ).

The mean  $P_{\max}$  values, were  $37.3 \pm 3.0m$  and  $31.1 \pm 2.0m$  for the HE and LE treatments, respectively ( $P=0.2$ ), and  $37.2 \pm 3.2m$  and  $32.3 \pm 5.3m$  for the HE+NTX and LE+NTX treatments ( $P=0.5$ ; Figure 5.7). The mean  $O_{\max}$  values were  $7,067 \pm 859m$  and  $6,417 \pm 464m$  with and without NTX, respectively ( $P=0.4$ ), and  $5,634 \pm 477m$  and  $7,851 \pm 662m$  for the HE and LE treatments ( $P=0.001$ ). The alpha values, derived from the exponential model of demand, did not differ between treatments ( $P>0.1$ ; Figure 5.8).

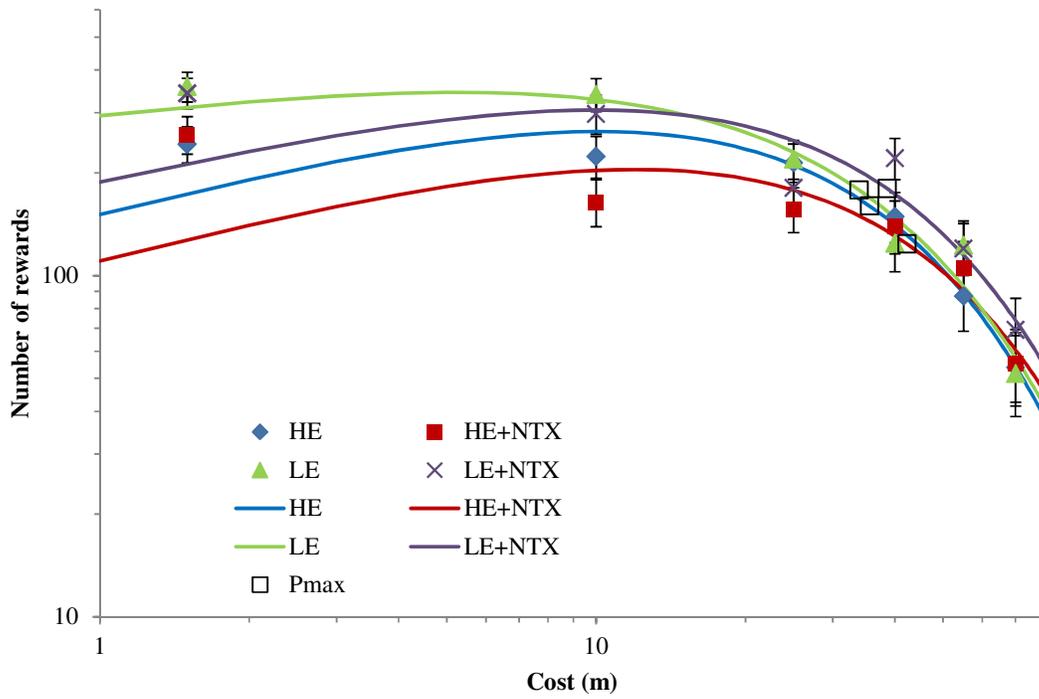


Figure 5.7 Linear elasticity demand curve showing the back transformed mean number of rewards as a function of cost on a log-log scale for the high energy (HE, blue diamond) and low energy (LE, green triangle) treatments and for the high energy food with naltrexone (HE+NTX, red square) and low energy food with naltrexone (LE+NTX, purple cross) treatments. The differences between treatments at  $P_{\max}$  are represented by open boxes ( $n=10$ ).

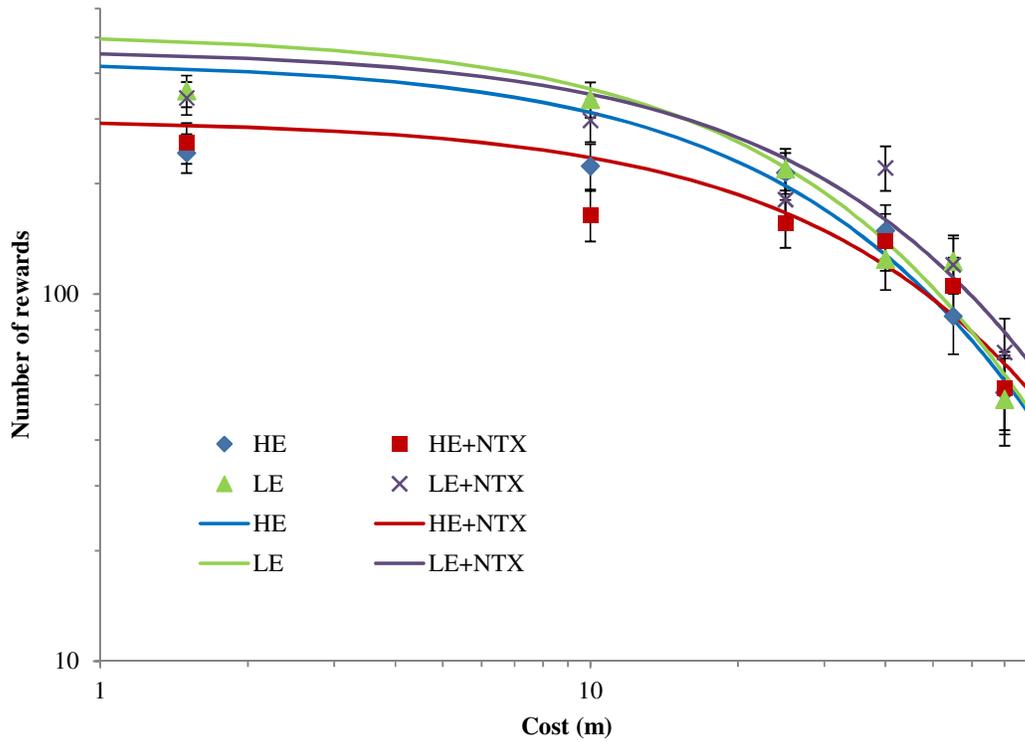


Figure 5.8 Exponential demand curve showing the back transformed number of rewards as a function of cost on a log-log scale for the high energy (HE, blue diamond) and low energy (LE, green triangle) food types and for the high energy food with naltrexone (HE+NTX, red square) and low energy food with naltrexone (LE+NTX, purple cross) treatments ( $n=10$ ).

Further analysis indicated that the cost at  $P_{\max}$  was similar to the estimated cost at zero energy balance for all treatments ( $P>0.1$ ; Table 5.2).

Table 5.2 Differences between costs at which  $P_{\max}$  and the estimated zero energy balance occurred for high energy (HE), low energy (LE), high energy with naltrexone (HE+NTX) and low energy with naltrexone (LE+NTX) treatments ( $P>0.1$  for all comparisons).

