The detection of disease in beef cattle through changes in behaviour

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ABSTRACT

Health challenges that lead to subclinical or clinical diseases can increase treatment costs, reduce performance and affect animal welfare. These impacts can be reduced by starting treatment early. Since behaviour is known to be affected by the health status of an animal, an early diagnosis by monitoring changes in behaviour is a possibility. The objective of this thesis was to quantify the changes in behaviour that take place as a consequence of health challenges and to asses them for their suitability to be used for the early detection of (sub) clinical disease. The behaviours looked at were: feeding; drinking; activity; and posture.

In two experiments an acute health challenge was used, in the form of either a vaccination or a lipopolysaccharide bolus. These acute challenges, due to their transient nature, did not manifest as substantial changes in behaviour. A gastro-intestinal parasite (*Ostertagia ostertagi*) was used as a chronic challenge in three experiments. This health challenge affected several aspects of the behaviours measured. Due to its prolonged nature these effects increased over time, but were reversed after the challenge had been removed. The challenge also showed a dose dependency, demonstrating a threshold, rather than a gradient, when affecting behaviour for different levels of parasitic infection.

From the overall results it was concluded that activity, posture and in some cases feeding behaviour, were affected by the parasitic (*O. ostertagi*) health challenge. From these, activity and posture were found to have the greatest magnitude of change and to be the most consistently affected across experiments. These behavioural changes, however, started at the same time as a rise in faecal egg counts and pepsinogen levels. Nonetheless, due to the magnitude of the behavioural changes, behaviour could still be used as an indicator of health status, predominantly in animals that receive little visual monitoring.

Declaration

I hereby declare that this thesis is of my own composition and has not been accepted in any previous application for a degree. All sources of information have been specifically acknowledged by means of referencing.

Ollie Szyszka

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Publications and conference abstracts

Peer-reviewed publications

Szyszka, O., Tolkamp, B.J., Edwards, S.A., Kyriazakis, I. *In press*. The effects of acute versus chronic health challenges on the behavior of beef cattle. *Journal of Animal Science*.

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Conferences

Szyszka, O., Edwards, S.A., Kyriazakis, I. The detection of disease in beef cattle through changes behaviour. Proceedings of the 16th annual EAAP Conference, Heraklion, 23-27th August 2010, pp. 68. Theatre presentation.

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Szyszka, O., Tolkamp, B.J., Edwards, S.A., Kyriazakis, I. The temporal changes in behaviour of beef bulls during exposure to *Ostertagia ostertagi* and subsequent recovery from infection. Proceedings of the British Society of Animal Science and the Association of Veterinary Teaching and Research Work Annual Conference, Nottingham, 24-25th April 2012 pp. 4. Theatre presentation.

List of abbreviations

au	Absorbance units
AUC	Area under curve
BW	Body weight
^{o}C	Degrees Celsius
CI	Confidence interval
CP	Crude Protein
d	Day
DM	Dry matter
epg	Eggs per gram
FEC	Faecal egg count
g	Gram
h	Hour
iu	International units
kg	Kilogram
L	Litre
L3	Third stage larvae
LPS	Lipopolysaccharide
m	Meter
ME	Metabolisable energy
MFV	Minimum function value
Min	Minute(s)
MJ	Mega joule
ml	Millilitre
nm	Nanometre
PBS	Phosphate buffered saline
rpm	Rounds per minute
SED	Standard error of the difference
SEM	Standard error of the mean
STFB	Short term feeding behaviour
µg	Microgram

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Chapter 1: General Introduction

When farm animals encounter health challenges, this can result in consequences ranging from minor effects that manifest as small losses in performance, to major deterioration in health and even possible death. If, however, the health challenge is detected and treated early, this can reduce production losses and treatment costs, and improve animal welfare (González et al., 2008). However, the early detection of health and welfare problems can be difficult and tends to rely on subjective identification (González et al., 2008). This is made harder by recent developments in cattle farming systems that reduce human-animal contact, thus decreasing the chance of signs associated with the challenge being spotted at an early stage. Moreover, subclinical health challenges are by definition difficult to detect due to the absence of clinical signs and might therefore escape early diagnosis. Allowing subclinical health challenges to linger usually compromises welfare and performance, and can possibly lead to more severe problems. It has been estimated that subclinical disease costs several million pounds per annum to the UK cattle industry (EBLEX Beef Diseases Directory, 2008).

One of the first changes that may occur as a consequence of a health and/or welfare challenge is a change in behaviour (Sowell et al., 1999; Quimby et al., 2001; Urton et al., 2005; González et al., 2008). Such behavioural changes have been encompassed by the term sickness behaviour (Hart, 1988). Sickness behaviour is the response of an animal to a health challenge that enhances the affected animal's ability to cope. Common symptoms of sickness behaviour include a reduction in voluntary food intake, hence called (pathogen-induced) anorexia (Larson and Dunn, 2001; Kyriazakis, 2010), reduced activity (Edwards and Tozer, 2004; Reiner et al., 2009) and a change in social interactions (Edwards, 1988; Galindo and Broom, 2002). Anorexia can serve, amongst other functions, to prevent the intake of nutrients such as iron, which will inhibit bacterial reproduction as well as reduce metabolic energy costs (Hart, 1988), or to allow the animal to be more discriminatory in its selected diet (Kyriazakis et al, 1998). A reduction in activity may allow the animal to conserve energy and heat (Hart, 1988), and a change in social behaviour can prevent the disease from spreading to other animals (Hart, 1990), although the latter maybe a function of the other animals rather than of the affected animal. A more detailed account of the changes in behaviour during a health and welfare challenge in various livestock species is given in Chapter 2.

The changes in behaviour can be specific to the health challenge; for example, an increase in drinking in the case of metabolic health challenges (Cottee et al., 2004) would allow the animal to dilute the effects of deleterious metabolites, such as the accumulation of acids in the rumen or to counteract the increased ruminal osmolality (Owens et al., 1998). On the other hand, drinking behaviour can decrease for other health challenges, such as micro-parasitic (Plata-Salaman and Borkoski, 1993; Sowell et al., 1999) and macro-parasitic (Ferre et al., 1996) challenges. This may be a direct consequence of the anorexia because of the close association between feeding and drinking (Fitzsimons and Le Magnen, 1969), or a side effect of reduced activity (Hart, 1988). However, there may also be consistent changes in behaviour across health challenges, such as a decrease in activity for animals suffering from a micro-parasitic infection (Plata-Salaman and Borkoski, 1993; Borderas et al., 2008), macro-parasitic infection (Reiner et al., 2009), metabolic disorders (Edwards and Tozer, 2004) or lameness (O' Callaghan et al, 2003). Another good example is the pathogen induced anorexia, which seems to be a feature of most health challenges, and it has been suggested that for a wide range of doses and pathogen challenges, the reduction in the food intake can be of the same magnitude, i.e. 20% reduction (Sandberg et al, 2006).

Focussing on the behavioural changes for the detection of specific disorders can be useful at times when an animal is at risk; for instance, after calving cows can be monitored for an increased risk of metritis (Urton et al., 2005) or ketosis (Goldhawk et al., 2009) that may lead to specific changes in behaviour. However, it may be equally (or even more) beneficial to know which behavioural changes are common across health challenges, because these can predict the general health status of an animal rather than predicting specific disorders. Activity, for instance, is affected for different health challenges (as mentioned above), making it a more general indicator of health status. Despite the fact that all these different health challenges show similar reductions in behaviour, the reasons behind the change can differ between challenges as they may be a consequence of sickness behaviour (Hart, 1988) or as a direct consequence of discomfort (O'Callaghan et al., 2003).

When using behaviour as an indicator of a health challenge it is important to know at what rate behaviour changes. If the change is immediate or very fast post challenge, there would be clear benefits as action can be taken very quickly. More gradual changes may have reduced diagnostic value. This is an area of research that has received less attention,

as most research focuses on retrospective studies where the prediction of health challenges are relative to clinical diagnosis (Sowell et al., 1999; Quimby et al., 2001; Edwards and Tozer, 2004; González et al., 2008). The rate of recovery of behaviour after the health challenge has been treated is also an area that has received little attention. Knowledge of this may be beneficial, as it may for example be used to assess the effectiveness of a treatment. Only González et al. (2008) and Edwards and Tozer (2004) report a recovery in activity and feeding behaviour within a number of days after treatment for metabolic disorders, and a slower recovery of feeding after lameness. Kyriazakis et al. (1994), on the other hand, report an almost instantaneous recovery in food intake of previously parasitized sheep after treatment with an antiparasitic drug. The change in food intake was assumed to be associated with changes in the feeding behaviour. The rate and magnitude of change in behaviour is likely to depend to a certain degree on the challenge dose (Larson and Dunn, 2001; Sandberg et al., 2009), with subclinical challenges showing more subtle changes in behaviour (Kyriazakis and Tolkamp, 2010). These relationships may also depend on the extent of damage caused by the pathogen. This would be of particular relevance to how quickly behaviours can recover once the animal has recovered from the challenge. However, such relationships between dose and behaviour are currently unknown.

With the subtle changes in behaviour associated with subclinical disease and the reduction in interaction between the stockperson and the animals, it is important (and indeed necessary) that such changes in behaviour can be monitored automatically if they are to have any value (Kyriazakis and Tolkamp, 2010). It is currently possible to use automated means for the early detection of disease, such as the automated monitoring of feeding behaviour with the use of passive transponders which monitor presence at the feeder (Sowell et al., 1999; Quimby et al., 2001; Huzzey et al., 2007) or computerized individual feeders (González et al., 2008). Activity and posture can also be monitored automatically with the use of pedometers (O'Callaghan et al., 2003; Mazrier et al., 2006). Social interactions can be recorded through the use of contact sensors (Böhm et al., 2009) which log the distance between animals giving the duration and frequency of the contact, though not the nature of the interaction. This is an area of research that is currently yielding rapid developments and is considered to be part of the Precision Livestock Farming initiative.

1.1 Thesis Aims

Developments in livestock industry, such as the use of automated feeders or compulsory individual electronic tags, can offer the opportunity to automatically capture behaviour on farm and use them for the early detection of health and welfare challenges (Weary et al., 2009). This could lead to behavioural change being used as a non-invasive, early indicator of reduced health and welfare.

The overall aim of this thesis was to identify and quantify changes in behaviour that could be associated with the early onset of disease or subclinical disease in beef cattle, with these behaviours being monitored through automated means.

The specific objectives of the thesis were:

1. To evaluate through a literature review the behaviours most suitable for the early detection of different health challenges. These behaviours should also be suitable for monitoring through automated means (Chapter 2).
2. To identify the behavioural changes following a subclinical micro- and macro-parasitic health challenge and determine the magnitude of such changes (Chapter 3 and 4).
3. To address the differences in behavioural changes resulting from either an acute and or chronic subclinical health challenge, taking into account the magnitude and duration of the behavioural effects (Chapter 4).
4. To quantify the recovery of behaviour and physiological parameters from a chronic parasitic health challenge after the animals had received treatment relieving them from the challenge (Chapter 5).
5. To quantify the relationship between different doses of a macro-parasitic health challenge and the changes in the behaviour of cattle, and to compare this to predictions of two suggested models of response (Chapter 6).

Chapter 2: The Diagnostic Value of Changes in Animal Behaviour as a Consequence of Health Challenges

2.1 Introduction

In production farm animals, health challenges result in a continuum that ranges from minor performance losses to severe deterioration of health and eventually death. Health challenges that lead to subclinical or clinical diseases also incur treatment costs and reduce animal welfare (Quimby et al., 2001; González et al., 2008). Further economic losses can be caused by premature culling and increased labour input for monitoring and treating the animals. A way to minimize disease impact and reduce costs is to start treatment early. However, making an early diagnosis has proven difficult, especially in animals suffering from a subclinical disease, since they do not display any overt clinical signs of illness. It may be possible, however, to develop an early disease detection system by monitoring aspects of an animal's behaviour, where changes may indicate the early onset of a disease or a subclinical disease (Kyriazakis and Tolkamp, 2011).

The exact changes that take place in the different types of behaviour and the rate and magnitude of such changes are largely unknown, especially during a subclinical disease (Gougoulis et al., 2010). However, if it were possible to clearly identify such changes in the different behavioural traits that relate to a health challenge, a tool could be developed for on farm use for the early detection of diseases, both clinical and subclinical. The aim of this chapter is to review the changes in animal behaviour that occur as a consequence of a health challenge, with a focus on feeding, drinking, social behaviour and activity (including posture). Before doing so, the term health challenge is defined, as are the parameters or dimensions of the different behaviours. Because some of the confusion regarding the effects of health challenges may arise from how these behaviours are measured and interpreted, a significant part of the chapter deals precisely with this issue. The hope is that, by doing so, a conclusion can be reached on how health challenges affect behaviours and whether these changes can be used to detect early the onset of disease

2.2 Methodological approaches

2.2.1 Health challenge

A health challenge is used to describe a disorder inducing a potentially pathological change in the animal's physiology. This disorder can have different causes: micro- or macro-parasitic infections, physical challenges such as injury, or metabolic challenges. Micro-parasitic, named due to their small size, indicate pathogens that complete a full life cycle within the host and can be transmitted to conspecifics, whereas macro-parasites encompass parasites that do not spend their full life cycle in the (same) host. These challenges can lead to a subclinical or clinical illness. By subclinical it is meant that the disorder is present and influences the animals' performance, though without showing any visual or overt signs. The subclinical disease can then progress and manifest itself as a clinical disease, or can remain subclinical, making it harder to detect. When an animal is subject to a health challenge its behaviour may change. This is known to happen for clinically diseased animals, though not yet clearly defined for sub-clinically ill animals. The behavioural change might be an effect of the physiological responses (e.g. increase in level of circulating cytokines as a consequence of the immune response) that take place during infection (Hart, 1988; Dantzer et al., 2000; Greer et al., 2005) or related to sparing the affected area, as is most likely the case with physical disorders.

2.2.2 Behaviours considered

The different behaviours that are considered within this review are chosen for their record of showing a change when the animal is subject to a health challenge, and a few have already been proven to permit early detection of disease. These behaviours are feeding, drinking, social behaviour, complexity of behaviour, activity and posture. Feeding behaviour includes meal size, duration, frequency, feeding rate and feeding time; the same applies to drinking behaviour. Social behaviour includes social, agonistic, sexual and maternal interactions. The complexity of behaviour is basically the pattern in which certain behaviour(s) is (are) displayed, and activity is the extent of movement made by the animal. The potential of these individual behaviours, or a combination of them, to provide a reliable system for early detection of health and welfare challenges in livestock is discussed below.

2.3 Food intake and feeding behaviour

2.3.1 Definitions

Animals of most species do not feed continuously or randomly in time, but do so in concentrated bouts (Tolkamp et al., 1998). This means that, within bouts, actual feeding may be interrupted by short non-feeding intervals, while bouts (which can be called meals if they are properly identified) are separated by long non-feeding intervals (Tolkamp et al., 1998; Yeates et al., 2001; Tolkamp et al., 2011). These feeding bouts, or meals, are underlain by the concept of satiety, implying that the probability of an animal starting a meal will increase over time since the end of the last meal (Tolkamp et al., 1998). In the pure and formal sense, the aggregate of the feeding events throughout a day results in the daily food intake. Health challenges can affect either food intake, feeding behaviour or both. Anorexia, a variable reduction in the voluntary food intake, is one of the first notable behavioural changes in response to a health challenge (Exton, 1997; Quimby et al., 2001; González et al., 2008; Kyriazakis, 2010). Anorexia is present with an increasing intensity in both sub-clinically and clinically affected animals (Kyriazakis, 2003). If daily food intake decreases, then this must be mediated through changes in the feeding behaviour. However, this is not necessarily a two way relationship, as changes in feeding behaviour do not always result in changes in daily food intake (González et al., 2008); as discussed further in the following section.

2.3.2 Methodology

Data on both feeding behaviour and food intake can be captured in many different ways, although the methodologies used can differ in suitability between situations and species. For instance, it might be sufficient to measure the number of pellets consumed by laboratory rodents or domestic birds (Plata-Salamán, 1994), whereas this would be inappropriate for larger livestock, for which other methods have been developed to capture feeding behaviour. By weighing feed offered and refusals, daily food intake can be calculated (Kyriazakis et al., 1996; Borderas et al., 2008), although this method will be inappropriate for group housed animals. Concerning feeding behaviour, measurements can be taken at a basic level by visual observations, where time spent in feeding is recorded (Mayes and Duncan, 1986); this however is very labour intensive. There is also the possibility of recording the (intensity of) jaw movements with a monitoring system attached to the muzzle (Dado and Allen, 1994; Baumont et al., 2006), a method most frequently used in grazing animals or when rumination data are required. Finally, there is the option of using computerized feeders. These can combine measures of food intake and

feeding behaviour (Tolkamp et al., 1998; Yeates et al., 2001) and hence they are particularly attractive; for this reason their use is increasing. The system needs to recognize the individual, which is fitted with a transponder carried on either a collar or an ear tag. The presence of the transponder is recognized when within a certain radius of a recording aerial fitted at, or close to, the feeder and this provides a very high accuracy in recording feeding events (Sowell et al., 1998). Several systems only record presence at the feeder, as it is reasonably assumed that the vast majority of time at the feeder is spend feeding (Sowell et al., 1998; Quimby et al., 2001; Urton et al., 2005). Furthermore when measuring both behaviour and intake, individual feeders are required to weigh the food prior to and after an animal has consumed feed, or on an instantaneous basis. Clearly the different methodologies can lead to different outcomes regarding the accuracy and types of behaviour measured (Tolkamp et al., 1998, 2011). Whereas modern computer-based systems have the ability to be accurate, visual observations for instance may be subjective and contain larger errors, especially if multiple observers are used.

2.3.3 Analysis

The way the resulting data are handled during analysis has a large influence on the interpretation of feeding behaviour (Tolkamp et al., 1998; Tolkamp and Kyriazakis, 1999; Morgan et al., 2000; Yeates et al., 2003; Tolkamp et al., 2011). Although what is recorded are feeding events, short term feeding behaviour (STFB) can be structured into meals. Separate feeding events occur in bouts, as mentioned above, and meals are the biologically relevant unit of feeding behaviour (Tolkamp and Kyriazakis, 1999; Yeates et al., 2001; Tolkamp et al., 2011). In order to group separate feeding events into meals, a meal criterion needs to be defined. This meal criterion is a calculated estimation of the longest non-feeding interval that is still considered to be part of a meal (Yeates et al., 2001). By doing so, the intervals between feeding events can be divided into within- and between-meal intervals. The resulting dimensions of STFB from such an analysis are meal size: the intake during a meal; meal frequency: the number of meals or visits over a certain period of time; meal duration: time spent feeding over a certain period of time, and feeding rate: the amount of food eaten over a period of time (Tolkamp et al., 1998; Yeates et al., 2001; González et al., 2008).

Currently there are various methodologies that aim to define meal criteria for farm animals. These range from the subjective (Dado and Allen, 1994; Sowell et al., 1999) to ones where statistical criteria are fitted to the feeding behaviour data (Tolkamp et al.,

1998; Tolkamp and Kyriazakis, 1999; Yeates et al., 2003). Even in the latter category, the methodology applied (for example, in aggregation of data) can affect the conclusions reached, as the way data are analysed has a large effect on results. For example, the best fitting meal criterion for cows on conserved forage appears to be between 26.4 to 63.7 minutes, with an average of 41.8 minutes (Tolkamp et al., 1998). This calculated interval differs greatly from some estimated intervals of 5 minutes (Sowell et al., 1999) and 7.5 minutes (Dado and Allen, 1994) for the same species. Therefore, meal criteria used for structuring data should always be viewed critically as they can make the outcome less comparable between studies. Furthermore, not all dimensions of STFB can be derived from every methodology. Feeding rate can only be calculated if food consumed during a feeding bout is measured.

Once the data are grouped into meals, pooling might need to take place. If the data are pooled in an inappropriate manner, or the methodology applied does not take into account the biological basis of behaviour, feeding appears to occur randomly over time (Mayes and Duncan, 1986; Sibly et al., 1990; Morgan et al., 2000). This can, for instance, occur by pooling data across day and night (Morgan et al., 2000; Yeates et al., 2003). Cows (Yeates et al., 2003), pigs (Morgan et al., 2000) and rats (Plata-Salamán, 1994) all show diurnal variation that requires separate pooling of day and night data. This is because diurnal activity levels vary, thereby creating a different feeding pattern, as was seen in pigs where the frequency of meals during the night was considerably lower than during daytime (Morgan et al., 2000). Pooling data across day and night can also give an underestimation of the starting probability; i.e. the probability of an animal starting a meal. This is because day feeding intervals are usually shorter than those overnight (Yeates et al., 2003). In studies where data are pooled, this can lead to a quantitative underestimation of the increase in the starting probability with time, but not a qualitative misinterpretation of the direction of change (Yeates et al., 2003).

2.4 Changes in food intake as a consequence of health challenges

It is well established that when an animal is faced with a health challenge, daily food intake will decrease, leading to anorexia (Crompton, 1984; Hart, 1988; Plaizier et al., 2009). Present in both sub-clinically and clinically affected animals (Kyriazakis, 2003), the extent of anorexia depends on the health challenge. For infectious challenges it also depends on pathogen dose and possibly strain, and for non-infectious challenges it probably depends on challenge type and severity (Sandberg et al., 2006; Bach et al., 2007;

Kyriazakis and Doeschl-Wilson, 2009). Therefore the effect of different types of health challenge on food intake is briefly discussed below.

2.4.1 Micro-parasitic health challenges

Following a health challenge with micro-parasites food intake decreases (Hart, 1988) in ruminants (Steiger et al., 1999; González et al., 2008), non-ruminant mammals (Plata-Salamán and Borkoski, 1993; Johnson and von Borell, 1994; Warren et al., 1997; Aubert et al., 1997b) and birds (Johnson et al., 1993). An evolutionary perspective suggests that the animal would forage less to preserve heat and energy and reduce the chance of encountering a predator (Hart, 1988). Furthermore, it reduces the risk of consuming anything that could induce another health challenge, for pathogens transmitted via the faecal-oral route, and may starve pathogens of substrates (White, 1980; Hart, 1988).

Most research concerning micro-parasitic challenge has been conducted using an endotoxin such as lipopolysaccharide (LPS) (Plata-Salamán and Borkoski, 1993; Warren et al., 1997; Steiger et al., 1999). LPS is present on the surface of gram-negative bacteria and functions as a PAMP (Pathogen-Associated Molecular Pattern), being highly immunogenic. Furthermore LPS and other pathogens have common features making LPS a good model of a pathogenic challenge. The endotoxins contained in the pathogens are recognized quickly by the immune system (Houdijk et al., 2007) leading to a fast induction of anorexia. The decrease in food intake as a result of LPS administration in rats mostly took place during the night when the dose was administered in the evening prior to the active period (Plata-Salamán and Borkoski, 1993). Some compensatory intake occurred during the following day, though an overall decrease in daily food intake was still present. To a certain extent, anorexia can be induced more quickly and with a higher severity if the pathogen load is higher, with a faster replication rate, though duration does not seem to be affected (Houdijk et al., 2007; Kyriazakis and Doeschl-Wilson, 2009). Furthermore, other pathogens cause a similar decrease in food intake (Sandberg et al., 2006; Escobar et al., 2007).

The response to an endotoxin such as LPS is highly dependent on dosage and time of administration as the effects are not always long lived, especially with low dosages (Johnson and von Borell, 1994; Larson and Dunn, 2001; Jacobsen et al., 2005). For example, pigs administered a dosage of 0.5 μg/kg or 5 μg/kg returned to a normal feed

intake between 4 to 8 hours after the challenge was administered, whereas pigs given 50 μg/kg still experienced a reduced food intake beyond this time (Johnson and von Borell, 1994). The pigs administered 0.5 μg/kg even demonstrated a compensatory increase in feed intake 4-8 hours after administration (Johnson and von Borell, 1994). The dose dependent response can, however, differ between breeds, as is shown in pigs (Henryon et al., 2001). Apart from breed variation, there are also differences between individuals concerning the strength and duration of the response to an endotoxin, as demonstrated in cows (Vandeputte-Van Messom et al., 1993; Jacobsen et al., 2005). Though feed intake was not measured as such in these studies, the variation shown by the physical parameters measured, e.g. duration of fever and heart rate, it can be assumed that a similar variation would be present for feed intake. Furthermore, the route of administration can influence dosage needed and manifestation time for an effect; for instance a direct infusion into the brain (Plata-Salamán, 1994) will require a lower dosage and less time to take effect compared to an intravenous injection (Elasser et al., 1996).

2.4.2 Macro-parasitic health challenges

During a challenge with macro-parasites, food intake decreases for a wide variety of animals, such as ruminants (Coop and Holmes, 1996; Kyriazakis et al., 1996), pigs (Forsum et al., 1981; Balaji et al., 2000) and rodents (Roberts et al., 1999; Mercer et al., 2000), by up to 30-60% dependent on the circumstances (Poppi et al., 1990). However, in birds infected with *Ascaridia galli*, food intake was actually found to increase (Gauly et al., 2007). This might be more logical to expect, as it would allow the host to counter the debilitating consequences of a macro-parasitic infection (Kyriazakis et al., 1998). Why this is not so in the vast majority of cases remains to be investigated. Perhaps the suppressing effect of the parasite-induced infection on food intake is, in most cases, stronger than the urge to compensate any nutritional effects.

Although macro-parasites commonly induce a slower induction of anorexia compared to micro-parasites (Kyriazakis and Doeschol-Wilson, 2009), this differs between host species and possibly the type of macro-parasite. For example, lambs infected with *Teladorsagia circumcincta,* have a reduction in food intake which starts in the second week of infection (Zaralis et al., 2008), whereas rats infected with *Nippostrongylus brasiliensis* already show anorexia after 1 day (Mercer et al., 2000). In contrast to these reductions in food intake, there is not always a decrease. No changes were observed in mice infected with metacestodes of *Taenia crassiceps*, indicating that host species and/or

type of macro-parasite can have a major influence on the manifestation time and the extent of anorexia.

As with micro-parasitic infections, breed also appears to have a large effect on the extent of anorexia when it comes to a macro-parasitic infection. For example, there was a 13% food intake decrease in cross-bred lambs after an infection with *Teladorsagia circumcincta* compared to no significant reduction in similarly infected Scottish Blackface lambs (Zaralis et al., 2008).

Though intake has been decreased in most of the studies on macro-parasitic infections, the loss in weight and production also largely depend on the type of feed, as was shown by offering lambs a choice of feed with different protein contents (Kyriazakis et al., 1994; Kyriazakis et al., 1996). When infected with *Trichostrongylus colubriformis*, food intake decreases occur from week 4 or 5 onwards until resistance develops. These animals choose a feed with a higher protein content, stabilizing the protein intake despite having a decreased feed intake (Kyriazakis et al., 1994; Larson et al., 1996). However the extent of anorexia does not appear to be influenced by food protein contents in ruminants (Greer et al., 2005; Kyriazakis and Doeschl-Wilson, 2009), though in non-ruminants the debate is still open (Kyriazakis and Doeschl-Wilson, 2009). Food composition on the other hand does appear to have an influence on the duration of the anorexia (Kyriazakis and Doeschl-Wilson, 2009).

2.4.3 Metabolic health challenges

The vast majority of research regarding the consequences of metabolic challenges on food intake has been performed on cattle. Metabolic disorders have led consistently to a reduced food intake as a consequence of ketosis (Bareille et al., 2003; González et al., 2008), acidosis (Britton et al., 1990; Owens et al., 1998; Plaizier et al., 2009) and other digestive disorders (metabolic disorders not inducing diarrhoea or displaced abomasum) (Bareille et al., 2003). The feeding pattern is mainly characterized by a rapid decrease, reaching near to zero (no intake) values on the day of diagnosis (González et al., 2008). This is in accordance with expectations, as the animal is unlikely to consume more food when this can exacerbate the impact on its system.

2.4.4 Physical health challenges

The final health challenge considered, involves physical disorders which can either lead to a decrease in food intake or result in no change. There is an interesting contradiction between different studies in this respect. In a study of lameness in dairy cows, González et al. (2008) observed that the daily feed intake was unaffected by the challenge. This is a consequence of the type of disorder, as it does not directly influence the ability to consume food, but more the ability to get to the feeder and stand whilst feeding. Since physical disorders may cause discomfort, feeding behaviour can be altered, although apparently not always in a manner that result in food intake reduction. A decrease in food intake was, however, found to occur in other studies of lameness (Bach et al., 2007) and foot- and hock lesions (Bareille et al., 2003).

These contradictory results can be explained by the fact that the foot and hock lesions differ from the other types of lameness, although all affect mobility. For the differing results on food intake in lame cows, showing either a decrease (Bach et al., 2007) or no difference (González et al., 2008), severity could have played a part, as feed intake only decreases after the lameness becomes more severe (Bach et al., 2007). Other factors can also have an effect for example the position of the cow at the feeder (Bach et al., 2007), so perhaps the layout of the stable can influence intake levels, as well as the parous state of the cow (Bach et al., 2007). Furthermore, in the study by Bareille et al. (2003), the data were taken over a span of 10 years from cows with access to a pasture, which allows for grazing to influence the results. These factors could all influence the feed intake after the challenge, though it remains to be hypothesised what exactly caused these apparently contradictory results.

2.5 Changes in feeding behaviour as a consequence of health challenges

Changes in daily food intake must be caused by changes in some aspect of feeding behaviour. However, changes in feeding behaviour do not necessarily lead to a change in food intake, especially when the changes are subtle (González et al., 2008). Changes in one dimension of feeding behaviour (e.g. meal duration) can be compensated by changes in another dimension (e.g. feeding rate) with the result that daily food intake may remain unchanged. The effect of different health challenges on the dimensions of feeding behaviour is discussed below.

2.5.1 Micro-parasitic health challenges

A micro-parasitic infection induces a decrease in food intake, as described previously, which arises from changes in feeding behaviour.

Feeding time

Total daily feeding time is generally found to change in ruminants (Takeuchi et al., 1995; Sowell et al., 1998; González et al., 2008) and non-ruminants (Escobar et al., 2007), challenged by a micro-parasite, although this has not been the case in laboratory rodents. For example, morbidity in beef cattle caused by Bovine Respiratory Disease (BRD) was detected on average 4.1 days earlier as a reduction in feeding time, than through conventional methods based on the visual observations of the farm staff (Quimby et al., 2001). Furthermore, there is a decrease in feeding time for almost two weeks in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) (Escobar et al., 2007), cattle affected by infectious metritis spend 22 minutes less in the feeding alley (Urton et al., 2005), and morbid beef steers in one study spent 30% less time at the feed bunk although the latter finding was not repeatable (Sowell et al., 1998). Based on these findings, a reduction in feeding time is suggested as a possible tool for the early detection of micro-parasitic infections.

Meal and bout frequency

Meal frequency in rodents (Plata-Salamán and Borkoski, 1993; Plata-Salamán, 1994; Larson et al., 1996) and feeding bout frequency in ruminants (Sowell et al., 1998) are known to decrease during micro-parasitic infections. However, this effect does not appear to be consistent across studies; for example, González et al. (2008) found no change in the meal frequency of cows suffering from mastitis. Sowell et al. (1998) showed that the feeding bout frequency decreased by 3 to 3.7 times in morbid steers, which is a very substantial reduction. However, the reliability of the estimation of these bouts may be questioned given the 'meal' criterion used, which was based on visual observations and was arbitrarily estimated to be 5 min. The contradiction between studies in the effects of micro parasitic infections on meal or feeding bout frequency may therefore arise from the methodology of defining the meal criterion and subsequently analysing feeding bouts. However, as the decrease found by Sowell et al. (1998) was very substantial, the differences between studies may reflect effects of different types of infections on feeding bout frequency. Whereas Sowell et al. (1998) did not discriminate between micro-parasitic infections (their steers were simply defined as morbid), the observations of

González et al. (2008) are very pathogen-specific and were only observed during mastitis. The effects of micro-parasitic challenges on meal and bout frequency are therefore currently inconclusive.

Meal duration, size and feeding rate

Meal duration, meal size and feeding rate can also change following a micro-parasitic health challenge. In rats injected with Interleukin -1β (IL-1 β), a cytokine induced by such a challenge, all three of these feeding behaviour dimensions decrease (Plata-Salamán, 1994). On the other hand, the same investigators, when using the same experimental setup but challenging animals with LPS, found that only meal size and feeding rate decreased (Plata-Salamán and Borkoski, 1993). In another study where rats were challenged with LPS, meal size was also found to decrease, although any change in feeding rate was not mentioned (Larson et al., 1996). These findings have led to the assumption that animals challenged with LPS do not decrease their feeding time, although they consume less by eating at a slower rate. The same criticism applied above regarding the type of analysis applied to estimate meal criterion could apply here and account for inconsistencies between studies. To summarize feeding time, meal frequency, meal duration, size and feeding rate may all decrease as a consequence of a micro-parasitic challenge. The occurrence and extent may depend on host species, type of infection and the analysis applied to the data. This has yet to be investigated systematically. As the above dimensions of feeding behaviour would be expected to decrease during micro-parasitic infections, this makes them potential candidates for use in the early detection of the consequences of such health challenges.

2.5.2 Macro-parasitic health challenges

Studies regarding the effect of a macro-parasitic infection on feeding behaviour are even scarcer than those of micro-parasitic infections. It has been possible to identify only three, two on ruminants and one on pigs in the literature. The pig study reported a decrease in feeding time 2-4 weeks post infection with *Sarcocystis miescheriana* (Reiner et al., 2009). A decrease in feeding time of 56 minutes was also observed in heifers infected with larvae of *Cooperia oncophora* and *Ostertagia ostertagi* (Forbes et al., 2007). Furthermore a decrease in feeding time was also present in a study involving sheep infected with *Fasciola hepatica* metacercariae daily for six days (Ferre et al., 1996); this was accompanied by a reduction in the number of meals and, surprisingly, an increase in feeding rate. It is not known if this increase in feeding rate is a feature of this particular

infection, as it has not been measured in other studies; it is also contradictory to observations during micro-parasitic infections. The increase in feeding rate, if consistent, might be caused by the animal trying to compensate for the decrease in feeding time. Given the fact that a reduction in daily food intake is the outcome of all macro-parasitic infections (Kyriazakis, 2010) it is implied that the compensation is not complete. Therefore a decrease in food intake after a macro-parasitic health challenge is mainly caused by a decrease in feeding time and possibly a reduction in the number of meals, in spite of an increased feeding rate.

2.5.3 Metabolic health challenges

The only report in the literature that has measured such effects on feeding behaviour concerns dairy cows, which were found to show a decrease in total feeding time when affected by ketosis (González et al., 2008). There was also a decrease in feeding rate; however this was seen only shortly prior to diagnosis by farm staff. This decrease in feeding time could account for the observed reduction in food intake, as there was no observed change in daily feeding episode frequency.

2.5.4 Physical health challenges

Feeding time and rate, as well as the number of feeding events, were found to change in the case of lameness in dairy cows. Both feeding time and the number of visits decreased, however feeding rate was found to increase (González et al., 2008). This leads to the assumption that the animal eats at a faster rate in order to spend less time at the feeder, probably caused by locomotory discomfort that the animal seeks to minimize by reduced time spent standing or in other locomotory related activity (González et al., 2008). The same decrease in feeding time is mentioned by Bach et al. (2007) and Galindo and Broom (2002), although feeding rate was not measured in these cases. Feeding time was also found to decrease in proportion to the severity of the disorder (Bach et al., 2007), eventually leading to a decrease in feed intake, which was not the case in the study of González et al. (2008), possibly due to differences in severity. This is logical since the severity of lameness, as defined by visual observations, increases then standing at the feeder may become increasingly painful for the animal. Feeding time was furthermore found to decrease for cows lame due to inflammatory foot lesions (Almeida et al., 2008).

Apart from feeding time, rate and the number of visits, no other feeding behaviour

parameters have been reported to be affected by physical health challenges. It can be hypothesized, however, that as feeding time decreases and rate increases, less time needs to be spent at the feeder to reach the same level of food intake; basically the animal is having fewer but larger meals. Nevertheless, in a study of lame broiler chickens the number of visits declined, but meal duration increased so the same time was spent feeding (Weeks et al., 2000). However this consistency in feeding time was caused by the posture during feeding; the chickens had the ability to lie during feeding in order to reduce the weight on their legs. In support of this, the feeding time spent standing decreased, whereas feeding time spent lying increased. Thus the animal will try to minimize walking and standing activity, which is reflected in feeding behaviour and thereby possibly in intake.

2.5.5 Conclusion

When food intake is reduced as a consequence of a health challenge, the reduction in daily food intake can only be achieved through changes in some aspect of feeding behaviour. However, it is also possible that, although feeding behaviour may change dramatically, food intake remains unaffected. The components of feeding behaviour that may change include meal size, meal duration and frequency, feeding time and feeding rate. Food intake is found to be decreased as a result of all types of health challenge, though there is still some debate concerning the outcome of physical disorders. The dimensions of feeding behaviour that change vary depending on the different health challenges. As STFB shows the most change following all health challenges and food intake cannot change without a change in behaviour, this would be the most important aspect to measure. Furthermore, feeding behaviour can change without food intake changing, for instance by eating less often but larger meals, potentially making it a more sensitive indicator.

For a micro-parasitic infection all feeding behaviour dimensions change, with particular emphasis on feeding time. A macro-parasitic infection also changes feeding time, as well as giving an increase in feeding rate, possibly as a means of trying to compensate for the reduction in feeding time. Currently it is not known how the other dimensions of feeding behaviour are affected during macro-parasitic infections, but a similarity with micro-parasitic infections may exist. For metabolic challenges, only feeding time has been found to be effected. Physical challenges cause feeding time and rate to change. As with a

macro-parasitic health challenge, feeding rate shows an increase. In the case of the physical challenge this increase in rate can actually compensate for the decrease in feeding time, depending on the severity of the disorder and thereby the discomfort caused by walking and standing. These differences indicate that, for varying challenges, different behavioural elements can be altered in diverse ways, in conjunction with or separately from feed intake.

Gaps still exist in the knowledge of how health challenges affect food intake and, particularly, feeding behaviour. Macro-parasitic infections, physical disorders and metabolic challenges show the largest gaps in knowledge. Physical disorders might prove to be of specific interest, as the food intake does not change in all cases. Furthermore, the studies mentioned, apart from some notable exceptions (Ferre et al., 1996; Kyriazakis et al., 1996; Forbes et al., 2007), rely on a health challenge inducing clinical symptoms. More research is therefore needed into subclinical health challenges and their effects on food intake and behaviour.

2.6 Water intake and drinking behaviour

2.6.1 Methodology

Drinking takes place in bouts and is underpinned by the concept of satiety, in a manner similar to feeding (Kyriazakis and Tolkamp, 2011). Furthermore, feeding and drinking events are closely associated; around 75% of drinking in rats (Fitzsimons and Le Magnen, 1969) and pigs (Bigelow and Houpt, 1988) is in close association with a feeding event. Drinking can be measured in different ways. A commonly used method to measure intake is by measuring the water flow into the drinker (Dado and Allen, 1994; Cottee et al., 2004). Also, as with food intake, the water can be placed in a bowl mounted on an electronic balance, which weighs water disappearance during drinking events (Ferre et al., 1996).

Measuring drinking in individually housed animals is relatively straight forward, although difficulties arise with group housed animals that share a common drinking trough. In this case the animals need to be individually identified (Sowell et al., 1999). As with the measurement of feeding behaviour measurement of duration of a drinking event can be combined with a measurement of amount of water consumed to give drinking rate (Cardot et al., 2008). Drinking behaviour can furthermore be measured by visual observations

(Plata-Salamán, 1994; Pinheiro Machado Filho et al., 2004), although this is quite a labour intensive method. The analysis of drinking can be performed in the same way as for feeding behaviour, as the two show a similar pattern, influenced by the concept of satiety (Zorrilla et al., 2005).

2.6.2 Water intake and food intake association

The close relation between water and food intake, the amount of water drunk is positively correlated to the quantity of food ingested (Johnson and Johnson, 1990), makes it possible that the decrease in water intake following a health challenge is not caused by the health challenge *per se*, but is a consequence of the decrease in feed intake (Plata-Salamán and Borkoski, 1993; Plata-Salamán, 1994). Water intake is highly associated temporally with food intake (Fitzsimons and Le Magnen, 1969), with 75% of daily water intake in rats occurring within 10 minutes before, during, and up to 30 minutes after a feeding bout (Johnson and Johnson, 1990); this relates to the functions of water, such as maintaining homeostasis or providing 'lubrication' (Tolkamp and Kyriazakis, 2011). In goats, 84% of drinking was associated with a meal (Rossi and Scharrer, 1992) and pigs showed meal-related drinking, with 25% of drinking taking place prior to a meal (Bigelow and Houpt, 1988).

Despite this relationship, a direct effect of a health challenge on water intake cannot be excluded (Plata-Salamán and Borkoski, 1993; Plata-Salamán, 1994). An increase in water intake-to-food intake ratio after a health challenge would indicate a larger negative effect on food intake than on water intake, or even a direct increase in water intake, due for example to pyrexia (Plata-Salamán and Borkoski, 1993; Plata-Salamán, 1994). The converse, however, does not seem to be the case, i.e. where water intake is disproportionately affected by the health challenge.

2.6.3 Changes in water intake

A reduction in water intake following a health challenge is possible (Hart, 1988), however water intake does not seem to decrease as rapidly as food intake (Plata-Salamán, 1994). Although the decrease in water intake would happen for the same reasons as for food intake (Hart, 1988), there is a greater need for water to be maintained on a daily basis as it is more vital for many bodily functions (Kyriazakis and Tolkamp, 2011). Water intake has been shown to decrease after a micro-parasitic challenge in ruminants (Sowell et al.,

1999), non-ruminants (Plata-Salamán and Borkoski, 1993; Plata-Salamán, 1994; Cross-Mellor et al., 2000) and birds (Baert et al., 2005). Water intake was found to be negatively affected by diseases accompanied by fever (Lukas et al., 2008), which is a common effect of a micro-parasitic infection. Plata-Salamán (1994) showed that rats decrease their water intake during the night following administration of LPS and increase it during the day; however, this change in the distribution of behaviour still resulted in an overall decrease of water intake. The increase during the day could indicate some compensatory drinking. However, a decrease has not been always reported, since some other studies have found that water intake was not affected or even increased during micro-parasitic infections (Plata-Salamán et al., 1988; Yirmiya, 1995). Such was the case for pigs suffering from enteric disease that increased their water intake prior to visual diagnosis (Madsen and Kristensen, 2005). The reason for such differences between studies is unknown, although it could be due to the nature of the challenge or to an influence of other internal or environmental factors, such as ambient temperature.