	Treatment			
	HE	LE	HE+NTX	LE+NTX
<b>Cost at <math>P_{\max}</math> (m)</b>	37.3	31.1	37.2	32.3
SEM	3.1	2.0	3.2	5.3
<b>Estimated cost at zero energy balance</b>	35.5	28.4	29.4	29.8
SEM	3.9	3.3	5.0	4.6

The results of the overall preferences testing (both test 1 and test 2) showed that the mean percentage of HE food eaten by the sheep ( $60.6 \pm 4.6\%$ ) was higher than that of the LE food ( $39.4 \pm 4.6\%$ ) in the free choice test ( $P=0.039$ ). Individual analysis of the two tests showed that the sheep tended to eat a higher percentage of HE ( $58.5 \pm 6.5\%$ ) compared to LE food ( $41.5 \pm 6.5\%$ );  $P=0.07$ ) when tested prior to the behavioural demand experiment but no differences were found in consumption amounts at the conclusion of the demand experiment ( $P=0.2$ ; Table 5.3).

**Table 5.3 Individual food preferences of sheep in a choice test. Tests were conducted prior to undergoing behavioural demand (BD) testing (test 1) and following the completion of BD testing (test 2). Intake is given as a percent of the total intake for the high energy (HE) and the low energy (LE) food types.**

Sheep No.	Test 1 (pre BD)			Test 2 (post BD)			Overall Preference change
	High energy (HE) %	Low energy (LE) %	Preference	High energy (HE) %	Low energy (LE) %	Preference	
15	73.2	26.8	HE	66.8	33.2	HE	HE-HE
17	55.2	44.8	HE	55.4	44.6	HE	HE-HE
19	66.6	33.4	HE	74.2	25.8	HE	HE-HE
20	95.9	4.1	HE	93.2	6.8	HE	HE-HE
21	55.5	44.5	HE	4.2	95.8	LE	HE-LE*
22	58.4	41.6	HE	63.5	36.5	HE	HE-HE
25	62.6	37.4	HE	6.0	94.0	LE	HE-LE*
28	33.3	66.7	LE	60.9	39.1	HE	LE-HE*
29	63.3	36.7	HE	70.2	29.8	HE	HE-HE
30	21.3	78.7	LE	92.3	7.7	HE	LE-HE*

\*Denotes change in preference from test 1 to test 2

Linear regression analysis was then undertaken to better analyse the relationship between the preferences of individual sheep for food type in the free-choice preference test (zero cost) and the work done in the behavioural demand facility (increasing costs). This showed non-significant ( $P=0.23$ ) correlations between the base preference (established in preference test 1 prior to behavioural demand testing), and the number of rewards eaten for each food type by an individual sheep in the facility (HE:LE rewards) with 7.2% of variation accounted for by this relationship. The strength of this correlation was not changed by the administration of NTX ( $P=0.23$ ).

## 5.5 Discussion

The objective of this experiment was to assess the impact of an opioid receptor antagonist on the motivation of sheep to work for access to two different feed types in a behavioural demand environment. Cost, or distance walked, was the one factor that significantly affected the amount of work the sheep were willing to do to access both food types. The pattern of reduced feeding motivation at higher costs has been reported in both this thesis (Chapters 3 and 4) and in other studies (Dumont et al., 1998; Verbeek et al., 2011; 2012a).

Overall, NTX was effective at reducing the number of food rewards consumed by the sheep over a 6h time frame and contrast analysis showed that NTX decreased the feeding motivation at the shortest costs of 1.5 and 10m, suggesting that the opioid reward system is involved in ruminant motivation for food. This finding agrees with other studies that have reported decreases in intake following antagonist administration, not only in ruminants but also in rodents and monogastric species (Morley et al., 1983; Marks-Kaufman et al., 1984; Billington et al., 1985).

In the current experiment, the sheep receiving the LE+NTX treatment increased the number of rewards collected at the lowest cost during the 12-20h period. This result may possibly suggest a pattern similar to the compensatory eating seen in the dose response test (Appendix 1) where NTX appeared to dampen the motivation to eat immediately after administration. This was then followed by a recovery period of higher rates of intake between 6 and 12h post injection. Similar effects have also been described in an investigation of naloxone on the intake of *ad libitum* feed sheep (Alavi et al., 1991). In humans, NTX has a biological half-life of approximately 4h (Verebey, 1976a; Misra, 1980). Although the pharmacokinetics of NTX have not, to our knowledge, been characterised in ruminants we assumed that the ability of NTX to block endogenous opioid action in the sheep diminished within 4-6h of administration. Therefore, it is possible that the increase in intake exhibited between 12-20h at the 1.5m cost may have reflected the loss of NTX bioactivity due to both metabolism and elimination.

A secondary aim of this experiment was to assess if down regulating opioid signalling decreased the motivation for a preferred food or increased motivation for the food highest in energy and fat. The results suggest that administration of the opioid antagonist did not, definitively, do either. It has been suggested that sheep develop preferences for food which most rapidly provide high levels of satiety through an integration of the energetic and nutritional value of the food with the orosensory response of the animal (Baumont, 1996). In the behavioural demand testing sheep displayed a higher motivation for the LE than the HE treatment when the cost to obtain food was low. This indicated that sheep in this experiment did not work hardest for the food that was the highest in energy and fat. As noted by Figlewicz and Sipols (2010), palatability is assumed to be related to the taste, flavour and, particularly, to the fat content of the food as, when tested in rodents, these are the diets which are most preferentially over-consumed (e.g. Carey et al., 1981; Gosnell and Majchrzak, 1989; Gosnell and Patel, 1993). However, sheep displayed higher motivation for the LE feed type in the present experiment. This could have been due to differences in the components of the diets altering preferences or, as previously mentioned, recognition of the differences in energy availability of the food. Again, further work, possibly under behavioural demand test conditions, would be necessary to elucidate the differences in motivation the sheep exhibited towards both treatments.

As mentioned above the data showed a pattern where the sheep were willing to work harder for access to the LE when compared to the HE treatment at the lower costs (1.5 and 10m). This finding is very similar to the results produced by the previous experiment described in Chapter 4. As mentioned in Chapter 4, the sheep may have been able to recognise foods of different energy values and were more motivated to consume the lower energy feed when the costs were shorter. One possible explanation for this behaviour is that more of the LE food would be required to meet the levels necessary for nutrition and metabolic function compared to the HE alternative. The reduction in motivation for the LE treatment at longer costs suggests that once the sheep are required to walk further than approximately 10m for access to food, the energy content of the food is no longer the determining factor in feeding motivation. Illius et al. (1999) and Dumont et al. (1998) noted that it may be difficult and not be worth discriminating between foods of a similar value, and this may also be true of foods that both offer relatively high levels of energy gain. However, this experiment and previous results (Chapter 4) suggest that it may be worth discriminating

between two foods with high energy values as long as the cost of accessing the food does not outweigh the benefit gained.

Although overall preference testing showed sheep exhibited a significant preference for the HE over the LE treatment, the baseline testing showed only a tendency for the sheep to prefer the HE and this pattern was not repeated in the post-behavioural demand test or in previous preference tests (Chapter 4). However, a tendency to prefer one food but work harder for another is a different result from that generally reported in the rodent literature. Here, it is reported that animals will work harder for a preferred food and that administration of an opioid agonist or antagonist can alter the consumption of a preferred food, regardless of macronutrient content (Gosnell et al., 1990; Gosnell and Krahn, 1992). It is possible that the differences between the trends shown in this experiment and those of the rodent literature may be based on methodological variations. Generally, baseline testing and the testing following drug administration are undertaken in the same environment. For example, Glass et al. (1996) measured the intake of preferred foods before and after a 24h food restriction, as well as following naloxone administration, in free-choice tests. It is possible that baseline preferences tested in a free-choice environment may not be indicative of preferences in an operant environment, and therefore not a suitable comparison. However, further research on preferences of sheep for food in different test environments would be necessary to confirm this hypothesis.

Previous experiments, in both this thesis (Chapters 3 and 4) and other investigations (Hutson, 1991; Verbeek et al., 2011; 2012a), have assessed the relationship between energy balance estimates and feeding motivation and found that animals will sustain an energy deficit to gain additional food. This finding was repeated in the current experiment. Reasons for an animal's continued willingness to work for food once it enters an energy deficit have previously been discussed, although one possibility merits further consideration; the theory that the homeostatic regulation of energy balance can be overridden by hedonic processes (Berthoud, 2007; Figlewicz et al., 2007; Berthoud, 2011). In Chapter 3 of this thesis it was theorised that this process might be partially responsible for an animal's willingness to sustain an energy deficit to gain additional food. Within this framework, the theory purports that an animal might be gaining a hedonic reward sufficient to continue its willingness to work for food, although it is a state of negative energy balance. However, this use of

homeostatic versus hedonic regulation to explain feeding behaviour within the context of this thesis, differs slightly from that more commonly seen in the literature. Most often, this theory is used to explain why animals will preferentially over-consume a palatable food, above their dietary requirements (Figlewicz and Sipols, 2010), rather than to explain why an animal will continue to work for food when it is in an energy deficit. However, it is possible that a hedonic reward may be influencing the willingness of the sheep to continue working for food when in a state of energy deficit in this experiment.

The costs at zero energy balance and the cost at  $P_{\max}$  were similar and were between 28 and 38 metres, irrespective of treatment. This suggests that feeding motivation began to decline around the point of zero energy balance. In earlier experiments in this thesis, similar results were observed with  $P_{\max}$  and zero energy balance estimates between 30 and 41 metres, again, irrespective of treatment and possible reasons for this have been discussed previously (Chapters 3 and 4). Although these consistent findings indicate that this effect may be real, it is difficult to theorise which metabolic feedback system and/or nutritional parameters may be influencing feeding motivation. As this regulatory system appears to influence behaviour relatively quickly, a feasible starting point for further research could involve short-term hunger and satiety patterns and the variety of hormones and peptides which are involved in these systems.

In conclusion, the administration of the opioid antagonist, NTX, decreased feeding motivation in sheep, particularly over the initial 6h test period. This suggests that the opioid reward system may be, at least partially, regulating the motivation of sheep to work for access to food. Although overall, the HE treatment was more preferred in the free-choice testing, there was no interaction between NTX and energy treatment on feeding motivation, nor were clear preferences determined during baseline testing. However, in the behavioural demand facility, sheep were more motivated to access the LE compared to the HE treatment, at the lowest costs. The results suggest that a combination of energetic balance, and the opioid system, may alter the feeding motivation of sheep in a behavioural demand environment. However, further investigation would be needed to validate this theory and to elucidate the specific mechanisms that may be involved.

## Chapter 6

# General discussion and conclusions



## 6.1 Introduction

The overarching objective of this thesis was to develop a better understanding of the motivation of sheep for food and attempt to elucidate the implications this may have for welfare assessment. Specifically, this thesis aimed to better understand the underlying factors or mechanisms that influence the motivation of a ruminant for food in behavioural demand test, focusing on the impacts of homeostatic balance and hedonic reward. To further these aims the following aspects of motivation for food were studied:

1. Relationships between the level of motivation and energy balance after a short-term food deprivation.
2. How motivational levels are impacted by food rewards of differing energy densities.
3. How motivational levels for foods of differing energy densities are altered by the opioid reward system.

Another objective was to identify measures, derived from behavioural demand models, that provide the most accurate methods to assess motivation. The remainder of this section synthesises and discusses the major findings from this research, and explores possible links between these findings, motivation levels, and welfare assessment.

## 6.2 Comparisons of measures from different behavioural demand models

Animal welfare scientists have traditionally used the elasticity of demand as an indicator of strength of motivation (Dawkins, 1990). This has been somewhat unreliable in behavioural demand testing as it assumes that the elasticity is constant across costs (Kirkden et al., 2003). However, this is often not the case as demand for a resource is usually defended at low costs, with motivation decreasing as costs increase, as shown in the experiments in this thesis. The use of  $P_{\max}$  and  $O_{\max}$  measures in this thesis ensured that both the cost at which maximum responding occurred and the maximum work done at  $P_{\max}$  were measured. The use of the alpha, or essential value, which is derived from the exponential demand model, and measures the rate of decline of the curve was also assessed, as were the quantitative measures of maximum price paid (reservation price) and number of rewards consumed. In terms of detecting differences between treatments at the 0.05 level, the number of rewards consumed, the alpha value and the  $O_{\max}$  measure were the most successful, as the alpha value showed

significant treatment differences in Chapter 3, and  $O_{\max}$  detected treatment differences in Chapters 4 and 5. Other studies have also reported that  $O_{\max}$  (maximum work done at  $P_{\max}$ ) is a more reliable measure of motivation than  $P_{\max}$  (Madden et al., 2007a; 2007b), possibly because  $P_{\max}$  does not account for the differences in the levels of food consumption or actual work done (Verbeek et al., 2012a). These experiments also suggest that the alpha value may be of use when measuring motivation in a demand test, as it assesses the decreases in motivation by measuring the rate of decline of the curve, and it can be used independently of reward size or type (Christensen et al., 2008a). The least useful measure was the maximum price paid (MPP), but that was possibly due to the practical constraints of the specific tests used. For example, to reach MPP the costs needed to increase until consumption was reduced to zero. The MPP extrapolations suggested that to achieve this, the facility would have needed to have been 70m longer. Furthermore, an experiment with more cost levels would have taken considerably longer to complete and the use of MPP did not, in this situation, provide any additional advantage over measures that were more easily obtained (e.g. number of rewards consumed and  $O_{\max}$ ).

### **6.3 The impact of energy balance on feeding motivation**

The results of the experiments conducted in this thesis indicate that energy balance, combined with effort required (cost), are likely to be the main factors influencing the amount of work the sheep were willing to do to access food. These experiments showed that at shorter costs (distances walked), of less than 25m, sheep were willing to work harder to overcome an energy deficit, in the form of a 14h food deprivation (Chapter 3), and work harder for food lower in energy content (Chapters 4 and 5). At costs of less than 25m it seems that alterations in work rate can be used to regain, or maintain, an energy related homeostatic set point. The results in Chapters 4 and 5 also showed that at low costs sheep appeared to be able to differentiate between two levels of energy density and were willing to work harder for the food with the lower energy (LE) content. This suggests that at shorter distances sheep did not work as hard to obtain the high energy (HE) food, possibly because less of this food was required to meet levels necessary for sufficient nutrition and metabolic function. It has been noted that it may be difficult, and ultimately, not worthwhile for a ruminant to discriminate between foods of similar values (Illius et al., 1999), and it is possible that the same may be true for foods with high energy values. The differences between the metabolisable energy (ME) levels of the food used for these experiments was

real (~20%), although both the LE and the HE treatments could be considered as having a ME in the high range. However, the trends shown in the results show that the sheep appeared to be able to differentiate between the two foods at costs of less than 25m. This indicates that it may have been beneficial to discriminate between the two treatments, provided that the cost did not outweigh the benefit.