Water intake also decreases after a macro-parasitic infection (Ferre et al., 1996). Sheep that were sub-clinically infected with *Fasciola hepatica* decreased their water intake significantly at 6 and 13 weeks after infection (Ferre et al., 1996). It should be noted that such infections are not associated with fever (Kaufman, 1996). There is little information on the effect of physical health challenges on water intake, although a small trend towards a decrease in water intake has been shown for cows suffering with lameness (Kramer et al., 2009).

In contrast to these previous results, metabolic challenges appear to be associated with substantial increases in water intake (Cottee et al., 2004), even when food intake is substantially reduced. Cottee et al. (2004) showed that cows with acute rumen acidosis increased their water intake at the peak of the ruminal pH depression. This was shown to be an effective strategy, as ruminal pH increased after a drinking bout. It is concluded, therefore, that the effect of health challenges on water intake will depend dramatically on the nature of the challenge. Infectious challenges appear to lead to a decrease in water intake, most often due to the associated decrease in food intake, whereas the opposite is the case during metabolic health challenges.

2.6.4 Changes in drinking behaviour

Considerably less information is available on the effects of health challenges on the

dimensions of drinking behaviour. As water intake does not always change as a consequence of a (micro-) parasitic health challenge, small changes in drinking behaviour would be expected. There are no studies, however, directly related to micro-parasitic health challenges. A study on infected cattle, mostly affected by micro-parasitic health challenges, showed some potential change in drinking behaviour (Sowell et al., 1999). Drinking bouts per day were 0.7 more for healthy calves in the first trial, although no such effect was obvious in their second trial. Time spent at the drinker was not significantly affected by the challenge.

No difference was found in the total time spent drinking in broiler chickens suffering from lameness (Weeks et al., 2000). It would be expected, however, that when drinking requires standing up, time spent drinking would decrease, especially in physical disorders where standing is painful (González et al., 2008). The expectation would be that drinking would resemble feeding behaviour in this case, with animals spending a shorter time on their feet, but increasing the drinking rate in order to maintain water intake at a similar level.

A decrease in time spent drinking, and also in the number of drinking bouts per day, was found in sheep infected with *F. hepatica* (Ferre et al., 1996), although drinking rate was actually found to increase. The reason why this increase in rate occurred is not known. However, there was still an overall decrease in water intake, indicating that the impact on time spent drinking was greater than the increase in drinking rate. Drinking time was furthermore found to decrease in pigs infected with *Sarcocystis miescheriana* 2-4 weeks post infection (Reiner et al., 2009).

There is no information available with regard to the effects of metabolic health challenges on drinking behaviour. However, based on an increase in intake (Cottee et al., 2004), at least some behavioural dimensions of drinking would need to be increased. Given the substantial effects of metabolic health challenges on water intake, it is surprising that this has not been investigated.

2.6.5 Conclusion

As water consumption is highly associated with food intake, it is possible that a decrease in water intake is not caused directly by the health challenge, but also, or even mainly, caused indirectly by the decrease in food intake. However, due to the several functions of

water in maintaining homeostasis within the body, the decrease in water intake is less prevalent than the decrease in food intake during health challenges. A special case to consider is when a decrease in water intake due to a decrease in food intake is offset by an increase due to, for example fever. This situation does not seem to have been investigated systematically and it is mainly expected to arise during micro-parasitic health challenges. This will have consequences on the potential diagnostic usefulness of water intake. Metabolic health challenges lead to very clear increases in water intake, since the animal attempts to maintain homeostasis through this action. The effects of physical health challenges on water intake appear to be inconclusive. There is significantly less research on the effects of health challenges on the dimensions of drinking behaviour. Given the previously discussed effects on water intake, some effort into investigating effects on drinking behaviour may be warranted.

2.7 Social behaviour

2.7.1 Definition

Social behaviour entails interactions between animals (mainly) of the same species. The behavioural actions include affiliative, agonistic, maternal and sexual interactions (Dewsbury, 1978). Affiliative social interactions include, for example, body contact, grooming, sniffing and investigating. These social interactions can also be considered as part of exploratory behaviour which occurs when an animal meets non familiar individuals. This results in the taking up of an investigatory position, in cattle the head is extended and with the legs sloping forward, a quick retreat can be facilitated if necessary (Phillips, 2002). Besides social interactions there is the related issue of social facilitation, where the mere presence or behaviour of an animal results in a change in a behavioural pattern in another animal (Dewsbury, 1978). Examples of social facilitation are imitation, learning and also herd behaviour, cooperation, competition and affiliation and vocalization as a way of communication (Dewsbury, 1978). Through all these interactions, an animal establishes or maintains its position in the group as well as in the possible competition for resources or other fitness related activities (Barnard, 2004). It is assumed that an animal may try to sustain social behaviours in the first instance, when its health is challenged. However, as health challenges become more invasive it is more likely that behaviour changes to facilitate animal recovery (Hart, 1988).

2.7.2 Methodology

Observations of social interactions usually take place in the home environment of the animal, but a situation can also be staged by moving an animal to a novel area and allow it to interact with familiar or unfamiliar conspecifics (Larson and Dunn, 2001; Rex et al., 2004). Social behaviours are measured by scoring the frequency, and possibly the time, spent performing a certain behaviour. This is done either visually or with an automatic detection system. In the latter case, sensors fitted to animals are used that detect another sensor within a predefined distance (Swain and Bishop-Hurley, 2007). Such an automated system cannot differentiate between the different interaction types.

The type of social behaviours performed differs between species. In rats and mice, body contact or investigatory behaviour is measured by noting sniffing certain parts of the body, following and grooming behaviour (Edwards, 1988, Larson and Dunn, 2001; Renault et al., 2008). For pigs, body contact, anal nosing and pushing are also recorded as social interactions (Morrison et al., 2003). In cattle, allogrooming behaviour can be used as a measure of social activity (Galindo and Broom, 2002; Val-Laillet et al., 2009). For all behaviours it is important to note which animal is performing and which is receiving them; this can be influenced by rank or health status, as discussed below.

As far as agonistic behaviour is concerned, the aggressive behaviour can be divided into approach, threat and physical contact (Schein and Fohrman, 1955), whereas submissive behaviour manifests as a defensive, flight or freezing response (Dewsbury, 1978). In mice, aggressive behaviour is defined as a physical struggle between two animals, also including biting, tail-rattling and kicking amongst other behaviours (Edwards, 1988; Renault et al., 2008). Submissive behaviour furthermore is classified as standing upright facing the other animal, or looking sideways, whilst leaning backwards (Edwards, 1988; Renault et al., 2008). In pigs aggressive behaviour includes parallel pressing, head-to-head knocks and levering each other (Morrison et al., 2003). Aggressive behaviour in cows is characterized by a lowered head whilst facing the opponent, uninterrupted eye contact and head butting (Schein and Fohrman, 1955). As a response, the other animal can return the aggressive behaviour, flee or freeze (Schein and Fohrman, 1955).

When behavioural observations are used to determine rank, one should record the agonistic behaviours taking place and, importantly, which animal performs the behaviour and which animal receives it (Schein and Fohrman, 1955). When an animal ’wins’ an

aggressive interaction by the other animal backing off, this is noted as a win over that individual and as a loss for the other. If the outcome is the same between the two individuals for most of the time, it can be assumed that the overall winner is higher in rank (dominant, Schein and Fohrman, 1955).

2.7.3 Analysis

When computer–based detection is used, analysis is relatively straightforward as all contact frequency and duration can be easily analysed through the use of relevant statistical software (Edwards, 1988; Galindo and Broom, 2002; Gauly et al., 2007; Renault et al., 2008). When visual observations are used, a differentiation can be made between the different interaction types, thus making analysis more complicated. Visual observations are the only way of distinguishing between social and agonistic interactions. However, a combination of computerized and visual observations is also possible. The Observer program (Noldus, 1991) allows for recording of behaviour by directly entering the behavioural pattern or event in the computer program, which is then capable of analysis and generating an output that can be converted for use in statistical software programs.

2.8 Changes in social behaviour as a consequence of health challenges

Social exploration is decreased when illness is induced (Bluthé et al., 1992; Larson and Dunn, 2001). However, it is possible that the motivation to engage in certain activities might be so high that a change in behavioural expression is less likely to occur (Larson and Dunn, 2001). As shown by Aubert et al. (1997a), mice treated with LPS still showed pup retrieving behaviour and, at lower temperatures, maintained nest building behaviour in order to care for their young. These effects could be caused by evolutionary prioritization or the environmental stress increasing the levels of corticosterone. The latter can affect the behavioural outcomes of a health challenge, as fewer changes in investigatory behaviour were displayed by animals that were treated with corticosterone (Goujon et al., 1995).

2.8.1 Micro-parasitic health challenges

Social behaviour changes are observed after a health challenge with micro-parasites in ruminants (Takeuchi et al., 1995) and non-ruminants (Yirmiya, 1995; Deak et al., 2005; Renault et al., 2008). This could be either due to a decrease in activity of the challenged individual which stays in one place to conserve energy, or can be caused by being

engaged less in social interactions by other animals, possibly to avoid infection (Hart, 1990).

An example of reduced social behaviour initiated by the challenged individual is the reduction in social exploration in rats. This reduction lasted for approximately 4 to 6 hours after receiving a challenge with LPS (Bluthé et al., 1992; Deak et al., 2005), whereas body temperature continued to be elevated up to 36 h after the challenge, the effect being dose dependent (Deak et al., 2005). So, despite the physiological response lasting longer, the behavioural effects were only noted for the first period post challenge, and probably during the peak of this response.

Furthermore, sexual behaviour of the challenged individual can also decline, though this has been shown to be mostly sex dependent (Larson and Dunn, 2001). In rats, females seem to decrease their sexual receptivity after the administration of LPS, whereas males given the same treatment did not change their behaviour (Yirmiya et al., 1995). It can be speculated that this is because of the different costs associated with reproductive behaviour in each sex. For females these are much higher as copulation may be followed by pregnancy and caring for the young. Both these actions are associated with high investment. Male rats, on the other hand, only need to copulate and have no further costs with producing offspring as a high priority, sexual behaviour will continue even after a health challenge.

Besides a decrease in social behaviour by the challenged individual, the other healthy animals in the group can also decrease or change the type of social behaviour towards the challenged animal. Such changes include increased inter-individual distance, decreased physical contacts, and changes in the modalities of social exploration, as observed in mice (Renault et al., 2008). The changes in modalities manifest as an increase in the proportion of muzzle sniffing and decrease in the proportion of ano-genital sniffing by healthy individuals (Renault et al., 2008). This could be caused by the other animals trying to minimize contact to prevent possible transmission (Hart, 1990). It is possible that there is a vicious circle in these interactions: the challenged individual is less receptive, which in turn may lead to it being avoided by its conspecifics. The cause and effect in these relationships has yet to be defined.

2.8.2 Macro-parasitic health challenges

A macro-parasitic health challenge is known to influence social behaviour in non-ruminants (Edwards, 1988) and birds (Gauly et al., 2007), and appears to be dose dependent (Edwards, 1988). Macro-parasites seem to have the ability of affecting host social behaviour in two ways: by causing abnormal behaviour and by reducing the levels of normal behaviour (Edwards, 1988). Mice infected with *Trichinella spiralis* showed a reduced frequency of exploratory and social behaviour compared to uninfected mice (Edwards, 1988). Furthermore, these animals were less aggressive and also received less aggression (i.e. were involved in fewer agonistic interactions: Edwards, 1988). It is notable, however, that the majority of these changes only occurred around 16 or more days post infection. While a decrease in aggressive behaviour was shown in mice by Edwards (1988), chickens infected with *Ascaridia galli* displayed more agonistic behaviour compared to non-infected individuals (Gauly et al., 2007). A possible explanation for this is that, when agonistic behaviour is divided into aggressive and defensive behaviours, in mice infected with *Toxocara canis* aggression was found to decrease, whereas defensive and flight behaviours increased (Cox and Holland, 1998). The larger the *Toxocara* infestation of the brain, the greater the change in behaviour (Cox and Holland, 1998). As the place of infection is probably of influence in this case, since an infected brain is likely to change behaviour, these results might not be entirely extrapolatable to other macro-parasitic infections. Besides the place of infestation other factors can also play a role, therefore other macro-parasites can cause similar effects, but perhaps to differing extents.

Besides these differences, the degree of alteration is furthermore dependent on the familiarity that the animals have with the group in which their social behaviour is scored (Edwards, 1988). When mice were familiar with each other, less investigatory behaviour towards the cage mates took place by the infected individual and there was more passive body contact than with unfamiliar mice (Edwards, 1988). Investigatory behaviour of the challenged individual was, however, increased in both familiarity scenarios compared to a saline inoculated individual (control), though less touch was apparent (Edwards, 1988). Investigatory behaviour by healthy individuals towards the challenged individual was increased, which could perhaps be to recognize infection or to clarify variance between expected and observed behaviour (Edwards, 1988). The decrease in touching between the infected and uninfected animals perhaps results from the other individuals noticing a deviation from normal behaviour in the infected mouse and identifying it as something to

keep a distance from. The difference between familiar and unfamiliar mice could be caused by stress accompanying the meeting of unfamiliar individuals (Edwards, 1988) and possibly the urge to establish social contact with the unfamiliar animals. The pressure of meeting unfamiliar animals can alter the behaviour and perhaps suppress the influence of the parasite, therefore measurements taken with unfamiliar animals represent a different response than those taken with familiar animals.

2.8.3 Metabolic health challenges

The only study dealing with the consequences of a metabolic health challenge on social behaviour suggested that acidosis in rats increases anxiety and aggressive behaviours (Hanstock et al., 2004). Although it is not known how metabolic challenges may affect the other aspects of social behaviour, some parallels can be drawn from what is observed with other health challenges. However, it should be emphasised that, whilst infectious health challenges led to a decrease in aggression, the opposite was the case with acidosis.

2.8.4 Physical health challenges

Concerning the influence of physical health challenges on social behaviour, not much information is available. The only studies conducted involve lameness in cows. Lameness in cows leads to a reduction in aggressive interactions initiated by the challenged individual (Galindo and Broom, 2002; Mülleder et al., 2003). This can be expected as the cow's mobility is impaired and the participation in aggressive interactions is possibly more painful; reduction in interactions avoids risk of further injury. Though lame cows initiated fewer aggressive interactions, they received just as much aggression as non-lame cows (Galindo and Broom, 2002). For allogrooming there was no difference in duration although, lame cows were licked more frequently (Galindo and Broom, 2002). There is no direct evidence as to why this is the case. However, it does raise the possibility that the other animals recognize a difference about the affected individual and the fact that its condition is not contagious, as is the case for infectious health challenges where social behaviour of other animals towards the infected individual decreases.

2.8.5 Conclusion

Social exploration, the investigating of other unfamiliar individuals, initiated by a challenged individual is found to decrease when challenged by either a micro- or macro-parasitic infection. This decrease can be caused by a general sickness feeling, deterring

the animal from investing effort and energy in social exploration. Sexual behaviour is also found to decrease after a micro-parasitic infection. However this decrease is only present in females, possibly due to the higher parental investment required. For macro-parasitic challenges, challenged animals were found to display less social behaviour and less aggression, as well as to receive less aggression. This decrease in aggression does not, however, necessarily mean a decrease in agonistic behaviour, as another study showed an increase in defensive behaviours leading to a total increase of agonistic behaviours. This increase can perhaps be explained by the animal feeling weaker and more vulnerable and therefore perceiving a greater need to defend itself. Less aggression was initiated by an infected individual suffering from a physical disorder. However, in contrast to a macro-parasitic challenge, there was no decrease in the amount of aggression received, perhaps related to the nature and possibility of transmission to conspecifics of the challenge. Although macro-parasites are not necessarily transmitted from one host to another, the behavioural signals are similar to those following a micro-parasitic challenge. In contrast to the above, animals affected with a metabolic disorder increased their aggressive behaviour. The reasons for this increase are unknown, however it can be envisioned that it is perhaps caused by a general discomfort of the challenged animal.

The social response of the infected individual to other animals is also dependent on its familiarity with the other animals in the group, as a higher degree of change from control behaviour is observed when a challenged individual is introduced to unfamiliar animals. This can be a consequence of environmental stressors influencing the behavioural response or competing motivations between the urge to establish social contact with unfamiliar individuals and the suppressive effect on social behaviour caused by the health challenge. The behaviour of other animals towards the infected animal is also altered, as found after a micro- and macro-parasitic challenge and a physical health challenge, perhaps recognizing the type of health challenge that is involved as well as trying to prevent transmission. One change is the modification in the body areas were a micro-parasitic infected mouse is sniffed by others. This implies an understanding of the situation of the other animal and an attempt to reduce possible transmission, as facial sniffing increased whereas ano-genital sniffing decreased. However this is not the case for physical disorders, where grooming frequency actually increased, implying that the animals can sense the difference between non-infectious and contagious health challenges.

2.9 Activity and Posture

2.9.1 Definition

Activity encompasses movement, or its absence, performed by an animal. Activity includes walking (Weeks et al., 2000; Galindo and Broom, 2002; Borderas et al., 2008), running (Gauly et al., 2007), exploratory behaviour (Edwards, 1988) and more species-specific actions, such as ground pecking and dust bathing in birds (Weeks et al., 2000). Furthermore, additional information on the place where the behaviour takes place (Galindo and Broom, 2002), if clearly defined within the enclosure, or the posture the animal adopts during inactive behaviours (Borderas et al., 2008), for instance lying with the head up or down or lying on the abdomen or side, may be of value.

Foraging is commonly included as part of overall activity (Weeks et al., 2000; Galindo and Broom, 2002; Borderas et al., 2008). An animal needs to move in order to get to the feeder and the drinker (Rook and Huckle, 1997). This leads to a close relationship between activity, feeding and drinking behaviour, and possibly social behaviour as this also requires activity. Therefore a change in activity could perhaps be associated with a change in any of the other behaviours. These influences aside, activity is mainly measured in the most general terms including all movements with emphasis on locomotory behaviour.

2.9.2 Methodology

Visual observations can be used in order to measure activity (Johnson and von Borell, 1994; Galindo and Broom, 2002; Borderas et al., 2008), quantifying both its duration (Plata-Salamán and Borkoski, 1993; Rook and Huckle, 1997; Borderas et al., 2008) and frequency (Rook and Huckle, 1997; Borderas et al., 2008). Activity can also be defined into bouts (Tolkamp et al., 2010) and, if it is associated with feeding or drinking behaviour, would be underlain by the principle of satiety. However, activity bouts may depend on group structure and social status within the group. As activity can be related to feeding and drinking (Rook and Huckle, 1997), it may be wise to distinguish between feeding related activity and other activity. This was done in a study on dairy cows where activity was divided into feeding related activity, ruminating activity and ‘other’ activity based on visual observations (Rook and Huckle, 1997).

Besides visual observations, automated recordings of activity may also possible. A pedometer can be fitted to the leg of the animal, counting the number of steps it takes

(O'Callaghan et al., 2003; Edwards and Tozer, 2004; Mazrier et al., 2006). The pedometer technology is already widely used in farm practise, mainly for oestrus detection in dairy cows. In addition to measuring activity, some instruments also provide the possibility of simultaneously recording animal posture, i.e. whether an animal is standing or lying down. For smaller animals such as laboratory animals, photo beams, noting activity when disrupted by movement of the animal, can be used (Plata-Salamán and Borkoski, 1993). Video imaging offers substantial opportunities to capture activity, especially in laboratory animals (Noldus et al., 2001).

Exploratory behaviour is a part of locomotory behaviour and more specifically focuses on the interaction of an animal with its environment or conspecifics. Unlike locomotory behaviour, exploratory behaviour can mainly be distinguished by visual observations. Tests to measure investigative or exploratory behaviour, such as how the animal interacts with a new environment or a novel object, have been used (Larson and Dunn, 2001), although these would be of limited value in practical contexts. Finally, the position of the animal within large spaces, such as occur under range conditions, may be used as an indicator of exploratory behaviour (Swain et al., 2003). Such behaviour can be captured through Global Positioning Systems (GPS) and improvements in the technology have opened up several avenues for novel research.

2.9.3 Analysis

When the measurements are taken by visual observation, statistical software may be used to manage the data (Weeks et al., 2000; Galindo and Broom, 2002; Borderas et al., 2008). When an automatic activity measure is generated the data will usually be transferred directly to a related software system, such as ArcGis for GPS, where the data are converted, after which they can be used and perhaps processed through statistical software (O'Callaghan et al., 2003; Edwards and Tozer, 2004; Mazrier et al., 2006). The duration and frequency of an activity and posture, and possibly the timing, will be analysed to allow for a distribution pattern to be created. Here activity peaks and drops can be picked out, as is currently the case in dairy herds to detect oestrus. The patterns are generally expressed on a daily basis, but can also be analysed over longer periods of time when short term changes are not expected.

2.10 Changes in activity and posture as a consequence of health challenges

Activity is found to decrease after an animal has been subject to a health challenge (Hart, 1988; Larson and Dunn, 2001). However, whether the decrease in activity is merely a side effect of increased lethargy is unknown (Hart, 1988). A prolonged slow-wave sleep and sleeping during normal alert periods can be a consequence of a health challenge, depending on type and severity (Hart, 1988; Larson and Dunn, 2001). There is possibly some interaction between the decrease in activity and decreases in other behaviours.

2.10.1 Micro-parasitic health challenges

A micro-parasitic health challenge has been found to decrease overall activity in ruminants (Borderas et al., 2008), non-ruminants (Plata-Salamán and Borkoski, 1993; Yirmiya, 1995; Escobar et al., 2007) and birds (Baert et al., 2005). This has the possible function of preserving heat and energy (Hart, 1988), and potentially minimising risk of getting caught by predators. This decrease mostly involves a reduction in walking, standing and running behaviour (Baert et al., 2005; Escobar et al., 2007), or an increase in inactive behaviours, such as lying (Borderas et al., 2008). The normal activity pattern of the species involved, furthermore, has an influence on the change in activity expressed. As can be expected, the greatest decrease in activity is during the normally active period (Plata-Salamán and Borkoski, 1993); no real decreases have been found during the normally non-active periods, but the combination of the two has led to a decrease in the overall daily activity. Besides locomotory behaviour, exploratory behaviour has also been found to decrease during such challenges (Yirmiya, 1995).

2.10.2 Macro-parasitic health challenges

Activity is found to decrease following a macro-parasitic infection in non-ruminants (Edwards, 1988; Reiner et al., 2009) and birds (Gauly et al., 2007). This is possibly for the same evolutionary reasons as the decrease in activity following a micro-parasitic infection. Locomotion activity, walking, running, fluttering and flying, was found to decrease in birds infected with *Ascaridia galli* (Gauly et al., 2007). Pigs infected with *Sarcocystis miescheriana* had decreased activity and increased lying 2-4 weeks post infection (Reiner et al., 2009). Exploratory behaviour was also found to be decreased in mice infected with *Trichinella spiralis* (Edwards, 1988). Currently there is little information on how different macro-parasites or different doses of the same pathogen affect activity and posture. The only exception is a study by Edwards (1988) that suggests a relationship between challenge dose and a reduction in social exploration.

2.10.3 Metabolic health challenges

The issue of how metabolic challenges affect activity and posture has been only addressed in ruminants, perhaps due to the higher incidence of these conditions in high yielding dairy animals. Ketosis, displaced abomasum and general digestive disorders have been found to decrease activity (Edwards and Tozer, 2004). The mean walking activity for challenged dairy cows in these studies was decreased; however this decrease started at about 8 to 9 days prior to diagnosis, before which there was an overall increase in activity (Edwards and Tozer, 2004). The reason for this pattern is unknown; however it can be envisaged to be due to discomfort leading to initial restlessness in the cattle, which causes an increase in movement, before leading to the decrease. For species other than ruminants, a decrease in activity is also a likely consequence, although it has not been possible to find any available information.

2.10.4 Physical health challenges

Decreased activity caused by a physical health challenge is mainly observed in the case of lameness, as has been found in ruminants (O'Callaghan et al., 2003; Mazrier et al., 2006) and birds (Weeks et al., 2000). This is a logical consequence, since the animal will be less inclined to walk due to discomfort, as was shown in lame cows which had longer total lying times (Ward, 2001; Galindo and Broom, 2002). In broiler chickens, activity has been found to decrease with age, due to increased pressure on the legs, to a total of 3.3% of total daily time spent on walking (Weeks et al., 2000). However, in cases of severe lameness this can decrease to 1.5% (Weeks et al., 2000). Furthermore, animals have been found to spend more time lying down than on their feet. Although these studies only focus on lameness, it can be assumed that for other physical disorders activity may also decrease. The extent of decrease is possibly influenced by the type of disorder, as disorders affecting the legs or feet compromise direct mobility and thus are likely to result in more dramatic effects than other physical challenges, such as an injury in other parts of the body.

In addition to this, a more complex relationship between activity, social status and lameness may be present, as social status can influence the activity of an animal. For instance, lower ranking cows have been observed to stand for longer (Galindo and Broom, 2000). These cows were also found to spend more time standing halfway in the resting cubicles, probably to prevent agonistic interactions by avoiding higher ranking cows. This avoidance behaviour causes the cows to put more pressure on their hooves

leading to a higher number of soft tissue lesions and lameness (Galindo and Broom, 2000).

2.10.5 Conclusion

For all the different health challenges considered, activity was found to decrease. Exploratory behaviour was also decreased, but this was measured only in micro- and macro-parasitic challenges. These decreases were accompanied by an increase in lying (and a consequent decrease in standing). The most interesting finding has been the increase in activity prior to a subsequent decrease in metabolic health challenges. This seems to be a unique feature of such challenges. The onset of all these effects depends on the health challenge, with immediate effects seen during metabolic disorders and micro-parasitic challenges, and more gradual ones seen during physical and macro-parasitic challenges. During physical health challenges activity is decreased but the magnitude of this will depend on the extent of the physical disorder, as more severe cases will be accompanied by higher decreases in activity. An important feature of these behaviours appears to be their temporal pattern, with deteriorations in animal health being accompanied by further decreases in activity.

2.11 Complexity of behaviour

2.11.1 Definition

The behavioural activities of animals follow certain patterns. These patterns can be in movement (Russell et al., 1992; Fritz et al., 2003), or the alternation between two or more behaviours, for example eating and not eating (Alados et al., 1996; Alados and Huffman, 2000). The behavioural sequences are identified as time series, which can have a random or linear distribution, or anything in between. These behaviour patterns can then be assessed for their complexity. Complex behaviour is neither distributed in a random nor linear fashion, but will be in between, depending on the behavioural type. For instance, the average head lift frequency during feeding is the same for different animals within a group; however the distribution of these head lifts over time differs depending on their health status (Alados et al., 1996). The distribution becomes more complex, as it becomes more random. A higher complexity has advantages, for example in predator avoidance. Lowered complexity, furthermore, appears to be an indicator of stress (West and Goldberger, 1987; Bassingthwaighte et al., 1994).

2.11.2 Methodology

For movement patterns GPS (Fritz et al., 2003) and tags that quantify position (Viswanathan et al., 1996) can be used to map the displayed pattern. When the alternation between behaviours is studied, focal animal sampling is the most common method. By following an animal repeatedly for a period of time (Alados et al., 1996) or for as long as possible (Alados and Huffman, 2000) different behaviours are noted and their sequence provides a pattern. These patterns are not always visible over a long period of time; therefore they can be divided into shorter time-series where differences become apparent. For example the total number of head lifts may be the same for all animals, but the frequency distribution per minute differs between individuals (Alados et al., 1996). These behaviours can possibly also be measured automatically, as activity and social behaviour complexity can be measured with pedometers and active transponder collars respectively (Rutherford et al., 2003; Edwards and Tozer, 2004; Swain and Bishop-Hurley, 2007).

2.11.3 Analysis

The complexity patterns of behaviour can be analysed as a fractal structure. A fractal process happens when an event with a time series distribution shows a fractal structure (Alados and Huffman, 2000). This process uses the fractal dimension (D) to compare the observed pattern to a linear pattern, or a straight line as would be the case with movement patterns, and a random pattern (Russell et al., 1992; Atkinson et al., 2002, Fritz et al., 2003). The value of the fractal dimension then indicates whether the pattern is more linear or random, and by how much. Instead of a fractal structure, a detrended fluctuation analysis can be used (Alados and Huffman, 2000; Rutherford et al., 2003). This operates following the same system as per example from Alados et al. (1996), with the difference of prior classification of behaviour as vigilant or non-vigilant and subtracting a point for non-vigilant behaviour whilst adding one for vigilant behaviour. The fluctuation within a time-series is then plotted against the size of the time series. This method is somewhat more advanced and better equipped to deal with biological and time-series data.

2.11.4 Changes in the complexity of behaviour as a consequence of health challenges

An alteration in the complexity of behaviour during a health challenge has been found in ruminants (Alados et al., 1996) and non-ruminants (Motohashi et al., 1993; Alados and Huffman, 2000). This can be expected, as complex behaviour may require more energy. Therefore when there is a health challenge, and reallocation of resources takes place, the behavioural patterns are likely to become less complex. For parasitized Spanish ibex the

patterns of vigilance, measured as head lifts during feeding on a small time scale, were found to be less complex than in healthy individuals (Alados et al., 1996). The fractal dimension of social behaviour in chimpanzees was lowered when they were suffering from a disease, either micro- or macro-parasitic infection (Alados and Huffman, 2000). There was also a sex difference present, as males had more predictable behavioural sequences than females, and did not change their complexity when infected (Alados and Huffman, 2000). However, more complex behaviour is not always an indication of good health, as is the case of certain diseases in humans (Hausdorff et al., 1997), and raises the possibility that behaviour does not necessarily becomes less complex after a health challenge.

When behaviour patterns are altered, it does not necessarily mean that other standard measures such as duration or frequency of behaviours would also be affected. This was the case with the (macro-) parasitized Spanish ibex, where there was no difference in overall feeding time or head lift frequency (Alados et al., 1996), although the frequency within one minute time frames did differ showing a higher complexity for the healthy animals. Therefore analysing the complexity of behavioural patterns can provide more information on the health status of the animal than would be the case with more conventional behavioural measures.

2.11.5 Conclusion

The complexity of behaviour may change after a health challenge, although this has been studied only for micro- and macro-parasitic health challenges. In general there is a decrease in complexity after a health challenge and these changes occur even when other gross behavioural measurements do not change. The combination of the complexity of behaviour with the duration of an event and its frequency, may lead to a better overview of the behaviour affected by the health challenge, and possibly allows for an earlier detection of disease than when only total duration and frequency are measured.

2.12 Discussion

In this chapter the changes in certain behaviours of livestock that happen as a consequence of health challenges were reviewed. Several behaviours have been identified here that may be used as ‘indicators’ of disease in farm animals challenged by a wide range of health conditions. In the past, monitoring of such behaviours would have been time consuming, expensive and usually confined within the bounds of research facilities.

Recent changes in electronic applications now allow for the capture of different behaviours through simple automated means. Using such technologies very large datasets can be generated and methodologies to download, summarise and utilise such data have already been developed. The advances in these technologies are occurring at a very rapid pace indeed (Weary et al., 2009). For this reason, the time is ripe for the consideration of the use of animal behaviour as a means of disease detection on farm.

Feeding and drinking behaviour can now be monitored automatically with the use of passive or active transponders, which are detected through the use of equipment located by the feeder or drinker. A number of systems now capture these behaviours electronically either at an individual or group level (Sowell et al., 1999; Quimby et al., 2001; Urton et al., 2005). More sophisticated equipment is required for the instantaneous measurement of food and water intake (Tolkamp et al., 1998; Chapinal et al., 2007; González et al., 2008), but this is now routinely used for animals of 'value', such as in breeding stations. Measurements of activity and posture are also fairly straightforward, as the animals can simply be fitted with a pedometer or accelerometer that will record all movements and changes in posture made. Novel systems are now available to allow for defining the position of the animal within a husbandry system, including extensively kept animals (Swain et al., 2003). Complexity of behaviour and social behaviour may be more difficult to capture automatically. Although methodologies are in place, for instance through the use of pedometers for the complexity of standing or lying behaviour, and active collar transponders for the detection of social interaction (Swain and Bishop-Hurley, 2007, Böhm et al., 2009), the interpretation of the generated data is more challenging.

Interpretation and use of captured behavioural data is a usual bottleneck. This, for example, has been a stumbling block for the use of recorded feeding, drinking and standing behaviour. Although the technology to capture feeding and drinking behaviour has been available for several years, advances in how to utilise the generated data are much more recent (Tolkamp et al., 2011). The issue of what constitutes the biologically relevant unit of feeding and drinking behaviour has been a long standing one (Tolkamp et al., 1998), and the lack of consistent methodology has impeded their use for some time. The same applies to the use of lying behaviour (Tolkamp et al., 2010). The inconsistency in expressing the behaviour has led to some of the confusion over how health challenges affect the components of feeding behaviour.

Different behaviours change in varying ways in response to different challenges. Therefore, by measuring the behavioural dimension that is most likely to generate the largest magnitude of change, an early detection system, or importantly a system of detecting subclinical disease, can be developed. For feeding behaviour, the measure of total time spent feeding on a daily basis was shown to be the most susceptible to change across all health challenges, and has been used as a means of early detection in previous studies (Sowell et al., 1999; Quimby et al., 2001; González et al., 2008). There is less consistency over the usefulness of the other dimensions of feeding behaviour, such as number of meals (González et al., 2008). For drinking behaviour, overall drinking time and number of daily bouts are most likely to show change. However a decrease in drinking time is not always consistent and appears to be dependent on the type of health challenge. As previously discussed, drinking behaviour may be of limited use for the detection of micro-parasitic health challenges. The exception is metabolic health challenges, where water intake actually shows a substantial increase and will therefore have a great potential use.

Social behaviour initiated by the challenged individual shows a gradual decrease for all health challenges considered, with social exploration showing the steadiest decrease. The increase in defensive behaviours and the decrease in aggression are also prominent, although the latter behaviour can increase after a metabolic health challenge. The healthy animals within a group may also change their behaviour towards the challenged individual, altering body areas which are sniffed and possibly the frequency of grooming. However, currently there are no means of capturing these behaviours automatically and, as a consequence, their current usefulness is limited. Activity has been used previously for early detection disease and shows a consistent decrease (Edwards and Tozer, 2004) that is easily detectable by simple means. For the complexity of behaviour, the changes due to a health challenge appear to be more subtle and may therefore show a lower, albeit detectable, magnitude of change. An advantage of this method is that it shows a change before any of the other behavioural measures are affected, making it useful for early detection. Therefore based on the degree of change, feeding behaviour and activity have a proven track record of being detectable and should prove suitable measures for the early detection of disease. On the other hand, drinking and the complexity of behaviour show a lower or inconsistent response to health challenges. In addition, such changes have been based on experiments performed on clinically ill animals. The effect of a subclinical

health challenge may be considerably smaller and changes in them may be harder to detect.

An important issue is whether the changes in behaviour have a generic value or are health challenge-specific. Some changes and their magnitude appear to be consistent across different health challenges, with the reduction in food intake being a good case in point. As discussed and reviewed previously (Sandberg et al, 2006; Kyriazakis, 2010) food intake is reduced by ~20% in animals challenged by very different pathogens. It is reasonable to assume that these changes are reflected in changes in feeding behaviour; however the parameters that are altered can differ between challenges (González et al., 2008). The same may apply to the decrease in activity (Hart, 1988). On the other hand some behavioural changes appear to be health challenge-specific, with the increase in drinking behaviour and water intake during metabolic disorders being another good case in point. Both generic and health-challenge-specific changes will have their role in the detection of disease in livestock systems.

A further issue, associated with the above, is whether the changes in behaviour are of different magnitude for different levels (pathogen dose) of infection by the same pathogen. This has only been addressed to a very limited extent (Edwards, 1988; Johnson and von Borell, 1994; Bluthé et al., 1996; Skinner et al., 2009). It would be expected that high pathogen doses would be associated with more severe changes in behaviour; this would be consistent with the other effects (pathology, physiology etc.) on the animal. However, a particular issue is what the consequences on behaviour are over the pathogen doses that lead to subclinical disease. These issues constitute the focus of subsequent chapters.

Significantly less attention has been given in the literature to the time scale of behavioural changes in relation to the health challenges. Questions including whether these changes are gradual and whether they are apparent long before conventional means of disease detection, are very relevant to their potential diagnostic use. The study of González et al. (2008) is one of the exceptions that have investigated these questions. Their findings suggest that, in some cases, the changes occur long before any effects on animal performance are detected. If this applies across different health challenges, it will clearly have very significant consequences for early disease detection.

In conclusion, several animal behaviours may change as a consequence of a health challenge. Some of them appear to have potential use in the detection of disease and are able to be captured automatically for a large number of individuals at the same time. Advances in electronics may extend what can be captured in the future through such means. There are other steps that need to be considered in order to incorporate these changes into an early disease-detection system, and for such a system to become part of a livestock precision farming process, but these are outside the bounds of this review.

Chapter 3: The Behavioural Response of Beef Cattle to Vaccinations

3.1 Introduction

Subclinical health challenges have the disadvantage of being hard to detect, whilst still having the ability to affect the animal. This can lead to reduced welfare and production losses (Quimby et al., 2001; González et al., 2008). However if these challenges are detected on time and treatment commences immediately, these losses can be reduced. Monitoring behaviour can help in making an early diagnosis, as changes in the behaviour of the animal can indicate a subclinical disease (González et al., 2008). The focus of this study was on the effect of a subclinical challenge as represented by a vaccination, simulating the effect of an immune system stimulation by a pathogen challenge.

When animals experience a health challenge, many aspects of behaviour can change. All elements of feeding behaviour can change (Takeuchi et al., 1995; Sowell et al., 1998; González et al., 2008), including time, rate and frequency. Stimulation of the immune response through vaccination appears to lead to changes in feed intake (Walk et al., 2011) however what changes in feeding behaviour are responsible for this change is unknown. A decrease in activity was expected, as lethargy commonly accompanies sickness behaviour (Hart, 1988; Larson and Dunn, 2001; Borderas et al., 2008). Considering posture, a study by Escobar et al. (2007) showed an increase in inactive lying in pigs having been inoculated. Borderas et al. (2008) also showed an increase in time spent lying and standing inactive in calves. Water intake does not always change as a consequence of a micro-parasitic health challenge; therefore smaller changes in drinking behaviour were expected (Sowell et al., 1999). Consequently, the behaviours that may be useful as an indicator of a health challenge are activity, posture and feeding and drinking behaviour.

A variety of agents inducing immunological activation can be used to induce behavioural changes that mimic the consequences of a health challenge, including endotoxins and vaccinations. The latter have already shown promising results in medical science (Wright et al., 2005), when used for the induction of depression as a symptom of sickness behaviour. Vaccines can have a subclinical or sometimes even clinical effect on cattle as stated by Oirschot van et al. (1996) concerning a BHV-1 live-attenuated vaccine. The type of vaccine used has a sizeable effect on the magnitude of the response mounted, such as the nature of the strain used (Reichenberg et al., 2001; Wright et al., 2005), as well as

the attenuation of the vaccine; live attenuated strains are able to replicate once inside the host (Oirschot van et al., 1996; 1999). In addition, the type of adjuvant is important as this assists in stimulating the immune response (Johnson et al., 1963).

There is little information available on the extent to which changes take place for the different aspects of behaviour, and the time course of these changes, especially during a subclinical health challenge. The aim of this study was to quantify these changes and assess their potential for the early detection of (sub) clinical disease. Two types of vaccines were used for this purpose as stimulants of the immune response that were expected to have an effect on animal behaviour. The hypothesis was that activity, posture, feeding and drinking behaviour would show a rapid but transient change as a response to the vaccination, with possibly a greater and more extended response to the live-attenuated vaccine.

3.2 Material and Methods

3.2.1 Animals and housing

The animals used in this study were 24 Holstein-Friesian beef bulls aged between 5 and 11 months, obtained from a single source. They were housed in two separate pens, each containing 12 animals; each group of 12 animals were formed according to age in accordance with normal husbandry practise. Each pen measured 12 x 9.5 metres and contained straw bedding that was topped up three times a week (Mondays, Wednesdays and Fridays). Both food and water were available on an *ad libitum* basis with feed top-ups taking place once a week (Fridays). The food offered was a total mixed ration, consistent throughout the experiment, containing 62.5% barley, 12.5% sugar beet pellets, 10% soya bean meal, 7.5% molasses and 7.5% chopped barley straw on a fresh weight basis. The chemical composition of the food was 12.6 MJ ME per kg DM (estimated from AFRC (1993) feed tables) and 155 g CP per kg DM. No previous treatment or vaccinations had been given to the animals.

3.2.2 Experimental design

The experiment was considered to start when the animals received the vaccinations (designated as Day 0). Prior to the application of the health challenges (Day -9) the animals were weighed and fitted with a pedometer (IceTag, IceRobotics, South Queensferry, UK) on their left front leg; the pedometers were secured with Velcro. At the same time, video recordings of feeding behaviour started in order to provide background

data before the application of the challenge. The animals were also subjected to a novel object test to assess individual differences in temperament.

The animals were randomly assigned, whilst balancing for body weight and pen, to one of three treatments (n = 8 per treatment). The first treatment group, called the 'activated' treatment, was vaccinated with 5 millilitre (ml) of Rispoval 4 (Pfizer, Tadworth, UK), containing Para-influenza 3 (PI3) and Bovine Respiratory Syncytial Virus (BRSV), live-attenuated, as well as inactivated Bovine Herpes Virus (BHV-1), with an aluminium hydroxide adjuvant. The injection was administered intramuscularly in the right hindquarter. The second treatment group, called the 'deactivated' treatment, was vaccinated with 5 ml of Bovipast (Intervet, Milton Keynes, UK), containing three pathogens; *Mannheimia (Pasteurella) haemolytica*, Respiratory Syncytial Virus (RSV) and Para-influenza 3 (PI3), deactivated, with an aluminium hydroxide adjuvant. The injection was given subcutaneously in the right shoulder. The third treatment group functioned as the control and received 5 ml of Vitesel (Norbrook, Corby, UK), a multivitamin injection; the injection was administered intramuscularly in the right hindquarters. The treatments were administered to the animals on Day 0 between 9 and 10am. The trial finished on Day 15 when the animals were weighed again, had their pedometers removed and were also being submitted to a repeat of the novel object test.

3.2.3 Behavioural observations

The behaviours monitored were activity, posture and feeding and drinking behaviour. Activity and posture were measured with the use of the pedometers (IceTag, IceRobotics, South Queensferry, UK) that took second-to-second readings throughout the experiment, measuring the number of steps taken and the posture of the animal. All other behaviours were monitored with the use of video recordings that took place from 4am until 9pm, omitting the night hours due to the lower amount of activity and feeding behaviour taking place during this period (Yeates et al., 2003). The video material was watched and analysed with the use of the Observer behavioural recording system (Noldus, Wageningen, the Netherlands)

Continuous focal sampling of the feeding and drinking stations was used to monitor all feeding and drinking episodes. A feeding episode started when an animal crossed the feeding rail with its head; similarly a drinking event commenced when the animal crossed the edge of the drinker with its head. However, if an animal was at the feeder or the

drinker but performed behaviour other than feeding or drinking this was also noted. For feeding these were head lifts, which occurred when the animal was for instance looking across, or object manipulation that was also a regularly occurring behaviour at the drinker. Both feeding and drinking were scored for frequency and duration.

Besides the video recordings there was also an automated detection system in place to monitor the feeding behaviour. This system (RFID) worked using passive transponders that were fitted to the animal's eartags. These transponders were detected by antennae that were placed in front of the feeder. The range of the antennae was set as such that the transponders would only be picked up when the animal had its head in the feeder. This system was used throughout this thesis, however the results are not included due to sensitivity and specificity issues, which will be further debated in the discussion (Chapter 7).

3.2.4 Temperament assessment

Animal temperament was assumed to fall into one of two categories, 1) the more daring or active coping animals and 2) the more fearful or passive coping animals (Hopster, 1998). The temperament of the animals was examined using a restraint test and a novel object test. Data on the restraint test were gathered during the fitting and removal of the pedometer whilst the animal was in a crush. The response of the animal to this procedure was subjectively assessed according to the extent of movement, vocal response, kicking and posture. The movement score ranged from 0 (no movement) to 5 (struggles violently and attempts to escape). The vocal response was scored from 0 (none) to 3 (bellowing). If the animal kicked or kneeled 1 point was given; for lying down 2 points were given for each occasion. These scores were then added up to calculate an agitation score (Kilgour et al., 2006). Furthermore, there was also a subjective measure taken of the general impression the animal was giving; this could either be calm (0) or nervous (1).

For the novel object test, a structure of pool floaters with balloons fitted on the top was placed in an arena laid out in front of the weighing crate. The time it took for the animal to make first contact was noted as well as the number of touches to the object (Forkman et al., 2007). The distance of the animal to the object was measured with the use of a grid painted on the arena floor, with $2m^2$ squares and two circles surrounding the object with a diameter of 2m and 4m. The arena used was 21x5.70m making for a maximum distance from the object of 10 metres. The initial distance of the animal to the object was also

recorded, using the moment that the animal first stood still after exiting the crush, as well as the number of squares crossed during the test. The combination of more touches and shorter distance indicated the calmer animals, while greater distance and fewer touches indicated more fearful animals. The set time for this test was 5 minutes, after which the animal was allowed to leave the arena. Both the novel object test and the restraint test were performed at the start (Day -9) and finish (Day 15) of the experiment. There was a slight change in the second novel object test as the object was moved from the centre of the arena to stand next to the exit gate, as the first test showed that this was a place where the animals spent most of their time, due to possible visual contact with the other animals from this location.

3.2.5 Statistical analysis

The activity and posture data acquired from the IceTags were downloaded with the provided IceRobotics software in a format of one summary record per minute. Each record provided a date, time and percentage of time spent lying and standing and the number of steps taken during that minute. The lying and standing data were summarized into episodes with the use of purpose written FORTRAN programs (Tolkamp et al., 2010). These episodes were calculated by assuming that a continuous series of records that showed 100% of either lying or standing behaviour, were part of the same episode. When both lying and standing occurred in the same minute, it was assumed that this was a transition minute in which the behaviour during the first part of the minute was the same as that during the last part of the previous minute. Short lying episodes (those under 4 minutes) were deleted because these were previously verified with video footage not to correspond to real lying behaviour (Tolkamp et al., 2010). This resulted in a sharp reduction in the number of episodes without any considerable impact on the total lying and standing times, since many deleted episodes lasted only a few seconds. The analysis applied was on the total number of steps taken, the total lying or standing time (which are reciprocal), the frequency of lying episodes (which by definition is identical to the frequency of standing episodes) and the average duration of lying and standing episodes. These data were analysed on a daily basis apart from activity, as measured by the number of steps, which was also analysed on an hourly basis for the day of the challenge (Day 0).

For feeding and drinking behaviour the days compared were days -6, -4, 1 and 3. The first two days were chosen to provide baseline behaviour before the challenge and the final two to capture the behavioural response after the challenge. Furthermore the days reflect

farm management routines; on Saturdays (Day -6 and 1), no husbandry events took place and the animals were fed the day before, so they were expected to show normal, undisturbed behaviour. On Mondays (Day -4 and 3), the animals received new bedding in the form of straw that they could eat, therefore possible giving slightly different behavioural outcomes. Feeding and drinking behaviour episodes were grouped into meals (feeding) and bouts (drinking) using meal/bout criteria calculated after fitting mixed models to the frequency distribution of interval lengths between episodes. The best fit was obtained by a three population model consisting of two Gaussian and one Weibull distribution for both meals and bouts. These models, developed by Yeates et al. (2001), were fitted to the log-transformed interval lengths (expressed in seconds) between feeding and drinking episodes using the SAS 9.1 (SAS Institute Inc, Cary, USA) programs of González et al. (2008). The rationale for fitting a three population model is that there are three populations of between-feeding intervals, one between meals and two within meals, during which animals do or do not drink (Yeates et al., 2001). From the model parameters, the meal interval criterion was estimated at 14.38 minutes. The same model was fitted to intervals between drinking episodes and resulted in a drinking bout interval criterion estimate of 18.8 minutes. Feeding and drinking episodes separated by intervals shorter than the estimated criteria were subsequently grouped into feeding meals and drinking bouts. Both episodes and meals/bouts were analysed for their duration and frequency. The object manipulation taking place at the feeder or drinker and headlifts taking place at the feeder were also recorded and analysed for both duration and frequency.

The relationships between the different measures taken in the behavioural temperament tests were examined using a principal component analysis (PCA). These components incorporated the results of the restraint test, the novel object test and the general impression given by the animal. For the three components that explained the majority of variation, the weightings for each measure were multiplied by the original value and summed to create a component score for each animal. Each component score was then examined for correlations to the other behavioural parameters measured.

All data were analysed with the use of a repeated measures ANOVA in SPSS 15.0 (IBM, Armonk, NY, USA). The days were defined as a random variable and the treatment and pen were defined as a fixed variable. Analysis of activity and posture measures used the mean of the 8 days prior to the experiment (Day -8 until -1) as a covariate to account for

variation between individuals. For feeding and drinking behaviour the mean over the two days prior to administration of the challenge (-6 and -4) were used as the covariate. The following parameters were log-transformed for analysis in order to normalize the data: (i) total and average feeding episode duration, (ii) total and average meal and drinking bout duration, (iii) average and total lying and standing episode duration, (iv) total and average duration of object manipulation at the drinker, (v) the frequency of object manipulation at both the feeder and the drinker and (vi) the frequency, average and total duration of headlifts. These results are reported as back-transformed means with 95% confidence intervals (CI).

3.3 Results

3.3.1 General

One of the animals in the activated treatment group was removed from the analysis as this animal was diagnosed with lameness. This lameness was clinical and caused a significant effect on all behavioural measures.

3.3.2 Body weight

Body weight (BW) was significantly affected by time ($P = 0.005$), but there was no effect of treatment and no interaction between time and treatment ($P > 0.05$). Weight showed a significant Pen effect ($P < 0.001$) with the average BW at the start of the trial being 365±22 kg for Pen 1 and 241±15 kg for Pen 2. At the end of the trial means were 399±20 kg for Pen 1 and 280±16 kg for Pen 2, giving an average BW gain of 34±5.5 kg for Pen 1 and 39±1.37 for Pen 2.

3.3.3 Activity and posture

Activity throughout the experiment, as measured by the number of steps taken per day, showed a significant effect of the covariate ($P < 0.001$) indicating consistent individual differences. However this measure was not affected by time, treatment or time and treatment interaction ($P > 0.05$); there were also no significant effects when the data were analysed per h for Day 0. There was an increase in activity, as measured by the number of steps taken, immediately post injection (Day 1; Figure 3.1) across all treatment groups. This increase persisted for all groups and only returned to pre-challenge values more than a week after the injection was administered. The average number of steps per day across treatments before Day 0 was 3282 (SEM = 159) and after Day 0 until the end of the experiment it was 4565 (SEM = 278).

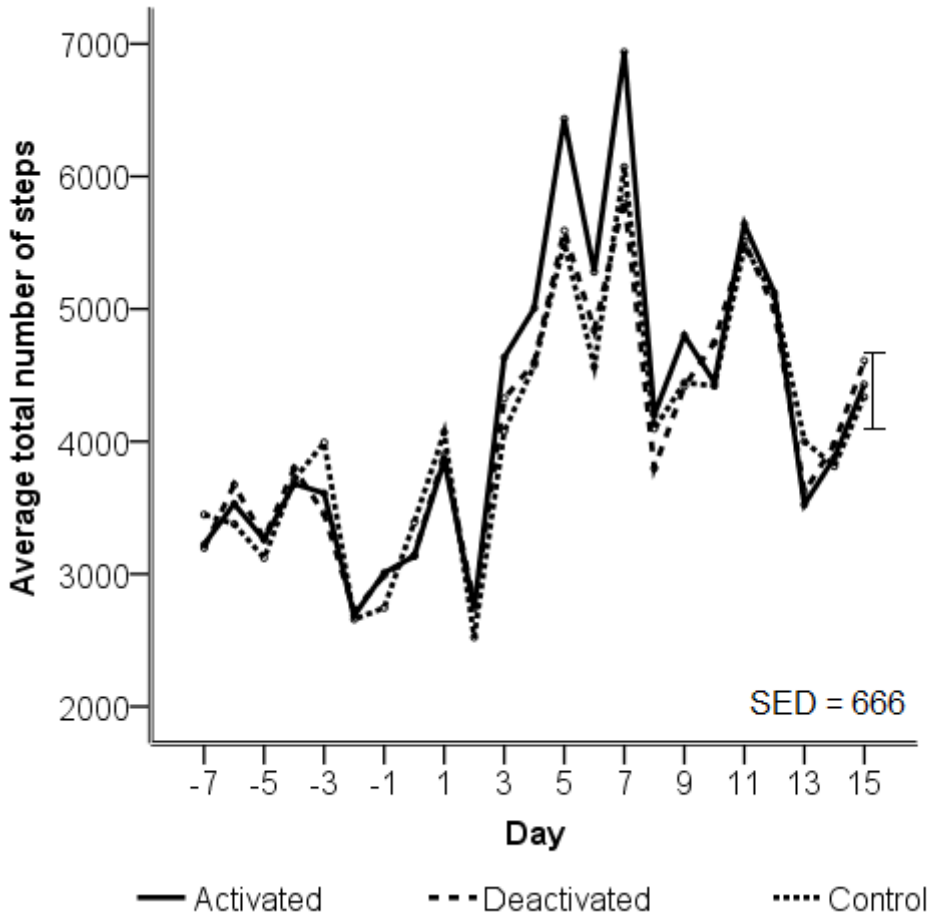

Figure 3.1: The number of steps taken per day against experimental day for unchallenged control (Control; n = 8) beef cattle and those presented with an activated (Activated; n = 7) or deactivated (Deactivated; n = 8) vaccine challenge on Day 0. The bar is the standard error of the difference (SED) and shown on the control treatment.

There was no significant effect of time, treatment or an interaction between time and treatment ($P > 0.05$) for the total and average time spent standing and lying, or the frequency of episodes (Table 3.1). All these behavioural measurement parameters were significantly affected by the covariate ($P < 0.002$). Total daily lying duration was 12.4 (CI: 11.6 – 13.1) h, and for standing this was 11.2 (CI: 10.5 – 11.9) h. The average episode duration was 40.5 (CI: 36.8 – 44.5) minutes for lying and 11.8 (CI: 9.4 – 14.8) minutes for standing episodes. The overall mean frequency of episodes, which was identical for both lying and standing, was 19.2 (SEM = 1.00) per day.

Table 3.1: The daily average number of steps taken, the average and total daily duration of time spent lying and standing and the frequency (freq), which is the same for both lying and standing, with the standard error of the difference (SED) and significance for time by treatment interaction, of beef bulls in one of three treatments: animals challenged with an activated (Activated; n = 7), a deactivated (Deactivated; n = 8) vaccine and unchallenged controls (Control; n = 8), on Day 0 of the trial.

	Activated	Deactivated	Control	SED	*P* - value
Number of steps (day)	4670	4727	4299	590	0.40
Episode[1] freq (day)	18.9	20.1	18.7	2.29	0.25
Lying					
Total duration (h)	12.7	12.1	12.3	0.86	0.32
Episode average (min)	42.3	40.5	38.9	4.80	0.08
Standing					
Total duration (h)	10.8	11.6	11.2	0.78	0.71
Episode average (min)	15.2	11.3	9.81	4.62	0.50

[1] Episode = Period of uninterrupted standing or lying

3.3.4 Feeding and drinking behaviour

There was no time or treatment effect and no time and treatment interaction ($P > 0.05$) for all measurements of feeding and drinking behaviour (Table 3.2). There was a Pen effect for feeding frequency which was significantly ($P = 0.011$) higher in Pen 2 and a covariate effect for average feeding duration ($P = 0.002$), total feeding duration ($P < 0.001$), feeding frequency ($P = 0.001$), drinking frequency ($P < 0.001$) and total drinking duration ($P = 0.032$). On average the total feeding duration was 91.9 (CI: 80.1 – 112) minutes per day, the average feeding episode duration was 1.03 (CI: 0.91 – 1.17) minutes, and frequency of feeding episodes was 92.6 (SEM = 4.59) per day. On average the total drinking duration was 9.77 (SEM = 0.94) minutes per day, the average drinking episode duration was 0.96 (SEM = 0.064) minutes per day, and frequency of drinking episodes was 11.0 (SEM = 1.21) per day.

Table 3.2: The average total daily duration of time spent feeding and drinking, the frequency (freq) and average duration of feeding episodes and meals and drinking episodes and bouts on Days 1 and 3, with the standard error of the difference (SED) and significance for time by treatment interaction, of beef bulls in one of three treatments: animals challenged with an activated (Activated; n = 7), a deactivated (Deactivated; n = 8) vaccine and unchallenged controls (Control; n = 8), on Day 0 of the trial.

	Activated	Deactivated	Control	SED	*P* - value
Drinking					
Total duration (min)	8.06	9.69	11.4	2 .33	0.53
Episode[1] freq (day)	11.3	10.6	11.0	3.00	0.78
Episode average (min	0.86	0.93	10.9	0.16	0.47
Bout[2] duration (min)	21.3	17.7	18.6	1.10	0.51
Bout freq (day)	7.14	7.19	7.94	1.16	0.77
Bout average (min)	2.85	2.64	2.54	0.90	0.16
Feeding					
Total duration (min)	99.3	94.1	84.1	17.7	0.89
Episode freq (day)	98.6	94.2	85.8	11.4	0.42
Episode average (min)	0.94	1.19	0.91	0.22	0.82
Meal[3] duration (min)	215	206	179	35.0	0.66
Meal freq (day)	11.5	11.3	11.1	2.38	0.23
Meal average (min)	18.2	18.2	16.5	3.08	0.33

[1] Episode = Period of uninterrupted feeding or drinking
[2] Bout = Group of drinking episodes that are divided by a bout criterion
[3] Meal = Group of feeding episodes that are divided by a meal criterion

When the feeding episodes were grouped into meals, there was no significant effect of time, treatment or time and treatment interaction ($P > 0.05$) for the total and average meal duration as well as the meal frequency. The covariate was significant for the total meal duration ($P < 0.001$) and the meal frequency ($P = 0.045$). The average total meal duration was 199 (CI: 173 – 228) minutes per day, the average meal duration was 17.6 (CI: 15.4 – 20.1) minutes and the average frequency was 11.4 (SEM = 0.497) meals per day.

With the drinking episodes grouped into bouts, there was no significant effect of time, treatment or time and treatment interaction ($P > 0.05$) for the total and average bout duration and the bout frequency. The covariate was significant for the total ($P = 0.002$) and the average ($P = 0.02$) bout duration as well as for the bout frequency ($P = 0.04$). The

average total bout duration was 19.0 (CI: 14.0 – 25.9) minutes per day, the average bout duration was 2.66 (CI: 2.12 – 3.33) minutes and the average bout frequency was 7.43 (SEM = 0.469) per day.

3.3.5 Object manipulation and headlifts

Behaviours taking place at the feeder and the drinker other than feeding and drinking were object manipulation as well as headlifts at the feeder. There was no significant effect of time, treatment and no time and treatment interaction ($P > 0.05$) for the average and total duration as well as frequency for any of these parameters. The covariate was significant for the frequency ($P = 0.002$) and total duration ($P = 0.001$) of headlifts, as well as for the total time spent on object manipulation at the drinker ($P = 0.045$). The average episode duration was 0.53 (CI: 0.43 – 0.65) minutes for headlifts, 1.25 (SEM = 0.13) minutes for object manipulation at the drinker and 0.47 (CI: 0.39 – 0.56) minutes for object manipulation at the feeder. The total daily duration was 2.79 (CI: 1.70 – 4.56) minutes for headlifts, 6.03 (CI: 3.87 – 9.40) minutes for object manipulation at the drinker and 2.96 (SEM = 0.48) minutes for object manipulation at the feeder. Average daily frequency was 5.31 (CI: 3.67 – 7.69) for headlifts, 5.43 (CI: 4.02 – 7.34) for object manipulation at the drinker and 5.29 (CI: 4.14 – 6.75) for object manipulation at the feeder. From the total amount of time spent at the drinker, 43.8% was spent on object manipulation rather than drinking. Most of the time spent at the feeder was spent eating (92.6%), only 2.71% was spent on object manipulation and 4.71% on headlifts.

3.3.6 Temperament

From the seven different scores of temperament taken (number of squares crossed, starting distance from the object and approach to the object from the novel object test; movement, vocalisations and kicks from the restraint test; and the impression given by the animal), three significant ($P < 0.001$) components were derived from the PCA (Table 3.3). The first component showed high positive weights for movement and impression; the second component showed high positive weights for the number of squares crossed and the starting distance from the object; and the third component showed a high positive weight for the approach to the object. Together these explained 89.7% of the variation (Table 3.3). For the first component the average score was 16.1±4.87, the second component had an average score of 44.68±13.6 and the third component an average of 2.61±4.00. There was no significant ($P > 0.05$) correlation between the components

defining temperament and any of the results on feeding and drinking behaviour or activity and posture in this experiment.

Table 3.3: The results of a principal component analysis of the temperament measures (impression given by the animal; movement made during restraint; number of squares crossed during the novel object test; starting distance from the object; approach to the object) showing the weightings and the % of variation explained by each component.

	Component		
	1	2	3
Impression	0.937	-.156	-.108
Movement	0.959	-.033	-.086
Squares crossed	0.332	0.844	0.103
Starting distance	-.163	0.770	-.493
Approach	0.076	0.299	0.913
%	38.8	28.4	22.1

3.4 Discussion

The objective of this study was to investigate which behavioural changes occurred in beef cattle subjected to a vaccination, to quantify these changes and to assess their potential in aiding the detection of (sub) clinical disease. In order to achieve this, beef cattle were injected with either an activated vaccine or a deactivated vaccine representing different degrees of an immune system stimulation challenge, with the control treatment receiving a multivitamin injection. The behaviours monitored for potential changes were activity, posture, feeding and drinking behaviour. A previous literature review (Chapter 1) had identified that these were the behaviours most likely to be affected by subclinical health challenges. The expectation was that vaccination will lead to some behavioural changes, with animals injected with the activated vaccine being affected more than ones challenged with the deactivated vaccine. This because the activated or "live" vaccine is more likely to induce sickness behaviour (Oirschot van et al., 1996) and can even lead to clinical signs (Oirschot van et al., 1996; 1999).

In the case of activity, as measured by the number of steps taken, there was no effect as a result of the treatments. The fact that there was no change in activity was contrary to expectations; a decrease was anticipated because animals affected by a health challenge tend to limit their movements and become more lethargic (Hart, 1988). For the same reason an effect on posture was also expected. Since increased lethargy reduces mobility

(Hart, 1988), the time spent lying was expected to increase whereas the standing duration was expected to decrease. However, no such effect was found. The only observed effect was caused by an increase in the number of steps taken after the challenges were presented on Day 0 for all treatments, including the control treatment. No explanations for this sudden and persistent increase could be detected from the videos taken. The only change in the management of the animals was the administration of the injection in all groups. The handling associated with this, involving moving the animals into the crush, was not a novel experience for the animals because it had already taken place on previous occasions. It was somewhat surprising that an injection could have had such a dramatic effect on activity, and even more so that this behavioural change persisted for a number of days. Vaccination is a routine husbandry practice and its consequence for the animals, including their behaviours, is frequently ignored making it difficult to hypothesise. However, if the effects can be attributed solely on the action of injection, this is something that may need to be taken into account in future experiments where behavioural observations are taken.

There was no change in either feeding or drinking behaviour; this included total daily duration, average episode duration and average daily episode frequency. For feeding behaviour, a change was expected (Sowell et al., 2001; González et al., 2008) to reflect a reduction in food intake, also known as anorexia, which is one of the first notable behavioural changes indicative of an activation of the immune system by pathogens (Quimby et al., 2001; González et al., 2008). Because the immune responses caused by vaccination evoke an increase in circulating cytokines, with anorexigenic effects (Plata-Salamán, 1998), a decrease in food intake was expected (Larson and Dunn, 2001), and has been observed for live attenuated vaccines (Walk et al., 2011). However, with no difference in BW between the treatments the possibility exists that anorexia either did not occur or was transient.

There were only Pen effects on BW and feeding episode frequency, likely caused by the differences in characteristics of the animals in the two pens, as these animals were divided according to age according to normal farm management procedures. Pen 1 contained the older, larger animals and this could possibly have caused the effect on feeding behaviour, because animals of different ages differ in their feeding behaviour (Albright, 1993). There were also no effects of treatment on drinking behaviour, for which a change was expected to accompany a possible raise in body temperature as a consequence of vaccination.

However such an effect could be compensated over the course of the day (Hart, 1988) because the intake of water is more vital to maintain bodily functions (Kyriazakis and Tolkamp 2011).

Besides feeding and drinking, other behaviours also took place at the feeder and the drinker. The largest part of this was represented by object manipulation at the drinker, which accounted for 43.8% of the total time spent at the drinker. Common forms of object manipulation were licking the wall or chewing the fence behind the trough. It has been suggested that such a behaviour can arise from lack of stimulation (Kyriazakis and Tolkamp 2011), or from lack of nutrients, such as salt which can lead to wall licking (Agricultural Board, 1970). With object manipulation representing such a large part of behaviours taking place at the drinker, this needs to be taken into account when using automated systems that only detect presence at the drinker, as this could result in a misrepresentation of drinking duration. A possible solution to this would be to link such a system to a water flow meter, to define when presence at the drinker was actually spent drinking.

Animals with different temperaments can show differences in their immune capability (Fell et al., 1999; Koolhaas, 2008), which is why temperament tests were implemented in this study. However, the temperament parameters did not relate to any of the other behaviours measured, possibly indicating that any influence of temperament was too small to detect. Another possibility is that the test results were influenced by factors other than temperament, such as the presence of distractions during the novel object test. In addition, there was no clear distinction in the response of animals of different temperaments; a nervous animal could freeze and stand still, or panic and move about a lot. Furthermore, the animals were not necessarily at the extremes of the two temperaments but somewhere in the middle, which makes detection harder, even by clearly defined tests (Hessing et al., 1993). A third possibility may be the inadequacy of the tests for this purpose. There is no well-defined temperament test for cattle compared to, for instance, a tonic immobility test for chickens (Jones et al., 1995), a backtest for pigs (Hessing et al., 1993) and a shock prod defensive burying test for rats (Koolhaas et al., 1999).

There could be various reasons as to why none of the behaviours showed treatment differences, the most obvious one being that the vaccines did not provide an immune

stimulation that was large enough to influence behaviour. Another possibility is that the individual variation in response between animals was too high and obscured any effects of the vaccinations on behaviour. Furthermore the effects were expected to be short-lived (Plata-Salamán and Borkoski, 1993; Plata-Salamán, 1994) and therefore could have been masked by fluctuations in behaviour, such as herd diurnal patterns (Hicks et al., 1989). As behaviour is known to change when an animal is faced with an acute health challenge (Plata-Salamán and Borkoski, 1993; Quimby et al., 2001; González et al., 2008) it is reasonable to assume that with a slightly stronger or more prolonged challenge behavioural changes will be detectable. The strongest challenge administered in this case was a live-attenuated vaccine; these vaccines can have a subclinical or sometimes even a clinical effect on cattle (Oirschot van et al., 1996), although as previously mentioned this did not manifest here.

It can be concluded that more research is needed to identify the changes in behaviour of beef cattle faced with a health challenge, so that the early detection of disease and the detection of subclinical disease can be made possible. As these results did not show clear treatment effects, a different approach will be needed. One method to achieve this could be to present the animals with a more severe or prolonged challenge. A more severe challenge could be administered in the form an endotoxin (e.g. lipopolysaccharide), and a prolonged challenge could be achieved by giving a repeated challenge, or inducing a chronic subclinical infection. Finally, it is important that the challenge does not lead to clinical effects, since this will defeat the purpose of monitoring changes before clinical signs manifest themselves and utilising them accordingly.

Chapter 4: The Effects of Acute Versus Chronic Health Challenges on the Behaviour of Beef Cattle

4.1 Introduction

Health challenges that lead to subclinical or clinical disease may result in performance losses, increase treatment costs, and lead to a reduction in animal welfare (Quimby et al., 2001; González et al., 2008). One way to minimize the impact of health challenges and reduce costs is to start treatment early. To allow for this, an early diagnosis is needed. Because animal behaviour might be one of the first things affected by a health challenge (Kyriazakis and Tolkamp, 2010), the possibility arises to use behavioural changes to detect the early onset of disease.

It is largely unknown what changes exactly take place for the different types of behaviour, and at what rate they develop during a health challenge, especially during subclinical disease. However, some broad descriptions of what changes during disease already exist (as summarized by Hart, 1988; Gougoulis et al., 2010; Kyriazakis and Tolkamp, 2010). It is possible that chronic challenges, such as a parasitic infection or lameness, may lead to gradual changes in behaviour, which can persist for long periods of time (Kyriazakis et al., 1994; González et al., 2008). The opposite may apply for the effects of acute challenges on behaviour, such as inflammation or metabolic disorders (Gougoulis et al., 2010). For this reason, the kind (direction) of behavioural changes that can aid in an early diagnosis may depend on the type of challenge. The objective of this experiment was to quantify the behavioural changes that arise from either an acute (i.e., lipopolysaccharide (LPS)) or a chronic (i.e., gastrointestinal parasitism) health challenge and to assess their potential for the development of an early disease detection system in beef cattle. The experiment focused on behaviours that have the potential to be captured automatically through advances in electronic sensors. The hypothesis was that the changes in behaviour as a response to the acute health challenge would be rapid but transient, whereas the chronic health challenge would lead to more gradual and prolonged changes. Although the experiment used beef cattle as the target animal, some of the principles developed here would be expected to apply to other animals exposed to health challenges.

4.2 Material and Methods

The experiment took place at the facilities of Newcastle University after approval of the experimental protocols by the Animal Experiments Committee and under license according to the UK Animals (Scientific Procedures) Act for experimental challenge and regulated procedures (licence number: PPL 60/4067).

4.2.1 Animals and housing

The animals used were 17 Holstein-Friesian beef bulls, aged between 4 and 11 months, which were obtained from the University farm. The sample size was determined using a power calculation based on the standard deviations associated with a previous experiment (Chapter 3). All animals were housed together in a single straw-bedded pen measuring 12 × 9.5 m^2 with a trough that provided 21 feeding spaces; artificial lighting was provided between 20.00 h and 05.00 h. Both feed and water were available on an *ad libitum* basis. The feed offered was a total mixed ration, consistent throughout the experiment, containing 62.5% barley, 12.5% sugar beet pellets, 10% soya bean meal, 7.5% molasses, and 7.5% chopped barley straw on a fresh basis. The chemical composition of the feed was 12.59 MJ/kg DM of ME (estimated from AFRC (1993) feed tables) and 155.2 g/kg DM of CP. The bulls had not received any prior challenges or vaccinations. Thirty days before the start of the experiment, all animals were treated with a long-acting anthelmintic (Noromectin, Norbrook, Carlisle, UK).

4.2.2 Experimental design

Bulls were randomly assigned to 1 of 3 treatments, balanced for BW and age. The experiment was considered to start when the bulls received the first challenge with the gastrointestinal parasite (designated as Day 0). Prior to the application of the health challenges (Day -8), bulls were fitted with a pedometer (Icetag, IceRobotics, South Queensferry, UK) on their left front leg; the pedometers were secured with Velcro.

The first treatment group consisted of 6 bulls. They were challenged with a LPS challenge (*E. coli* 0111:B4 endotoxin, Sigma Aldrich, Gillingham, UK). Lipopolysaccharide is one of the most common endotoxins used as a model challenge to induce sickness behaviour in a variety of animals, ranging from laboratory rodents (Bret-Dibat et al., 1995) to large farm animals (Borderas et al., 2008), as well as leading to reduced levels of water intake (Johnson and von Borell, 1994; Plata-Salamán and Borkoski, 1993) in some cases. The LPS challenge, which took place every other day over a week-long period (during week 3

of the experiment; Days 13, 15 and 17), started with a dose of 0.2 µg/kg LPS by intravenous bolus injection, as suggested by Elasser et al. (1995). This was increased by 0.025µg/kg in each subsequent injection in order to account for any habituation to LPS (West and Heagy, 2002), which would have reduced any effects shown by the animals. The LPS, which was in a lyophilized form, was reconstituted in sterile saline at a 1:1 ratio and kept in a refrigerator for 1 week, until it was required. Shortly before the challenge, the samples were diluted again with sterile saline at a 1:100 ratio. The bulls were also gavaged on Day 0 with 30 ml of water; this was to control for another treatment described below.

The second treatment group also consisted of 6 bulls. Each bull received a single dose of 200,000 L3 *Ostertagia ostertagi* larvae administered by gavage on Day 0 of the experiment. This dose has been shown to reduce feed intake for a number of days, as well as to increase circulating pepsinogen levels, which are considered to be an indicator of abomasal damage (Fox et al., 2002). The parasitism induced by this dose was expected to be subclinical. The parasite was not expected to lead to any effects on BW or feeding behaviour for the first 2 weeks post treatment because the larvae need time to mature**.** The L3 larvae used for the challenge were obtained from Ridgeway Research (Gloucestershire, UK) and were of an Ivermectin-susceptible strain that was isolated in South Gloucestershire 2 months before use (reference label OOSG10). Upon arrival, the 1.2 million larvae were split into 2 groups and diluted in 500 ml each of water, which was changed every other day until use. Just before dosing, 410 ml of surplus water was removed from the top leaving 3 doses of 30 ml containing 200,000 L3 per dose, 1 of which was administered to each animal by gavage. A 10 ml saline injection was also given repeatedly every other day during week 3 (Days 13, 15 and 17) to control for the other treatment described previously.

The third treatment group, which acted as unchallenged controls, consisted of 5 bulls. These bulls were given a gavage containing 30 ml of water on Day 0, as well as a repeated saline injection of 10 ml during week 3 (Days 13, 15 and 17). Thus, the experimental design subjected all animals to the same handling procedures. The LPS was given during week 3 for the purpose of contemporary comparison because the effects of the parasite challenge were unlikely to be seen before this point.

The experiment continued for 4 weeks after Day 0, and the bulls were weighed at every handling occasion on Days 0, 13, 15, 17, 20, 27, and 31; the rectal temperature of each bull also was taken on every handling occasion to minimize additional disturbance. The animals were weighed and faecal samples were collected at the start of the experiment (Day 0). Both faecal and blood samples were collected during the third week every other day (Days 13, 15 and 17), before the LPS injection, as well as on Day 20. Additional faecal samples were collected on Day 27. All animal handling took place in the morning between 10.00 h and noon. The faecal samples were collected from the rectum and directly placed in labelled pots. The FEC took place on the same or the next day, in which case the samples were kept in the refrigerator overnight. The blood samples (10 ml) were collected from the jugular vein with a plain tube (Vacutainer, BD, Franklin Lakes, New Jersey, USA) for serum collection, and the samples were left overnight in the refrigerator at 4^{o}C, after which they were centrifuged for 15 min at 1500rpm at room temperature (18^{o}C). At the end of the experiment (Day 31), bulls in all treatment groups were treated with a broad-spectrum anthelmintic (Noromectin, Norbrook, Carlisle, UK).

4.2.3 Behavioural observations

Behavioural levels of activity, posture, feeding, and drinking were monitored. The focus was on these cattle behaviours because they can be automatically monitored and are potentially affected by health challenges (Quimby et al., 2001; Borderas et al., 2008; González et al., 2008). Activity levels and posture were measured with the use of the pedometers which took second-to-second readings throughout the experiment, measuring the number of steps taken and the posture of the animal. Feeding and drinking behaviour were monitored with 24-h video recording equipment. Feeding episodes were considered to start when an animal put its head into the feeder. Similarly, drinking episodes started when the animal placed its head above the drinking trough. The video material was watched using continuous sampling, and analysed for the duration and frequency of feeding and drinking episodes. Drinking behaviour was recorded during 4 d prior to the challenge (Day -4 to Day -1), as well as for 6 d during the third week (Day 13 to Day 18). Analysis of feeding behaviour focused on Days -4, 13, 15, 17, and 19. These days were chosen because they coincided with the LPS challenge and the expected start of the *O. ostertagi* effects.

4.2.4 Blood sample analysis

The collected blood samples were used to assess LPS serum antibodies and pepsinogen levels. Antibodies were assessed by indirect ELISA. Medium-binding 96-well ELISA plates (NUNC, Roskilde, Denmark) were coated with LPS identical to that used in the health challenge at a dilution of 1:100 in coating buffer (1.59 g Na_2CO_3 L^{-1}, 2.92 g $NaCHO_3$ L^{-1}) at 100 µL per well and left overnight at 4^{o}C. The following day, the plates were washed twice with a washing solution (PBS with 0.5% Polysorbate 20) after which the non-specific binding sites were blocked with a Normal Horse Serum (NHS) solution (100% sterile PBS, 0.5% NHS, and 0.05% Tween 20) at 200 µL per well and left to incubate for 1 h at 37°C. The plates were then washed twice with washing solution after which the serum was added in triplicate wells at a dilution of 1:10,000 in sterile PBS at 100 µL per well. This dilution had been considered as ideal from a pilot study that utilized different dilutions. The plates were then incubated for 2 h at 37°C, after which they were washed twice. Secondary antibody (goat polyclonal secondary antibody to bovine IgG – H&L (HRP); Abcam, Cambridge, MA) was added at a dilution of 1:25,000 (concentration also derived from a pilot study), diluted with sterile PBS, 100 µL per well and left to incubate for 2 h at 37°C. Plates were washed 3 times after which the tetramethylbenzidine with horseradish peroxidase (TMB HRPO) (T118, Leinco Technologies Inc., St Louis, Missouri) was added at 100 µL per well to visualize the reaction. The plates were left in the dark at room temperature for 20 min. To stop the reaction, 50 µL of H_2SO4 was added to each well after which the absorbance was read at 450 nm. The intra-assay variation had a SED of 0.135 of absorbance at 450 nm. Plasma pepsinogen concentration (iu/L) was determined using the modified method of Paynter (1992).

4.2.5 Faecal egg counts

Faecal egg counts were expressed as the number of eggs per gram of collected fresh faeces. They were assessed by the flotation method, modified from the method detailed in the Ministry of Agriculture "Manual of veterinary parasitological laboratory techniques" (Ministry of Agriculture, 1977). Briefly, 4.5 g of faeces was added to 45 ml of water. The mixture was shaken until all the faecal matter was dissolved. It was then poured through a wire mesh screen (pore diameter of 0.15 mm) and the strained fluid caught in a bowl. The debris was discarded. A sample (15 ml) of the strained fluid was centrifuged for 2 min at 1500rpm at room temperature (18^{o}C) and the supernatant removed. The tube was then filled with saturated salt solution and the sediment was loosened and stirred. The tube was then filled to the brim until a convex meniscus stood above the top of the tube. A standard

thick plastic round cover was placed on the tube, covering the top completely with no air bubbles trapped beneath it. The tube was then centrifuged at 1000rpm for 2 min at room temperature (18^{o}C). The plastic cover to which the eggs had adhered was removed from the tube by lifting it vertically with a deliberate movement. The cover was placed on a microscope slide and the eggs were counted under a 50× magnification. Because approximately 1/6 of the eggs are expected to be lost in the process of flotation (Ministry of Agriculture, 1977), a correction factor of 6/5 was applied. The consistency of the faecal samples was assessed visually and found to be of a constant consistency for all treatments.

4.2.6 Statistical analysis

The data acquired from the IceTags was downloaded with the provided IceRobotics software in a format of 1 summary record per minute, for lying and standing analysis, and per day for the analysis of number of steps taken. Each record provided a date, time, percentage of time spent lying and standing, and the number of steps taken. The lying and standing data were summarized into episodes with the use of purpose-written Fortran programs (Tolkamp et al., 2010). These episodes were calculated by assuming that a continuous series of records that showed either 100% lying or standing behaviour, are part of the same episode. When both lying and standing occurred in the same minute, it was assumed that this was a transition minute in which the behaviour during the first part of the minute was the same as that during the last part of the previous minute. Short-lying episodes (those under 4 min) were deleted because these were previously verified with video footage not to correspond to real lying behaviour (Tolkamp et al., 2010). This resulted in a sharp reduction in the number of episodes without any considerable impact on the total lying and standing times, because many episodes were < 60 sec.

Feeding and drinking behaviour episodes were grouped into meals (feeding) and bouts (drinking) using meal/bout criteria calculated after fitting mixed models to the frequency distribution of interval lengths between episodes. The best fit was obtained by a 3-population model consisting of 2 Gaussian distributions and 1 Weibull distribution for both meals and bouts. These models, developed by Yeates et al. (2001), were fitted to the log-transformed interval lengths (expressed in seconds) between feeding and drinking events using SAS 9.1 programs (SAS Institute Inc, Cary, NC) of González et al. (2008). The rationale for fitting a 3-population model was that there are 3 populations of between-feeding intervals; 1 between meals and 2 within meals during which animals do

or do not drink (Yeates et al., 2001). From the model parameters, the meal interval criterion was estimated at 18.4 min. The same model was fitted to intervals between drinking episodes and resulted in a drinking bout interval criterion estimate of 6.79 min. Feeding and drinking episodes separated by intervals shorter than the estimated criteria were subsequently grouped into feeding meals and drinking bouts. Both episodes and meals/bouts were analysed for their duration and frequency.

Body weight, LPS antibody concentration, pepsinogen levels, activity, posture, and feeding and drinking behaviour were analysed with the use of a repeated measures ANOVA using SPSS 15.0 (IBM, Armonk, NY) with treatment as a fixed effect and day as a random effect. For BW and BW gain analysis, the Day 0 value was used as a covariate, whereas for LPS antibody levels the Day 13 value was used as a covariate. Activity, as measured by the number of steps taken, and posture used the mean of the 8 d before the experiment (Day -8 to Day -1) as a covariate to account for variation among individuals. Mean drinking behaviour over Day -4 to Day -1 and feeding behaviour on Day -4 were used as covariates for the analyses of drinking and feeding behaviour, respectively. Furthermore, the data on the LPS antibodies, pepsinogen, average and total lying and standing episode duration, and drinking bout duration were log transformed prior to analysis to normalize their distributions. These results are reported as back-transformed means with 95% confidence intervals (CI).

4.3 Results

4.3.1 Body weight gain

The change in animal BW throughout the experiment is shown in Figure 4.1. There was a strong interaction between treatment and time ($P < 0.001$) on BW; and an effect of the covariate ($P < 0.001$). There was no difference between the BW of the control and LPS-treated bulls at any time point ($P > 0.05$). From Day 16, the BW of the bulls in the other groups started to diverge from the bulls on the parasite treatment who were gaining weight at a slower rate ($P < 0.001$); at the end of the experiment (Day 31), parasitized bulls had a mean BW of 343 kg, whereas control and LPS-treated bulls had mean BW of 364 kg and 370 kg (SED = 2.47 kg), respectively. Up to Day 16, bulls in all treatment groups gained weight at a similar rate ($P > 0.05$), which was 1,365 g/d (SEM = 100), data not shown. The growth rates of the animals from Day 16 to the end of the experiment were 1,653 g/d (control), 1,844 g/d (LPS treated), and 444 g/d (parasite treated; SED = 148) ($P < 0.01$). The growth rates of the bulls over the whole experimental period were

1,519, 1,630, and 896 g/d (SED = 230; $P < 0.022$) for the control, LPS, and parasite treatments, respectively.