It may be useful to consider energy balance, and the resulting motivation for food, in terms of optimal foraging theory. This theory predicts that an animal will make choices to maximise net energy gain per unit of feeding time (MacArthur and Pianka, 1966; Stephens and Krebs, 1986), and that patterns of food intake are determined by both food quality and taste, as well as the costs involved in accessing the food (Pyke, 1984). This theory has been used, with some success, to assess the levels of motivation, and resultant feed intake, of ruminants for differing qualities and quantities of food (Dumont et al., 1998), and for food after varying levels of restriction (Schütz et al., 2006). A logical prediction of optimal foraging theory is that an animal would reduce (or even stop) work done when the costs of consumption of food outweighed the benefits. Each experiment in this thesis showed a decrease in the work done when sheep reached the approximate point of zero energy balance (the point where energy intake equaled energy expended). Comparisons between the cost at which zero energy balance occurred and the cost at which  $P_{\max}$  (cost at maximum responding) occurred were between 29 and 41m, irrespective of treatment or experiment. This suggests that the work output of the sheep began to decline around the point of zero energy balance. Although these findings were very consistent, it is difficult to identify which metabolic feedback system and/or nutritional parameters may account for these results. This behaviour is also more difficult to explain in a ruminant than a monogastric animal due to the constant availability of energy from rumen content. As this system appears to regulate behaviour relatively quickly, a feasible starting point for further research could involve short-term hunger and satiety patterns, as well as the variety of hormones and peptides which are involved in these systems.

It is interesting to note that in the current experiments the sheep rarely ceased walking for food. The number of rewards collected decreased as distance increased, but only two of the sheep reached a 'break point' and this was only at the longest distance used (105m) (Chapter 3). Sheep continued to work for access to food even after they apparently entered

into a state of energy deficit and, therefore, it seems that a model where choices are explained by the energy gain maximisation hypothesis may not be useful in explaining the results of the current experiments, particularly at costs of greater than 25m. Possible reasons for the continued willingness to walk in when energy expended exceeded energy intake and future research directions have previously been discussed (Chapters 3, 4 and 5).

The above section indicates that there is a homeostatic component involved in determining the motivation for food within an operant test environment (where homeostatic regulation can involve short-term energy and nutrient related cues as well as longer term, metabolic related hormonal and neuronal cues) (Ferenczi et al., 2010).

#### **6.4 The opioid reward system and feeding motivation**

This thesis supports other studies (e.g. Alavi et al., 1991; Alavi et al., 1993) by providing evidence that there is a hedonic or reward related component to the feeding motivation of a ruminant. Chapter 5 reported that the opioid antagonist, naltrexone hydrochloride (NTX), was able to decrease short-term feeding motivation at the 1.5m and 10m cost over a 6h period. However, the impact of NTX on the intake of a preferred food could not be definitively determined as consistent preference patterns were not displayed by the sheep toward either LE or HE treatments.

It has been proposed that the hedonic reward system may be able to override the homeostatic regulation (Berthoud, 2007; 2011) of feed intake. The finding that hedonic reward is involved in feeding motivation in a behavioural demand test was not able to provide further information as to the importance of the hedonic system relative to homeostatic regulation. Further questions regarding this relationship could include whether homeostatic regulation is the main determinant of food intake with the hedonic reward system acting as a reinforcer, ensuring that intake is continued at levels required to maintain long term energy balance. Alternatively, the two systems may be of equal importance and understanding to what, if any, extent one system can override the other may provide a better understanding of the impacts of these systems on the adaptive ability of an individual.

## 6.5 Exploring possible links between feeding motivation, adaption, welfare and welfare assessment

The overarching aim of this thesis was to better understand the motivation of an animal for food and attempt to elucidate how this might impact welfare and welfare assessment. The motivation of an animal for a specific resource is often used to help determine its welfare state based on the assumption that an animal which is prevented from accessing a resource, that it values highly, is likely to have compromised welfare. However, little work has been done to empirically investigate this assumption, nor is it clear how an animal's efforts relate to the degree to which it needs a resource (Patterson-Kane et al., 2011). It was hypothesised that by better understanding the factors influencing motivation it might be possible to gain insight into the relationship between level of motivation, adaptive ability and coping strategies and, possibly, welfare and welfare assessment.

Unfortunately, the experiments undertaken for this thesis were not able to provide conclusive evidence as to how factors that underpin behavioural motivation might influence adaption and/or welfare. It may be useful to undertake further motivation studies, focusing on investigating adaptation and adaptive ability, to better understand the possible relationships between these components. To that end, the remainder of the current section attempts to synthesise recent literature, as well as results from the current experiments where possible, to explore this relationship and to suggest directions for future research.

Perhaps, the most obvious link which might enable motivation to reflect welfare is the behaviour that occurs when motivation is impeded, typically described as frustration. Panksepp (2005) has theorised that animals have a 'seeking/expectancy system' where seeking drives behaviours and, once experience with a reward has been gained, the seeking behaviour is followed by some comprehension, or expectancy, of being able to access the reward. When the actual outcome does not meet the expected outcome, frustration is the term generally used to explain the response of the animal (Greiveldinger et al., 2011).

Frustration behaviours vary between species (e.g. pawing, biting, increased vocalisations, head movements, displacement behaviours, escape behaviours and various stereotypic behaviours) and have been reported in lambs (Napolitano et al., 2003), goats (Carbonaro et al., 1992), boars (Bishop et al., 1999), dairy cows (Sandem et al., 2002; 2006),

horses (Goodwin et al., 2007) and domestic fowl (Duncan and Wood-Gush, 1972; Bokkers et al., 2004). However, it appears that the relationship between short term frustration and welfare compromise is not particularly well-defined. It would seem reasonable to assume that frustration is an aversive affective or emotional state (Toates, 1986) and therefore, may cause a welfare compromise when the view is taken that any adverse affective state is undesirable. However, if viewed as part of an adaptive process, short term frustration may simply be an animal ensuring that it has tried all options to obtain a resource.

A review of energy balance of mammals from an ecological perspective (Stubbs and Tolkamp, 2006) has noted that sustained, high motivation may also confer an evolutionary advantage. Therefore, high levels of motivation may not necessarily reflect reliable information regarding welfare compromise (Verbeek, 2010) as it may be part of normal, adaptive behaviour. Throughout ruminant evolution, few species would have had continued access to high-quality foods due to seasonal variations and environmental events and would have needed to spend considerable time and effort foraging. When food was abundant (when supply exceeded demand), it is likely that motivation remained high until energy deposits were restored (Stubbs and Tolkamp, 2006). If it is an adaptive advantage to be highly motivated to seek and ingest food, it is conceivable that animals exhibiting lower levels of motivation may not be as adaptively fit and may have poorer welfare than their more highly motivated conspecifics.