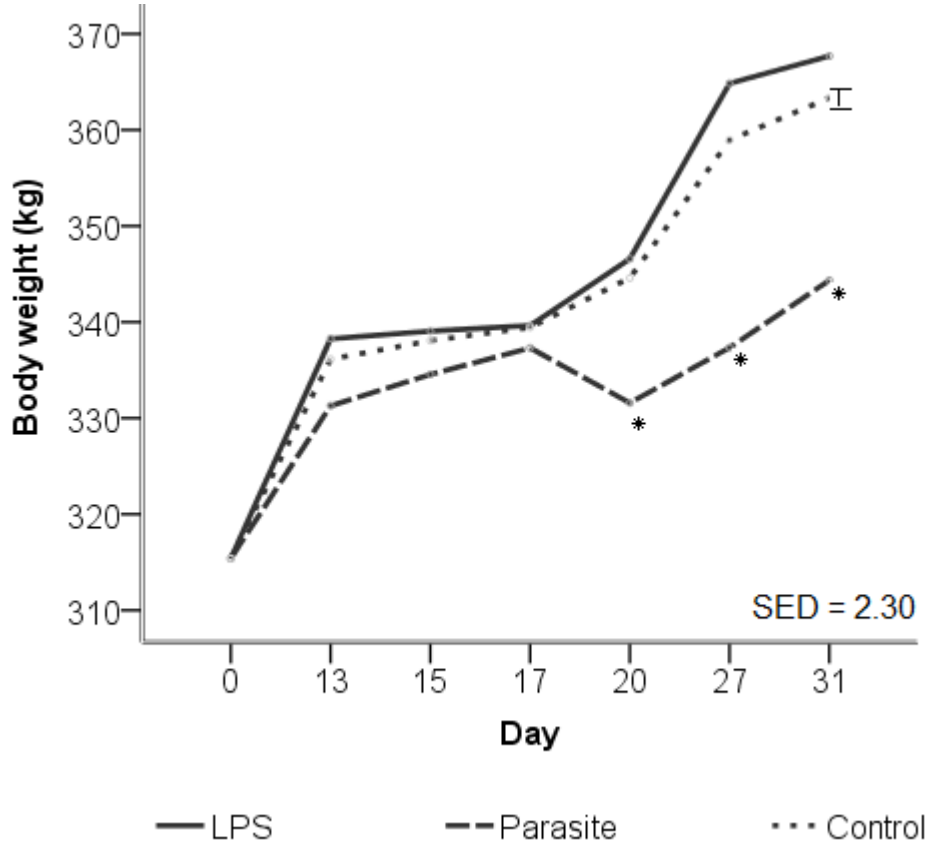

Figure 4.1: Body weight of beef bulls by experimental day for unchallenged controls (Control; n = 5), bulls challenged with lipopolysaccharide (LPS; n = 6) starting dose of 0.2µg/kg and increasing by 0.025µg/kg in subsequent doses on Days 13, 15, and 17, and bulls challenged with 200,000 L3 larvae of *Ostertagia ostertagi* (n = 6) on Day 0 of the experiment. The bar is the SED and shown on the control group.
* = $P < 0.05$

4.3.2 Lipopolysaccharide antibodies

There was a treatment by time interaction ($P = 0.003$) on blood LPS IgG antibodies, caused by a rise in antibodies in the LPS-treated group on Day 20 (7 days after the first challenge; Figure 4.2). The back-transformed means at Day 20 for the LPS-treated group was 0.762 absorbance units (au) (CI: 0.379 - 1.03), 0.325 au (CI: 0.243 - 0.400) for the parasite treatment, and 0.326 au (CI: 0.285 - 0.367) for the control group. There was an effect of the covariate ($P < 0.001$), due to a lower level in the LPS treated group before treatment compared to the other 2 treatments. There was no difference between treatments for Day 15 and 17 ($P > 0.05$), although the level of antibodies were numerically slightly greater for the LPS-treated group.

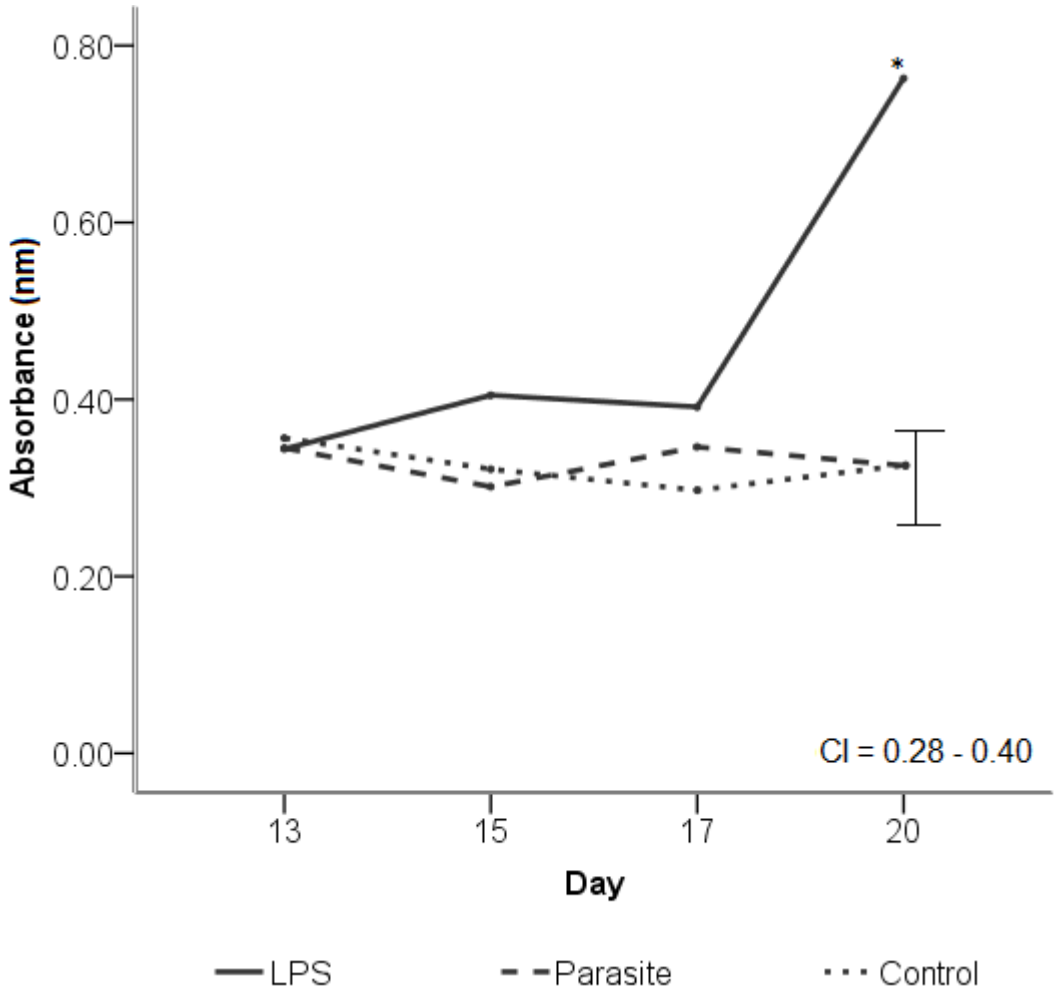

Figure 4.2: Back-transformed means of lipopolysaccharide (LPS) IgG blood antibody levels of beef bulls, as measured by absorbance at 450 nm on Day 13, 15, 17, and 20 of the experiment. Treatments included unchallenged controls (Control; n = 5), bulls challenged with lipopolysaccharide (LPS; n = 6) starting dose of 0.2µg/kg and increasing by 0.025µg/kg in subsequent doses on Days 13, 15, and 17, and bulls challenged by 200,000 L3 larvae of *Ostertagia ostertagi* (Parasite; n = 6) on Day 0 of the experiment. The bar represents the confidence interval (CI) associated with the back-transformed means and is shown with the control group.
* = $P < 0.05$

4.3.3 Faecal egg counts

The FEC taken on a weekly basis were positive for the parasitized group from Day 20, which was 3 weeks after infection. On average, the back-transformed mean FEC of the parasitized animals was 179 eggs/g on Day 20, (CI: 92 –194). The FEC for control and LPS-treated bulls remained 0 throughout the study (data not shown).

4.3.4 Pepsinogen

The levels of pepsinogen showed a treatment by time interaction effect ($P = 0.011$), as well as a treatment effect ($P < 0.001$). Parasite-treated bulls had elevated pepsinogen levels by Day 13, which increased over time, with a sharp rise between Day 17 and 20, as depicted in Figure 4.3. The back-transformed pepsinogen levels by Day 20 were 1.18 iu/L (CI: 0.78 – 1.77) for the control, 1.02 iu/L (CI: 0.82 – 1.27) for the LPS treatment, and 3.95 iu/L (CI: 3.73 – 4.16) for the parasite treatment.

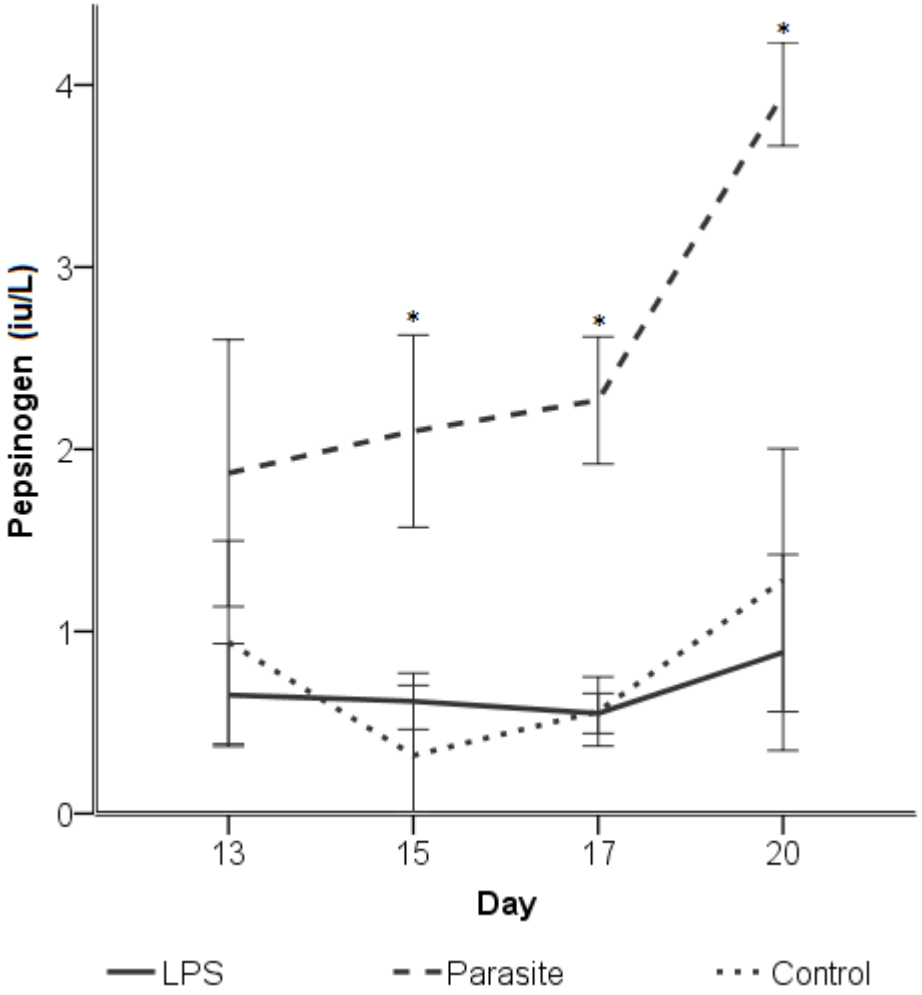

Figure 4.3: Back-transformed mean blood pepsinogen levels (international units / L (iu/L)) of beef bulls at four time points, Days 13, 15, 17 and 20 for unchallenged controls (Control; n = 5), animals challenged by lipopolysaccharide (LPS; n = 6) starting dose of 0.2µg/kg and increasing by 0.025µg/kg in subsequent doses on Days 13, 15 and 17 of the experiment and animals challenged by 200,000 L3 larvae of *Ostertagia ostertagi* (Parasite; n = 6) on Day 0 of the experiment. The bars are the confidence intervals for the back-transformed means.
* = $P < 0.05$

4.3.5 Rectal temperature

There was no difference in the rectal temperature measured. The average temperature was 39.1^{o}C (SEM = 9.49) across treatments.

4.3.6 Activity and posture

Overall activity as measured by the number of steps taken on a daily basis showed no treatment by time interaction and no significant main treatment effects ($P > 0.05$). However, when activity was analysed on an hourly basis within a day, there was a tendency for a treatment by time interaction during Day 13 ($P = 0.057$). This was caused by a lower activity in the final 3 h of the day in the LPS-treated animals (Figure 4.4). The average number of steps taken by the LPS-treated animals from 22.00 h onwards (around 10 h post challenge) was 156, whereas it was 306 steps for the parasite-treated bulls and 268 steps for the controls (SED = 74.2), showing a 49.0% difference. There was no effect for any of the other challenge days ($P > 0.05$).

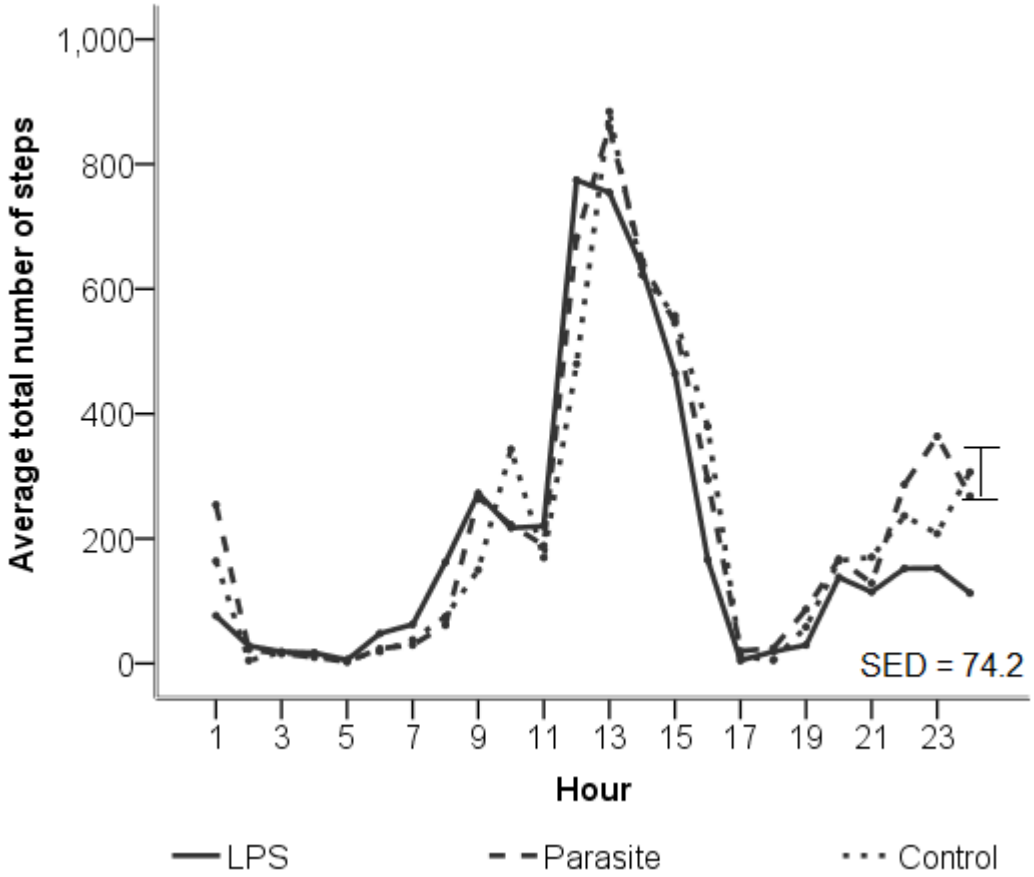

Figure 4.4: Activity of beef bulls as measured by the number of steps taken over 24-h for Day 13 for unchallenged controls (Control; n = 5), animals challenged by lipopolysaccharide (LPS; n = 6) starting dose of 0.2µg/kg and increasing by 0.025µg/kg in subsequent doses on Days 13, 15 and 17 and animals challenged by 200,000 L3 larvae of *Ostertagia ostertagi* (Parasite; n = 6) on Day 0 of the experiment. The LPS challenge was administered between 11.00 and 12.00 h. The bar is the standard error of the difference (SED) and shown on the control treatment.

There was no effect of treatment or treatment by time interaction on the total time spent lying or standing ($P > 0.05$) (Table 4.1). The frequency of the lying and standing episodes (which by definition are identical) showed an interaction between treatment and time ($P = 0.043$) and an effect of treatment ($P = 0.003$). This was due to a decrease in frequency by the parasitized animals after Day 19 (Figure 4.5). The average frequencies of episodes before Day 19 were 18.2, 16.3, and 15.2 (SED = 3.17) for the control, LPS treatment, and parasite treatment, respectively. After Day 19, the averages for the same treatments were 20.2, 19.1, and 13.7 (SED = 2.33), equivalent to a 9.87% overall decrease for the parasite treatment, or a 32.2% difference for the parasite treatment after Day 19 (data not shown).

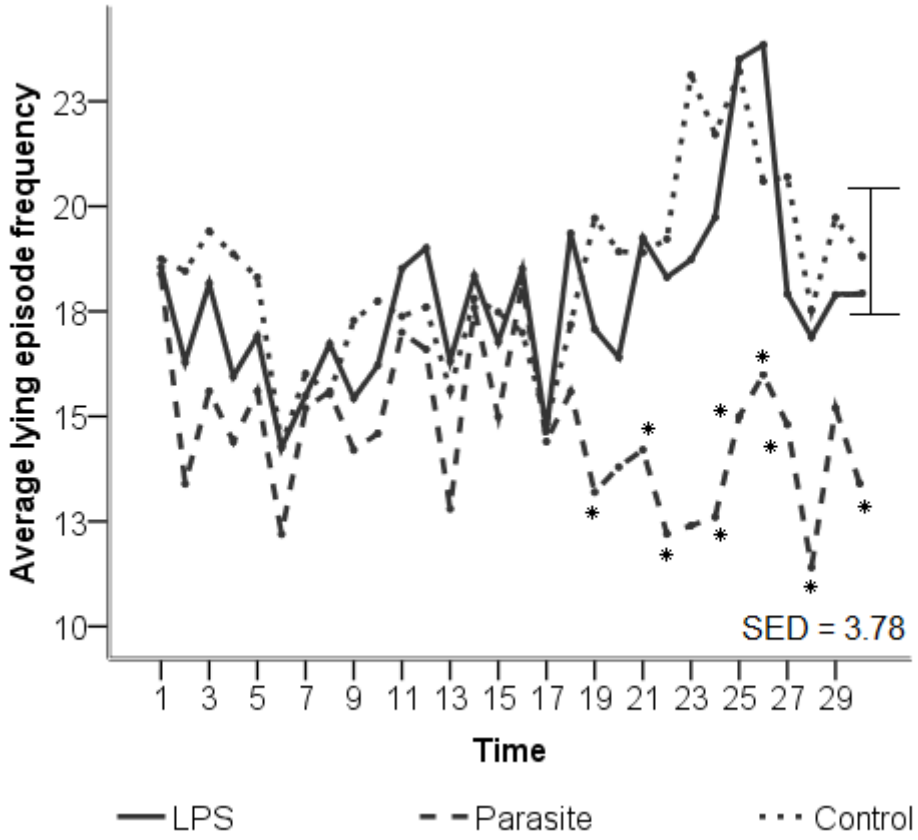

Figure 4.5: Average lying episode frequency (number per day) of beef bulls against experimental day for unchallenged controls (Control; n = 6), animals challenged by lipopolysaccharide (LPS; n = 5) starting dose of 0.2µg/kg and increasing by 0.025µg/kg in subsequent doses on Days 13, 15 and 17 and animals challenged by 200,000 L3 larvae of *Ostertagia ostertagi* (Parasite; n = 6) on Day 0 of the experiment. The bar is the standard error of the difference (SED) and shown on the control treatment.
* = $P < 0.05$

Average lying episode duration was affected by treatment ($P = 0.038$), which was due to an increase in these variables for the parasite-treated bulls. The increase in average lying

time started on Day 19 and persisted until the end of the experiment (Figure 4.6). The back-transformed mean for lying time per episode up to Day 19 was 38.1 min (CI: 27.3 – 53.2) across treatments. After Day 19, the back-transformed means for lying time were 34.2 min (CI: 25.8 – 45.4), 35.0 min (CI: 27.6 – 44.5), and 48.4 min (CI: 38.3 – 61.1) for the control, LPS treatment, and parasite treatment respectively. This is equivalent to an increase in lying time for the parasitized animals of 19.3% (data not shown). There was also a covariate effect ($P = 0.004$) with the 3 groups being slightly dispersed before treatment. Average standing duration also showed a numerical increase for the parasite treatment, however this was not significant ($P = 0.254$). Thus, the analysis of posture showed that the parasite treatment resulted, on average, in fewer and longer lying episodes compared to the other 2 treatments; the consequence of this combination was that the total lying time was not different among the treatments.

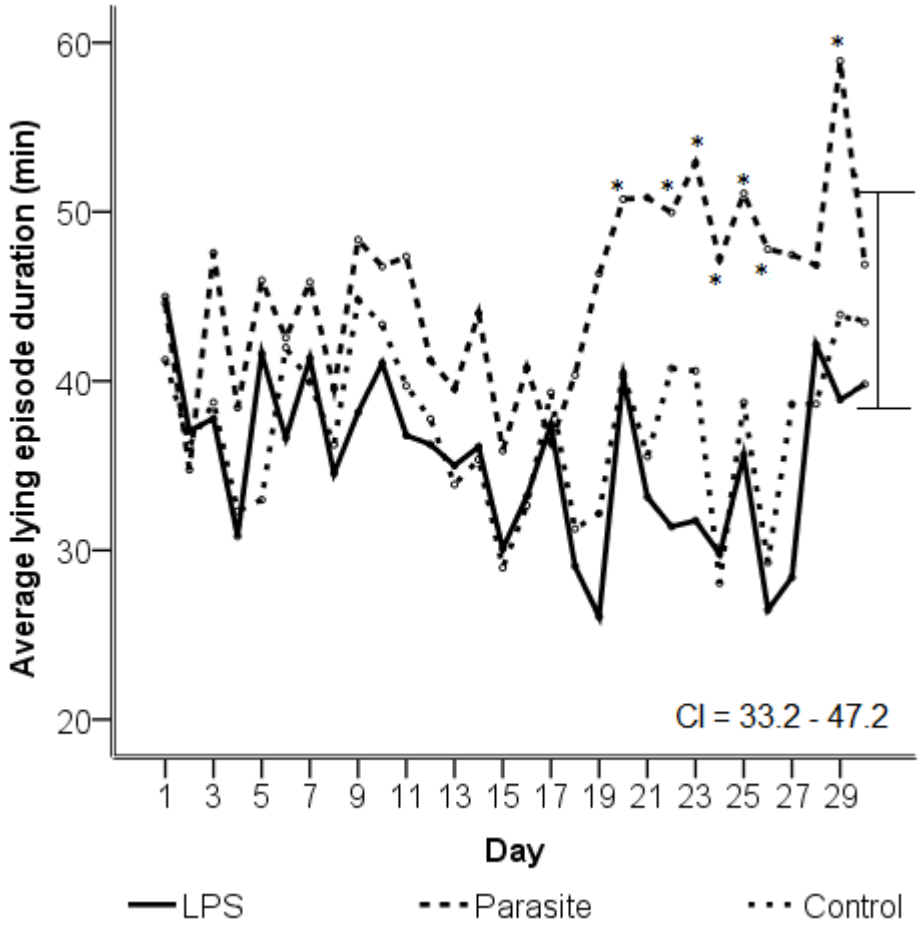

Figure 4.6: Duration of back-transformed average lying episode in minutes for unchallenged controls (Control; n = 5), animals challenged by lipopolysaccharide (LPS; n = 6) starting dose of 0.2µg/kg and increasing by 0.025µg/kg in subsequent doses on Days 13, 15 and 17 and animals challenged by 200,000 L3 larvae of *Ostertagia ostertagi* (Parasite; n = 6) on Day 0 of the experiment. The bar is the confidence interval (CI) associated with the back-transformed means and shown on the control treatment.
* = $P < 0.05$

4.3.7 Feeding and drinking behaviour

Table 4.1 summarizes the feeding and drinking behaviours measured. Both feeding and drinking behaviour showed no ($P > 0.05$) treatment by time interaction or main treatment effect on total episode, meal and bout duration, or frequency. However, there was a time by treatment interaction ($P = 0.013$) for the average feeding meal duration (Figure 4.7). This was caused by a greater average duration for the parasite group compared to the controls on Day 19. The mean durations before Day 19 were 19.9, 17.1, and 18.4 min/d (SED = 4.27) for the control, LPS-treated, and parasite-treated bulls, respectively. On Day 19, these were 15.8, 17.9, and 28.1 min/d (SED = 4.07) for the control, LPS-treated, and parasite-treated bulls, respectively. This is equivalent to a difference of 34.5% for the parasitized animals.

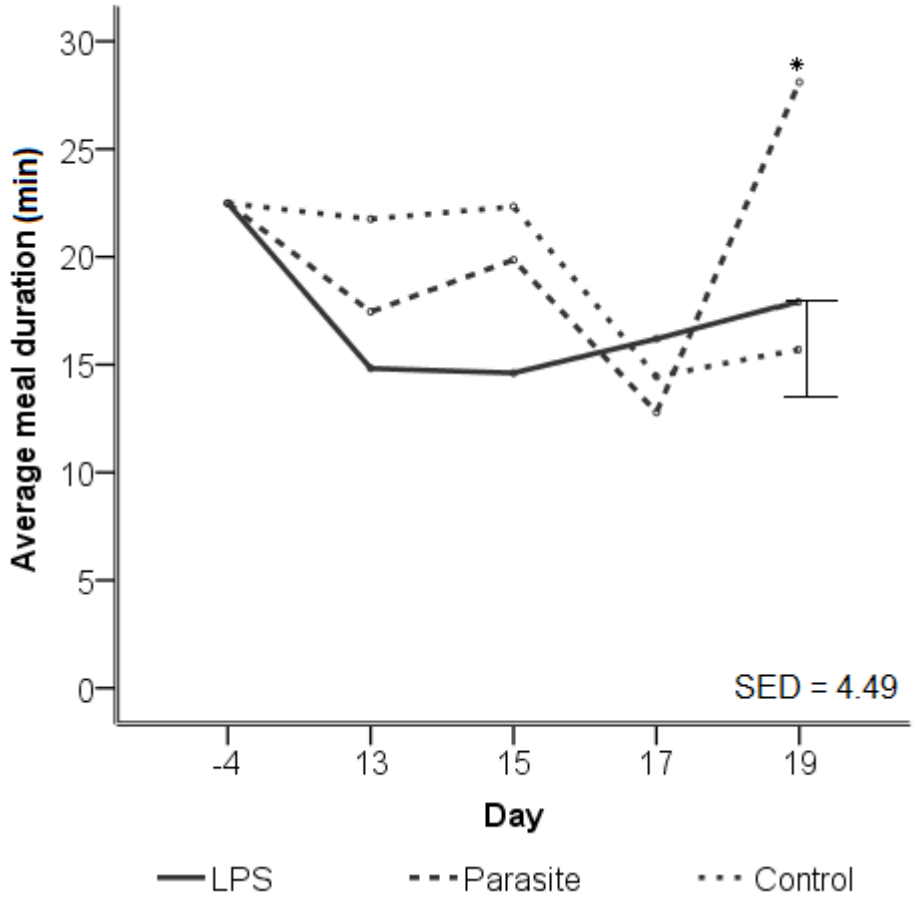

Figure 4.7: Average feeding meal duration (min) of beef bulls against experimental day for unchallenged controls (Control; n = 5), animals challenged by lipopolysaccharide (LPS; n = 6) starting dose of 0.2µg/kg and increasing by 0.025µg/kg in subsequent doses on Days 13, 15, and 17 and animals challenged by 200,000 L3 larvae of *Ostertagia ostertagi* (Parasite; n = 6) on Day 0 of the experiment. The bar is the standard error of the difference (SED) and shown on the control treatment.
* = $P < 0.05$

On average for all treatments, feeding episodes showed a total daily duration of 92.1 min (SEM = 10.6), with an average episode lasting 3.53 min (SEM = 0.559), and a total daily frequency of 28.4 episodes (SEM = 3.51). For drinking, the total daily duration was 13.6 min (SEM = 6.17), with an average episode taking 0.835 min (SEM = 0.472), and a total daily frequency of 30.5 (SEM = 7.38). When the episodes had been grouped into meals for feeding and bouts for drinking behaviour, the daily averages showed a slight numerical increase, as the time spent on within feeding and drinking intervals were included in the meal and bout analysis. This led to a total daily meal time for feeding behaviour of 189 min (SEM = 25.8) and a frequency of 9.46 meals/d (SEM = 0.792). For drinking, these were a total of 20.0 min (SEM = 5.64), with an average bout lasting 2.40 min (SEM = 0.537), and a total frequency of 8.59 drinking bouts/d (SEM = 1.90).

Table 4.1. The average feeding and drinking behaviours and total standing and lying times of beef bulls as unchallenged controls (Control) (n = 5), bulls challenged with lipopolysaccharide (LPS) (n = 6) starting dose of 0.2µg/kg and increasing by 0.025µg/kg in subsequent doses on Days 13, 15, and 17, and bulls challenged with 200,000 L3 larvae of *Ostertagia ostertagi* (Parasite) (n = 6) on Day 0 of the trial, with P-values representing the time and treatment interaction.

Behavior		**LPS**	**Parasite**	**Control**	**SED**	**P-value**
Standing, h		10.8	10.6	10.2	0.60	0.74
Lying, h		13.2	13.4	13.8	0.61	0.58
Drinking						
	Episode[1] duration, min	13.3	15.3	13.7	8.36	0.85
	Episode freq	26.1	33.3	32.7	10.0	0.54
	Episode average, min	1.10	0.77	0.65	0.64	0.45
	Bout[2] duration, min	16.5	20.9	23.2	7.63	0.88
	Bout freq	8.44	8.14	9.30	2.57	0.35
	Bout average, min	2.06	2.67	2.48	0.72	0.49
Feeding						
	Episode duration, min	90.4	89.0	99.3	14.4	0.54
	Episode freq	28.2	31.1	25.4	4.75	0.26
	Episode average, min	3.32	3.20	4.09	0.76	0.40
	Meal[3] duration, min	181	195	190	34.9	0.085
	Meal freq	9.83	9.37	9.12	1.43	0.71
	Meal average, min	17.3	20.3	19.1	4.49	0.013

[1] Episode = Period of uninterrupted feeding or drinking
[2] Bout = Group of drinking episodes that are divided by a bout criterion
[3] Meal = Group of feeding episodes that are divided by a meal criterion

4.4 Discussion

The objective of this study was to contrast the behavioural changes that arise from either an acute or a chronic health challenge in growing beef cattle. Both challenges were expected to be subclinical, defined as unidentifiable by the animal's keeper using visual observation. The behaviours focused on were activity, measured by the number of steps taken, animal posture, and feeding and drinking behaviours. The hypothesis was that the effect on behaviour would be strong in magnitude, but relatively short lived for the acute health challenge. In contrast the chronic health challenge was expected to show a more gradual response, with longer term and persistent changes in behaviour due to the infection pattern of *O. ostertagi*. Both health challenges were expected to have similar effects on the direction of change of the behaviours measured. Feeding and drinking behaviours were expected to decrease in their duration, and possibly frequency (González et al., 2008). The overall activity as measured by number of steps taken was expected to decrease (Larson and Dunn, 2001; Reiner et al., 2009), and be accompanied by a longer duration of lying behaviour in the challenged animals (Borderas et al., 2008; Reiner et al., 2009).

Parasite infection was associated with an increase in circulating pepsinogen levels and excretion of parasite eggs in the faeces. Pepsinogen is considered to be an indicator of abomasal mucosal damage, as blood and serum levels increase in a response to the damage (Paynter, 1992)**.** The FEC taken on a weekly basis were positive for the parasite treated animals from Day 20 onwards, 3 weeks after infection. This delay was expected because the dosed larvae needed time to mature before they started reproducing (Rose, 1969). There were no systematic effects on the consistency of the faeces of any of the experimental animals, and therefore no visual indication of parasite infection. On the basis of the above results, it can be concluded that (subclinical) abomasal parasitism was successfully established in these animals and this was consistent with the desired chronic infection. The infection was associated with lower average daily weight gain from Day 17 onwards, resulting in lighter BW at the end of the experiment. This was an expected consequence of a reduction in feed intake that accompanies parasitic infections (Fox et al., 1989; Fox, 1993), and possibly damage to the gastrointestinal tract (Fox, 1993).

The only effect of LPS administration was a rise in LPS antibodies on Day 20, which was 7 days after the first challenge and 3 days after the final challenge. Although a rise in antibody levels was anticipated, the response was nevertheless later than expected. The

delay could be due to the fact that the antibodies measured were IgG, which generally take longer to manifest (Tizard, 2004). Rectal temperature, taken at every handling occasion, showed no difference among treatments in this study. This could be due to the fact that bulls were only handled every other day, thus probably missing any increase in rectal temperature that was only expected to last between 2 to 8 h after administration of the LPS (Borderas et al., 2008).

The BW of the LPS-treated bulls was not different from that of the unchallenged controls at any time. This was expected, given the infrequency of and the errors associated with measuring BW (e.g. rumen fill) and the short-lived nature of the challenge. The absence of obvious performance and physiological effects on the LPS-treated bulls was accompanied by an absence of clear effects on their behaviour, the exception being the activity on the first day of administration. This was possibly because the effects of LPS were smaller than expected or masked by individual bull variation. The dose used in the experiment was designed to avoid the manifestation of any clinical signs and, therefore, was relatively low.

A single LPS bolus with a dosage of 0.2 µg/kg BW previously resulted in a decrease in feed intake by 60% 24 h after administration, and animals took another 68 h to return to normal feeding levels (Elasser et al., 1995). The dosage and the cattle size were comparable between the study of Elasser et al. (1995) and this study, it is not known why similar effects were not observed here. Borderas et al. (2008) found a decrease in feeding time after a dose of 0.025 µg LPS/kg BW or 0.05 µg/kg BW via an indwelling jugular catheter. Although LPS effects are known to be mostly dose dependent (Johnson and von Borell, 1994; Larson et al., 1996), there are also individual differences among animals in their responses (Jacobsen et al., 2005). A change in feeding rate in rats challenged with LPS was previously observed by Plata-Salamán and Borkoski (1993). Bulls challenged with LPS could, therefore, have consumed less feed by eating at a slower rate rather than by decreasing their feeding time (González et al., 2008). Given that any such effects of LPS administration are transient, it is not surprising that they were not reflected in the performance of these animals.

Drinking behaviour was not affected by LPS administration. Usually, drinking behaviour tends to be less affected by health challenges than feeding behaviour (Hart, 1988). There is a greater need for it to be maintained because water is more vital in maintaining bodily

functions than food in the short term (Kyriazakis and Tolkamp, 2011). A study on infected cattle, classified as morbid, showed some possible changes in drinking behaviour (Sowell et al., 1999). It was found that the number of drinking episodes per day was slightly greater for healthy than morbid calves, although total drinking time was unaffected. Furthermore, water intake was shown to decrease in cows presenting with a mastitis-related fever (Lukas et al., 2008).

Lying and standing behaviour of the LPS-treated animals was unaffected by treatment. However, an effect on their activity was found in the number of steps taken, approximately 10 h after the first challenge. This decrease was expected to be a potential consequence of an acute health challenge (Baert et al., 2005; Escobar et al., 2007). However, the response was slightly later than expected, as the effect of LPS on physiological parameters was expected to last only between 2 to 8 h after challenge (Borderas et al., 2008). The observed delay in the decrease was possibly caused by a nadir in activity for all treatments approximately 6 h after the challenge was administered. Nine hours after challenge overall activity started to increase again, with the LPS-treated group showing less of an increase compared to the other treatments. A reason for there not being a repeat of this result could be that the increase in the dose for the repeated challenge could not overcome habituation to the challenge by the animals.

The animals given the *O. ostertagi* infection, which represented the chronic health challenge, showed significant behavioural effects in their posture. Lying and standing episodes were longer and less frequent in the parasitized animals. Because there was no change in total lying and standing times, this means that the animals changed behaviour less frequently. A possible explanation for this could be lethargy as a behavioural response to the health challenge (Hart, 1988). A decrease in the pattern distribution of behaviour sequences (complexity of behaviour) has been observed in parasitized wild goats (ibex; Alados et al., 1996). Because there was no change in total lying or standing times, an increase in average episode duration could be caused by the animals spending less time standing idle between lying episodes, causing longer and less frequent episodes. These changes, once established, persisted for the duration of the experiment (10 days), which is consistent with the hypothesis that gradual but persistent changes in the behaviour of the parasitized animals were to be expected. Therefore, these subtle but significant changes could be used for the detection of subclinical disease. Such changes

cannot be detected readily, unless continuous observations are taking place, but can easily be established with the use of automated recordings.

Overall activity, as measured by the number of steps taken, was not affected by parasitism. The latter was unexpected, as activity is generally expected to decrease when an animal is faced with a health challenge (Hart, 1988; Larson and Dunn, 2001; Forbes et al, 2004). This could possibly be a result of the subclinical nature of the health challenge, allowing the animals to engage in necessary activities associated with feeding and drinking, as well as activity connected to social behaviour.

Only one dimension of feeding behaviour was affected by parasitism which was an increase in average meal duration, although it has to be emphasized that only 2 of the 3 components of feeding behaviour were measured (i.e., the number of episodes and duration, but not feeding rate). This is in contrast with the findings of Forbes et al. (2004), who found that following treatment with an anthelmintic, dairy cattle increased meal duration and total feeding time compared to animals that continued to be parasitized (i.e., the opposite from what was found here). However, the methodology used to define meals by Forbes et al. (2004) was arbitrary and hence different from what was used here for meal analysis (Yeates et al., 2001). In addition, as Forbes et al. (2004) were measuring the response of parasitized cattle to the removal of the health challenge through the treatment with the anthelmintic, it is possible that the increase in total feeding time was associated with compensatory increases in feed intake during recovery (Sandberg et al., 2006).

As there was a change in the rate of BW gain from Day 16 onwards, this was expected to be reflected in changes in feeding behaviour and feed intake (Kyriazakis, 2010). Although the amount of feed consumed was not recorded, there is consistent evidence that feed intake is reduced during gastrointestinal parasitism in ruminants (Kyriazakis et al., 1998; Fox et al., 2002; Kyriazakis and Tolkamp 2010). This reduction in intake, usually referred to as anorexia, is considered mainly responsible for the reduction in animal performance (Kyriazakis, 2010). A significant effect of *O. ostertagi* parasitism on feed intake of calves was previously seen by Fox et al. (2002) who found that a reduction in feed intake took place between Day 24 and 27 in calves given 200,000 L3 as a single dose. A response in aspects of feeding behaviour that would result from an anticipated reduced feed intake was expected in this study. However, this was inconsistent with the observed increase in average meal duration 3 weeks after infection. Such an inconsistency could be accounted

for by a change in feeding rate as hypothesized by Hutchings et al. (2000). They reported that the biting rate of parasitized sheep was reduced when compared to non-parasitized controls and hypothesized that this leads to parasite-induced anorexia. González et al. (2008) demonstrated the importance of considering all aspects of feeding behaviour as an aid to diagnose the consequences of health challenges, as feeding rate of cattle was negatively affected by some health problems, such as ketosis, but positively by others, such as lameness.

Some of the behaviours in this experiment were captured using automated means. The automated capture of behaviours is preferable, as it does not require a lot of time to be spent with the animals. However, feeding and drinking behaviours were captured by video cameras, which were not automated. This requires extra time input to view the images taken. This could be overcome by using an automated feeding behaviour capture system. With the use of a passive transponder fitted to the animal, for instance as an ear tag, a system can be developed to recognize individuals when they are at the feeder or drinker. At the moment, however, these systems are still expensive and only used for research, rather than for commercial purposes.

Activity and posture were measured using IceTag pedometers. Pedometers are commonly used for dairy cows, but less commonly for beef cattle. These pedometers measure posture, as well as the number of steps taken, which increases their applications. It is possible that other behaviours than the ones addressed here could be captured by automated means and used for the purposes of disease detection. For example, social behaviour, as captured by contact-loggers that record proximity to other animals (Prange et al., 2006; Swain and Bishop-Hurley, 2007; Marsh et al., 2011) and spatial movements measured outside using GPS-like positioning systems (Swain et al., 2003) or indoors using Bluetooth wireless technology (Togersen et al., 2010) are potential candidates. Although these systems do not give information about the nature of the interactions, they may well capture information that is useful for early detection of disease (Renault et al., 2008). This could be supplemented with additional automated measurements of physiological indicators, for instance body temperature as measured by ruminal boli (Alzahal et al., 2011; Timsit et al., 2011) or injectable microchips (Torrao et al., 2011).

When considering early diagnosis and diagnosis of subclinical disease, there is a clear difference between acute and chronic health challenges. The changes in behaviour caused

by the acute health challenge in this study were not strong enough or were too short lived to show a significant effect, and hence to have diagnostic value. A more promising outcome arose from the changes in behaviour caused by a chronic health challenge in the form of gastrointestinal parasitism. However the changes in posture as measured here (13% increase in lying time and 15 % decrease in lying and standing episode frequency) and the observed variation among days may not be enough to detect low-responding individuals and avoid false positives. The use of a fuzzy logic model (Kramer et al., 2009) that includes information on posture, as well as (feeding) behaviour, possibly in combination with automated recording of physiological indices, could well provide a more reliable indicator of chronic subclinical disease.

Chapter 5: The Diagnostic Value of Changes in Behaviour for the Detection of Parasitism in Beef Cattle and the Recovery of these Behaviours after Treatment

5.1 Introduction

Animal behaviour may be one of the first things that change when an animal is affected by a health or welfare challenge, and can precede any clinical signs of stress or disease (Kyriazakis and Tolkamp, 2010). A number of recent papers have demonstrated that several aspects of cattle behaviour may be modified by (mainly) bacterial challenges (Quimby et al., 2001; Borderas et al., 2008; González et al., 2008; Fogsgaard et al., 2012) and that these changes may have diagnostic value. González et al. (2008), for example, have shown that measurable changes in the (feeding) behaviour of dairy cattle occur in cases of lameness and ketosis several days before anything can be detected by farm personnel, which allows for early action to be taken. The rate of change in such behaviours is dependent on the nature of the health challenge (González et al., 2008).

The preliminary and short term study (Chapter 4) is the only one that has investigated several behavioural changes that may occur in beef cattle parasitized by helminths. It was found that several aspects of cattle posture and activity, such as lying and standing behaviour, and the number of steps taken, were affected to a variable extent by subclinical parasitism with *Ostertagia ostertagi*. In the present study the focus was on the temporal aspects and the magnitude of similar changes in behaviour that might take place during such infections. In addition to measurements of activity and posture, feeding behaviour was also measured because (i) a reduction in food intake is a consistent feature of such infections (Fox et al, 1989; Kyriazakis et al., 1998) and (ii) changes in the feeding behaviour of dairy cattle as a consequence of parasitism have been reported by Forbes et al. (2007). The interest was in whether such changes arise before conventional signs of parasitism or clinical symptoms occur and also how quickly these changes are reversed when animals are dosed with an anthelmintic. Some abomasal damage by the parasites can occur before any eggs appear in the faeces, concurring with the development of the larvae (Lawton et al., 1996), which might manifest as changes in animal behaviour detection before current diagnostic methods. In addition, Kyriazakis et al. (1996) have shown that the food intake of sheep parasitized with *Trichrostonglylus coubriformis* recovered within a couple of days post dosing with an anthelmintic. Thus knowledge of

such temporal changes may have diagnostic value for the assessment of the effectiveness of a treatment. However, such behavioural changes must be of sufficient magnitude if they are to have a disease detection value (González et al., 2008). The hypotheses tested here were, therefore: (1) changes of sufficient magnitude in aspects of behaviour due to parasitism will appear before any clinical signs, or the presence of eggs in the faeces and (2) such changes will be reversed rapidly after dosing with an anthelmintic.