When food access is thwarted it is likely that there is another consequence for the animal. Bokkers et al. (2004) restricted the food intake of broilers and found that the cohort of birds fed 50% of an *ad libitum* diet showed greater motivation for food rewards than those fed 75% of an *ad libitum* diet. These authors also measured frustration behaviours after the last food reward was obtained and reported that birds showed significant levels of these behaviours, such as re-directed pecking, indicating that the animals were still hungry. This negative subjective state may indicate compromised welfare, although there is little evidence of the level of hunger that equates to poorer welfare. Verbeek (2010) has noted that strong motivation for food only indicates whether the animal 'wants' the resource or not and does not provide insight into the affective state that having or not having the resource creates. To better understand the impact of hunger on welfare state it would be necessary to have knowledge of the level of feeding restriction at which an animal experiences hunger and to

understand how experiencing hunger impacts the affective state of the animal (Verbeek, 2010). It would also be necessary to understand the implications and differences between short term hunger, or the motivation for food as it changes with diurnal cycles, with those of longer term hunger, as it is possible that adaptation to the state of ‘constant hunger’ occurs over time (i.e. through the downregulation of intake systems).

It may be that decreases in motivation provide more information regarding welfare state than initial levels. For example, sheep in the experiments described in this thesis exhibited high levels of motivation for food, provided that the cost of access did not outweigh the benefit gained. However, when behaviour options to alter intake rate (e.g. increasing reward size) were not possible, motivation gradually decreased with increasing costs. This may suggest that the initial responses (e.g. continuing to work harder to access food) were no longer beneficial. This fits with Panksepp’s (2005) seeking/expectancy model where animals exhibit high motivation and frustration-driven behaviours to attain goals and when this does not succeed the behaviour is eventually extinguished. Although only 2 sheep stopped walking for food in the experiments described for this thesis, it is possible that more may have extinguished this behaviour if longer costs had been used. Given an animal’s range of available responses, when certain responses are no longer effective, other responses are likely to be employed. An ‘adaptation cascade’ might include behavioural changes as a first response followed by physiological and then metabolic changes, although these changes are likely to overlap. Extrapolating this further, it is possible that the increased feeding motivation, seen in Chapters 4 and 5 for the LE over the HE treatments, was both a behavioural and physiological adaptation strategy to ensure that sufficient energy intake was achieved. Given the lack of behavioural options available to the sheep in the operant environment (e.g. the sheep could not, effectively, increase bite rate or reward size), motivation eventually decreased for both treatments as the costs increased. Further research may have indicated whether the sheep were making any other physiological or metabolic adaptations to the demand environment (e.g. changes in blood parameters or utilising various energy reserves), and this may be an area of further research which could allow a better understanding of adaptation and adaptive ability.

If desired goals are repeatedly unfulfilled, it may be more likely that longer-term motivational frustration, and possible associated welfare compromise, is experienced.

Learned helplessness and behavioural stereotypies, (e.g. bar-biting, tongue rolling, pacing, swaying) are possible outcomes of this, although there are varying reports of the impact of stereotypies on welfare state. Nevison et al. (1999) noted that stereotypies generally form from a functional goal orientated behaviour which is unfulfilled and frustrated and becomes fixed in a specific behavioural pattern. There is evidence to suggest that a stereotypy can be linked with physiological measures which may indicate a sub-optimal or negative welfare state (Von Borell and Hurnik, 1991; Vestergaard et al., 1997). In contrast, other studies have reported no changes in physiological indicators between stereotypic and non-stereotypic animals (Pell and McGreevy, 1999), as well as increases in stereotypies following improvements in housing conditions (Korhonen et al., 2001). In an attempt to clarify these anomalies, Mason and Latham (2004) reviewed stereotypical behaviour and classified it as having a negative effect on welfare, a neutral effect or a positive effect (where negative welfare was associated with likely signs of fear, stress or depression). Their results indicated that approximately 68% of the situations that caused or increased stereotypies also decreased welfare. However, they also found that within that stereotypy-inducing environment the animals exhibiting the highest levels of these behaviours were likely to be the least welfare compromised. This was the situation in 60% of individuals studied, although there were exceptions. This indicates that in some situations an animal exhibiting a stereotypy may have better welfare than those without this behaviour, as the stereotypy may confer better adaptation to the specific environment.

It could be proposed that while high levels of motivation may not directly reflect a change in the welfare of an animal, it is still possible to assume that this type of motivation will mean an eventual welfare compromise, provided a valued resource remains restricted. As with most measures of welfare the issue then becomes deciding upon the point at which welfare is compromised, or at what threshold intervention should occur. It may be possible to address this by assessing the length of time which a valued resource is restricted. For example, if an animal has restricted access to food for a short period of time (hours to days), it is likely that it can use adaptive processes such as behavioural and, possibly, physiological changes, ensuring that it can cope with and recover from the challenge. If a food restriction is longer term (weeks to months), it is possible that the animal will exhibit reduced motivation to feed and may suffer welfare compromise. However, without further research it is not possible to make definitive conclusions with regard to this hypothesis.

The above discussion indicates that high levels of motivation (provided the cost of the motivation is minimal and/or short term) would seem to confer an adaptive advantage to an animal and therefore, may indicate an animal's abilities to cope with a challenge, rather than reflect a welfare compromise. As with many welfare measures, this indicates that high levels of motivation alone may not be a reliable indicator of welfare state. The measurement of animal welfare can be somewhat subjective and if we are to have confidence in the suite of measures that we use to assess welfare, and if motivation is to be one of them, then empirical evidence would be very useful to better understand this measure. There are many possible directions which could be taken to further this research, as have previously been mentioned, and two have particular promise. These are, firstly, investigating what, if any, levels of biological dysfunction occur when an animal is deprived of a valued resource, particularly with reference to longer term deprivation, and secondly, examining whether changes in motivation levels (high to low) can be used as an early warning sign that an animal is no longer coping with a challenge, and whether this lack of coping and reduced adaption results in a welfare compromise.

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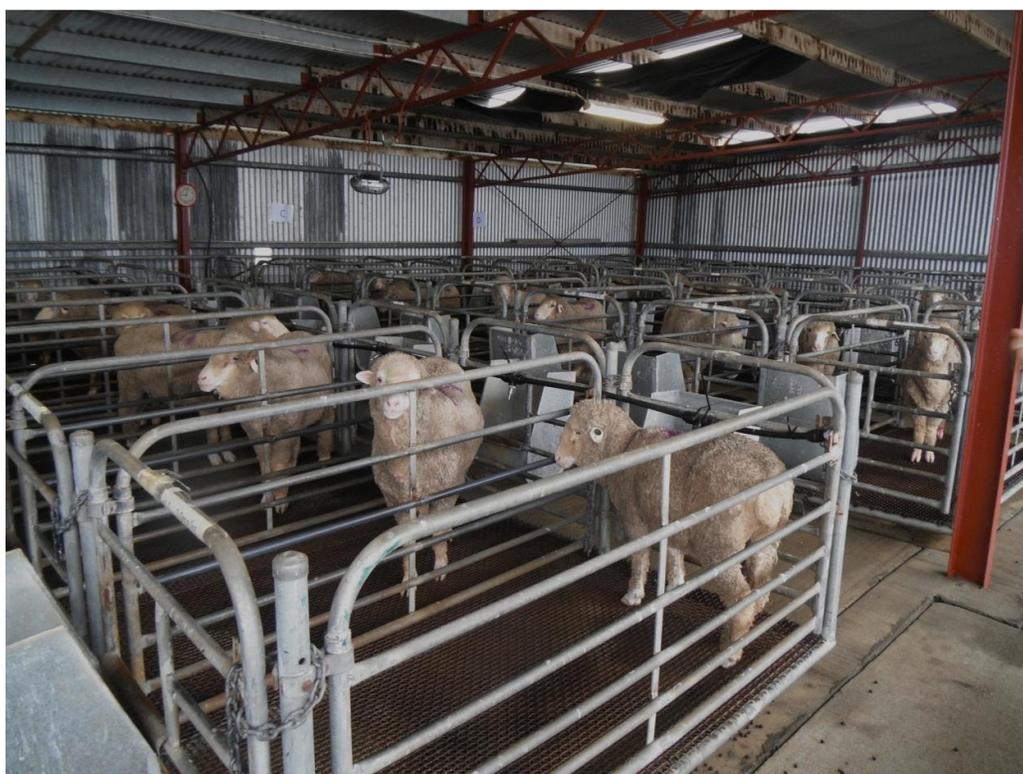
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**Appendix 1**