5.2 Material and Methods

The experiment took place at the facilities of Newcastle University after approval of the experimental protocols by the Animal Experiments Committee and under license according to the UK Animals (Scientific Procedures) Act for experimental challenge and regulated procedures (licence number: PPL 60/4067).

5.2.1 Animals and housing

The animals used were 26 Holstein-Friesian beef bulls, approximately 3 months of age, with an average weight of 117 ± 25kilograms (kg), derived from a commercial farm. The sample size was determined using a power calculation based on the standard deviations associated with a previous experiment (Chapter 3). All animals were housed together in a single straw-bedded pen measuring 10 x 4 m^2. Both food and water were available on an *ad libitum* basis. The food offered was a total mixed ration, consistent throughout the experiment, containing 31.25% barley, 18.45% sugar beet pellets, 15.98% soya bean meal, 13.42% barley cereal, 9.76% distillers maize, 7.41% molasses and 3.75% chopped barley straw. The chemical composition of the food was 13.01 MJ ME and 197 g CP per kg DM as estimated from AFRC (1993) feed tables. The animals had not received any prior challenge with parasites and were treated with 2 millilitre (ml) of the anti-inflammatory dexamethasone (Rapidexon, Eurovet, Cambridge, UK) and 8 ml of the antibiotic florfenicol (Nuflor, Shering-Plough, Milton Keynes, UK) on Day 0 to reduce the risk of potentially confounding bacterial infections and were not expected to affect the parasitology (Greer et al., 2005).

5.2.2 Experimental design

The animals were randomly assigned, taking into account their initial body weight, to one of four treatments. The experiment was considered to start when the animals received the first challenge (designated as Day 0). Prior to the application of the health challenges

(Day -8) animals were fitted with a pedometer (IceRobotics, South Queensferry, UK) secured with Velcro on their left front leg, and video recordings of feeding behaviour started in order to provide background data before the parasite challenge. Also on the same day (Day -8) the animals received 7.5 mg/kg body weight of the anthelmintic albendazole (Albenil, Virbac, Woolpit, UK).

The first treatment group (P) consisted of seven animals, which received a trickle dose of 300,000 L3 *Ostertagia ostertagi* larvae in total, administered by gavage in doses of 100,000 L3 on Day 0, 7 and 14 of the experiment. The dose and its expected consequences on feeding behaviour, activity and posture had been established in a previous experiment (Chapter 4). Trickle dosages of *O. ostertagi* have been previously reported in literature (Fox et al., 1989; Forbes et al., 2009), to mimic a repeated challenge, which is more likely to be encountered in the field than a single infectious dose. The animals in this group remained infected for the duration of the experiment (Day 45). The second treatment group (PA) also consisted of seven animals and received the same trickle infection as group P. However, on Day 31 of the experiment, these animals received 7.5mg/kg of the broad-spectrum anthelmintic ablendazole (Albenil, Virbac, Woolpit, UK).

The third (C) and fourth (CA) treatment groups, which acted as unchallenged controls, each consisted of six animals. They were given a gavage with 20 ml water on Day 0, 7 and 14. Group CA was also treated with the anthelmintic on Day 31 (Albenil, Virbac, Woolpit, UK) to coincide with the drenching of the second treatment group, thereby controlling for any potential behavioural side effects of the anthelmintic dosing on the animals. The L3 larvae used as the challenge were obtained from Ridgeway Research (Gloucestershire, UK) and were of an Ivermectin susceptible strain that was isolated in South Gloucestershire, UK, 3 months before use (reference label OOSG10). Upon arrival the 3.6M larvae were split in six glass beakers and diluted in 500 ml of water each, which was changed every other day until use. Just prior to dosing, 410 ml of surplus water was removed from the top leaving six doses of 15 ml with 100,000 L3 per dose per beaker. Each dose was topped up with 5 ml of water and administered to each animal by gavage.

The experiment lasted for 45 days, throughout which faecal samples and body weight measurements were taken twice a week, and blood samples were taken once a week; the same measurements were taken on the last day of the experiment. All animal handling

took place in the morning, between 9.30 and noon and their frequency ensured that the animals were accustomed to the procedures. The faecal samples were taken from the rectum and their consistency was recorded prior to being placed in labelled pots. The counting of the number of eggs in the faeces (FEC) took place on the same or the next day of sampling, in which case the samples were kept in the refrigerator overnight. The 10 ml blood samples were taken from the jugular vein with a plain tube (Vacutainer, BD, Franklin Lakes, New Jersey, USA) for serum collection, and the samples were left overnight in the fridge, after which they were spun for 15 min at 1500rpm at room temperature (18°C); the serum samples were frozen for subsequent analysis. At the end of the experiment all animals were treated with anthelmintic (Albenil, Virbac, Woolpit, UK).

5.2.3 Behavioural observations

The behaviours observed were posture, levels of activity and feeding. The focus was on these behaviours because they can be automatically monitored and are potentially affected by health challenges in cattle (Quimby et al., 2001; González et al., 2008, Chapter 4). Posture and activity levels were measured with the use of IceTag (IceRobotics, South Queensferry, UK) pedometers that took second-to-second readings throughout the experiment, measuring the number of steps taken and the posture of the animal. These data were read at the end of the experiment when the pedometers were removed. Feeding behaviour was monitored with 24-h video recording equipment, which was watched using continuous focal sampling. Observations during Days 7-9 (period 1, pre-patent parasitism), 31-33 (period 2, peak of parasitism) and 39-41 (period 3, post dosing with anthelmintic) were considered in the analysis of the results. Feeding episodes were considered to start when an animal put its head into the feeder and end upon withdrawal. The animals were distinguished by their individual markings. All behaviours were analysed for duration and frequency.

5.2.4 Blood and faecal measurements

The collected serum samples were used to assess pepsinogen levels. Plasma pepsinogen concentration was determined using the modified method of Paynter (1992) and expressed in international units (iu), per L. The samples analysed were those taken on Days -5, 21, 28, 35 and 42.

The FEC were expressed as the number of eggs per gram (epg) of collected fresh faeces. They were assessed by the flotation method, as described in the Ministry of Agriculture

"Manual of veterinary parasitological laboratory techniques" (Ministry of Agriculture, 1977) and detailed in Chapter 4.

5.2.5 Statistical analysis

The CA treatment was used to control for any potential effect of the anthelmintic treatment *per se* on the PA treatment group. However, it was found that there was no significant ($P > 0.05$) effect of the anthelmintic treatment *per se* on the control animals, as all measurements taken were similar between the two control groups. For this reason, C and CA groups were combined for subsequent analysis.

The activity and posture data acquired from the IceTags were downloaded with the IceRobotics software in a format of one summary record per min. Each record provided a date, time and percentage of time spent lying and standing and the number of steps taken. The lying and standing data were consolidated into episodes with the use of purpose written FORTRAN programs (Tolkamp et al., 2010). These episodes were calculated by assuming that a continuous series of records that showed 100% either lying or standing behaviour, were part of the same episode. When both lying and standing occurred in the same min, it was assumed that this was a transition min in which the behaviour during the first part of the min was the same as that during the previous min. Short lying episodes (those under 4 min) were deleted because these were previously verified with video footage not to correspond to real lying behaviour (Tolkamp et al., 2010, Chapter 4). This resulted in a sharp reduction in the number of episodes without any considerable impact on the total lying and standing times, since many deleted episodes lasted only a few seconds. The analysis applied was on the total number of steps taken, the total lying or standing time (which are reciprocal), and the frequency of standing episodes (which by definition is identical to the frequency of lying episodes) and duration of lying and standing episodes per day.

Feeding behaviour episodes were grouped into meals using meal criteria calculated after fitting mixed models to the frequency distribution of interval lengths between episodes. Both a three population model, consisting of two Gaussian distributions and one Weibull distribution, when compared to a two population model with one Gaussian and one Weibull distribution were fitted to the data. These models, developed by Yeates et al. (2001), were fitted to the log-transformed interval lengths (expressed in seconds) using the SAS 9.1 (SAS Institute Inc, Cary, USA) programs of González et al. (2008). The

rationale for fitting a three population model is that there are three populations of between-feeding intervals, one between meals and two within meals, during which animals do or do not drink (Yeates et al., 2001). The fit of both models were expressed in a "minimum function value" (MFV, i.e. twice the negative log-likelihood), where the three population model showed a MFV of 19835 and the two population model a MFV of 20233. A chi-square test showed that the addition of the third population resulted in a significantly ($P < 0.001$) better fit. From the model parameters, the meal criterion was estimated at 19.41 min and all intervals shorter than this were considered as within meal intervals. Both episodes and meals were analysed for their duration and frequency. Feeding behaviour data were averaged per animal across the three days within each period considered.

Body weight (BW), pepsinogen levels, posture, activity and feeding behaviour were all analysed with the use of a repeated measures ANOVA (SPSS 15, IBM, Armonk, NY, USA) with treatment as a fixed and day (or period) as a random effect. For BW and BW gain analysis, the Day 0 BW value was used as a covariate. Activity as well as lying and standing behaviour used the mean of the 8 days prior to the experiment (Day -8 until -1) as a covariate to account for variation between individuals. Furthermore the data on the activity, as number of steps taken, FEC, average meal duration, and lying and standing episode duration were log-transformed prior to analysis in order to normalize their distribution. These results are reported as back-transformed means with 95% confidence intervals (CI).

In addition, the area under the curve (AUC) (Matthews et al., 1990) was calculated for the FEC for each individual on the P and PA treatments over specified time periods. The time periods used were Day 21-31 for both the P and PA treatments, and Day 31-45 and Day 21-45 for the P treatment only. The AUC values were correlated to the corresponding mean for individual activity and posture measurements indicated above through a Pearson correlation (SPSS 15, IBM, Armonk, NY, USA).

5.3 Results

5.3.1 General

One animal from treatment P was removed from the experiment due to poor growth even before parasitism developed and its data were treated as a missing value.

5.3.2 Faecal egg counts

The consistency of the faeces varied throughout the trial and between treatments: up to Day 17 the faeces were similarly solid for all treatments. However, after Day 17 a number of animals in both P and PA treatments showed signs of diarrhoea. On Day 17 this was 4 out of 14 animals, 7 on Day 21, 8 on Day 28, 8 on Day 31, 7 on Day 35, 6 on Day 38, 2 on Day 42 and 5 on Day 45. Faecal consistency returned to normal in the PA animals post dosing with the anthelmintic. The egg counts for the control animals remained at zero throughout the experiment. The FEC of the parasitized animals were positive from Day 17, almost three weeks after infection. Between Days 17-31 the average FEC was 190 eggs/g (CI: 70.2 – 516) for the P and 183 eggs/g (CI: 76.7 – 439) for the PA treatment ($P > 0.05$). After the PA treatment group was drenched on Day 31, their FEC returned to, and remained at 0 throughout (Figure 5.1). The FEC for the P treatment continued to be high until the end of the experiment, having an average of 527 eggs/g (CI: 286 – 968), from Days 31-45.

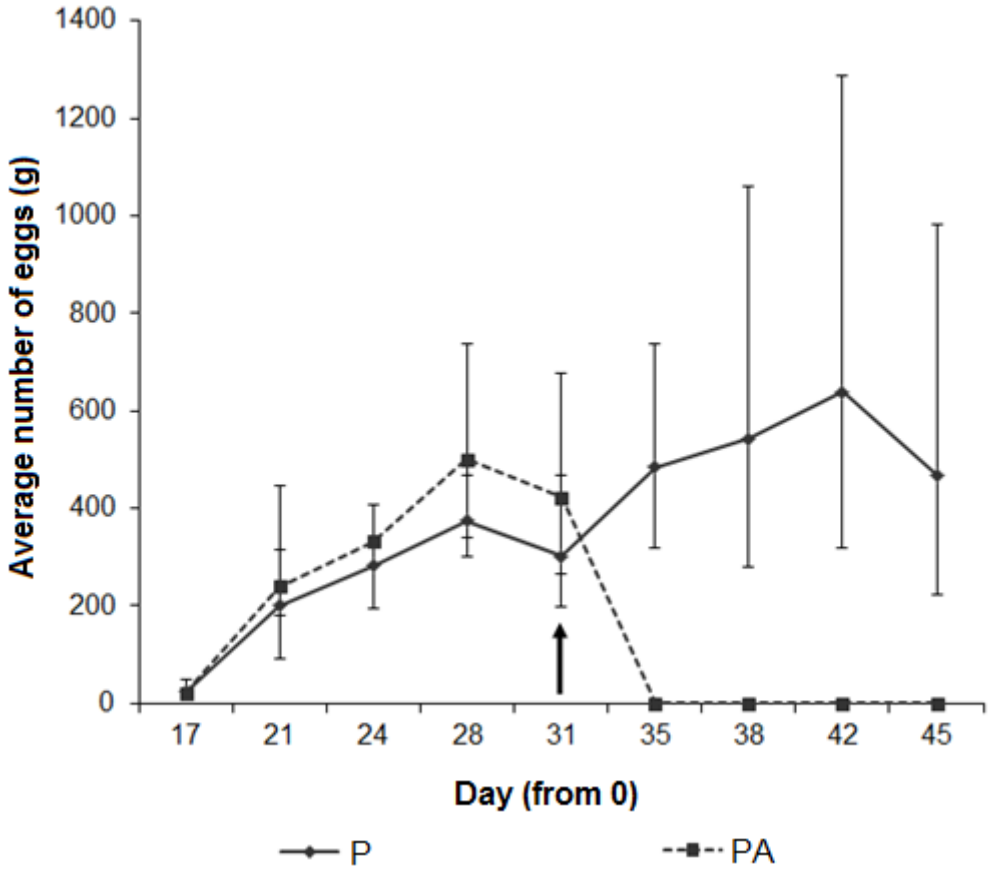

Figure 5.1: Eggs per gram of fresh faeces against experimental day with confidence intervals for beef bulls challenged with 100,000 L3 larvae of *Ostertagia ostertagi* on Day 0, 7 and 14 of the experiment. The PA (parasite interrupted; n = 7) group were drenched with an anthelmintic on Day 31, whereas P (parasitized; n = 7) group remained undrenched throughout. Drenching is indicated by an arrow.

5.3.3 Pepsinogen

The pepsinogen levels in the serum showed a significant ($P < 0.001$) time effect, treatment effect and treatment by time interaction. Animals on the P and PA treatment had elevated pepsinogen levels by Day 21, 2.17 and 2.19 iu/L for the P and PA respectively, while pepsinogen levels of the controls remained low at 0.49 (SED = 0.34) iu/L. For the P treatment these remained elevated throughout the experiment, whereas for the PA treatment levels started to decrease post dosing (Day 42), as shown in Figure 5.2. Despite this decrease, they had not returned to control levels by the end of the trial.

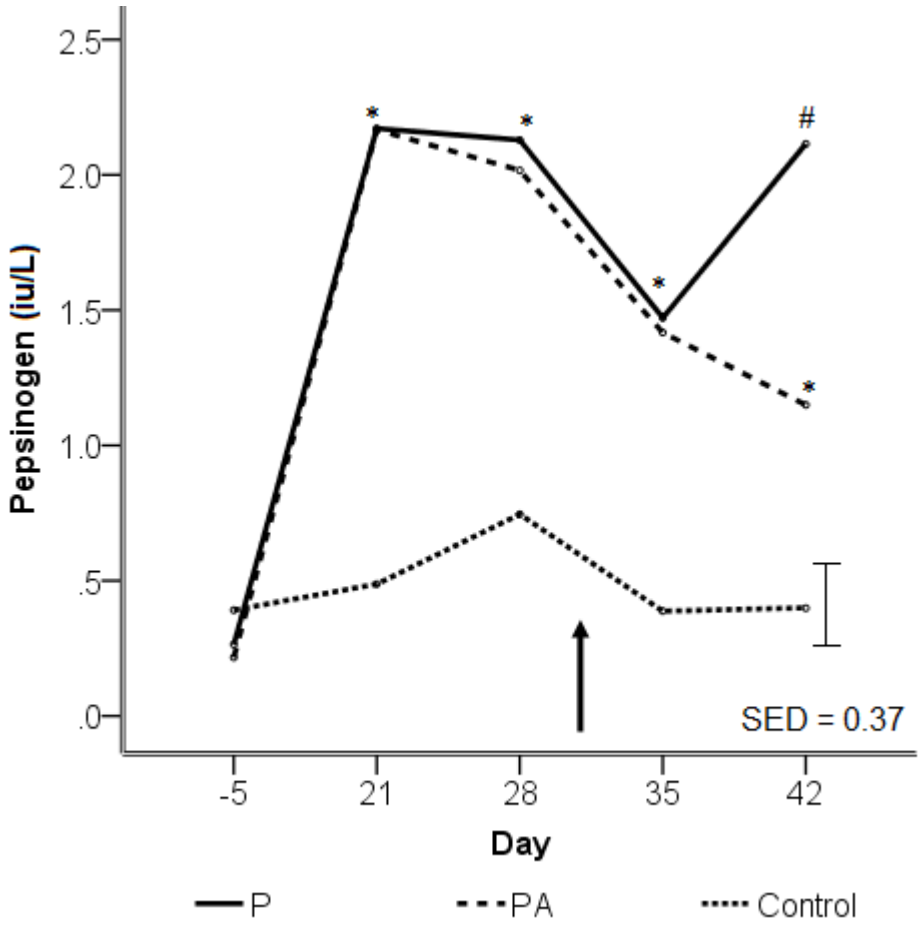

Figure 5.2: Pepsinogen levels (iu/L) against experimental day for unchallenged controls (Control; n = 12) and beef bulls challenged with 100,000 L3 larvae of *Ostertagia ostertagi* (P and PA) on Day 0, 7 and 14 of the experiment. The PA (parasite interrupted; n = 7) group were drenched with an anthelmintic on Day 31, whereas P (parasitized; n = 7) group remained undrenched throughout. The bar is the standard error of the difference (SED) and shown on the control treatment. Drenching is indicated by an arrow.
* = $P < 0.05$ P and PA compared to control
# = $P < 0.05$ P compared to PA

5.3.4 Body weight gain

The change in BW throughout the experiment is shown on Figure 5.3. There was no significant time effect ($P > 0.05$), but there was a significant ($P = 0.05$) treatment effect and a highly significant interaction between treatment and time ($P < 0.001$) on BW; the effect of the covariate was also significant ($P < 0.001$). Up to Day 21 animals in all treatments gained weight at a similar rate ($P > 0.05$), which was 935 (SEM = 81.5) g/d. From Day 21 the BW of the animals started to diverge with infected bulls gaining weight at a slower rate ($P < 0.001$) than the controls. The growth rate of the animals from Day 21 to Day 31 was -33, -357 and 1775 (SED = 364) g/d for the P, PA and control respectively. For the same treatments the growth rate from Day 31, when the PA group was treated with the anthelmintic, until the end of the experiment was -200, 895 and 1172 (SED = 231) g/d, respectively. The growth rate of the animals over the whole experimental period was 314, 584 and 1287 (SED = 181; $P = 0.002$) g/d for the P, PA and control respectively. At the end of the experiment (Day 45) P treatment animals weighed 132 kg, whereas PA treatment animals were 142 kg, the controls differed significantly from the P animals ($P = 0.004$) with an average final weight of 172 kg (SED = 3.79).

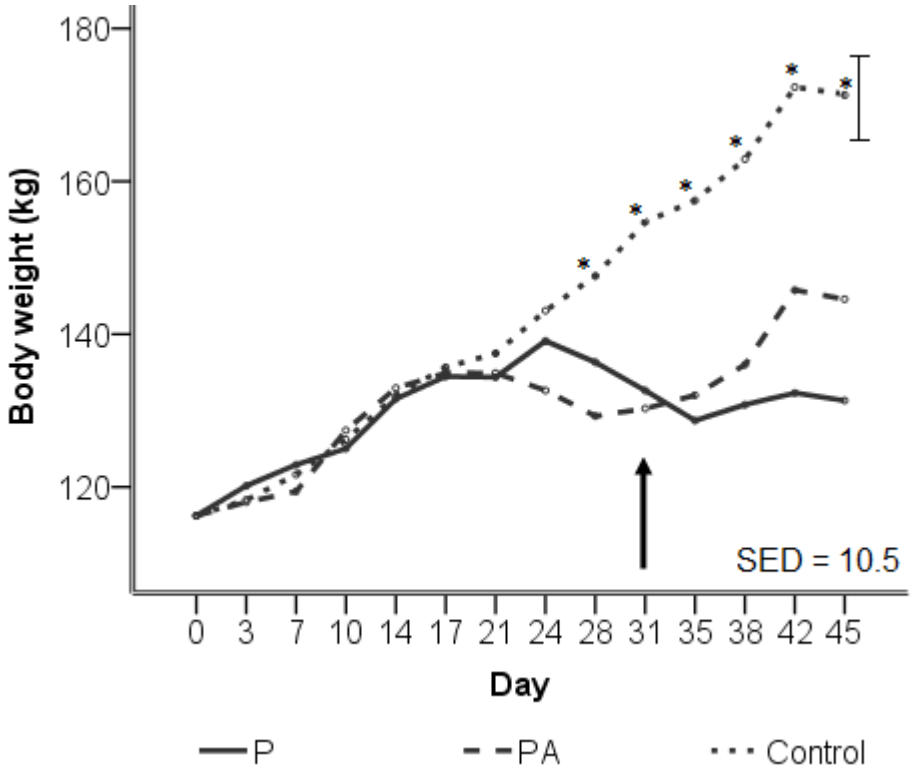

Figure 5.3: Body weight in kilograms (kg) against experimental day for unchallenged controls (Control; n = 12) and beef bulls challenged with 100,000 L3 larvae of *Ostertagia ostertagi* (P and PA) on Day 0, 7 and 14 of the experiment. The PA (parasite interrupted; n = 7) group were drenched with an anthelmintic on Day 31, whereas P (parasitized; n = 7) group remained undrenched throughout. The bar is the standard error of the difference (SED) and shown on the control treatment. Drenching is indicated by an arrow.
* = $P < 0.05$ P and PA compared to control

5.3.5 Activity and posture

Overall activity, as measured by the number of steps taken per day, was not affected by time or treatment ($P > 0.05$); however it did show a significant decrease for both the P and PA treatments from Day 21, which resulted in a significant time by treatment interaction ($P < 0.001$) (Figure 5.4); in addition the effect of the covariate was significant ($P < 0.001$). Before Day 21 the average number of steps per day was similar across treatments at 2150 (CI: 1830 – 2525). Between Day 21 and Day 31 this was 1391 (CI: 1044 – 1853), 1206 (CI: 918 – 1585) and 1866 (CI: 1555 – 2239) steps per day for the P, PA and control treatments respectively, and continued to be lower for the parasitized treatments after Day 31 at 1252 (CI: 892 – 1756), 1156 (CI: 861 – 1551) and 1934 (CI: 1610 – 2324) steps per day, respectively. There was no significant difference between the P and PA treatments after dosing ($P > 0.05$).

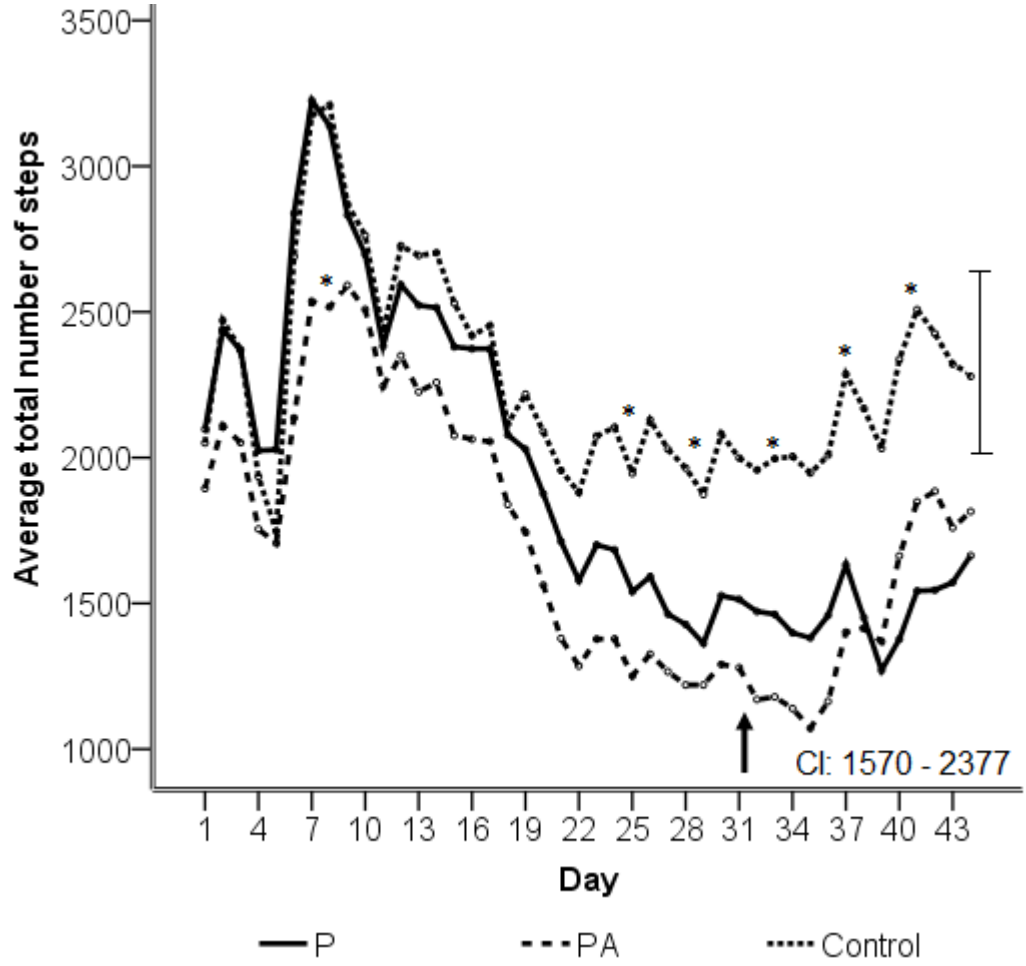

Figure 5.4: The 3-day rolling mean for the total number of steps taken per day for unchallenged controls (Control; n = 12) and beef bulls challenged with 100,000 L3 larvae of *Ostertagia ostertagi* (P and PA) on Day 0, 7 and 14 of the experiment. The PA (parasite interrupted; n = 7) group were drenched with an anthelmintic on Day 31, whereas P (parasitized; n = 7) group remained undrenched throughout. The bar is the confidence interval (CI) associated with the back-transformed means and shown on the control treatment. Drenching is indicated by an arrow.
* = $P < 0.05$ P and PA compared to control

There was no effect of time and no time by treatment interaction on total standing and lying time, however there was a significant effect of treatment ($P = 0.043$) (Figure 5.5) on total standing time and the same applied for total lying time ($P = 0.05$). In addition, the effect of the covariate was significant for both total standing and lying time ($P < 0.002$). Because an increase in total standing time implies a decrease in total lying time, the latter is not dealt with to any further extent. The effect of treatment was due to the P and PA groups having an increase in total standing time compared to control. The total daily standing time was 526 (CI: 452 – 613), 527 (CI: 456 – 610) and 484 (CI: 449 – 521) min per day for the P, PA and control treatments respectively.

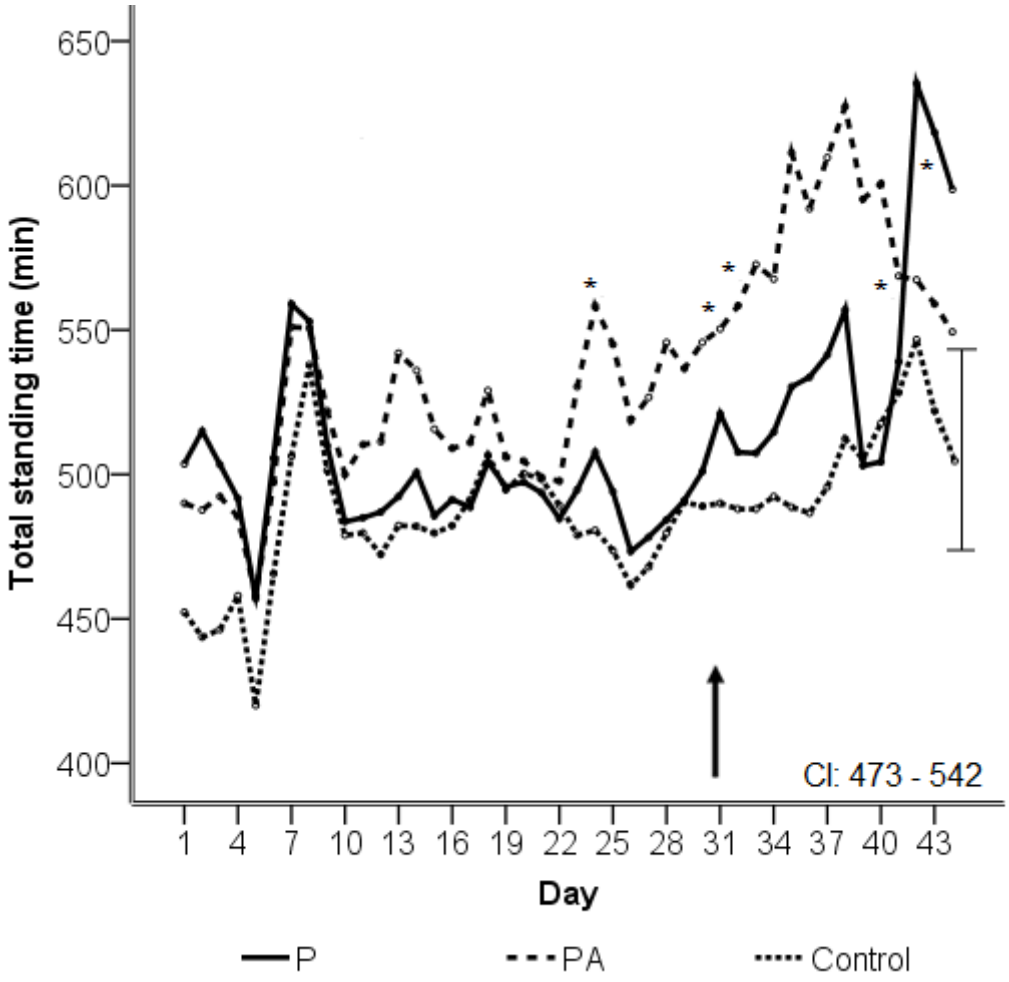

Figure 5.5: The 3-day rolling mean for total daily standing time in minutes against experimental day for unchallenged controls (Control; n = 12) and beef bulls challenged with 100,000 L3 larvae of *Ostertagia ostertagi* (P and PA) on Day 0, 7 and 14 of the experiment. The PA (parasite interrupted; n = 7) group were drenched with an anthelmintic on Day 31, whereas P (parasitized; n = 7) group remained undrenched throughout. The bar is the confidence interval (CI) associated with the back-transformed means and shown on the control treatment. Drenching is indicated by an arrow.
* = $P < 0.05$ P or PA compared to control
# = $P < 0.05$ P compared to PA

The frequency of the lying and standing episodes, which by definition are identical, was significantly ($P < 0.001$) affected by time, treatment and the interaction between treatment and time; the effect of the covariate was also significant ($P < 0.001$). The interaction was due to a decrease in frequency by the P and PA animals after Day 21 (Figure 5.6). The average frequency of episodes before Day 21 was 16.8 episodes per day (SEM = 0.95) across treatments. Between Day 21 and Day 31 the average frequency of episodes was 12.5, 10.7 and 19.7 (SED = 1.58) for the P, PA and control treatments respectively. For the same treatments after Day 31, the average frequencies were 9.02, 12.1 and 18.6 (SED = 1.99), respectively. The PA group had returned to the same values as the control by Day 39.

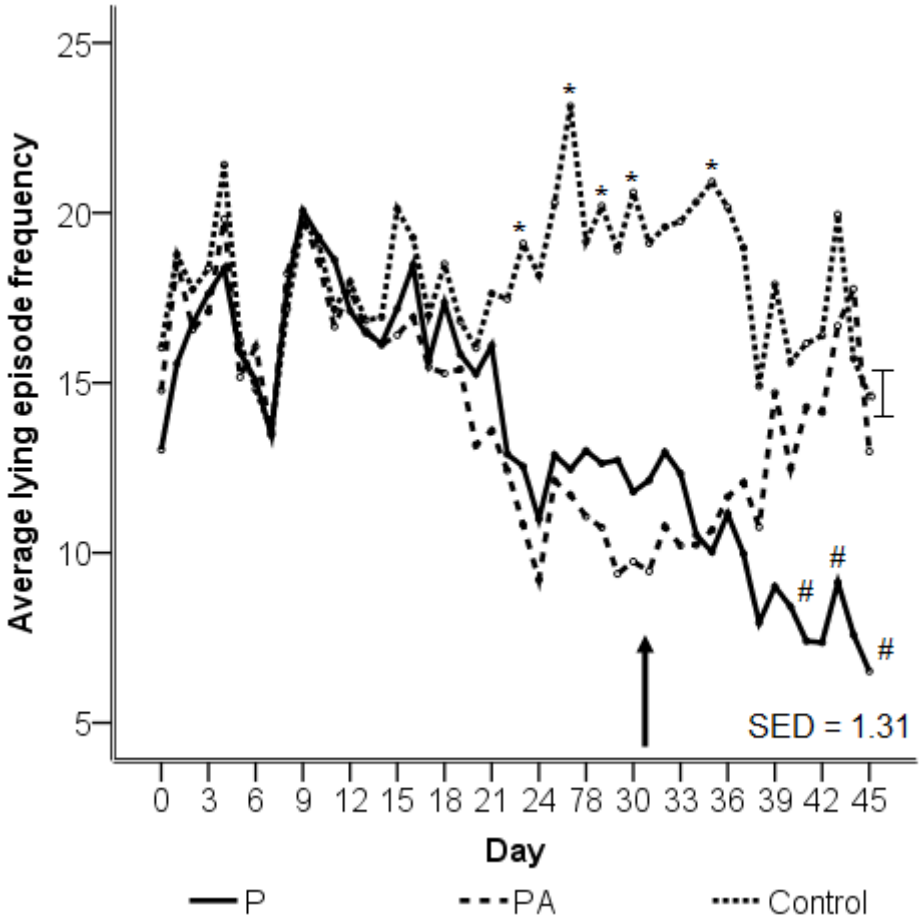

Figure 5.6: Average lying episode frequency (number per day) against experimental day for unchallenged controls (Control; n = 12) and beef bulls challenged with 100,000 L3 larvae of *Ostertagia ostertagi* (P and PA) on Day 0, 7 and 14 of the experiment. The PA (parasite interrupted; n = 7) group were drenched with an anthelmintic on Day 31, whereas P (parasitized; n = 7) group remained undrenched throughout. The bar is the standard error of the difference (SED) and shown on the control treatment. Drenching is indicated by an arrow.
* = $P < 0.05$ P and PA compared to control
# = $P < 0.05$ P compared to PA

There was a significant time effect ($P < 0.001$) for the average lying, however not for the average standing episode duration. Average lying ($P = 0.002$) and standing ($P = 0.013$) duration were significantly affected by treatment, with a time by treatment interaction ($P < 0.001$); the effect of the covariate was also significant ($P < 0.001$). The time by treatment interaction was due to an increase in average standing and lying episode duration for the P and PA treatment, which started on Day 22 and persisted whilst infestation continued (Figure 5.7). The average lying time per episode up to Day 22 was 43.1 (CI: 37.7 – 49.2) min and the average standing time per episode was 15.0 (CI: 12.3 – 18.2) min across treatments. Between Day 22 and Day 31, the average lying time was 60.1 (CI: 47.8 – 76.3), 72.6 (CI: 50.4 – 105) and 37.0 (CI: 31.0 – 42.9) min, and the average standing time was 21.5 (CI: 14.2 – 32.6), 25.2 (CI: 16.6 – 38.3) and 11.0 (CI: 8.8 – 13.8) min for the P, PA and control respectively. For the same treatments post Day 31, the average lying episode durations were 69.4 (CI: 38.3 – 126), 55.0 (CI: 33 – 91.9) and 35.9 (CI: 28.1 – 45.8) min, and the average standing episode durations were 34.0 (CI: 20.2 – 57.2) 27.0 (CI: 15.7 – 46.3) and 12.4 (CI: 9.7 – 15.9) min, respectively. The PA treatment had returned to the same average lying and standing episode values as the control by Day 39. The analysis of activity therefore showed that the P and PA treatments resulted, on average, in less frequent and longer lying and standing episodes, showing less alteration between the postures.

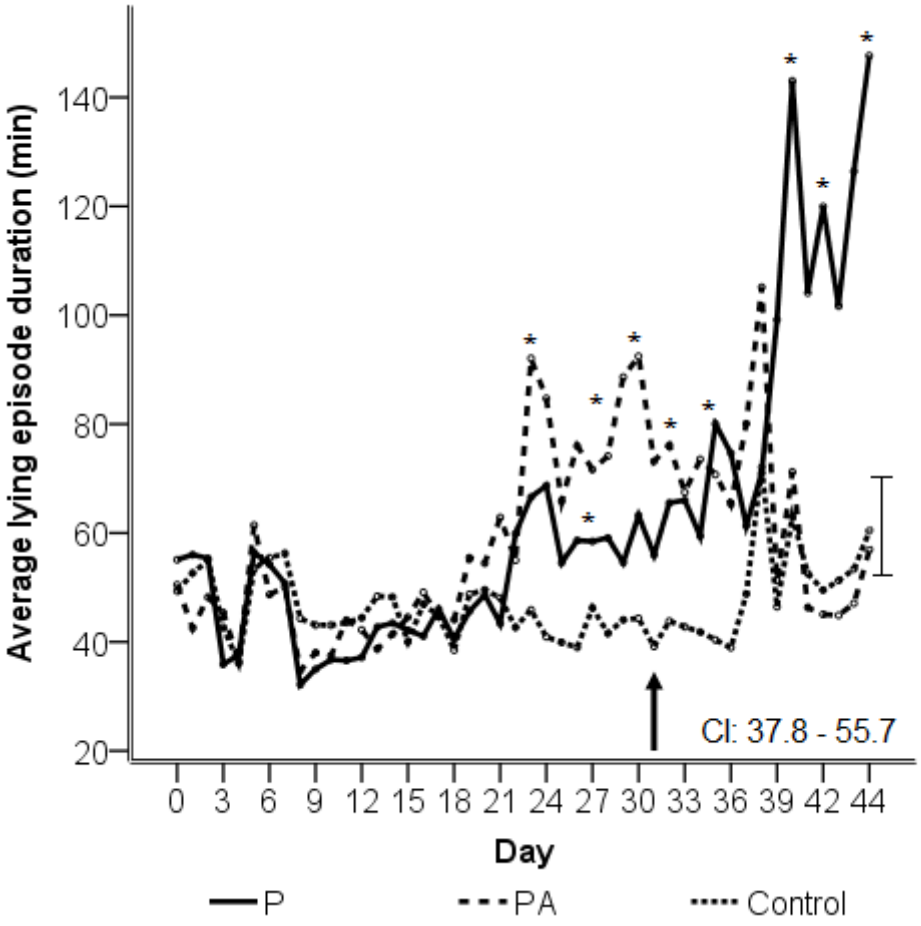

Figure 5.7: Duration of average lying episodes in minutes against experimental day for unchallenged controls (Control; n = 12) and beef bulls challenged with 100,000 L3 larvae of *Ostertagia ostertagi* (P and PA) on Day 0, 7 and 14 of the experiment. The PA (parasite interrupted; n = 7) group were drenched with an anthelmintic on Day 31, whereas P (parasitized; n = 7) group remained undrenched throughout. The bar is the confidence interval (CI) associated with the back-transformed means and shown on the control treatment. Drenching is indicated by an arrow.
* = $P < 0.05$ P or PA compared to control

5.3.6 Feeding behaviour

Feeding behaviour was analysed per period for both feeding episodes and meals. There was a significant ($P < 0.001$) time effect for both the average and total feeding episode duration; this was not the case for the feeding episode frequency ($P > 0.05$). There was no significant effect of treatment and no interaction between treatment and time ($P > 0.05$) on average and total feeding episode duration, as well as on feeding episode frequency. The average feeding episode duration was 14.9, 16.5 and 17.9 (SEM = 0.8) min; total feeding episode duration was 73.4, 99.6 and 115 (SEM = 8.19) min; and the frequency of

feeding episodes was 24.8, 22.6 and 22.6 (SEM = 2.20) episodes per day for period 1, 2 and 3 respectively.

There was only a time effect on average meal duration ($P = 0.003$) and total meal duration ($P < 0.001$). Treatment ($P > 0.05$) and treatment by time interaction ($P > 0.05$) did not significantly affect these measurements. The average meal duration was 17.7 (CI: 12 – 26), 18.5 (CI: 12 – 29) and 21.5 (CI: 15 – 30) min and the total meal duration was 120, 138 and 165 (SEM = 11.1) min per day for period 1, 2 and 3 respectively. However there was a significant time ($P = 0.027$) and time by treatment interaction ($P = 0.039$) on meal frequency. This was caused by a decrease in the meal frequency in the third period for the P treatment (Figure 5.8), whereas meal frequency for the PA and control animals continued to rise, leading to 6.6, 7.8 and 8.1 (SED = 0.67) meals per day for P, PA and control respectively during the third period.

5.3.7 Correlations between FEC and activity and posture

The AUC of the FEC showed a significant ($P = 0.007$) positive correlation with the average lying episode duration between Days 21-31 ($r = 0.71$). Negative correlations were seen between the AUC of the FEC over the time period between Days 31-45 and activity (number of steps taken) ($r = -.882$; $P = 0.02$) on the one hand, and lying and standing frequency on the other ($r = -.891$; $P = 0.017$) indicating that animals with higher FEC were less active and changed posture less frequently. None of the other correlations considered, including those between Days 21-45, were significant ($P > 0.05$).