**Dose response investigation of levels of  
naltrexone hydrochloride suitable for use in  
sheep**



## **A 1. Introduction**

The aim of the dose response study was to investigate what dose level of naltrexone hydrochloride (NTX) could be safely administered to sheep and would be sufficient to create a difference in feed intake without impairing the animal's ability to make decisions or unduly affect other behaviours.

## **A 2. Materials and methods**

This study was approved by the CSIRO's Animal Ethics Committee and conformed to the standards in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Animal Research Authority Number 11/22). The testing was undertaken at the CSIRO in Armidale, NSW at the Chiswick FD McMaster Laboratory. All animals were closely monitored during and after the experiment and no adverse long-term effects on welfare or health of the animals was observed.

### **A 2.1 Animals and housing**

Thirty two mature 3-year old Merino wethers were used for the dose response portion of this experiment. The sheep were weighed weekly and over the 8 weeks of the dose response study had a mean body weight of  $48.2 \pm 0.7$ kg and a mean body condition score of  $2.8 \pm 0.4$  when scored from 1-5 (Russel, 1984).

All wethers were born and reared on the same experimental farm and were grazed on pasture and supplemented with concentrate sheep animal house (AH) pellets (11.9MJ/kg DM). During this supplementation period the animals were habituated to the presence of human handlers. Nutritional supplementation was slowly increased over a 5 week period. Once habituated to both the dietary changes and the presence of trainers the wethers were moved to a partially enclosed housing environment, allocated to individual pens (2m x 1.0m) and given another 10 days to habituate the new environment. They had continual access to water through nipple drinkers and were provided with additional water during the first 4 days of being placed in individual pens while they learnt to use the drinkers. The sheep were fed once daily between 7:00 and 07:30am and initially received a theoretical maintenance ration (AFRC, 1995; Primary Industries Standing Committee, 2007) of 700g of pellets and 100g of oaten chaff (8.9MJ/kg/DM) per sheep each day. As *ad libitum* feed would be provided to the

sheep during the intake testing as part of the protocol for the dose response investigation, an attempt was made to feed the sheep *ad libitum* amounts of food. To achieve this, the maintenance ration was slowly increased. However, at 200% of theoretical maintenance (1400g of AH pellets per day), 75% of the sheep were still eating all of the food they received in the first 6h and large gains in weight over the experimental period and the impact of this on the effects of the administered drug were a concern. Consequently, the amount of feed was decreased to an estimated 170% of a theoretical maintenance ration which was 1200g AH pellets and 100g oaten chaff per day (AFRC, 1995; Primary Industries Standing Committee, 2007). This provided an amount of feed close to the *ad libitum* levels the sheep would receive during the feed intake testing, but produced a slower rate of weight gain over the period the animals would be in the facility. The animals were kept in this housing environment on this ration for the remainder of the dose response experiment. On completion of this portion of the experiment the animals were returned to a paddock and continued to receive AH pellets at a reduced rate until they were weaned off the supplemented feed. Following this the animals were returned to the experimental farm.

### **A 2.2 Drug administration and treatments**

Naltrexone hydrochloride (NTX; Tocris, Bristol UK) is a general opioid receptor antagonist and functions by blocking access of agonists to the opioid receptor sites at the molecular level. Although, to our knowledge naltrexone has been utilised infrequently in sheep, it was chosen for this experiment as it has a relatively long acting blood plasma half-life, compared to other opioid antagonists, such as naloxone, and opioid antagonists have been used to successfully to alter feeding motivation in sheep (Alavi et al., 1994). When taken orally NTX has an initial phase half-life of approximately 4 hours and terminal phase of approximately 10 hours and takes up to 72 hours before almost no traces of the drug are found in the blood plasma (Verebey, 1976b; Misra, 1980). As there are no agonists present in the drug it has minimal side effects in humans (nausea and, rarely, some slight abdominal pain). It has also been found that abrupt discontinuation does not cause changes typically characteristic of agonists. Martin et al. (1973) also demonstrated that tolerance does not develop to the antagonist action of NTX and it is non-addicting. These data and information suggested that NTX would be a suitable drug to use to assess the feeding motivation within, at least, the first 6h of a behavioural demand test and to be administered to sheep repeatedly, with little to no adverse effects.

In humans the recommended oral clinical dose is 1mg/kg; however, studies have indicated that oral doses of at least 20mg/kg have not been found to be toxic in rats, dogs or monkeys when given either intravenously, subcutaneously or orally (Misra, 1980). The few studies which have used NTX in sheep have varied greatly in their purpose and dose rates. For example, NTX has been administered intravenously in either a 50mg or 150mg single dose to assess if the drug delays the onset of maternal behaviour in the ewe (Caba et al., 1995). In comparison, Maas et al. (1986) has investigated the effect of opioids on rumen motility and used between 20 and 200 mcg/kg, again administered intravenously. As NTX is most often used to assess the rewarding properties of the various components of food (e.g. fat and sucrose), feed intake and feeding patterns most studies in the literature have administered the drug to rats using a variety of methods (intravenously, intraperitoneally or via intracerebroventricular cannula) and in a wide range of doses from 25mcg to 30mg/kg (Sanger and McCarthy, 1982; Kirkham and Blundell, 1987; Naleid et al., 2005; Naleid et al., 2007).

The treatment levels chosen for this experiment were 0.1 (low), 0.5 (medium) and 1.0 (high) mg/kg as well as a control (sham injected) group (injection of saline to the same volume as the treatment groups). It was thought that within this range behavioural differences may be observed and both short and long term detrimental impacts on the sheep avoided. The drugs were dissolved in sterile water to a concentration of 10mg/mL and were administered intramuscularly into the gluteus muscle. This route of administration was chosen for the ease of repeated administration, a reduction in handling and stress when administered to sheep used to being handled and to allow slow the release of the drug into the body. This was important as the animals would be tested in the behavioural demand facility for periods of 20h. Sheep were randomly allocated to one of 4 treatment groups, low, medium, high or sham and these groups were balanced for weight and body condition score. For ease of handling and testing, sheep were further split into 4 cohorts; A, B, C and D with each cohort containing all 4 treatment groups. Dose response sheep had a minimum of 10 days between drug treatments and each sheep in the dose response trial received NTX twice; once for the feed intake test and once for the approach/avoidance conflict motivation test (see below).