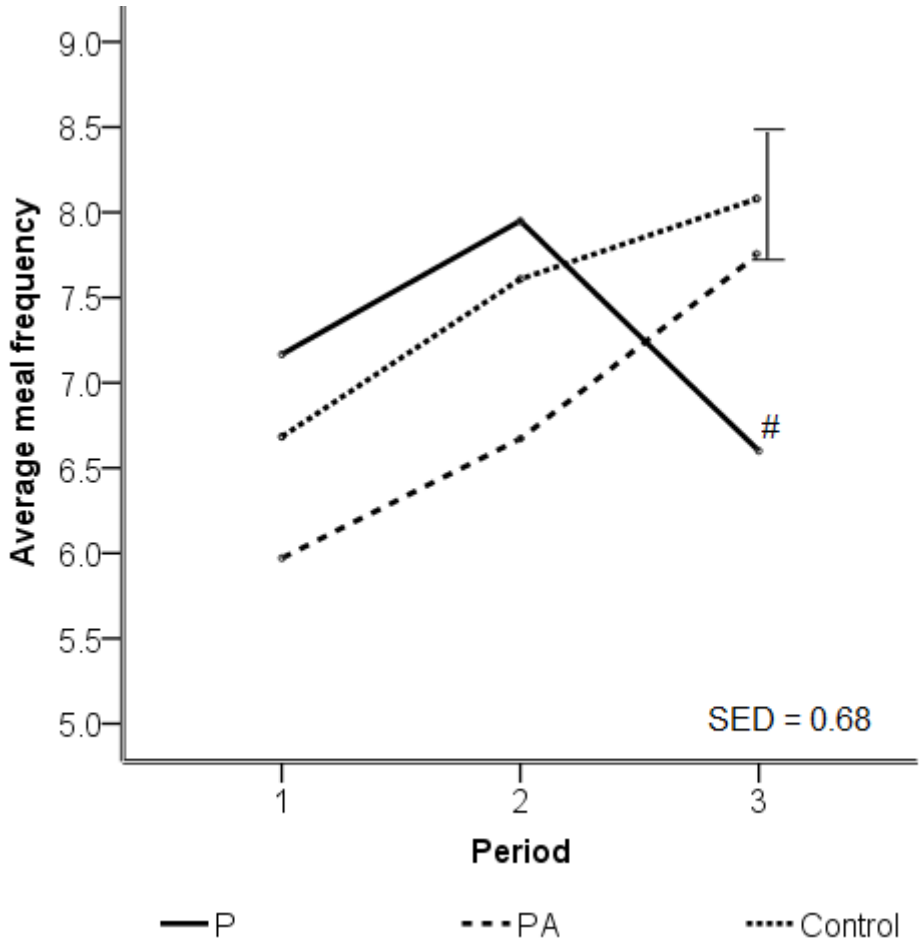

Figure 5.8: Meal frequency against experimental day for unchallenged controls (Control; n = 12) and beef bulls challenged with 100,000 L3 larvae of *Ostertagia ostertagi* (P and PA) on Day 0, 7 and 14 of the experiment. The PA (parasite interrupted; n = 7) group were drenched with an anthelmintic on Day 31, whereas P (parasitized; n = 7) group remained undrenched throughout. The bar is the standard error of the difference (SED) and shown on the control treatment.
= $P < 0.05$ P compared to PA and control

5.4 Discussion

The objectives of the study were to identify any temporal changes that occur in several types of behaviour of cattle during abomasal parasitism and to quantify the magnitude of such changes. The latter was undertaken with the aim to assess the diagnostic value of these behavioural changes. The device of interruption of parasitism through dosing with an anthelmintic was used to test how quickly behaviours potentially affected by parasitism returned to 'normal' levels (Kyriazakis et al., 1996). Fast recovery in the behaviours tested could also have diagnostic use for the assessment of the effectiveness of treatments (Forbes et al., 2004).

The effects of parasitism on the standard diagnostic indicators of FEC and pepsinogen levels were not apparent until Day 21 post infection, when both were significantly elevated. Because abomasal damage, of which pepsinogen is an indicator, concurs with the establishment of infective larvae (Lawton et al., 1996), a rise in pepsinogen is likely to occur before Day 21 (Forbes et al., 2009; Chapter 4) and before any eggs appear in the faeces. Differences between treatments in BW were seen from around Days 21-24 as a result of the absence of gain or even BW loss in infected bulls and persisted until the end of the experiment. The effect of infection on BW gain was more severe than expected, both on the basis of the literature (Fox et al., 2002) and prior experience (Chapter 4). This, in combination with the detection of low dry matter faeces in some animals, shows that the established infection developed into a clinical one. The removal of infection through the administration of the anthelmintic had the expected immediate effect both on FEC and BW gain. The issue is how the changes in these measurements relate to any changes in the behaviours measured.

The focus was on measurement of overall activity and posture, captured through automated means, and feeding behaviour, captured through the use of video cameras. The latter could also be captured by automated means, because the technology to achieve this is available in confined animals (Sowell et al., 1999; Quimby et al., 2001; Weary et al., 2009). The expectation was that changes in activity and/or posture would be observed at an earlier stage than any more conventional signs of parasitism (such as FEC and decreased BW gain). This was based on the expectation that behaviour would be affected by, for example, damage caused by the establishment of infective larvae, which generally occurs within a few days (Lawton et al., 1996). This expectation however, did not materialize. The changes caused by parasitism in all measurements of activity and posture were observed to begin at approximately Day 22 post infection. In some cases the differences between infected and control animals attained formal significance only a few days later, presumably due to variations in individual responses. These effects, manifested as a time by treatment interaction, were in summary: (i) a decrease in the number of steps taken, (ii) a decrease in lying and standing episode frequency and (iii) an increase in average lying and standing episode duration in infected compared to control animals. In addition, there was an increase in total standing time due to parasitism, and this effect was consistent over time. The decrease in steps taken was expected, because activity levels frequently decrease when an animal is faced with a health challenge (Hart, 1988; Forbes et al, 2004; Reiner et al., 2009; Chapter 4), although the occurrence of the decrease may

depend on the nature of the pathogen challenge (Hart, 1990). It is still unclear what causes this decrease in activity during parasite or other infectious challenges. It has been suggested that it may arise from the lethargy that accompanies the physiological changes associated with "sickness behaviour" (Hart, 1988). Animals may try to conserve energy during exposure to pathogens, especially because sickness behaviour is frequently accompanied by a reduction in voluntary food intake (see below; Sandberg et al., 2006). The effect of parasitism on posture resulted in a reduction in transition between the lying and standing postures, thereby decreasing the energy requirements for posture changes (Hart, 1988) and the pattern distribution of behaviour sequences when compared to the controls. This pattern distribution if viewed over time, also known as the complexity of behaviour, has previously been observed in environmentally challenged animals, such as parasitized wild goats (ibex, Alados et al., 1996) and food deprived chickens (María et al., 2004). A decrease in behavioural complexity is considered an indicator of stress that arises, including health challenges (Alados et al., 1996).

The above changes in activity and posture due to parasitism persisted throughout the infection period and in some cases they increased over time, as was the case for lying and standing episode frequency and average episode duration. Furthermore there was a correlation between the level of the FEC and the degree to which activity and posture were affected, indicating that animals with higher FEC took fewer steps, lay for longer and changed posture less frequently. The infection established in this experiment, was for a number of animals at least, clinical. A previous study (Chapter 4) showed similar effects, but of lesser magnitude on the behaviour of beef cattle with a subclinical *O. ostertagi* infection. All the above raised the possibility that the magnitude of behavioural changes observed may be dose dependent. There is a dose dependent response in behaviour during micro-parasitic health challenges on social exploration (Larson and Dunn, 2001) and activity (Skinner et al., 2009). The effects of increased doses of macro-parasites on the behaviour of animals have yet to be systematically addressed. It is possible that the relationship between parasite dose and effect on behaviour is of the form suggested by Sandberg et al., (2006), where they assume changes in feed intake to occur at a gradient dependent on dose.

Drenching parasitized animals with an anthelmintic failed to result in the expected and hypothesised rapid recovery in activity and posture. Although after anthelmintic dosing the behavioural levels for the average standing and lying episode duration returned to

similar values to those of the uninfected animals, this occurred approximately one week post-dosing. For the total frequency of lying and standing, the recovery started immediately, though taking the same amount of time to return to control levels. The hypothesis was based on the findings that certain behaviours such as feeding behaviour, seem to recover within a couple of days post administration of an anthelmintic in parasitized sheep (Kyriazakis et al, 1996). It is possible, however, that the time course of recovery of the behaviour post-treatment depends on the severity of the pathology caused by the parasite challenge and hence the physiological recovery, and is thus different for different health challenges.

Feeding behaviour was measured through the use of video cameras and the measurements were taken over three periods, each three days long. Period 1 covered Days 7-9, to provide baseline measurements because the behaviour should not be different between the treatments at this stage as was shown in a previous study (Chapter 4). Period 2 (Days 31-33), was chosen with the expectation that the effects of parasitism on activity and posture would then peak. Finally, period 3 (Days 39-41), was selected because by this time the bulls in the PA treatment were expected to have recovered from any possible changes in behaviour due to parasite infection. The hypothesis was that duration and possibly frequency of the feeding events would decrease during infection, as suggested by González et al. (2008) in cows suffering from subclinical ketosis or lameness, and by Forbes et al. (2000; 2004; 2007) in parasitized grazing calves and dairy cattle. Such changes would be consistent with an expected decrease in food intake of cattle parasitized with *O. ostertagi* (Fox et al., 1989; 2002).

However, the only effect seen on feeding behaviour was a decrease in meal frequency in period 3 for the animals parasitized throughout the trial, seven weeks after the initial challenge. The reduction was around 17% in the P group compared with the other two (parasite-free) treatments. This potentially complements previous findings where a significant increase in average meal duration in animals challenged by the same parasite was observed 19 days after the challenge was administered (Chapter 4). A decrease in meal frequency with an increase in average duration could maintain the same feed intake in infected animals, though no effect on average meal duration was found in this experiment.

A rapid effect on feeding behaviour following the administration of the anthelmintic was also expected, based on the study of Kyriazakis et al. (1996) who found very rapid recovery in food intake of parasitized sheep post-dosing. Forbes et al. (2004) likewise recorded an increase of feeding episode duration in animals that had received preventive treatment with an anthelmintic compared to infected animals. Although there was an increase in meal frequency seven days after the anthelmintic treatment in the PA treatment compared to the untreated parasitized animals, the meal frequency of the control animals also continued to increase over time, albeit at a slower rate. In view of the rapid advances in the monitoring of beef cattle feeding behaviour (Weary et al., 2009), the absence of effects of infection on feeding behaviour in this study were disappointing.

The magnitude of behavioural changes in activity and posture was large enough to be statistically significant which suggests that they might be suitable candidates for disease detection. Activity, as measured by the number of steps taken, showed a 41% decrease in the parasitized animals once established. There was an increase by 52% in average lying and by 55% in standing episode duration, while the frequency of lying and standing episodes decreased by 44%. González et al. (2008) suggested that changes of at least 2.5 standard deviations from the previous 7-day rolling average of total individual daily feeding time have strong diagnostic value. They were able to identify more than 80% of cows with acute disorders at least 1 day before diagnosis by farm staff. The changes in the behaviours observed in this experiment are of greater magnitude than such deviations. Although, in this experiment, the changes in the behaviour coincided with the onset of clinical measurements, such as FEC, they might have a diagnostic value where clinical signs are not apparent (Forbes et al., 2004; 2007).

Chapter 6: The Relationship between Macro-Parasite Dose and Behavioural Change in Cattle

6.1 Introduction

It is now well established that health challenges, such as infection with a pathogen or other stimulation of an immune response, may lead to changes in animal behaviour (Hart, 1990; Larson and Dunn, 2001; Weary et al., 2009; Chapter 4). Such behavioural changes may have diagnostic value, as they precede any clinical signs of the condition (Quimby et al., 2001; Huzzey et al., 2007; González et al., 2008; Kyriazakis and Tolkamp 2010), and may enable early intervention. The question whether the extent of the behavioural changes is related to the magnitude of the health challenge, such as the infection dose of a pathogen, largely remains unanswered. Knowledge of such relationships has obvious diagnostic advantages, as it allows targeted interventions and will therefore enhance animal health as well as welfare.

Currently there is little information about the relationship between pathogen dose and change in behaviours. The few exceptions are studies, mainly in rodents, which suggest a relationship between challenge dose and a reduction in social exploration (Edwards, 1988; Bluthé et al., 1996), anxiety (Bassi et al., 2012) or activity (Johnson and von Borell, 1994; Skinner et al., 2009). There is also indirect evidence of a possible relationship between pathogen dose and the different dimensions of feeding behaviour (González et al., 2008). In these experiments, however, infection either occurred naturally, or only a narrow range of infective doses was used. As a consequence, they do not allow conclusions to be drawn about the shape of the relationship.

There are at least two possible forms of such a relationship, which are depicted in Figure 6.1. The first is that above a certain dose the change in behaviour is of the same magnitude for a wide range of doses that may lead to (subclinical) diseases. Only at high pathogen doses that may lead to clinical disease, further changes in behaviour become apparent and may be linearly related. This expectation comes from the studies on pathogen-induced anorexia; these have demonstrated that over a wide range of infective doses with macro- or micro-parasites the reduction in food intake is very similar for a variety of animals and pathogens (as summarised by Kyriazakis et al., 1998; Sandberg et al., 2006; Kyriazakis, 2010). The second possible form is that increasing pathogen doses

lead to increasing changes in behaviour. These two relationships would have different (intervention) consequences when using behaviour as a predictor of the level of infection. The objective of this study was to investigate the relationship between the infective dose of a macro-parasite, *Ostertagia ostertagi*, and a number of behaviours in growing cattle. *O. ostertagi* is the most significant parasite affecting cattle in temperate climates (Anderson, 1988; Rinaldi and Geldof, 2012). As the selected range of macro-parasite doses was expected to lead to subclinical infections (Chapter 4 and 5), the hypothesis was that the relationship would be of the first form, i.e. that the effects on the behaviours would be of similar magnitude across all doses.

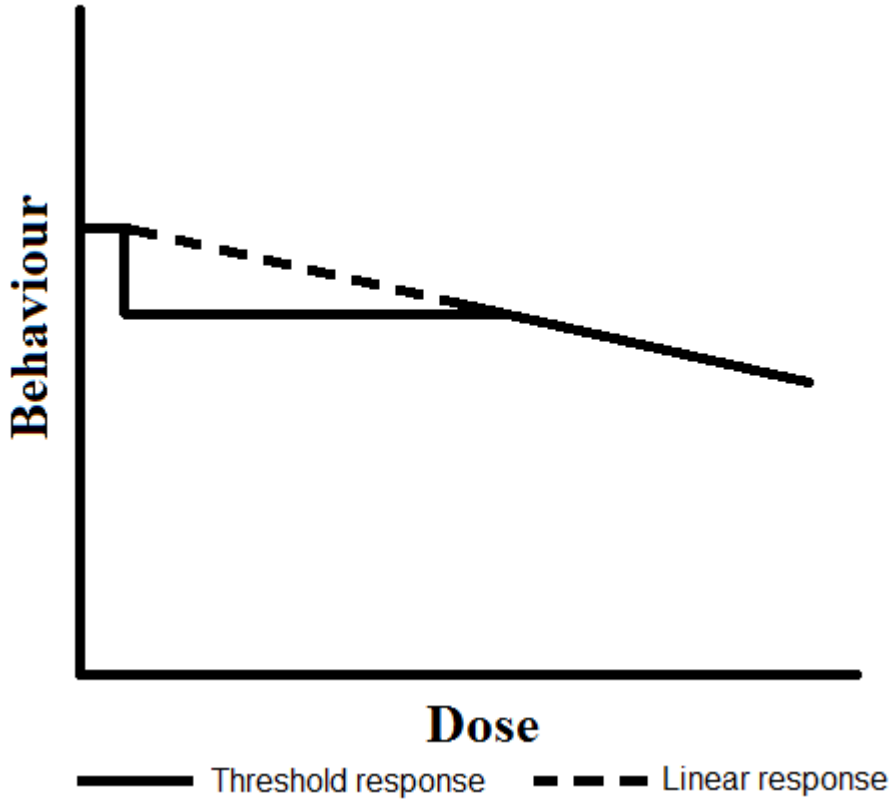

Figure 6.1: Two potential relationships between a pathogen infective dose and the effect on host behaviour. Both assume that no effects will be observed at very low doses. The solid line suggests that a lower range of intermediate infective doses will have similar effects on behaviour initially. Only at very high pathogen doses does the relationship between the two become dose dependent. The dotted line suggests that increasing pathogen doses lead to increasing effects on behaviour. Although a decrease in behaviour is represented here, an increase is also possible depending on the type of behaviour.

6.2 Material and Methods

The experiment took place at the facilities of Newcastle University after approval of the experimental protocols by the Animal Experiments Committee and under license according to the UK Animals (Scientific Procedures) Act for regulated procedures (licence number: PPL 60/4067).

6.2.1 Animals and housing

The animals selected for the study were 24 Holstein-Friesian bull calves aged between 5 and 6 months, with an average weight of 210 ± 30 kilograms (kg) that were obtained from a single source. The sample size was determined using a power calculation based on the standard deviations associated with a previous experiment (Chapter 3). All animals were housed in a single, straw-bedded pen, measuring 116m^2. Both food and water were available *ad libitum*. The food offered was a total mixed ration, consistent throughout the experiment, containing 31.25% barley, 18.45% sugar beet pellets, 15.98% soya bean meal, 13.42% crushed barley, 9.76% distillers maize, 7.41% molasses and 3.75% chopped barley straw. The nutrient composition of the food was 13.01 MJ ME and 197 g CP per kg DM as estimated from AFRC (1993) feed tables. The animals had not received any prior challenge with parasites and were treated with 2 millilitre (ml) of the anti-inflammatory dexamethasone (Rapidexon, Eurovet, Cambridge, UK), 8 ml of the antibiotic florfenicol (Nuflor, Shering-Plough, Milton Keynes, UK) and 7.5 mg/kg body weight (BW) of the anthelmintic albendazole (Albenil, Virbac, Woolpit, UK) two weeks before the experiment started, to prevent interference from other potential health challenges.

6.2.2 Experimental design

The experiment was considered to start when the animals first received a parasite challenge (designated as Day 0). Prior to the application of the challenge (Day -8) animals were fitted with a pedometer (Icetag, IceRobotics, South Queensferry, UK) on their right front leg; the pedometer was secured with a Velcro strap. Video recordings of their behaviour started at the same time in order to provide background data before the start of the experiment.

The animals were randomly assigned, whilst balancing for BW, to one of four treatments (n = 6 per treatment). The first three treatment groups, High (H), Medium (M) and Low (L) received a total dose of 300,000, 150,000 or 75,000 L3 *O. ostertagi* larvae

respectively, which was administered on three occasions by gavage on Days 0, 7 and 14 of the experiment in three equal amounts. Dose H had been used in previous experiments (Chapter 5) and had led to significant changes in the behaviour of bulls during the course of the infection. The parasitism induced by all doses was expected to be subclinical (Chapter 4 and 5). Animals remained infected for the duration of the experiment (up to Day 55). The fourth treatment group acted as an unchallenged control (C). Animals on this treatment were given a gavage with 20 ml water on Days 0, 7 and 14. The infections were not expected to cause any effects on BW and feeding behaviour for the first 2 weeks post-treatment (Fox et al., 1989; Chapter 4 and 5), because the larvae need time to mature and exert their effects on their host.

The third stage larvae (L3) used for the challenge were obtained from Ridgeway Research (Gloucestershire, UK) and were of an Ivermectin susceptible strain isolated in South Gloucestershire 2 months before use (reference label OOSG10). Upon arrival, the 3.15 million larvae were split into six pots and each diluted in 500 ml of water, which was refreshed every other day until use. Just prior to each dosing occasion, 395 ml of surplus water was removed from two of those pots leaving 42 doses of 5 ml with 25,000 L3 per dose; these were subsequently distributed between doses of 100,000, 50,000, and 25,000 L3. The smaller doses were topped up with water to make up a 20 ml volume per dose, and each dose was administered to an animal by gavage on the prescribed day.

The experiment lasted for 8 weeks, with BW, faecal samples and blood samples taken at the start of the experiment (Day 0). The BW and faecal samples were subsequently taken twice a week, and blood samples taken once a week. The frequency of sampling ensured that animals became accustomed to these procedures. The faecal samples were taken from the rectum and directly placed in labelled pots; a faecal consistency score on a qualitative scale of 1 (very liquid) to 3 (solid) was assigned for each sample. The enumeration of the number of parasite eggs in the faeces took place on the same or the following day, in which case the samples were kept in the refrigerator overnight. The blood samples were taken from the jugular vein into plain tubes (Vacutainer, BD, Franklin Lakes, New Jersey, USA), and the samples were left overnight in the fridge, after which they were spun for 15 minutes at 1500rpm at room temperature (18^{o}C) and the resultant serum was taken for subsequent analysis. At the end of the experiment (Day 55) all animals were treated with a broad-spectrum anthelmintic (Albenil, Virbac) and returned to farm stock after a health check.

6.2.3 Behavioural observations

The behaviours measured were levels of activity, posture and feeding. The focus was on these behaviours because they can potentially be affected by the investigated health challenge administered and are considered as part of 'sickness behaviour' (Quimby et al., 2001; González et al., 2008, Chapter 4 and 5). Activity and posture were measured with the use of the pedometers (Icetag, IceRobotics, South Queensferry, UK) that took second-to-second readings throughout the experiment, measuring the number of steps taken and the posture of the animal (lying or standing). Feeding behaviour was monitored with 24-h video recording equipment, which was watched using continuous focal sampling. Feeding episodes were defined as starting when an animal put its head into the feeder and to end upon withdrawal from the feeder. Cattle were distinguished by their individual markings. Observations during Days 7-9 (prior to any parasite effects) and 27-29 (when the first effects on performance were expected to be observed (Chapter 5)) were considered in the analysis of the results. All behaviours were analysed for duration and frequency.

6.2.4 Blood and faecal measurements

The collected serum samples were used to assess pepsinogen levels, which are an indicator of the abomasal damage caused by the parasite (Paynter, 1992). Plasma pepsinogen concentration was determined using the modified method of Paynter (1992) and expressed in international units (iu) per L. The samples analysed were those taken on Days 0, 20, 27, 34, 41 and 55 as these were expected to best illustrate the parasite effects.

The faecal egg count (FEC) was expressed as the number of eggs per gram of collected fresh faeces. This was assessed by the flotation method, modified from the method described in the Ministry of Agriculture "Manual of veterinary parasitological laboratory techniques" (Ministry of Agriculture, 1977) and detailed in Chapter 4.

6.2.5 Statistical analysis

The posture and activity data acquired from the IceTags was downloaded with the provided IceRobotics (South Queensferry, UK) software in a format of one summary record per minute, for lying and standing, and per day for the number of steps taken. Each record provided a date, time and percentage of time spent lying and standing and the number of steps taken. The lying and standing data were summarized into episodes with the use of purpose written FORTRAN programs (Tolkamp et al., 2010). These episodes were calculated by assuming that a continuous series of records that showed either 100%

lying or standing behaviour, were part of the same episode. When both lying and standing occurred in the same minute, it was assumed that this was a transition minute in which the behaviour during the first part of the minute was the same as that during the last part of the previous minute. Short lying episodes (those under 4 minutes) were deleted because these were previously verified with video footage not to correspond to real lying behaviour (Tolkamp et al., 2010, Chapter 4). This resulted in a sharp reduction in the number of episodes without any considerable impact on the total lying and standing times, since many deleted episodes were less than 60 seconds.

Feeding behaviour episodes were grouped into meals using meal criteria calculated after fitting mixed models to the frequency distribution of interval lengths between episodes. Both a three population model, consisting of two Gaussian distributions and one Weibull distribution, and a two population model with one Gaussian and one Weibull distribution were compared for their fit to the data (Tolkamp et al., 2011). These models, developed by Yeates et al. (2001), were fitted to the log-transformed interval lengths (expressed in seconds) using the SAS 9.1 (SAS Institute Inc, Cary, USA) programs of González et al. (2008). The rationale for fitting a three population model is that there are three populations of between-feeding intervals, one between meals and two within meals, during which animals do or do not drink (Yeates et al., 2001).The fit of both models were expressed in "minimum function values" (MFV, i.e. twice the negative log-likelihood), where the three population model showed a MFV of 7610 and the two population model a MFV 7734. When these MFV were compared by X^2 test, the addition of the third population was shown to provide a significantly ($P < 0.005$) better fit. From the model parameters, the meal interval criterion was estimated at 26.0 minutes, meaning that all intervals shorter than the estimated criterion were grouped as within-meal intervals. Both episodes and meals were analysed for their duration and frequency. Feeding behaviour data were averaged per animal across the three days within each period considered.

The BW, pepsinogen levels, activity, posture and feeding behaviour were all analysed with the use of a repeated measures ANOVA SPSS 15.0 (IBM, Armonk, NY, USA) with treatment as a fixed and Day (or period) as a random effect. For BW and BW gain analysis, the Day 0 value was used as a covariate. Activity, as measured by the number of steps taken, as well as lying and standing behaviour used the individual mean of the 8 days prior to the experiment (Day -8 until -1) as a covariate to account for variation between individuals. For the pepsinogen levels, the linear and quadratic effects of the

treatments were also tested. Furthermore the data on activity, as number of steps taken, FEC, average and total lying and standing episode duration were log-transformed prior to the analysis in order to normalize their distribution. These results are reported as back-transformed means with 95% confidence intervals (CI).

6.3 Results

6.3.1 Faecal egg counts

The consistency of the faeces varied throughout the experiment both within and between treatments: up to Day 23 the faeces were similarly solid for all treatments. However, after Day 23 a number of animals in the H treatment showed signs of diarrhoea, this was 3 out of 6 animals on Day 23, 4 on Day 27, 3 on Day 30, 4 on Day 34, 5 on Day 37, 3 on Day 41, 1 on Day 44, 4 on Day 48 and 2 on Day 51. Animals in the M treatment also showed some minor signs of diarrhoea with 1 out of 6 animals on Day 23, 3 on Day 27, 3 on Day 34 and 1 on Day 48. This was not the case for the L and C treatments. The FEC of the C animals remained at zero throughout the experiment. For the other treatments, eggs were detected in the faeces from Day 20, almost three weeks after the first challenge (Figure 6.2), and remained present throughout the experiment. The FEC of the parasitized animals were affected significantly by time ($P < 0.001$), however there was no significant ($P > 0.05$) effect of treatment and no time and treatment interaction.

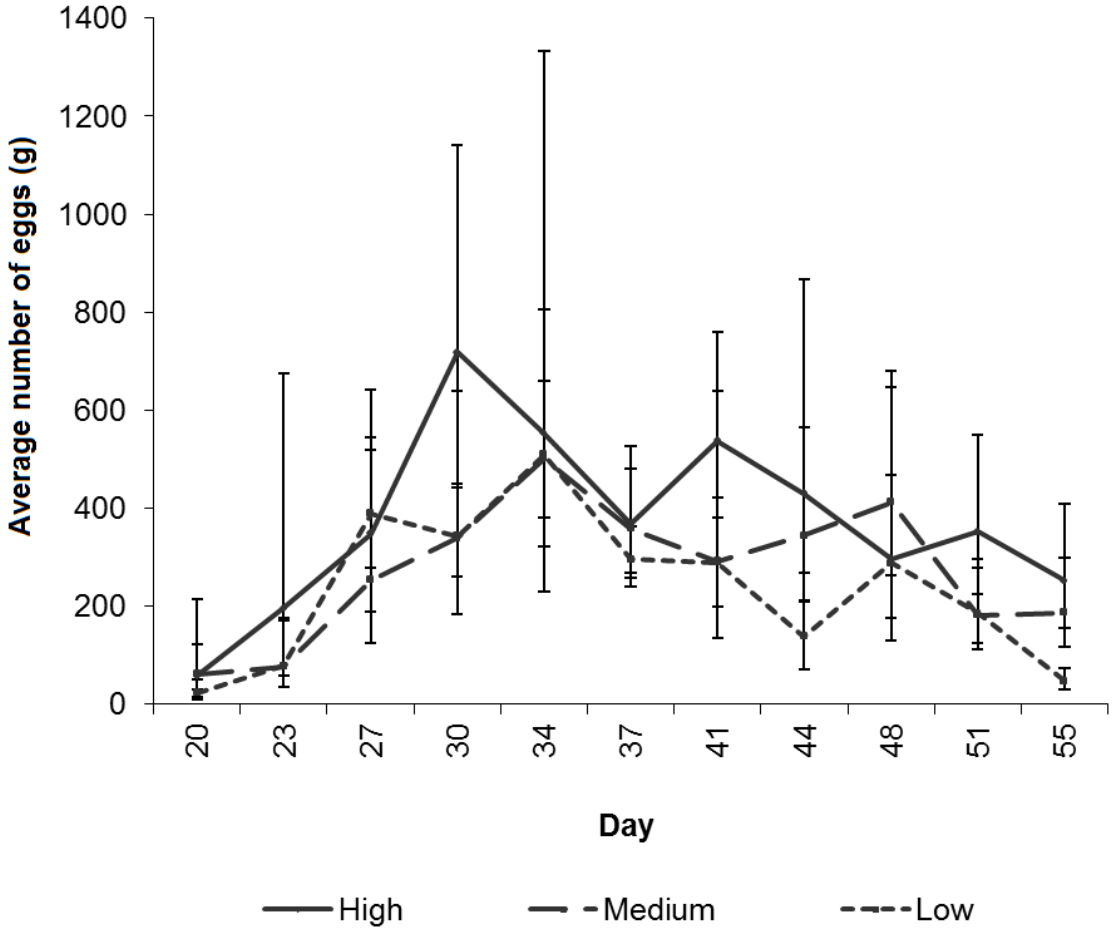

Figure 6.2: Faecal Egg Counts (FEC, number of parasite eggs per g of fresh faeces) of beef bulls against experimental day for animals challenged by 300,000 (High), 150,000 (Medium) or 75,000 (Low) L3 larvae of *Ostertagia ostertagi*; the parasites were administered in three equal doses on Days 0, 7 and 14 of the experiment. The FEC of the uninfected control animals remained at zero throughout the experiment (line cannot be shown).

6.3.2 Pepsinogen

The levels of pepsinogen were significantly affected ($P < 0.001$) by both time and treatment; the time by treatment interaction effect on pepsinogen levels was similarly significant. Animals on the H, M and L treatments had elevated pepsinogen levels by Day 20, which continued to increase with time for treatments H and M and plateaued for treatment L, as shown in Figure 6.3. The pepsinogen levels of the control animals did not change throughout the experiment. The treatment effect was significant at every sampling point post Day 20 ($P < 0.001$) and was essentially linear ($P < 0.001$).

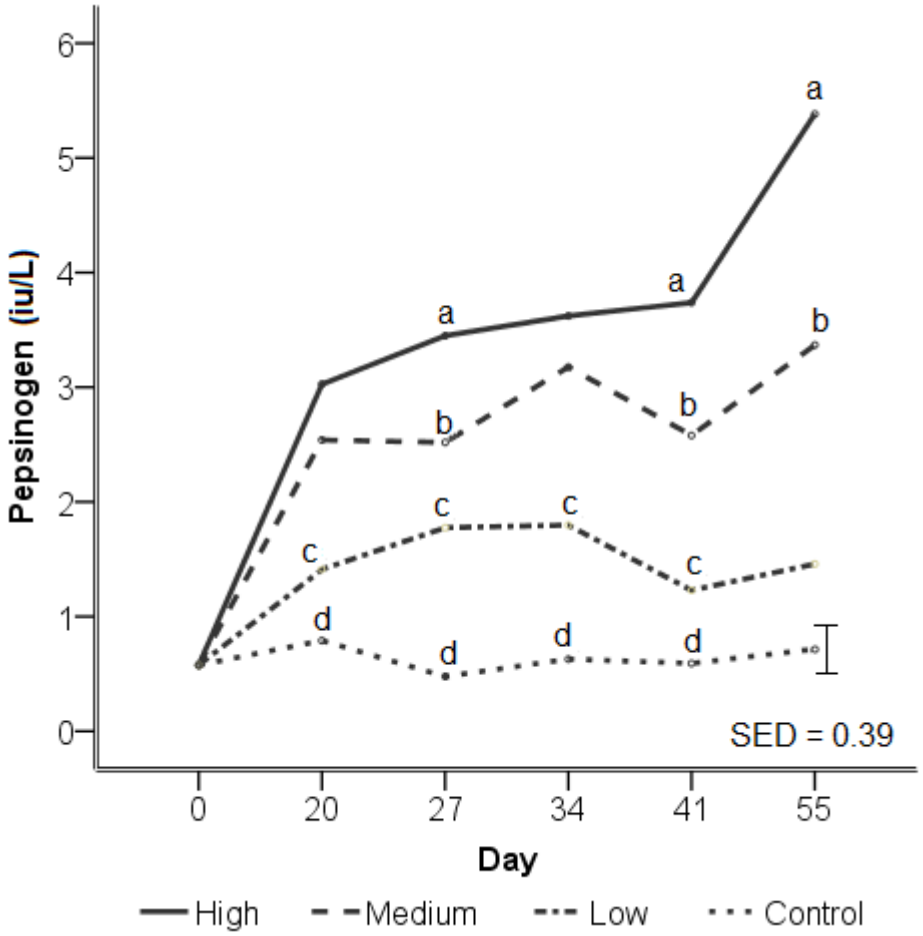

Figure 6.3: Blood pepsinogen levels (iu/L) of beef bulls against experimental day for non-parasitized controls and animals challenged with 300,000 (High), 150,000 (Medium) or 75,000 (Low) L3 larvae of *Ostertagia ostertagi*; the parasites were administered in three equal doses on Days 0, 7 and 14 of the experiment. The bar is the standard error of the difference (SED) and shown on the control treatment.

6.3.3 Body weight gain

The change in animal BW throughout the experiment is shown in Figure 6.4. There was a significant effect of time ($P = 0.015$) and treatment ($P < 0.001$), and a significant interaction between time and treatment ($P < 0.001$) on BW; furthermore the effect of the covariate on BW was found to be significant ($P < 0.001$). Up to Day 27, animals in all treatments gained BW at a similar rate ($P > 0.05$), which was 1463 (SEM = 13.3) g/d. After Day 27 the BW of the animals started to diverge, with animals on the H and M parasite treatments gaining BW at a slower rate ($P = 0.003$) than the other two treatments. However, BW returned to control levels by Day 37 for the M treatment, whereas the H animals continued to grow at a slower rate until the end of the experiment. There was no difference between the BW of the L and the control animals throughout the experiment. The growth rate from Day 27 to the end of the experiment was 429, 929, 1310 and 1274

(SED = 210) g/d for the H, M, L and C animals respectively, with the growth rate of the H animals being significantly ($P < 0.001$) lower than that of the L and C treatment. At the end of the experiment (Day 55) the H animals had an average BW of 270 kg, whereas animals on the other treatments weighed 288, 304 and 302 kg (SED = 10.8) for the M, L and C respectively; the latter three did not differ statistically from each other ($P > 0.05$). For the same treatments the growth rate of the animals over the whole experimental period was 924, 1227, 1477 and 1485 (SED = 110) g/d.

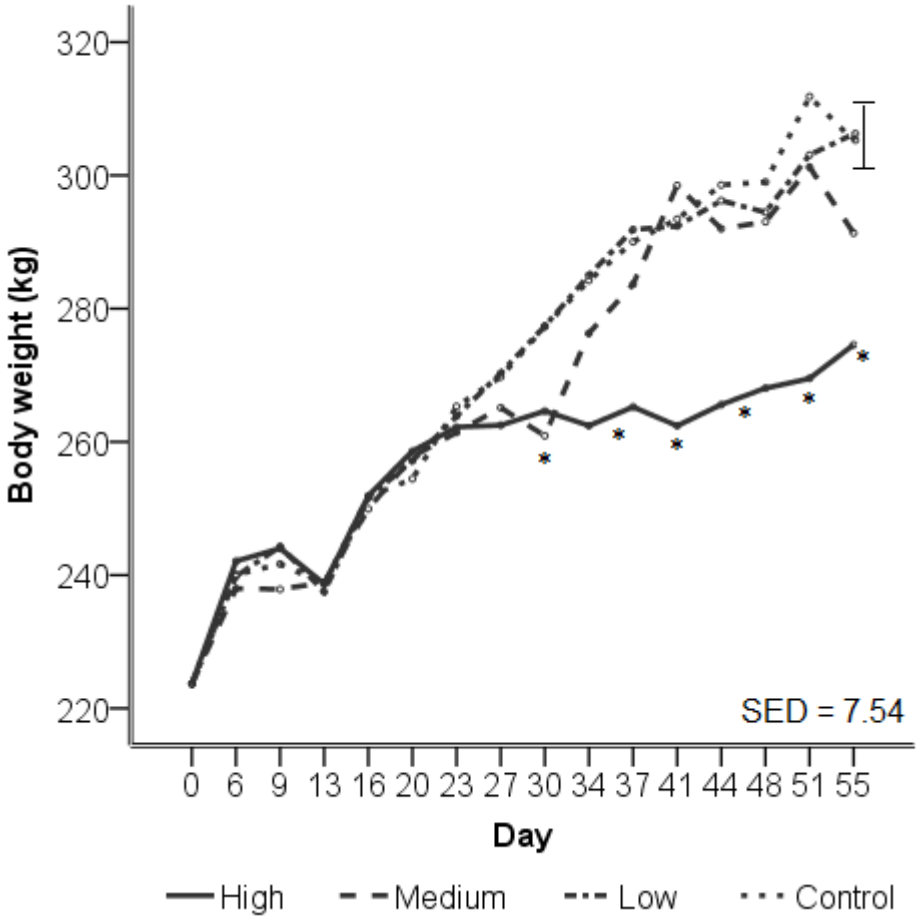

Figure 6.4: Body weight (in kilograms) of beef bulls over time for non-parasitized controls and animals challenged with 300,000 (High), 150,000 (Medium) or 75,000 (Low) L3 larvae of *Ostertagia ostertagi*; the parasites were administered in three equal doses on Days 0, 7 and 14 of the experiment. The bar is the standard error of the difference (SED) and shown on the control treatment.
* = $P < 0.05$, between Medium or High and Control

6.3.4 Activity and posture

Activity, as measured by the number of steps taken, was significantly affected by time ($P = 0.03$) and treatment ($P = 0.007$), and there was a significant interaction between time and treatment ($P = 0.008$); the covariate effect was also significant ($P < 0.001$) (Figure

6.5). The interaction between time and treatment was mainly caused by a significant increase in activity between Days 36 and 46 for the M, L and C treatments, which was not shown to the same extent by the animals on the H treatment. After Day 46 the other treatments decreased the number of steps and returned to H treatment levels. The average number of steps taken before Day 36 across treatments was 2834 (CI: 2485 – 3233); from Day 36 until Day 55 these were 2882 (CI: 2139 – 3885), 3968 (CI: 2829 – 5566), 4602 (CI: 3587 – 5905) and 3971 (CI: 2903 – 5431) for the H, M, L and C treatments respectively.

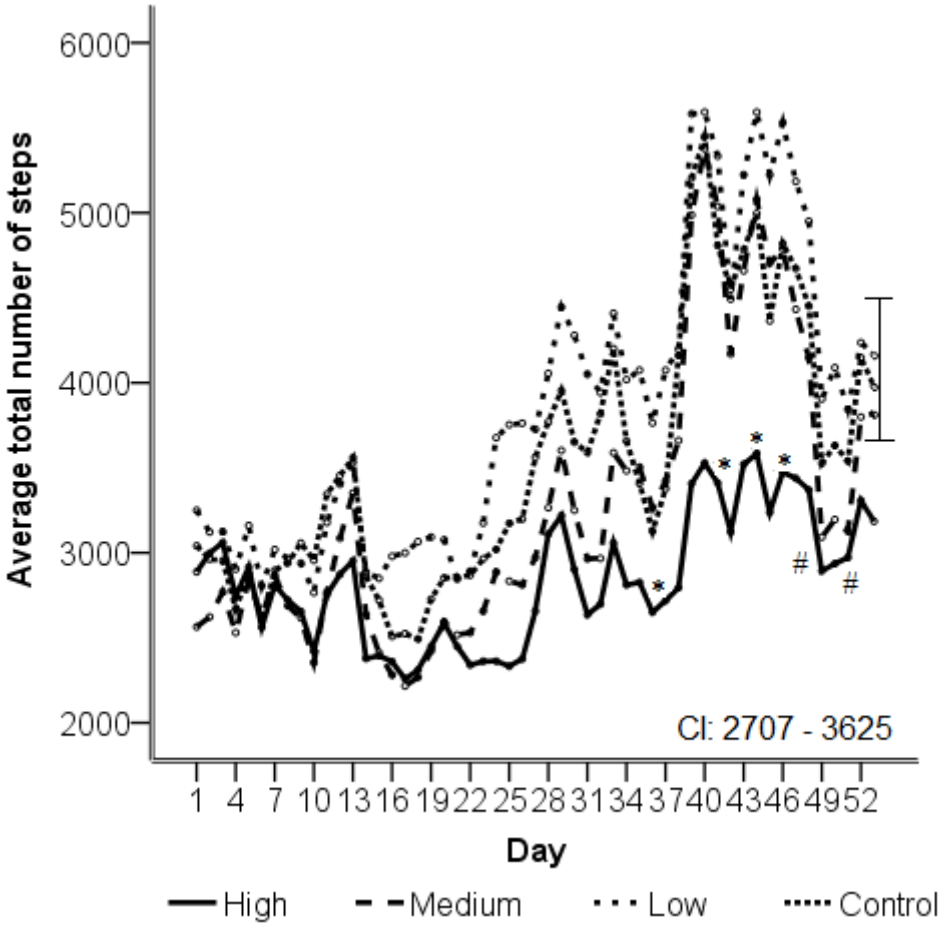

Figure 6.5: Rolling mean (over 3 days) of the average number of steps of beef bulls against experimental day for non-parasitized controls and animals challenged with 300,000 (High), 150,000 (Medium) or 75,000 (Low) L3 larvae of *Ostertagia ostertagi*; the parasites were administered in three equal doses on Days 0, 7 and 14 of the experiment. The bar is the confidence interval (CI) associated with the back-transformed means and shown on the control treatment.
* = $P < 0.05$, between High and Control
# = $P < 0.05$, between High and Low

Concerning posture, the lying or standing episode frequency, which is identical as the animal fluctuates between these two behaviours, was not affected by either time or treatment ($P > 0.05$). However there was a significant time and treatment interaction ($P = 0.038$) effect and the covariate effect was also significant ($P = 0.013$) (Figure 6.6). The interaction effect was due to the lower episode frequency observed in animals in treatment H from Day 29 onwards and persisted until the end of the experiment. This difference was statistically significant between Days 31 to 39. Before Day 31 the average frequency of lying or standing across treatments was 18.7 (SEM = 2.24) per day. After Day 31 this was 13.4, 18.9, 21.0 and 18.1 (SED = 3.46) per day for the H, M, L and C treatment respectively.