### **A 2.3 Food intake test**

The food intake test was repeated twice, 5 days apart, so that both baseline measures of intake and intake following drug administration could be assessed. For practicality, 2 cohorts were tested each day. Feed bins were cleared of any remaining food at 6:30am the day of the test and the first animal in the first cohort was fed 1000g of fresh AH pellets at 0h (7:00am), immediately followed by the second animal. Each of the remaining 6 animals in that cohort were fed, followed by the 8 animals in the second cohort. The feeding order was maintained throughout the day and for each test. On drug administration test days, the first animal in the first cohort was injected at 0h (6:59am), followed by the immediate presentation of 1000g of fresh pellets followed by the remaining 15 animals in the 2 cohorts. The same order as baseline testing was maintained. Feed remaining in the bins was removed after 20min (7:20am for the first animal), followed by the immediate presentation of another 1000g of fresh pellets. The weight of remaining food was measured at 20min intervals for the first hour followed by hourly measures until 3h at which time it reverted to 20min intervals until the 7h mark. This was followed by measures at 8, 9, 12 and 24h. The placement of 20min intervals corresponded to periods of higher levels of feed intake patterns shown by sheep in previous behavioural demand experiments (Chapter 3).

### **A 2.4 Approach / avoidance conflict motivation test**

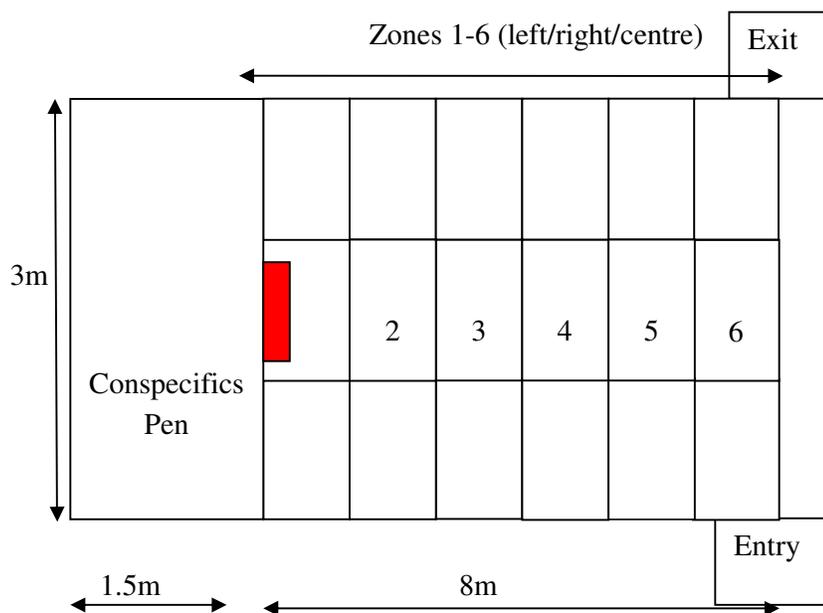
The conflict motivation test was used to aid in assessing changes in motivation in the sheep caused by NTX. A conflict motivation test generally involves a motivational choice for the sheep (Kilgour, 1998) in that it produces an approach/avoidance conflict decision for the animal. The test involves creating a conflict between the social attraction of conspecifics at the end of the arena (Figure A.1), versus the fear of a human observer, positioned in front of the conspecifics in the arena (Erhard, 2006)

The test arena was 3m wide by 8m long with the conspecifics pen at one end measuring 3m x 1.5m. Five sheep from a different cohort to that which was being tested were placed in the conspecifics pen prior to the testing to remove underlying preferences an animal may have for known familiar conspecifics. The steel panels used for the arena were 1.2m high and all panels were covered with recycled rubber split belt, except the panel dividing the arena proper, and the conspecifics pen to allow visual contact (Figure A.1 and A.2). A series of squares approximately 1m apart were marked on the concrete floor with pavement chalk

allowing for the quantification of the sheep's movement. The squares were number 1-6 (front (observer) to back (entry/exit point) and left, right or centre. Each test sheep was first habituated to the arena in groups of 5 for 3 x 20min periods to ensure that the resulting data were not confounded by the novelty of the experience. The in-arena observer was positioned approximately 0.5m in front of the conspecifics pen in the centre of the arena and was in position when the test sheep entered the pen. The same person (in-arena observer) wearing the same clothing was used for all tests and this person had previously not interacted with the sheep in any way.

Initial analysis of the feed intake data indicated that differences in intake were only found in sheep receiving the highest level of the drug (see results) so to avoid unnecessary dosing of the animals only the control sheep (sham injected) and the sheep receiving the high (1.0mg/kg) treatment underwent conflict motivation testing. Therefore, 4 animals from each cohort (2 x control, 2 x high drug treatment) were tested giving a total of  $n = 16$  animals tested in the conflict motivation test. On the day of testing all the animals were fed at 7:00am and the first animal to be tested was administered NTX or sham injected at 8:00am. The remainder of the injections, sham or otherwise, were administered at 15min intervals (e.g. 8:15am for second sheep being tested). All animals were tested in the arena 1h after drug administration. The animals were moved quietly from their individual pens to the arena and the test began once the animal had entered the arena. The animal was left in the arena for 3min and during this time video recordings (Sony HDR-XR550, Sony Corporation, Tokyo, Japan) of the animal's movements were made from an elevated hide positioned behind the in-arena observer.

Measurements were also made of the number of vocalisations, urinations, defecations, escape attempts (any sudden movement where the animal jumped, reared or ran towards a wall), attempts to join conspecifics (rearing, jumping toward internal panel between arena and conspecifics), touching nose to ground, nose to wall, nose to in-arena observer, nose to conspecifics and staring at the in-arena observer. From the video recordings the total number of zones crossed (a sheep was considered to have entered a zone when its front legs had crossed a line), number of zones crossed in the first 30s, mean distance from the conspecifics (calculated by multiplying the distance from the conspecifics pen by the proportion of time spent there) and the approximate total distance travelled over the 3min test were measured.



**Figure A 1.** Schematic of the approach/avoidance conflict motivation test arena. In-arena observer positioned in box in front of conspecifics pen in zone 1.



**Figure A 2.** Testing of individual sheep in the approach/avoidance conflict motivation test. The pen of conspecifics is located in the foreground.

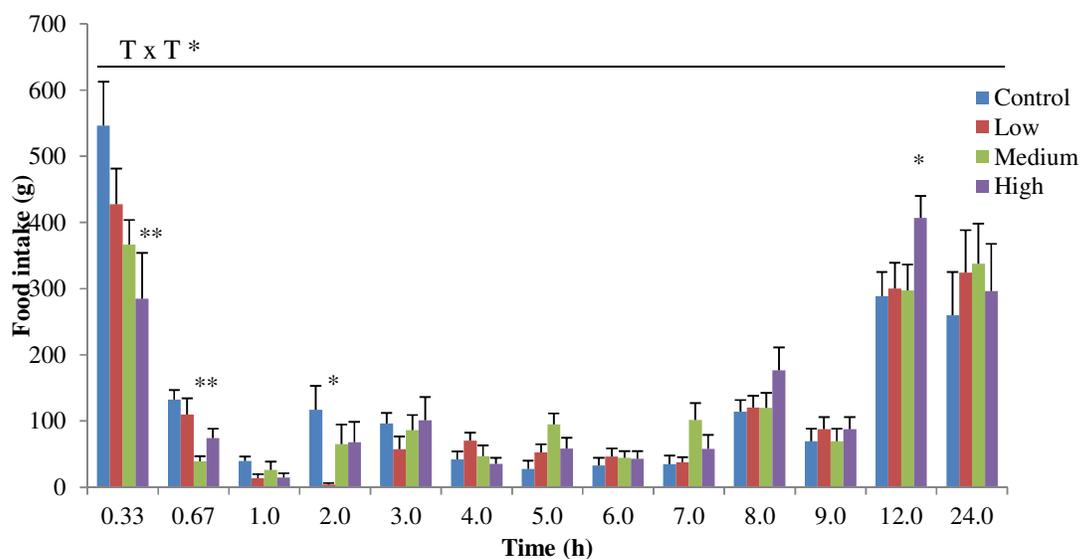
### **A 2.5 Statistical analysis**

Statistical procedures were all carried out in GenStat 15 (VSN International Ltd., Hemel Hempstead, U.K.). All data were checked for normality and transformed when necessary (square root transformation was used for all food intake data). Data consumed at time points from the food intake test were analysed using a repeated measures ANOVA to test for differences between drug levels over time. Sheep was included as a random effect and the total food intake from the baseline testing was included as a covariate. Individual time points for the food intake test were also analysed using ANOVA and corresponding baseline values were included as covariates. All *posthoc* contrast analyses were Bonferroni corrected. Data from the conflict motivation tests were analysed using the Kruskal-Wallis one way ANOVA procedure and all data are presented as actual mean  $\pm$  standard error of the mean (SEM) unless otherwise stated.

### **A 3. Results**

Following administration of NTX there was a significant time by NTX interaction for food intake ( $P=0.036$ , Figure A.3). There were also significant effects of NTX 20min after administration with control sheep eating more than the high dose group ( $P<0.005$ ). Sheep given the high dose of NTX continued to have significantly reduced feed intake when compared to controls 40min after administration ( $P=0.02$ ), although this effect was no longer visible after 1h ( $P>0.1$ ). Forty minutes after administration of NTX the medium dose sheep ate less than the controls ( $P=0.001$ ) and at the 2h measure the low dose sheep were also eating less than the control animals ( $P=0.01$ ). At 8h the high dose sheep tended to eat less than the controls ( $P=0.08$ ) and significant differences in feed intake appeared again 12h after administration with high dose sheep eating less than control sheep ( $P=0.03$ ), as well as the medium dose ( $P=0.03$ ) and low dose sheep ( $P=0.02$ ). No differences were found between intake of low and medium dose levels at 12h or at any of the drug levels 24h after treatment.

Analysis of the approach/avoidance conflict motivation test data showed no significant differences between treatments for any of the parameters measured although the sheep administered NTX tended to move close enough to sniff the conspecifics more often than the control animals ( $P=0.06$ ). However, no differences were found in the mean distance from conspecifics ( $P=0.6$ ) between treatments.



**Figure A.3** The effects of the opioid antagonist naltrexone (NTX) on food intake over a 24h period ( $n=32$ ). \* indicates a treatment difference at individual time points between dose rate and control ( $*P<0.05$ ,  $**P<0.005$ ). TxT indicates an overall treatment x time interaction of the NTX on feed intake ( $P<0.05$ ). Control: sham injection; Low; 0.1mg/kg NTX; Medium; 0.5mg/kg NTX; High 1.0mg/kg NTX.

#### A 4. Discussion

The aim of the dose response trial was to investigate which, if any, of the proposed dose rates would be appropriate for use in a behavioural demand test. To be suitable the dose needed to be high enough to impact on levels of food intake without interfering with decision making abilities or other behaviours. Given the results mentioned above the 1.0mg/kg dose was thought to be the most suitable as it reduced food intake within the first hour following administration, appeared to cause compensatory eating later in the test (12h), indicating an earlier suppression of intake had likely been achieved, and had no long lasting negative impacts on the sheep used in the experiment. The use of the 1.0mg/kg dose is further supported by the results of the conflict motivation test which found no behavioural differences between the control and treatment sheep at the highest dose rate. There is evidence that suggests that opioid antagonists reduce social and exploratory activity in rodents (File, 1980; Rodgers, 1982; Rodgers et al., 1984) and ruminants (Gates et al., 1990). This may have impacted on the willingness of sheep to move away from and toward flock mates, as was required in the behavioural demand test facility. However, this seemed unlikely to be problematic as the above results suggest that the highest dose tested was low enough to prevent this from occurring.

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## Appendix 2

### Publications arising from this thesis

#### Peer reviewed scientific journals

Doughty, A.K., Hinch, G.N., Ferguson, D.M., and Matthews L.R. 2012. A comparison of analytical methods to assess the strength of demand for feed rewards in sheep. Behavioural Processes (Submitted to journal)

Doughty, A.K., Ferguson, D.M., Matthews L.R and. Hinch, G.N 2012 Energy balance plays a role in the motivation of sheep for food in a behavioural demand environment. Applied Animal Behaviour Science (Submitted for internal review).

Doughty, A.K., Ferguson, D.M., Matthews L.R and. Hinch, G.N 2012 Sheep in a motivation test work harder for access to low energy food. Applied Animal Behavior Science (Draft completed).

Doughty, A.K., Ferguson, D.M., and Hinch, G.N., 2012 Opioid reward systems may influence the willingness of sheep to walk for food in a motivation test. Physiology and Behavior (Draft completed).

Doughty, A.K., Ferguson, D.M., Matthews, L.R and Hinch, G.N., 2012 A note on suitable levels of opioid antagonist, naltrexone hydrochloride, for use in sheep. Physiology and Behavior (Draft completed).

#### Peer reviewed conference abstracts

Doughty, A.K., Ferguson, D.M., Matthews L.R and Hinch, G.N. 2011. Using motivation to assess welfare. Are we measuring what we think we are measuring? Proceedings of the RSPCA Australian Scientific Seminar, 2011, Canberra, Australia.

Doughty, A.K., Hinch, G.N., Ferguson, D.M., and Matthews L.R. 2011. Energy balance and feeding motivation in a demand test. Proceedings of the 45<sup>th</sup> Congress of the International Society for Applied Ethology (ISAE), 2011, Indianapolis, USA.

Doughty, A.K., Ferguson, D.M., Matthews L.R and. Hinch, G.N. 2011. Improving the practicality of measuring feeding motivation in ruminants. Proceedings of the 5<sup>th</sup> International Conference on the Assessment of Animal Welfare at the Farm and Group Level, Guelph, Canada.

Doughty, A.K., Ferguson, D.M., Matthews L.R and Hinch, G.N. 2012. Opioid systems may influence willingness of sheep to walk for food in a motivation test. Proceedings of the Regional Meeting of the ISAE, Australasia and Africa, Melbourne, Australia.