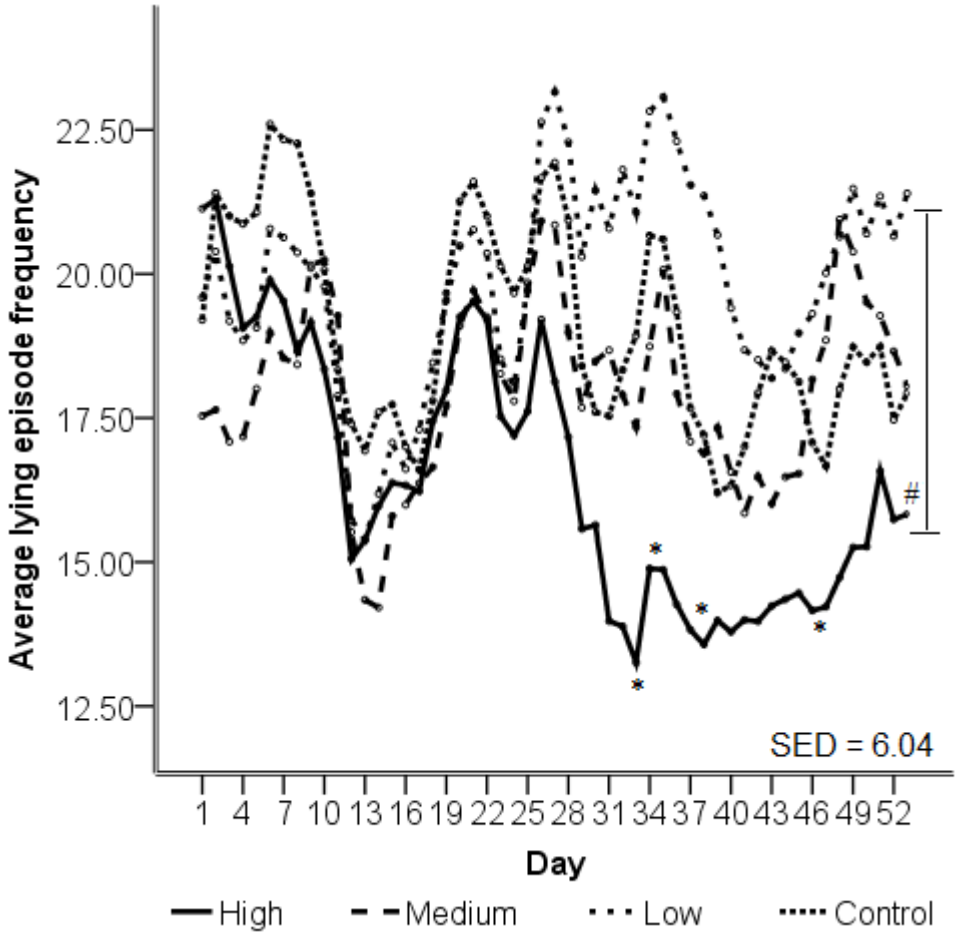

Figure 6.6: Rolling mean (over 3 days) of the lying (and standing) episode frequency of beef bulls against experimental day for non-parasitized controls and animals challenged with 300,000 (High), 150,000 (Medium) or 75,000 (Low) L3 larvae of *Ostertagia ostertagi*; the parasites were administered in three equal doses on Days 0, 7 and 14 of the experiment. The bar is the standard error of the difference (SED) and shown on the control treatment.
* = $P < 0.05$, between High and Control
# = $P < 0.05$, between High and Low

There was no significant effect of time or treatment ($P > 0.05$) on the average lying and standing episode duration. There was however a time by treatment interaction for average lying duration between the H and L treatment ($P = 0.011$) (Figure 6.7); also the covariate effect was significant ($P = 0.026$). The difference between treatments occurred between Days 29 to 39 and was due to an increase in average lying episode duration for the H treatment group ($P = 0.026$). Before Day 29 the average lying episode duration was 35.6 (CI: 28.5 – 37.2) minutes. Between Day 29 and 39 these were 47.7 (CI: 36.0 – 63.2), 32.8 (CI: 27.2 – 39.4), 28.9 (CI: 22.4 – 37.2) and 32.4 (CI: 26.3 – 39.9) minutes for the H, M, L and C treatments respectively. After Day 39 the average episode duration for the H treatment, tended ($P = 0.055$) to be different between treatments, with averages of 38.2 (CI: 30.2 – 48.4) minutes for the H and 30.4 (CI: 23.9 – 38.7), 27.9 (CI: 21.7 – 35.8) and 29.2 (CI: 22.9 – 37.3) minutes for the M, L and C treatment respectively. There was no significant interaction between time and treatment ($P > 0.05$) on average standing episode duration, despite a numerical increase in the H treatment group after Day 29. The average standing episode duration before Day 29 was 13.4 (CI: 11.0 – 16.4) minutes per day; after Day 29 this was 17.3 (CI: 11.2 – 27.0), 13.3 (CI: 8.8 – 20.0), 10.4 (CI: 6.8 – 15.8) and 12.0 (CI: 8.1 – 17.9) minutes per day for the H, M, L and C treatment respectively.

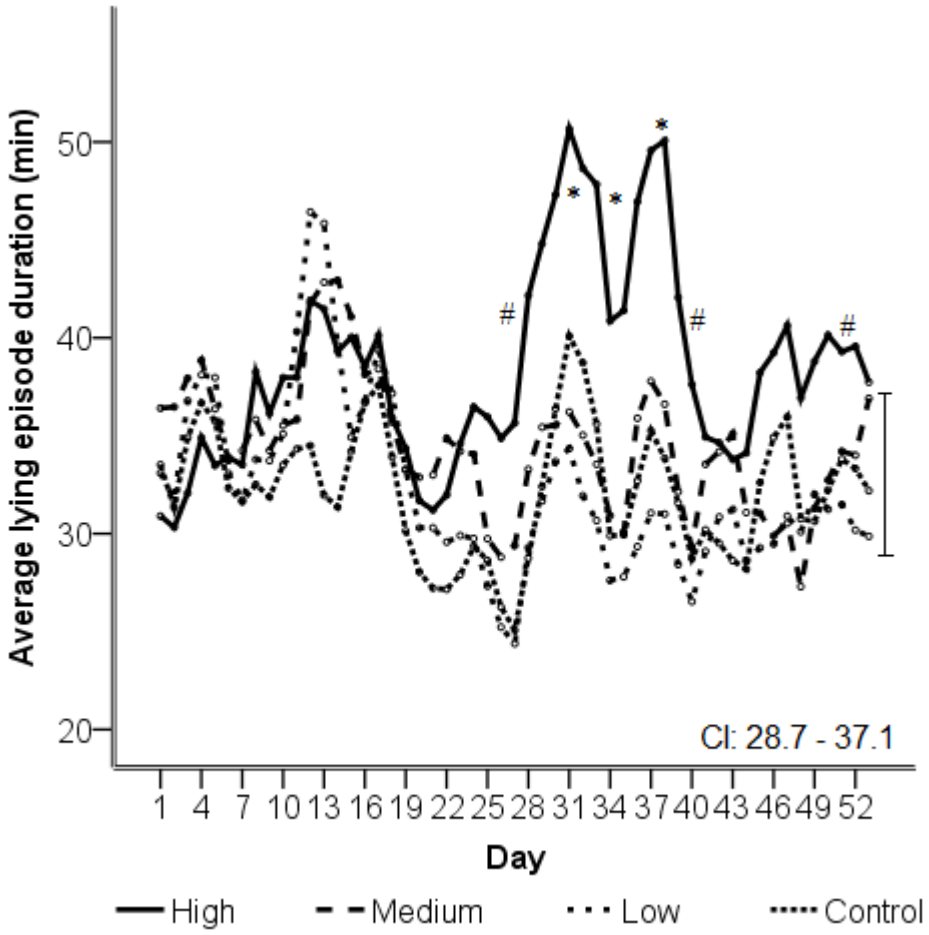

Figure 6.7: Rolling mean (over 3 days) of the average lying episode duration in minutes of beef bulls against experimental day for non-parasitized controls and animals challenged with 300,000 (High), 150,000 (Medium) or 75,000 (Low) L3 larvae of *Ostertagia ostertagi*; the parasites were administered in three equal doses on Days 0, 7 and 14 of the experiment. The bar is the confidence interval (CI) associated with the back-transformed means and shown on the control treatment.
* = $P < 0.05$, between High and Control
# = $P < 0.05$, between High and Low

For total daily standing duration there was no time or treatment effect, and no time and treatment interaction ($P > 0.05$). There was a significant time effect caused by a decrease in total daily lying time duration ($P = 0.005$) as the experiment progressed; there were no other effects on total daily lying duration ($P > 0.05$). The covariate effects were significant on both total daily standing and lying duration ($P < 0.005$). Overall, average total daily standing duration was 10.6 (CI: 9.8 – 11.5) h, the average total daily lying duration was 13.1 (CI: 12.3 – 13.9) h.

6.3.5 Feeding behaviour

There was a significant time effect over the two time periods considered, caused by a decrease in both the average ($P = 0.003$) and total ($P = 0.006$) episode duration. There were no such effects on average episode frequency. None of these behaviours were affected by treatment or by the time and treatment interaction ($P > 0.05$). The average episode duration was 4.99 (SEM = 0.42) minutes, the total episode duration 100 (SEM = 7.44) minutes per day and the frequency 20.8 (SEM = 1.06) episodes per day. When the feeding episodes were grouped into meals, there was a significant time effect on meal frequency ($P < 0.002$) which decreased, but there was no effect on the average and total meal duration ($P > 0.05$). There was no treatment effect or time and treatment interaction ($P > 0.05$) for any of the meal dimensions considered. The average meal duration was 20.1 (SEM = 0.85) minutes, the total meal duration 162 (SEM = 7.56) minutes per day, and the frequency 8.27 (SEM = 0.35) meals per day.

6.4 Discussion

The objective of this study was to investigate the relationship between the macro-parasite dose and a number of behavioural responses that are encompassed by the term 'sickness behaviour' (Hart, 1988) in growing cattle. The health challenge used was an *O. ostertagi* infection, which had been previously shown to affect several aspects of the behaviours addressed here (Forbes et al., 2007; Chapter 4 and 5). Three different infective doses of this parasite were used; as their range was expected to lead to subclinical infections, the null hypothesis was that their effects on behaviour would be of similar magnitude across doses. With these changes quantified, an assessment could be made as to their usefulness for the early detection of disease, as well as the ability to predict the level of infection by the behavioural response.

The effects of the parasitic challenge on the standard diagnostic indicators of parasitism, FEC and serum pepsinogen were not evident until Day 20 post infection. This delay in the rise of FEC was as expected, because larvae need time to mature and start reproducing (Rose, 1969). Pepsinogen levels were likely elevated before Day 20 (Lawton et al., 1996; Fox et al., 2002), but were not assessed prior to this time point. There was no significant difference between the three different parasite doses in their effects on FEC. There was an indication that towards the end of the experiment (Day 55) there was a decline in FEC in all three parasite treatments, suggesting that there was development of immunity (Smith et al., 1987; Hilderson et al., 1993). A difference in FEC between the three parasite

treatments was expected (Hilderson et al., 1993), but may not necessarily appear (Michel, 1969a). This could be due to the large variation in FEC between individuals (Michel, 1969b), or to a density dependent relationship between numbers of parasites and their fecundity (Smith et al., 1987; Kidane et al., 2009). A clear linear relationship was shown between parasite dose and levels of serum pepsinogen post Day 20. Since pepsinogen is considered a reliable indicator of abomasal damage in previously parasite naïve cattle (Claerebout et al., 1997), it is safe to assume that the effect on abomasal integrity was also linear. Whilst pepsinogen levels remained low and constant for the uninfected controls, these continued to rise for the other treatments. The exception was the Low parasite dose treatment that declined in level post Day 34, suggesting a containment of the parasitism in these animals from that point onwards.

Differences between treatments in BW were seen from around Day 24 for both the M and H treatment compared to the uninfected controls; there were no effects on the BW of the bulls infected by the lowest parasite dose (L). The absence of an effect on BW at this lowest level of parasitism is consistent with the findings of Michel (1969a) and Burggraaf et al. (2007), and suggests that a threshold parasite level within the host needs to be exceeded before any effects on host performance can be seen (Sandberg et al., 2006). The effect of treatment M on BW was short-lived, up to Day 30, and animals on this treatment also appeared to show compensatory gain post Day 30, since their BW was similar to the uninfected controls by the end of the experiment. The duration of this effect on BW was comparable to that seen by Fox et al. (2002) for a similar level of parasitism, possibly suggesting a containment of the infection within a short period of time. However, the experiment by Fox et al. (2002) finished shortly after the start of the reduction and therefore a recovery could not be confirmed. The reduction in BW gain by the H animals persisted until the end of the experiment and, although it was in a similar direction to that seen in Chapter 5 for the same level of infection, it was not as severe. This probably reflected the higher initial BW of the animals in this experiment. Taken together, the relationship between parasite dose and its consequences for BW gain is similar to the general one suggested by Sandberg et al. (2006).

On the basis of the above results it is perhaps not surprising that there were no effects on the behaviours observed for the L animals. This suggests that there is probably a threshold dose before any effects on behaviour can be measured as a consequence of a parasite challenge and is consistent with the suggestions of Sandberg et al. (2006) and Kyriazakis

and Doeschl-Wilson (2009). What is surprising however is the absence of any measurable effects on the behaviour of M animals, despite the effects of infection on BW gain and serum pepsinogen, indicative of abomasal damage. There are two possible explanations for the absence of effects on behaviour. The first is that this was due to the short lived effects of parasitism, discussed above. The first effects on behaviour for the H treatment were only apparent at the time when the bulls in the M treatment had recovered BW gain. In a previous experiment (Chapter 5), it was found that changes in the behaviour of parasitized animals were also gradual and took some time to develop and be detected. There are several reasons to account for these gradual changes in behaviour, including that the effects on animal physiology are also gradual rather than abrupt (Fox et al., 1989). Repair of tissues damaged by the parasites may take several days (Murray et al., 1970).

The second explanation that could account for the absence of an effect may be due to the fact that the relevant behaviours that were affected by parasitism were not measured. The focus of the behavioural measurements was on activity, measured by the number of steps taken, posture and feeding behaviour. Both activity and posture were captured using automated means, whereas feeding behaviour was captured using video cameras. The latter may also be captured by automated means, because the technology to achieve this is available (Sowell et al., 1999; Quimby et al., 2001) and being perfected (Weary et al., 2009). There are suggestions that in addition to the above behaviours, (gastrointestinal) parasitism can affect social behaviours and exploration (Edwards, 1988), and also the complexity of behaviour, e.g. the temporal patterns of behaviour, such as the transitions between two different types of behaviour (Alados et al., 1996; Alados and Huffman, 2000). It should be emphasised, however, that these effects are not normally considered as part of 'sickness behaviour' (Hart, 1988; Dantzer, 2001; Weary et al., 2009). In the study of Edwards (1988), the reduction in the frequency of exploration and interactions with conspecifics was dependent on the infective dose of *Trichinella spiralis* in mice. There are now novel developments in sensors that would allow changes in such behaviours to also be captured automatically. Böhm et al. (2009), for example, have used proximity data-logging devices to quantify contacts between individuals in a herd of cattle, although this was not done within the context of exposure to pathogens.

There were clear effects on behaviour at the highest parasite dose, which occurred from around Day 30 for posture and between Days 36-46 for activity. As previously observed,

the effects occurred with some delay from the moment when abomasal damage on the host was expected (Chapter 4 and 5). Activity changes manifested as a difference in the number of steps taken, whereas posture showed a decrease in lying and standing episode frequency as well as an increase in average lying episode duration compared to the other treatments. Although the expectation was a reduction in activity of the H animals, the difference was actually caused by an increase in the number of steps taken in the stated period for the other three treatments, causing a difference of 34% between them and the H treatment. The expectation was based on the findings of previous experiments where activity levels often decrease when an animal is faced with a health challenge (Forbes et al., 2004; Reiner et al., 2009; Chapter 4 and 5). It is unknown what caused the increase in activity for the other treatments, but the source was most likely age or related to environment or management. With the rest of the herd changing its behaviour but not the bulls of the H treatment, this clearly can have diagnostic value. Knowledge of these herd dynamics is, therefore, very important for the detection of health and welfare challenges as demonstrated here. Such knowledge could also account for any sudden changes in the behaviour of the individual caused by external factors rather than health challenges, and therefore assist in avoiding false positives.

Posture was affected because bulls in the H treatment had less frequent but longer lying episodes. This effect by the H treatment was similar to results observed previously in Chapter 4 and 5. This reduction in frequency, combined with an increase in average lying episode duration, may well be a consequence of sickness behaviour, whereby energy is conserved by limiting movement (Hart, 1988). The magnitude of change for the H treatment was a 25% increase for the average lying episode duration and a 22% decrease in the lying and standing episode frequency. The total daily lying and standing times were not affected, showing that the increase in average episode duration was largely balanced by the decrease in episode frequency (Chapter 4). Parasitized animals may still continue to engage in other activities such as feeding or drinking when standing, but the frequency of doing so can be reduced (González et al., 2008). The magnitudes of these changes were sizeable enough to show statistically significant differences and could therefore have diagnostic value for the detection of parasitic infections.

Feeding behaviour measurements were taken over two periods. The assumption was that the duration, and possibly frequency, of the feeding episodes and/or meals would be affected by parasitism (Forbes et al., 2004, 2007; Chapter 4 and 5). Such changes would

be consistent with an anticipated decrease in food intake of cattle parasitized with *O. ostertagi* (Fox et al., 1989; 2002); this would be in keeping with the decrease in BW gain seen in treatment H. Such a reduction, also known as parasite-induced anorexia, is considered to be the main contributor to the reduction in animal performance (Fox et al., 1989; Kyriazakis et al., 1998; Fox et al., 2002; Kyriazakis, 2010). It was, therefore, expected that feeding behaviour would be influenced accordingly (Kyriazakis et al., 1998). However, there was no effect on feeding behaviour in any of the treatments. During previous research an effect of parasite infection on average meal duration was found (Chapter 4) and average meal frequency (Chapter 5) and the reason why no significant changes were observed here is unclear. Effects of parasitism (Forbes et al., 2007; Kyriazakis and Tolkamp, 2011) as well as other health challenges (Gonzalez et al., 2008) on feeding rate are well documented. Because treatment significantly affected BW gain, it is possible that a reduction in feeding rate resulted in a decreased feed intake even though feeding time and frequency remained unaffected.

The results of the experiment discount the suggestion that changes in (sickness) behaviours as a consequence of parasitism are directly dose dependent. Instead, the data show that for a range of doses the behaviour is unaffected despite significant effects on clinical measurements and BW. High parasite doses affect behaviour significantly, although these seem to follow the clinical signs with a delay of a few days. Thus, these findings are more in line with the hypothesis put forward in the Introduction (6.1), rather than the suggestion that the relationship between the pathogen dose and behavioural change is simply linear. The point of the transition between the two phases has yet to be defined. Although the potential of detecting health challenges by the use of changes in behaviour is well established (Sowell et al., 1999; Quimby et al., 2001; González et al., 2008; Weary et al., 2009), useful application seem to depend on the level of infection or challenge dose. Mild subclinical infections are hard to detect while high levels of infection result in clinical signs which obviate the need for behavioural detection. This leaves the possibility, however, that it may be feasible to detect subclinical disease between these extremes through the use of behavioural changes. The automated detection of behaviour utilising recently developed in sensors, once set up, requires less input and may therefore be useful as well for extensively kept animals or systems where the contact between the animal and its keeper may be very limited.

7. General discussion

Changes in behaviour are acknowledged to accompany clinical disease and such characteristic changes are commonly referred to as sickness behaviour (Hart, 1988). However, less is known about the ability of behaviour to indicate the early stages of disease or subclinical disease. Therefore, the aim of this thesis was to identify and quantify changes in behaviour that could be associated with the early onset of disease or subclinical disease in beef cattle. This was achieved by identifying the behavioural changes that accompany acute (Chapters 3 and 4) and chronic (Chapters 4, 5 and 6) health challenges, with greater focus on changes related to the chronic health challenge which resulted in more significant changes in behaviour.

Three underlying assumptions were made at the start of the thesis. The first assumption was that there would be common behavioural indicators across different health challenges that would be associated with subclinical disease. Despite the fact that the behavioural changes observed were consistent across experiments for the chronic health challenge, the hypothesis could not be confirmed with great certainty. The only changes demonstrated here were those associated with a chronic macro-parasitic (*Ostertagia ostertagi*) infection. From the literature it can be deduced that changes in activity (Edwards and Tozer 2004; Mazrier et al., 2006), posture (Galindo and Broom, 2002; Escobar et al., 2007) and feeding behaviour (Quimby et al., 2001; González et al., 2008) as found in the case of the *O. ostertagi* infection occur for a variety of different health challenges. These changes may differ in magnitude between challenges (González et al., 2008) and, as shown for the acute challenge, might only manifest as short-lived effects that would be hard to detect (Borderas et al., 2008). Thus, even though the type of behaviours affected seem to be consistent, the magnitude and duration of these may vary, depending on the type of challenge.

The second assumption was that the behavioural changes would occur quickly after the health challenge and that recovery of behavioural normality after treatment would be equally fast (Kyriazakis et al., 1996). For all experiments involving *O. ostertagi* challenges used in this thesis, a change in behaviour coincided with a reduction in BW gain and with detection of FEC at around three weeks after the initial infection. This was a more gradual behavioural change than expected, which may be explained by the nature of the infection (Smith et al., 1987; Fox et al., 2002). Despite the gradual onset, the

magnitude of the behavioural changes was sufficiently great to be used for the detection of the disease. Furthermore, the recovery in behaviours to pre-challenge levels, against expectations, took around one week after treatment. The recovery of the behaviours measured in this thesis does, however, provide an insight into the recovery process of behavioural normality that was previously undocumented.

The final assumption was that the rate and magnitude of behavioural change would be dose-dependent. The reasoning for this assumption was based on the suggestions of Sandberg et al. (2006), which followed an extensive review of the literature. This relationship was confirmed (Chapter 6) since the lower, subclinical, doses did not result in any effects on behaviour whereas the highest dose leading to clinical symptoms caused significant changes in behaviour as seen in previous experiments (Chapter 4 and 5). Although this relationship between dose and behaviour was assumed (Kyriazakis et al., 1998; Sandberg et al., 2006), it had not been previously tested. The current results give a clear insight into the relationship between challenge dose and behavioural response, although more research is needed to confirm this relationship and the dose-dependency of the magnitude and duration of behavioural change.

The overriding conclusion arising from this thesis was that a chronic health challenge, caused by *O. ostertagi*, caused a gradual change in behaviour, leading to a significant difference in the level of activity and adopted postures from the non-infected animals. These behavioural changes proved that it was possible by these means to detect both subclinical disease as well as the onset of clinical disease. These changes in behaviour could therefore be used for the early detection of disease in an automated manner, as will be discussed below, creating the possibility of an on-farm application.

7.1 Behaviour as an indicator of disease

Both activity and posture were affected consistently after infection with *O. ostertagi* throughout the experiments (Table 7.1). The number of steps, lying episode duration and lying and standing episode frequency showed the largest magnitude of change. Changes ranged from around 14% in the case of the single, subclinical dose to around 38% for the trickle, higher doses. These results were shown to be reproducible between the different experiments and are therefore the most likely to be of practical use when used on farm situations. Previous research has commented on the value of activity to detect disease (Edwards and Tozer, 2004; Skinner et al., 2009), but also other physiological states, such

as oestrus detection in dairy cows (Kiddy, 1977). With a reduction in activity shown for a variety of health challenges, ranging from lameness (O'Callaghan et al., 2003) to micro-parasitic infections (Johnson and von Borell, 1994; Skinner et al., 2009) and metabolic disorders (Edwards and Tozer, 2004), it may be possible to use this behaviour to detect a wide variety of health challenges rather than just gastrointestinal parasitism. A similar logic applies to posture, with an increase in lying, or lying inactive, for animals having received a micro-parasitic (Escobar et al., 2007; Borderas et al., 2008) or macro-parasitic health challenge (Reiner et al., 2009) as well as in cases of lameness (Galindo and Broom, 2002). Since both activity and posture were measured by the same device (a pedometer), this creates the possibility that these behaviours can be automatically monitored and used for health screening (this is discussed further in section 7.4).

Table 7.1: Overview of the time, as measured in days, from when a reduction in average weight gain or changes in posture, activity and feeding behaviour were shown by the animals after receiving a dose of *Ostertagia ostertagi* L3 larvae on Day 0 across three different experiments, as referred to by the corresponding chapters.

	Dose (L3)	**Weight**	**Posture**	**Activity**	**Feeding**
Chapter 4	200.000	Day 16 onwards	Day 19 onwards	x	Day 19 onwards
Chapter 5	3x100.000	Day 21 onwards	Day 22 onwards	Day 21 onwards	Day 39-41
Chapter 6	3x25.000	x	x	x	x
	3x50.000	Day 27-34	x	x	x
	3x100.000	Day 27 onwards	Day 29	Day 36-46	x

x = no difference between the treatment and the control group

Despite the promising role suggested for behavioural change, in the form of activity and posture, in the early detection of disease, the issue is whether these would be affected before changes in the performance of the animals or clinical signs are detected. This was shown not to be the case in the final experiment (Chapter 6; Table 7.1), where the

medium dose infection group showed a transient effect on performance, which was not accompanied by any effects on behaviour. In practise, this means that such a growth change would be noticed for animals that are closely monitored. For animals that received the lowest subclinical dose there was no measurable effect on performance or behaviour, suggesting that these animals were able to cope with the level of infection. When the dose was increased to a high level though, there was a prolonged effect on performance and behaviour over a similar time course. The observed relationships between a health challenge and effects on performance and behaviour show that the absence of effects on behaviour does not necessarily indicate a disease-free individual.

7.2 Improvements to the experimental design

7.2.1 Increasing the quantity and quality of feeding behaviour data

The feeding data presented in this thesis were generated from videos that were watched manually. Due to this labour- and time-intensive method of analysis, not all days within the experiments could be analysed. An improvement that could therefore be made is the capture of feeding behaviour data by an automatic detection system. Despite the fact that such a system was attempted throughout the experiments, the data generated proved not to be suitable for the intended purpose. This was because the system continuously underestimated the average feeding episode duration by 38% and the total feeding episode duration by 51% when compared to the video recordings, which were considered the golden standard.

The automatic detection system which was utilised relied on the animals carrying passive transponders (RFID), attached to eartags, which would be recorded by a set of two antennae placed in front of the feeder when within range. Problems arising from the instability of RFID transponders are common (Ranasinghe and Cole, 2008). If this system was to be used, either experimentally or in practice, then it would need perfecting to generate reliable data. However, a possible way of exploiting the captured data would be to link the feeding data captured by the RFID to the activity data captured by the pedometers. In doing so, gaps in the feeding episodes could be joined up to form continuous episodes by deleting the between episode intervals within which the animal is not recorded to be active. Such inactivity would reasonably suggest that the animals have remained stationary in their previous location, the feeder. This could be a worthwhile option as this RFID system is much more cost effective then some of the other systems currently available (Tolkamp and Kyriazakis, 1997; Sowell et al., 1998; Huzzey et al.,

2007). With the system generating reliable data, it would be possible to have daily data on feeding behaviour throughout the experiments, which could possibly lead to the discovery of temporal changes in feeding behaviour that were missed in the current studies due to the limited number of days analysed.

Even more data could be generated by using equipment that monitors intake as well as all aspects of feeding behaviour. This could provide valuable additional data because both intake (Johnson and von Borell, 1994; Kyriazakis et al., 1996; Kyriazakis and Doeschl-Wilson, 2009) and feeding rate (Plata-Salamán and Borkoski, 1993; Plata-Salamán, 1994; Nielsen, 1999; González et al., 2008) are known to be affected by health challenges. Also, a decrease in feeding rate and thereby feed intake could account for the reduction in BW gain observed in the final experiment (Chapter 6), when no changes for the other parameters of feeding behaviour, such as feeding episode or meal duration and number of feeding episodes or meals within a day, were found. Similarly, monitoring daily food intake could reveal a decrease in feeding rate that could explain the increase in meal duration detected in Chapter 4 where, despite the increase in time spent at the feeder, there was still a decrease in BW gain for the infected animals. These extra data on food intake, however, comes at a price, with these systems which measure intake costing more than those that only measure feeding duration and frequency. Therefore, there would be a trade-off between the additional information provided and what the researcher or farmer could afford.

7.2.2 The occurrence of clinical signs

Part of the aim of this research was to focus on identifying and quantifying behavioural changes associated with subclinical disease. The detection of subclinical parasitic infections using behaviour was demonstrated in Chapter 4 and previously in the literature (Ferre et al., 1996; Forbes et al., 2004; 2007), albeit to a limited extent. However, for the final two experiments (Chapter 5 and 6) there were unintended clinical signs of the infection. This was most likely caused by the young age and therefore smaller size of the animals used in this experiment, in combination with an increase in infective dose. The increase in dose would have resulted in a higher number of parasites, because around 70% of each dose establishes itself in the abomasum (Durham and Elliot, 1976). However, although the loose faeces were a sign of clinical infection, in a low monitoring situation this condition is not expected to be noticed, especially if only a few animals are affected out of the whole herd.

In future experiments, due care needs to be given to ensure that the used dose (or doses) lead only to subclinical effects, although it is still possible, due to individual variation in physiological responses (Jacobsen et al., 2005), that some individuals can show clinical signs. The highest subclinical dosage used in this thesis was 200,000 L3 larvae administered as a single dose (Chapter 4). When considering this, the size of the animals will also need to be taken into account as the smaller animals which received a trickle dose of 150,000 L3 larvae (Chapter 6) did show some clinical signs of infection. Whether the infection results in clinical disease can also depend on factors other than dose and animal size, such as the quality of the feed provided (Coop and Kyriazakis, 1999; Forbes et al., 2009) and this would need to be taken into account. Although the results generated are of interest, the final experiment (Chapter 6) does raise a few questions in relation to dose. The two lower dosages showed no effect on behaviour, even though the higher of these two did show a transient effect on performance. This may imply that there is only a limited window within which subclinical disease affects behaviour to a detectable level. Clearly this would limit the value of monitoring behaviour as means of detecting disease.

7.2.3 The variation between animals

A final way to improve the methodology relates to the age and size of the animals; differences in these parameters can cause variation in the results. Between trials, and sometimes within trials, there was some variability in the origin, size, age and weight of the animals. The latter three can affect their ability to deal with a health challenge, as the larger, older and heavier animals are less affected by the same dosage when compared to smaller, younger animals (Herlich, 1980) which have a higher metabolic demand and lower energy reserves (Fisher et al., 2009). The origin (farm) of the animals is important because the previous management as well as the stress associated with the mixing of animals, can affect their health status and condition (Duff and Galyean, 2007). Even though time was allowed before the experiments began for the animals to re-establish social stability after regrouping, more agonistic behaviours were observed during casual observations in animals that were mixed two weeks before the trial, compared to those that had already been part of the same group before allocation to the experiment. In future, it would be beneficial to ensure that the animals originate from a single source and, more importantly, have been part of the same cohort. The age and weight of the animals also needs to be as closely grouped as possible to ensure less variation in the experimental outcomes, as well as reduced stress for the smaller animals associated with a low position in the hierarchy (Philips, 2002) which may be associated with bullying by

larger, more dominant animals.

7.3 Scope for future research

7.3.1 Natural infection

Following on from the results presented in this thesis on the timescale, magnitude and rate of change of the behaviours resulting from an 'artificial' parasite infection, the logical next step would be to look at the patterns generated by a natural infection. Within this thesis the method of dosing applied was that of a single, or trickle parasite dose applied on three separate occasions. However if the infection was acquired naturally it would be expected that the animal takes in a lower number of larvae on a greater number of occasions (Mansour et al., 1992) as well as a mixture of parasite species (Forbes et al., 2004). Therefore, in order to eventually progress to the early detection and the detection of subclinical parasite infection through behaviour, the magnitude and rate of change in behaviours that accompany a natural infection will need to be quantified. When the animals are infected naturally, regular faecal egg counts will need to be taken to determine the level of infection. Faecal egg counts are the normal standard for determining the level of infection (Vecruysse and Claerebout, 2001) and, even though the fecundity of the parasites is density dependent (Smith et al., 1989), it remains the most suitable method due to the mixture of species. Because it can be difficult to distinguish between species by the eggs, faecal cultures can be set up to determine the species of parasite involved and pasture samples can be taken for direct larval counts (Forbes et al., 2004). The infective species would most likely involve a combination of *Ostertagia, Cooperia* and *Trichostrongylus* (Forbes et al., 2004). A constraint arising from a natural method of infection is the variation in the response that can occur between individuals due to varying levels of infection (Stear et al., 2006). Although this is of course the closest to an on-farm scenario, it might be preferred to use a controlled environment first, where the animals are infected with a known number and combination of parasitic larvae.

Currently the only information available on changes in behaviour following a natural infection relates to feeding behaviour (Forbes et al., 2004; 2007). However, the main behavioural effects seen within the current study were on activity and posture. The effects of a natural infection on behaviour are expected to be the same as those generated here, although the magnitude and rate of the behavioural changes might differ. Depending on the species of parasite, the larvae could mature at varying timescales therefore the composition of parasite species as well as the infection level could determine the rate and

magnitude of behavioural change. When rate and magnitude of the behavioural changes follow a natural infection are known, it can be determined if these changes in activity and posture could be used for the early detection of disease.

7.3.2 Measurement of different behaviours

The behaviours measured in this thesis were chosen for their likelihood to show a measurable change when animals are faced with a (sub) clinical health challenge (Chapter 2). Furthermore, these behaviours have the potential to be monitored using automated systems which, in the future, could be applied on farm. There are, however, other aspects of behaviour with the potential to indicate disease that were not measured within this thesis due to lack of time and available equipment. Two further types of behaviour that have such potential are social behaviour and the complexity of behaviour.

Social behaviour is known to be affected by health challenges (Edwards, 1988; Gauly et al., 2007) with the responses sometimes being dependent on the type of challenge. For example, social licking was increased for dairy cows suffering from lameness (Galindo and Broom, 2002); however contact was decreased in the case of a parasitic infection in mice (Edwards, 1988). In general a reduction in social behaviours, either initiated by the infected animal or by the other unchallenged animals, is the outcome of health challenges (Renault et al., 2008). Social behaviours are most regularly monitored from video recordings using behavioural observation software such as Observer (Noldus, Wageningen, The Netherlands). However recent developments mean that there are now also automatic contact detection systems available, called proximity loggers (Swain and Bishop-Hurley, 2007; Böhm et al., 2009). These devices log the contact between an animal and other animals within its group, providing the frequency and duration of the interaction (Sirtrack Ltd., Havelock North, New Zealand). The minimal distance for readings to take place can be set between 7 to less than 1 meter between the animals (Swain and Bishop-Hurley, 2007). These data loggers do not provide information on the nature of the interaction; however a general reduction in contact (Chapter 2) between animals suffering from a micro- or macro-parasitic health challenge and their con-specifics might still prove to be of use in disease detection.

Another possible route of investigation is measurement of the complexity of behaviour. This is the pattern distribution created by the alternation between different behaviours.

This pattern tends to lose complexity (i.e. become less random) if the animal is stressed (Rutherford et al., 2003; María et al., 2004) or suffering from a parasitic infection (Alados et al., 1996; Alados and Huffman 2000). These patterns can be related to social behaviour (Alados and Huffman, 2000), the number of headlifts during feeding (Alados et al., 1996) and activity (María et al., 2004) for example. The latter could be particularly useful, as a reduction in lying and standing frequency was already apparent as a consequence of the *O. ostertagi* challenges measured in this thesis. The advantage of looking at the complexity of behaviour is that it can be affected before any difference is shown for the other, more conventional, measures of behaviour such as duration and frequency (Alados et al., 1996), and could therefore be applicable to the detection of more subtle cases of subclinical disease. However the constraint to an approach based on complexity is that this requires complex data analysis which does not, at the time of this thesis, make it suitable for automatic detection.

7.3.3 Type of health challenge

In this thesis the focus was on the changes in behaviour resulting from a chronic health challenge represented by a gastrointestinal parasite and, to a lesser extent, on those from an acute health challenge represented either by a vaccine or LPS challenge. The gastrointestinal parasite served as an example of a macro-parasitic health challenge and the LPS as an example of a micro-parasitic health challenge. Of course *O. ostertagi* does not represent all macro-parasites, and there could be a difference in the behavioural response depending on the species of parasite (Chapter 1). LPS is not actually a micro-parasite, but simply induces the same immune responses. However, there will be differences since micro-parasites that reproduce within the host can cause a more prolonged infection.

Other types of health challenges, such as a metabolic or physical, are different in their nature from the health challenges investigated in this thesis. They might therefore be expected to result in a different magnitude and rate of behavioural change because of their different effects on animal physiology. There has already been some research into the use of behaviour for the early detection of physical and metabolic health challenges, including acidosis, ketosis, and lameness (Edwards and Tozer, 2004; Mazrier et al., 2006; González et al., 2008). For both metabolic and physical health challenges, changes in activity and posture are to be expected (Galindo and Broom, 2002; Edwards and Tozer, 2004; Mazrier et al., 2006), both as a consequence of 'sickness' behaviour, but also as a sign of physical

discomfort (Weir and McNish, 1960; Weeks et al., 2000) caused by these challenges. A change in feeding behaviour would also be expected and, indeed, has been observed previously for both metabolic and physical health challenges (González et al., 2008). A difficulty accompanying research into these health challenges, however, is that their severity is more difficult to control. Therefore, with greater variance in the expression of the challenge, greater numbers of animals would be necessary in order to be able to relate the magnitude and rate of change of behaviours to the severity of the challenge.

7.4 Value to the industry

The value of this research to the industry lies mainly in the potential for capturing changes in behaviour through automated means and using these data for the purposes of disease detection. With activity and posture showing the most consistent change in response to disease, there is the possibility of monitoring these with the use of pedometers. In dairy cattle, the use of pedometers to detect activity is already widespread for oestrus detection; the output could therefore be upgraded to monitor posture as well and used in the early detection of disease. When activity level monitoring is used for the detection of oestrus, there are various ways to determine the significance of the increase in activity. Three common methods are: an increase by 1 or 2 times the standard deviation (Williams et al., 1981; Schofield et al., 1991), the percentage by which activity is increased (Eradus et al., 1992; Redden et al., 1993) or when activity passes a pre-defined value (Yang, 1998). Despite the sharp increase in activity associated with oestrus, sometimes up to 400% (Kiddy, 1977), there is still a relatively large prevalence of errors resulting in false positives, the number of which is dependent on the method used (Firk et al., 2002). This highlights a possible problem when using a similar system for the early detection of disease. Due to the nature of the experiments, this research has not looked at the diagnostic validity of the behaviour measured by conducting a sensitivity and specificity analysis. It is, however, possible that both of these can be improved if changes in a greater spectrum of behaviours are combined as means of disease detection.

The early detection of disease through activity is already commercially available and can be provided by the company producing the pedometers used in this thesis (CattleGrid, IceRobotics, South Queensferry, UK). However, the exact method which is used to achieve this is unspecified. Furthermore, such systems based on individual behavioural change would benefit from some adjustments, for example by taking into account herd dynamics or social facilitation. In order to do this, the behaviour of an individual would

need to be contrasted to that of the herd, since diurnal variations in the management or changes in the environment are likely to have significant effects on the behaviours of a group of animals (Hicks et al., 1989). The changes in the behaviour of an individual can then be contrasted with the changes that occur in the group. Another issue associated with the existing systems is the extent to which the raw data have been manipulated for quality control. The importance of this has been illustrated in research demonstrating that the short lying episodes do not always represent actual lying behaviour (Tolkamp et al., 2010). Inclusion of such episodes in the analysis of standing behaviour is likely to lead to very different conclusions as far as the behaviour of the animal is concerned. Even with the further development of automated detection systems, these errors are still likely to occur and would need to be taken into account.

When considering beef cattle, the biggest gain in the early detection of disease is to be made for animals that otherwise receive little visual monitoring. However the implementation of an automated system for the early detection of disease is an expensive affair. The pedometers that measure posture as well as activity are still in the early stages of development, which means they are still an expensive investment in a commercial setting. This could change in the future as the demand for automated monitoring increases, due to a lower number of interactions between the stockperson and the animals. Some return on this investment should accrue from the early detection of disease and the possibility of tailor made treatment of specific individuals. The benefits from targeted selective treatments have been shown for several diseases, especially in relation to reducing the development of pathogen resistance to pharmaceuticals (Wyk van et al., 2006). A variety of companies have already started producing these new generation pedometers for the commercial market, such as AfiTag (AfiMilk, Kibbutz Afikim, Israel), ENGS watch ID (ENGS, Rosh Pina, Israel) and Crystal (Fullwood, Ellesmere, UK). However, currently the pedometers used in this study (IceTag, IceRobotics, South Queensferry, UK) and possibly those mentioned above are currently not a cost effective investment for a farmer because the lifetime of the battery, if in constant use, is between 12 to 18 months. The option of changing the batteries does not yet exist, contributing towards the costs. Therefore the regular application of these systems on farm will depend on a reduction in price and/or the ability to extend their working lifetime.

Another complication that presents itself is that of the data extraction. In order to calculate the changes in behaviour to predict the early onset of disease, daily data extraction and analysis would be necessary. This is simple enough in dairy cattle as sensors can be read at the parlour, however in beef cattle such a routine is absent. A possible solution would be to construct either individual drinkers or spaces around the communal drinker with readers fitted at leg height that download the data every time the animal goes to the drinker. Such a system could work in both housed (feed lot) and outdoor (grazing) systems. Another option could be to invest in a remote monitoring system that can download information from the tags over a long range (ENGS, Rosh Pina, Israel). When cattle are kept extensively, care would need to be taken that the pedometers do not get lost or become too tight if fitted at a young age. Therefore, regular checks would be advised.

Automated monitoring of feeding behaviour is also something that could be applied on-farm. There are individual feeders which register a tag or collar fitted on the animals and measure individual feed intake and all aspects of feeding behaviour (Hoko Farm RIC feeders, Insentec BV, Marknesse, The Netherlands; GrowSafe Systems Ltd, Airdrie, Alberta, Canada). When only feeding behaviour is measured, group feeders can be used without scales in the trough (Sowell et al., 1998; De Vries et al., 2003). The advantages of both systems were discussed in paragraph 7.2.1. These systems are, of course, only applicable for indoor kept animals. The systems currently available provide data on feeding episodes, however it is not known whether they can also provide the possibility of meal data. This could be a welcome addition, as it was shown in this thesis that although feeding episodes may not show any change as a consequence of a health challenge, meal duration and frequency can be affected. However this does require the addition of some complicated algorithms that determine the meal criterion (Tolkamp et al., 2000). Furthermore, a baseline of normal feeding behaviour data would need to be taken, which could be then be used to provide the meal criterion. However, since feeding behaviour will change as the animals grow (Albright, 1993), the baseline would need to be repeatedly adjusted. It can be concluded that the automated detection of meals is unlikely to be available in the near future due to its complexity and the fact that greater change shown in activity and posture as a response to a health challenge will make it a lower priority.

From the above it can be concluded that the automated measurement of behaviour for the early detection of disease using activity and posture monitoring will be possible in the near future. One of the main factors determining the wide application of this, however, will be the system price. There are already some systems commercially available, but it is unclear which criteria these use for defining a sick animal as well as the percentage of false positives that are generated. These systems will therefore need perfecting, taking into account herd dynamics and integrating multiple behaviours, such as both activity and posture, to develop clear criteria for determining 'sickness' that provides the most reliable results. Provided that these further improvements are incorporated, automated identification of early disease onset could be a realistic prospect for the industry.

7.5 Conclusion

The contribution to science generated by the experiments within this thesis arises from a number of novel results. The timescale, magnitude and rate of change in activity and postural behaviours to an *O. ostertagi* challenge have not been described previously. Furthermore, the rate of recovery of behaviour after treatment of the animals and its diagnostic value is entirely novel information. Finally, the dose dependent nature of the behavioural response, although hypothesised upon by others, had not previously been tested. The behavioural changes associated with the *O. ostertagi* infections only started three weeks after the initial challenge, at the same time as changes in faecal egg counts and a rise in pepsinogen levels. Nonetheless, due to the magnitude of the behavioural changes, behaviour could still be a useful indicator of health status, particularly in animals that receive little visual monitoring, provided that the data can be captured by automated means. It can be concluded that for certain aspects of behaviour, namely activity and posture, there is a sizeable change when an animal is subject to a health challenge; this can aid in the early detection of disease. The possibilities exist to monitor these behaviours automatically in the future, making routine on-farm application a realistic prospect, with significant associated benefits for health management, production economy and animal welfare.

References

AFRC. 1993. *Energy and Protein Requirements of Ruminants: An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients*. CAB International, Wallingford, UK.

Agricultural board, subcommittee on beef cattle nutrition, committee on animal nutrition, national research council. 1970. *Nutrient requirements of beef cattle*. National academy of sciences, Washington, USA.

Alados, C.L., Escos J.M. and Emlen, J.M. 1996. Fractal structure of sequential behaviour patterns: An indicator of stress. *Animal Behaviour*, 51, 437-443.

Alados, C.L. and Huffman, M.A. 2000. Fractal long-range correlations in behavioural sequences of wild chimpanzees: A non-invasive analytical tool for the evaluation of health. *Ethology*, 2, 105-116.

Albright, J.L. 1993. Feeding behavior of dairy cattle. *Journal of Dairy Science*, 76, 485-498.

Almeida, P.E., Weber, P.S.D., Burton, J.L. and Zanella, A.J. 2008. Depressed DHEA and increased sickness response behaviors in lame dairy cows with inflammatory foot lesions. *Domestic Animal Endocrinology*, 34, 89-99.

Alzahal, O., Alzahal, H., Steele, M.A., Kyriazakis, I., Duffield T.F. and McBride, B.W. 2011. The use of a radio-telemetric ruminal bolus to detect body temperature changes in lactating dairy cattle. *Journal of Dairy Science*, 94, 3568-3574.

Anderson, N. 1988. Aspects of the biology of *Ostertagia ostertagi* in relation to the genesis of ostertagiasis. *Veterinary Parasitology*, 27, 13-21.

Atkinson, R.P.D., Rhodes, C.J., Macdonald, D.W. and Anderson, R.M. 2002. Scale-free dynamics in the movement patterns of jackals. *Oikos*, 98, 134-140.

Aubert, A., Goodall, G., Dantzer, R. and Gheusi, G. 1997a. Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. *Brain, Behavior, and Immunity*, 11, 107-118.

Aubert, A., Kelley, K.W. and Dantzer, R. 1997b. Differential effect of lipopolysaccharide on food hoarding behavior and food consumption in rats. *Brain, Behavior, and Immunity*, 11, 229-238.

Bach, A., Dinarés, M., Devant, M. and Carré, X. 2007. Associations between lameness and production, feeding and milkin attendance of Holstein cows milked with an automatic milking system. *Journal of Dairy Research*, 74, 40-46.

Baert, K., De Boever, S.,Duchateau, L. and De Backer, P. 2005. Sodium salicylate attenuates lipopolysaccharide (lps)-induced adipsia, but not hypophagia, in broiler chickens. *British Poultry Science*, 46, 144-148.

Balaji, R., Wright, K.J., Hill, C.M., Dritz, S.S., Knoppel, E.L. and Minton, J.E. 2000. Acute phase responses of pigs challenged orally with *Salmonella typhimurium*. *Journal of Animal Science*, 78, 1885-1891.

Bareille, N., Beaudeau, F., Billon, S., Robert, A. and Faverdin, P. 2003. Effects of health disorders on feed intake and milk production in dairy cows. *Livestock Production Science*, 83, 53-62.

Barnard, C. 2004. *Animal behaviour: Mechanism, development, function and evolution*. Pearson Education Ltd, Harlow, UK.

Bassi, G.S., Kanashiro, A., Antin, F.M., Souza de, G.E.P., Nobre, M.J. and Coimbra, N.C. 2012. Lipopolysaccharide-induced sickness behaviour evaluated in different models of anxiety and innate fear in rats. *Basic and Clinical Pharmacology and Toxicology*, 110, 359-369.

Bassingthwaighte, J.B., Liebovitch, L.S. and West, B.J. 1994. *Fractal physiology*. Oxford University Press, New York, New York. USA.

Baumont, R., Doreau, M., Ingrand, S. and Veissier, I. 2006. Feeding and mastication behaviour in ruminants. In: *Feeding in Domestic Vertrebates* (Ed. by V. Bels), pp. 241-261. CAB International, Wallingford, UK.

Bigelow, J.A. and Houpt, T.R. 1988. Feeding and drinking patterns in young pigs. *Physiology & Behavior*, 43, 99-109.

Bluthé, R., Dantzer, R. and Kelley, K.W. 1992. Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rats. *Brain Research*, 573, 318-320.

Bluthé, R.M., Michaud, B., Kelley, K.W. and Dantzer, R. 1996. Vagotomy blocks behavioural effects of interleukin-1 injected via the intraperitoneal route but not via other systemic routes. *NeuroReport*, 7, 2823-2827.

Böhm, M., Hutchings, M.R. and White, P.C.L. 2009. Contact networks in a wildlife-livestock host community: Identifying high-risk individuals in the transmission of bovine TB among badgers and cattle. *PLoS ONE*, 4, e5016.

Borderas, T.F., Passilé, A.M. and Rushen, J., 2008. Behavior of dairy calves after a low dose of bacterial endotoxin. *Journal of Animal Science*, 86, 2920-2927.

Bret-Dibat, J., Bluthé, R., Kent, S., Kelley, K.W. and Dantzer, R. 1995. Lipopolysaccharide and interleukin-1 depress food-motivated behavior in mice by a vagal-mediated mechanism. *Brain Behaviour and Immunity*, 9, 242-246.

Britton, R., Stock, R.A., Sindt, M., Oliveros, B. and Parott, C. 1990. New feed additive and technique to evaluate acidosis in cattle. *University of Nebraska, Agricultural Experiment Station*, 56, 55-58.

Burggraaf, V.T., Brooky, A.R. and Boom, C.J. 2007. Response of beef calves to different levels of postweaning ingestion of gastrointestinal parasite larvae. *Australian Journal of Experimental Agriculture*, 47, 1297-1303.

Cardot, V., Le Roux, Y. and Jurianz, S. 2008. Drinking behavior of lactating dairy cows and prediction of their water intake. *Journal of Dairy Science*, 91, 2257-2264.

Chapinal, N., Veira, D.M., Weary, D.M. and Von Keyserlingk, M.A.G. 2007. Technical note: Validation of a system for monitoring individual feeding and drinking behaviour and intake in group-housed cattle. *Journal of Dairy Science*, 90, 5732-5736.

Claerebout, E., Hilderson, H., Shaw, D.J. and Vercruysse, J. 1997. The presence of an early L4 population in relation to the acquired resistance of calves naturally infected with *Ostertagia ostertagi*. *Veterinary Parasitology*, 68, 337-346.

Coop, R.L. and Holmes, P.H. 1996. Nutrition and parasite interaction. *International Journal for Parasitology*, 26, 951-962.

Coop, R.L. and Kyriazakis, I. 1999. Nutrition-parasite interaction. *Veterinary Parasitology*, 84, 187-204.

Cottee, G., Kyriazaki, I., Widowski, T.M., Lindinger, M.I., Cant, J.P., Duffield, T.F., Osborne, V.R. and McBride, B.W. 2004. The effects of subacute ruminal acidosis on sodium bicarbonate-supplemented water intake for lactating dairy cows. *Journal of Dairy Science*, 87, 2248-2253.

Cox, D.M. and Holland, C.V. 1998. The relationship between numbers of larvae recovered from the brain of Toxocara canis-infected mice and social behaviour and anxiety in the host. *Parasitology*, 116, 579-594.

Crompton, D.W.T. 1984. Influence of parasitic infection on food intake. *Federation Proceedings*, 43, 239-245.

Cross-Mellor, S.K., Kent, W.D.T., Kavaliers, M. and Ossenkopp, K.P. 2000. Examining the effects of lipopolysaccharide and cholecystokinin on water ingestion: Comparing intake and palatability. *Brain Research*, 861, 220-232.

Dado, R.G. and Allen, M.S. 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. *Journal of Dairy Science*, 77, 132-144.

Dantzer, R., Konsman, J.P., Bluthé, R. and Kelley, K.W. 2000. Neural and humoral pathways of communication from the immune system to the brain: parallel of convergent? *Autonomic Neuroscience: Basic and Clinical*, 85, 60-65.

Dantzer, R. 2001. Cytokine-induced sickness behavior: Where do we stand? *Brain Behavior and Immunity*, 15, 7-24.

Deak, T., Bellamy, C. and Bordner, K.A. 2005. Protracted increases in core body temperature and interleukin-1 following acute administration of lipopolysaccharide: Implications for the stress response. Physiology & Behavior, 85, 296-307.

DeVries, T.J., von Keyserlingk, M.A.G., Weary, D.M. and Beauchemin, K.A. 2003. Measuring the feeding behavior of lactating dairy cows in early to peak lactation. *Journal of Dairy Science*, 86, 2254-3361.

Duff, G.C. and Galyean, M.L. 2007. Recent advances in management of highly stressed, newly received feedlot cattle. *Journal of Animal Science*, 85, 823-840.

Dewsbury, D.A. 1978. *Comparative animal behaviour*. McGraw-Hill, New York, New York, USA.

Durham, P.J.K. and Elliot, D.C. 1976. Experimental ostertagia spp. infection of sheep: development of worm populations and lesions resulting from different dose-levels of larvae. *Veterinary Parasitology*, 2, 157-166.

Edwards, J.C. 1988. The effects of *Trinchinella spiralis* infection on social interactions in mixed groups of infected and uninfected male mice. *Animal Behaviour*, 36, 529-540.

Edwards, J.L. and Tozer, P.R. 2004. Using activity and milk yield as predictors of fresh cow disorders. *Journal of Dairy Science*, 87, 524-531.

Elasser, T.H., Caperna, T.J. and Rumsey T.S. 1995. Endotoxin administration decreases plasma insulin-like growth factor (igf)-i and igf-binding protein-2 in angus x hereford

steers independent of changes in nutritional intake. *Journal of Endocrinology*, 144, 109-117.

Elasser, T.H., Richards, M., Collier, R. and Hartnell, G.F. 1996. Physiological responses to repeated endotoxin challenge are selectively affected by recombinant bovine somatotropin administration to calves. *Domestic Animal Endocrinology*, 13, 91-103.

Eradus, W.J. Rossing, W., Hogewerf, P.H. and Benders, E. 1998. Signal processing of activity data for oestrus detection in dairy cattle. *Proceeding of the International Symposium on Prospects for Automatic Milking*, 65, 360-369.

Escobar, J., Van Alstine, W.G., Baker, D.H. and Johnson, R.W. 2007. Behaviour of pigs with viral and bacterial pneumonia. *Applied Animal Behavioural Science*, 105, 42-50.

Exton, M.S. 1997. Infection-induced anorexia: Active host defence strategy. *Appetite*, 29, 369-383.

Fell, L.R., Colditz, I.G., Walker, K.H. and Watson, D.L. 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. *Australian Journal of Experimental Agriculture*, 39, 795-802.

Ferre, I., González-Gallego, J., Rojo-Vázquez, F.A. and Barrio, J.P. 1996. Effects of experimental subclincal fasciolosis on the feeding behaviour of sheep. *Journal of Animal and Feed Sciences*, 5, 135-145.

Firk, R., Stamer, E., Junge, W. and Krieter, J. 2002. Automation of oestrus detection in dairy cows: a review. *Livestock Production Science*, 75, 219-232.

Fisher, A.D., Colditz, I.G., Lee, C. and Ferguson, D.M. 2009. The influence of land transport on animal welfare in extensive farming systems. *Journal of Veterinary Behavior: Clinical Applications and Research*, 4, 157-162.

Fitzsimons, T.J. and Le Magnen, J. 1969. Eating as a regulatory control of drinking in the rat. *Journal of Comparative and Physiological Psychology*, 67, 273-283.

Fogsgaard, K.K., Røntved, C.M., Sørensen, P. and Herskin, M.S. 2012. Sickness behaviour in dairy cows during *Escherichia coli* mastitis. *Journal of Dairy Science*, 95, 630-638.

Forbes, A.B., Huckle, C.A., Gibb, M.J., Rook, A.J. and Nuthall, R. 2000. Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake and growth in young grazing cattle. *Veterinary Parasitology*, 90, 111-118.

Forbes, A.B., Huckle, C.A. and Gibb, M.J. 2004. Impact of eprinomectin on grazing behavior and performance in dairy cattle with sub-clinical gastrointestinal nematode infections under continuous stocking management. *Veterinary Parasitology*, 125, 353-364.

Forbes, A.B., Huckle, C.A. and Gibb, M.J. 2007. Evaluation of the effect of eprinomectin in young dairy heifers sub-clinically infected with gastrointestinal nematodes on grazing behaviour and diet selection. *Veterinary Parasitology*, 150, 321-332.

Forbes, A.B., Warren, M., Upjohn, M., Jackson, B., Jones, J., Charlier, J. and Fox, M.T., 2009. Associations between blood gastrin, ghrelin, leptin, pepsinogen and *Ostertagia ostertagi* antibody concentrations and voluntary feed intake in calves exposed to a trickle infection with *O. ostertagi*. *Veterinary Parasitology*, 162, 295-305.

Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E. and Jones, R.B. 2007. A critical review of fear tests used on cattle, pigs sheep poultry and horses. *Physiology & Behavior*, 91, 531-565.

Forsum, E.,Nesheim, M.C. and Crompton, D.W.T. 1981. Nutritional aspects of Ascaris infection in young protein-deficient pigs. *Parasitology*, 83, 497-512.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gil M. and Gale, D.L. 1989. *Ostertagia ostertagi* infection in the calf: Effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. *Research in Veterinary Science*, 47, 294-298.

Fox, M.T. 1993. Pathophysiology of infection with *Ostertagia ostertagi* in cattle. *Veterinary Parasitology*, 46, 143-158.

Fox, M.T., Uche, U.E., Vaillant, C., Ganabadi, S. and Calam, J. 2002. Effects of *Ostertagia ostertagi* and omeprazole treatment on feed intake and gastrin-related responses in the calf. *Veterinary Parasitology*, 105, 285-301.

Fritz, H., Said, S. and Weimerskirch, H. 2003. Scale-dependent hierarchical adjustments of movement patterns in a longe-range foraging seabird. *Proceedings of the Royal Society B: Biological Sciences*, 270, 1143-1148.

Galindo, F. and Broom, D.M. 2000. The relationship between social behaviour of diary cows and the occurrence of lameness in three herds. Research in Veterinary Science, 69, 75-79.

Galindo, F. and Broom, D.M. 2002. The effects of lameness on social and individual behavior of dairy cows. *Journal of Applied Animal Welfare Science*, 5, 193-201.

Gauly, M., Duss, C. and Erhardt, G. 2007. Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (*Gallus gallus domesticus*). *Veterinary Parasitology*, 146, 271-280.

Goldhawk, C., Chapinal, N., Veira, D.M., Weary, D.M. and Von Keyserlingk, M.A.G. 2009. Prepartum feeding behaviour is an early indicator of subclinical ketosis. *Journal of Dairy Science*, 92, 4971-4977.

González, L. A., Tolkamp, B.J., Coffey, M.P., Ferret, A. and Kyriazakis, I. 2008. Changes in feeding behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. *Journal of Dairy Science*, 91, 1017-1028.

Gougoulis, D.A., Kyriazakis, I. and Fthenakis, G.C. 2010. Diagnostic significance of behaviour changes of sheep: A selected review. *Small Ruminant Research*, 92, 52-56.

Goujon, E., Parnet, P., Aubert, A., Godall, G. and Dantzer, R. 1995. Coricosterone regulates behavioral effects of lipopolysaccharide and interleukin-1β in mice. *American*

Journal of Physiology – Regulatory Integrative and Comparative Physiology, 269, 154-159.

Greer, A.W., Stankiewicz, M., Jay, N.P., McAnulty, R.W. and Sykes, A.R. 2005. The effect of concurrent corticosteroid induced immune-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on food intake and utilization in both immunologically naïve and competent sheep. *Animal Science*, 80, 89-99.

Hanstock, T.L., Clayton, E.H., Li, K.M. and Mallet, P.E. 2004. Anxiety and aggression associated with the fermentation of carbohydrates in the hindgut of rats. *Physiology & Behavior*, 82, 357-368.

Hart, B.L. 1988. Biological basis of the behavior of sick animals. *Neuroscience & Biobehavioral Reviews*, 12, 123-137.

Hart, B.L. 1990. Behavioral adaptations to pathogens and parasites: Five strategies. *Neuroscience & Biobehavioral Reviews*, 14, 273-294.

Hausdorff, J.M., Mitchell, S.L., Firtion, R., Peng, C.K., Cudkowiczm, M.E., Wei, J.Y. and Goldberger, A.L. 1997. Altered fractal dynamics of gait: Reduced stride-interval correlations with aging and Huntington's disease. *Journal of Applied Physiology*, 82, 262-269.

Henryon, M., Berg, P., Jensen, J. and Andersen, S. 2001. Genetic variation for resistance to clinical and subclinical diseases exists in growing pigs. *Animal Science*, 73, 375-387.

Herlich, H. 1980. *Ostertagia ostertagi* infection and reinfection in cattle of different ages. *American Journal of Veterinary Research*, 41, 259-261.

Hessing, M.J.C., Hagelsø, A.M., Beek van, J.A.M., Wiepkema, R.P., Schouten, W.G.P. and Krukow, R. 1993. Individual behavioural characteristics in pigs. Applied Animal Behaviour Science, 37, 285-295.

Hicks, R.B., Owens, F.N. and Gill, D.R. 1989. Behavioral patterns of feedlot steers. *Miscellaneous publication - Agricultural Experiment Station, Oklahoma State University*, MP-127, 94-105.

Hilderson, H., Vercruysse, J., de Graaf, D.C., Bastiaensen, P., Fransen, J. and Berghen, P. 1993. The presence of an early L_4 larvae population in relation to the immune response of calves against *Ostertagia ostertagi*. *Veterinary Parasitology*, 47, 255-266.

Hopster, H. 1998. Coping strategies in dairy cows. PhD thesis.

Houdijk, J.G.M., Campbell, F.M., Fortomaris, P.D., Eckersall, P.D. and Kyriazakis, I. 2007. Effects of sub-clinical post weaning colibacillosis and dietary protein on acute phase proteins in weaner pigs. *Livestock Science*, 108, 182-185.

Hutchings, M.R., Gordon, I.J., Robertson, E., Kyriazakis, I. and Jackson, F. 2000. Effects of parasitic status and level of feeding motivation on the diet selected by sheep grazing grass/clover swards. *Journal of Agricultural Science*, 135, 65-75.

Huzzey, J.M., Veira, D.M., Weary, D.M. and Von Keyserlingk, M.A.G. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. *Journal of Dairy Science*, 90, 3220-3233.

Jacobsen, S., Toelboell, T. and Andersen, P.H. 2005. Dose dependency and individual variability in selected clinical, haematological and blood biochemical responses after systemic lipopolysaccharide challenge in cattle. *Veterinary Research*, 36, 167-178.

Johnson, P., Neal, R.A. and Gall, D. 1963. Protective effect of killed trypanosome vaccines with incorporated adjuvants. *Nature*, 200, 83.

Johnson, R.F. and Johnson, A.K. 1990. Light-dark cycle modulates drinking to homeostatic challenges. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology*, 259, 1035-1042.

Johnson, R.W., Curtis, S.E., Dantzer, R., Bahr, J.M. and Kelley, K.W. 1993. Sickness behavior in birds caused by peripheral or central injection of endotoxin. *Physiology & Behavior*, 53, 343-348.

Johnson, R. W. and von Borell, E. 1994. Lipopolysaccharide-induced sickness behaviour in pigs is inhibited by pretreatment with indomethacin. *Journal of Animal Science*, 72, 309-314.

Jones, R.B., Blokhuis, H.J. and Beuving, G. 1995. Open-field and tonic immobility responses in domestic chicks of two genetic lines differing in their propensity to feather peck. *British Poultry Science*, 36, 525-530.

Kaufman, J. 1996. *Parasitic infections of domestic animals*. pp. 92. Birkhäuser, Basel, Switzerland.

Kidane, A., Houdijk, J.G.M., Tolkamp, B.J., Athanasiadou, S. and Kyriazakis, I. 2009. Consequences of infection pressure and protein nutrition on periparturient resistance to *Teladorsagia circumcincta* and performance in ewes. *Veterinary Parasitology*, 165, 78-87.

Kiddy, C.A. 1977. Variation in physical activity as an indication of estrus in dairy cows. *Journal of Dairy Science*, 60, 235-243.

Kilgour, R.J., Melville, G.J. and Greenwood, P.L. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans, restraint and novelty. *Applied Animal Behaviour Science*, 99, 21-40.

Koolhaas, J.M., Korte, S.M., Boer de, S.F., Vegt van der, B.J., Reenen van, C.G., Hopster, H., Jong de, I.C., Ruis, M.A.W. and Blokhuis, H.J. 1999. Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, 23, 925-935.

Koolhaas, J.M. 2008. Coping style and immunity in animals: Making sense of individual variation. *Brain Behavior and Immunity*, 22, 662-667.

Kramer, E., Cavero, D., Stamer, E. and Krieter, J. 2009. Mastitis and lameness detection in dairy cows by application of fuzzy logic. *Livestock Science*, 125, 92-96.

Kyriazakis, I., Oldham, J.D., Coop, R.L and Jackson, F. 1994. The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. *British Journal of Nutrition*, 72, 665-677.

Kyriazakis, I., Anderson, D.H., Oldam, J.D., Coop, R.L. and Jackson, F. 1996. Long-term subclinical infection with *Trichostrongylus colubriformis:* Effects on food intake, diet selection and performance of growing lambs. *Veterinary Parasitology*, 61, 297-313.

Kyriazakis, I., Tolkamp, B.J. and Hutchings, M.R. 1998. Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Animal Behaviour*, 56, 265-274.

Kyriazakis, I. 2003. The control and prediction of food intake in sickness and in health. In: *Perspectives in Pig Science*, (Ed. by J. Wiseman, M.A. Varley, and B. Kemp), pp. 381-404. Nottingham University Press, Nottingham, UK.

Kyriazakis, I. and Doeschl-Wilson, A. 2009. Anorexia during infection in mammals: variation and its sources. In: *Voluntary feed intake in pigs* (Ed. by D. Torrallardona & E. Roura), pp. 307-321. Wageningen Academic Publishers, Wageningen, The Netherlands.

Kyriazakis, I. 2010. Is anorexia during infection in animals affected by food composition? *Animal Feed Science and Technology*, 156, 1-9

Kyriazakis, I. and Tolkamp, B.J. 2010. Disease. In: *The encyclopedia of applied animal behaviour and welfare* (Ed. by D.S. Mills), pp. 176-177. CAB International, Wallingford, UK.

Kyriazakis, I. and Tolkamp, B.J. 2011. Hunger and thirst. In: *Animal Welfare* (Ed. by M.C. Appleby, J.A. Mench, I.A.S. Olsson & B.O. Hughes), pp. 44-63 CAB International, Wallingford, UK.

Larson, S.J., Collins, S.M. and Weingarten, H.P. 1996. Dissociation of temperature changes and anorexia after experimental colitis and LPS administration in rats. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 271, 967-972.

Larson, S. J. and Dunn, A.J. 2001. Behavioral effects of cytokines. *Brain Behaviour and Immunity*, 15, 371-387.

Lawton, D.E.B., Reynolds, G.W., Hodgkinson, S.M., Pomroy, W.E. and Simpson, H.V. 1996. Infection of sheep with adult and larval *Ostertagia circumcincta*: effects on abomasal pH and serum gastrin and pepsinogen. *International Journal for Parasitology*, 26, 1063-74.

Lukas, J.M., Reneau, J.K. and Linn, J.G. 2008. Water intake and dry matter intake changes as a feeding management tool and indicator of health and estrus status in dairy cows. *Journal of Dairy Science*, 91, 3385-3394.

Madsen, T.N. and Kristensen, A.R. 2005. A model for monitoring the condition of young pigs by their drinking behaviour. *Computers and Electronics in Agriculture*, 48, 138-154.

Mansour, M.M., Dixon, J.B., Rowan, T.G. and Carter, S.D. 1992. Modulation of calf immune responses by *Ostertagia ostertagi*: the effect of diet during trickle infection. *Veterinary Immunology and Immunopathology*, 33, 261-269.

María, G.A., Escos, J. and Alados, C.L. 2004. Complexity of behavioural sequences and their relation to stress conditions in chickens (*Gallus gallus domesticus*): a non-invasive technique to evaluate animal welfare. *Applied Animal Behaviour Science*, 86, 93-104.

Marsh, M.K., Hutchings, M.R., McLeod, S.R. and White, P.C.L. 2011. Spatial and temporal heterogeneities in the contact behaviour of rabbits. *Behavioral Ecology and Socio-biology*, 65, 183-195.

Matthews, J.N.S., Altman, D.G., Campbell, M.J. and Royston, P. 1990. Analysis of serial measurements in medical research. *British Medical Journal*, 300, 230-235.

Mayes, E. and Duncan, P. 1986. Temporal patterns of feeding behaviour in free-ranging horses. *Behaviour*, 96, 105-129.

Mazrier, H., Shlomit, T., Aizinbud, E. and Bargai, U. 2006. A field investigation of the use of the pedometer for the early detection of lameness in cattle. *Canadian Veterinary Journal*, 47,883-886.

Mercer, J.G., Mitchell, P.I., Moar, K.M., Bissett, A., Geissler, S., Bruce, K. and Chappell, L.H. 2000. Anorexia in rats infected with the nematode, *Nippostrongylus brasiliensis*: experimental manipulations. *Parasitology*, 120, 641-647.

Michel, J.F. 1969a. Some observations on the worm burdens of calves infected daily with *Ostertagia ostertagi*. *Parasitology*, 59, 575-95.

Michel, J.F. 1969b. The regulation of egg output by *Ostertagia ostertagi* in calves infected once only. *Parasitology*, 59. 767-74.

Ministry of Agriculture, Fisheries and Food. 1977. *Manual of veterinary parasitological laboratory techniques*. London, UK.

Morgan, C.A., Tolkamp, B.J., Emmans, G.C. and Kyriazakis, I. 2000. The way in which the data are combined affects the interpretation of short-term feeding behavior. *Physiology & Behavior*, 70, 391-396.

Morrison, R.S., Hemsworth, P.H., Cronin, G.M. and Campbell, R.G. 2003. The social and feeding behaviour of growing pigs in deep-litter, large group housing systems. *Applied Animal Behaviour Science*, 82, 173-188.

Motohashi, Y., Miyazaki, Y. and Takano, T. 1993. Assessment of behavioral effects of tetrachloroethylene using a set of time-series analysis. *Neurotoxicology and Teratology*, 15, 3-10.

Mülleder, C., Troxler, J. and Waiblinger, S. 2003. Methodological aspects for the assessment of social behaviour and avoidance distance on dairy farms. *Animal Welfare*, 12, 579-584.

Murray, M., Jennings, F.W. and Armour, J. 1970. Bovine ostertagiasis: structure, function and mode of differentiation of the bovine gastric mucosa and kinetics of the worm loss. *Research in Veterinary Science*, 11, 417-427.

Nielsen, B.L. 1999. On the interpretation of feeding behaviour measures and the use of feeding rate as an indicator of social constraint. *Applied Animal Behaviour Science*, 63, 79-91.

Noldus, L.P.J.J. 1991. The Observer: A software system for collection and analysis of observational data. *Behavior Research Methods, Instruments & Computers*, 23, 415-429.

Noldus, L.P.J.J., Spink, A.J. and Tegelenbosch, R.A.J. 2001. EthoVision: A versatile video tracking system for automation of behavioral experiments. *Behavior Research Methods*, 33, 398-414.

O'Callaghan, K.A., Cripps, P.J., Downham, D.Y. and Murray, R.D. 2003. Subjective and objective assessment of pain and discomfort due to lameness in dairy cattle. *Animal Welfare*, 12, 605-610.

Oirschot van, J.T., Kaashoek, M.J. and Rijsewijk, F.A.M. 1996. Advances in the development and evaluation of bovine herpesvirus 1 vaccines. *Veterinary Microbiology*, 53, 43-54.

Oirschot van, J.T., Bruschke, C.J.M. and Rijn van, P.A., 1999. Vaccination of cattle agains bovine viral diarrhoea. *Veterinary Microbiology*, 64, 169-183.

Owens, F.N., Secrist, D.S., Hill, W.J. and Gill, D.R. 1998. Acidosis in cattle: A review. *Journal of Animal Science*, 76, 275-286.

Paynter, D.I. 1992. Pepsinogen activity; determination in serum and plasma. In: *Australian Standard Diagnostic Techniques for Animal Diseases*. Csiro, Australia.

Philips, C. 2002. *Cattle behaviour & welfare*. pp. 105. Blackwell science ltd., Oxford, UK.

Pinheiro Machado Filho, L.C., Teixeira, D.L., Weary, D.M., Von Keyserlingk, M.A.G. and Hötzel, M.J. 2004. Designing better water troughs, Dairy cows prefer and drink more from larger troughs. *Applied Animal Behaviour Science*, 89, 185-193.

Plaizier, J.C., Krause, D.O., Gozho, G.N. and McBride, B.W. 2009. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *The Veterinary Journal*, 176, 21-31.

Plata-Salamán, C.R., Oomura, Y. and Kai, Y. 1988. Tumor necrosis factor and interleukin-1β: suppression of food intake by direct action in the central nervous system. *Brain Research*, 448, 106-114.

Plata-Salamán, C.R. and Borkoski, J.P. 1993. Centrally administered bacterial lipopolysaccharide depresses feeding in rats. *Pharmacology Biochemistry and Behavior*, 46, 787-791.

Plata-Salamán, C.R. 1994. Meal patterns in response to the intracerebroventricular administration of interleukin-1β in rats. *Physiology & Behavior*, 55, 727-733.

Plata-Salamán, C.R. 1998. Cytokine-induced anorexia: Behavioral, cellular, and molecular mechanisms. *Annals of the New York Academy of Sciences*, 856, 160-170.

Poppi, D.P., Sykes, A.R. and Dynes, R.A. 1990. The effect of endoparasitism on host nutrition – The implications for nutrient manipulation. *Proceedings of the New Zealand Society of Animal Production*, 50, 237-243.

Prange, S., Jordan, T., Hunter, C., and Gehrt, S.D. 2006. New radiocollars for the detection of proximity among individuals. *Wildlife Society Bulletin*, 34, 1333-1344.

Quimby, W. F., Sowell, B.F., Bowman, J.P.G., Branine, M.E., Hubbert, M.E. and Sherwood, H.W. 2001. Application of feeding behaviour to predict morbidity of newly received calves in a commercial feedlot. *Canadian Journal of Animal Science*, 81, 315-320.

Ranasinghe, D.C. and Cole, P.H. 2008. Networked RFID systems. In: Networked RFID systems and lightweight crypography (Ed. by D.C. Ranasinghe and P.H. Cole), pp. 45-58. Springer, Heidelberg, Germany.

Redden, K.D., Kennedy, A.D., Ingalls, J.R. and Gilson, T.L. 1993. Detection of oestrus by radiotelemetric monitoring of vaginal and ear skin temperature and pedometer measurements of activity. *Journal of Dairy Science*, 76, 713-721,

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A. and Pollmächer, T. 2001. Cytokine-associated emotional and cognitive disturbances in humans. *Archives of General Psychiatry*, 58, 445-452.

Reiner, G., Hübner, K. and Hepp, S. 2009. Suffering in diseased pigs as expressed by behavioural, clinical and clinical-chemical traits, in a well defined parasite model. *Applied Animal Behaviour Science*, 118, 222-231.

Renault, J., Gheusi, G. and Aubert, A. 2008. Changes in social exploration of a lipopolysaccharides-treated conspecific in mice: Role of environmental cues. *Brain Behavior and Immunity*, 22, 1201-1207.

Rex, A., Voigt, J.P., Gustedt, C., Beckett, S. and Fink, H. 2004. Anxiolytic-like profile in Wistar, but not Sprague-Dawley rats in the social interaction test. *Psychopharmacology*, 177, 23-34.

Rinaldi, M. and Geldof, P. 2012. Immunologically based control strategies for ostertagiosis in cattle: Where do we stand? *Parasite Immunology*, 34, 254-264.

Roberts, H.C., Hardie, L.J., Chappell, L.H. and Mercer, J.G. 1999. Parasite-induced anorexia: Leptin, insulin and corticosterone responses to infection with the nematode, *Nippostrongylus brasiliensis*. *Parasitology*, 118, 117-123.

Rook, A.J. and Huckle, C.A. 1997. Activity bout criteria for grazing dairy cows. *Applied Animal Behaviour Science*, 54, 89-96.

Rose, J.H. 1969. The development of the parasitic stages of *Ostertagia ostertagi*. *Journal of Helmintology,* 63, 173-184.

Rossi, R. and Scharrer, E. 1992. Circadian patterns of drinking and eating in pygmy goats. *Physiology & Behavior*, 51, 895-897.

Russell, R.W., Hunt Jr, G.L., Coyle, K.O. and Cooney, R.T. 1992. Foraging in a fractal environment: Spatial patterns in a marine predator-prey system. *Landscape Ecology*, 7, 195-206.

Rutherford, K.M.D., Haskell, M.J., Glasbey, C., Jones, R.B. and Lawrence, A.B. 2003. Detrended fluctuation analysis of behavioural responses to mild acute stressors in domestic hens. *Applied Animal Behaviour Science*, 83, 125-139.

Sandberg, F.B., Emmans, G.C. and Kyriazakis, I. 2006. A model for predicting food intake of growing animals during exposure to pathogens. *Journal of Animal Science,* 84, 1552-1566.

Schein, M.W. and Fohrman, M.H. 1955. Social dominance relationships in a herd of dairy cattle. *The British Journal of Animal Behaviour*, 3, 45-55.

Schofield, S.A., Phillips, C.J.C. and Owens, A.R. 1991. Variation in milk production, activity rate and electrical impedance of cervical mucus over the oestrus period of dairy cows. *Animal Reproduction Science,* 24, 231-248.

Sibly, R.M., Nott, H.M.R. and Fletcher, D.J. 1990. Splitting behaviour into bouts. *Animal Behaviour*, 39, 63-69.

Skinner, G.W., Mitchell, D. and Harden, L.M. 2009. Avoidance of physical activity is a sensitive indicator of illness. *Physiology & Behavior*, 96, 421-427.

Smith, G., Grenfell, T. and Anderson, R.M. 1987. The regulation of *Ostertagia ostertagi* in calves: density-dependent control of fecundity. *Parasitology*, 95, 373-388.

Sowell, B.F., Bowman, J.P.G., Branine, M.E. and Hubbert, M.E. 1998. Radio frequency technology to measure feeding behaviour and health of feedlot steers. Applied Animal Behaviour Science, 59, 277-284

Sowell, B.F., Branine, M.E., Bowman, J.P.G., Hubbert, M.E., Sherwood, H.E. and Quimby, W.F. 1999. Feeding and watering behavior of healthy and morbid steers in a commercial feedlot. *Journal of Animal Science*, 77, 1105-1112.

Stear, M.J., Abuagob, O., Benothman, M., Bishop, S.C., Innocent, G., Kerr, A. and Mitchell, S. 2006. Variation among faecal egg counts following natural nematode infection in Scottish Blackface lambs. *Parasitology*, 132, 275-280.

Steiger, M., Senn, M., Altreuther, G., Werling, D., Sutter, F., Kreuzer, M. and Langhans, W. 1999. Effect of a prolonged low-dose lipopolysaccharide infusion on feed intake and metabolism in heifers. *Journal of Animal Science*, 77, 2523-2532.

Swain, D. L., Wilson, L.A. and Dickinson, J. 2003. Evaluation of an active transponder system to monitor spatial and temporal location of cattle within patches of a grazed sward. *Applied Animal Behaviour Science*, 84, 185-195.

Swain, D.L., and Bishop-Hurley, G.J. 2007. Using contact logging devices to explore animal affiliations: Quantifying cow-calf interactions. *Applied Animal Behaviour Science*, 102, 1-11.

Takeuchi, Y., Kikusui, T. and Mori, Y. 1995. Changes in the behavioral parameters following the lipopolysaccharide administration in goats. *Journal of Veterinary Medical Science / Japanese Society of Veterinary Science*, 57, 1041-1044.

Timsit, E., Assie, S., Quiniou, R., Seegers, H.and Bareille, N. 2011. Early detection of bovine respiratory disease in young bulls using reticulo-rumen temperature boluses. *Veterinary Journal*, 190, 136-142.

Tizard, I. 2004. *Veterinary immunology: An introduction*. pp. 148. W.B. Saunders Co, Philadelphia, Pennsylvania.

Togersen, F. A., Skjoth, F., Munksgaard, L. and Hojsgaard, S. 2010. Wireless indoor tracking network based on Kalman filters with an application to monitoring dairy cows. *Computers and Electronics in Agriculture*, 72, 119-126.

Tolkamp, B.J. and Kyriazakis, I. 1997. Measuring diet selection in dairy cows: effect of training on choice of dietary protein level. *Animal Science*, 64, 197-207.

Tolkamp, B.J., Allcroft, D.J., Austin, E.J., Nielsen, B.L. and Kyriazakis, I. 1998. Satiety splits feeding behaviour into bouts. *Journal of Theoretical Biology*, 194, 235-250.

Tolkamp, B.J. and Kyriazakis, I. 1999. To split behaviour into bouts, log-transform the intervals. *Animal Behaviour*, 57, 807-817.

Tolkamp, B.J., Schweitzer, D.P.N. and Kyriazakis, I. 2000. The biologically relevant unit for the analysis of short-term feeding behavior of dairy cows. *Journal of Dairy Science*, 83, 2057-2068.

Tolkamp, B.J., Haskell, M.J., Langford, F.M., Roberts, D.J. and Morgan, C.A. 2010. Are cows more likely to lie down the longer they stand? *Applied Animal Behaviour Science*, 124, 1-10.

Tolkamp, B.J., Allcroft, D.J., Barrio, J.P., Bley, T.A.G., Howie, J.A., Jacobsen, T.B., Morgan, C.A., Schweitzer, D.P.N., Wilkinson, S., Yeates, M.P. and Kyriazakis, I. 2011. The temporal structure of feeding behavior. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology*, 301, R378-393.

Torrao, N. A., Hetem, R.S., Meyer, L.C.R. and Fick, L.G. 2011. Assessment of the use of temperature-sensitive microchips to determine core body temperature in goats. *Veterinary Record*, 168, 328-334.

Urton, G., Von Keyserlingk, M.A.G. and Weary, D.M. 2005. Feeding behavior identifies dairy cows at risk for metritis. *Journal of Dairy Science*, 88, 2843-2849.

Val-Laillet, D., Guesdon, V., Von Keyserlingk, M.A.G., Passillé, A.M. and Rushem J. 2009. Allogrooming in cattle: Relationships between social preferences, feeding displacements and social dominance. *Applied Animal Behaviour Science*, 116, 141-149.

Vandeputte- Van Messom, G., Burvenich, C., Roets, E., Massart-Leën, A.M., Heyneman, R., Kremer, W.D. and Brand, A. 1993. Classification of newly calved cows into moderate and severe responders to experimentally induced *Escherichia coli* mastitis. *Journal of Dairy Research*, 60, 19-29.

Vercruysse, J. and Claerebout, E. 2001. Treatment vs non-treatment of helminth infections in cattle: defining the threshold. *Veterinary Parasitology*, 98, 195-214.

Viswanathan, G.M., Afanasyev, V., Buldyrev, S.V., Murphy, E.J., Prince, P.A. and Stanley, H.E. 1996. Lévy flight search patterns of wandering albatrosses. *Nature*, 381, 413-415.

Walk, C.L., Cowieson, A.J., Remus, J.C., Novak, C.L. and McElroy, A.P. 2011. Effects of dietary enzymes on performance and intestinal goblet cell number of broilers exposed to a live coccidian oocyst vaccine. *Poultry Science*, 90, 91-98.

Ward, W.R. 2001. Lameness in dairy cattle. *Irish Veterinary Journal*, 54, 129-139.

Warren, E.J., Finck, B.N., Arkins, S., Kelley, K.W., Scamurra, R.W., Murtaugh, M.P. and Johnson, R.W. 1997. Coincidental changes in behavior and plasma cortisol in unrestrained pigs after intracerebroventricular injection of tumor necrosis factor-α. *Endocrinology*, 138, 2365-2371.

Weary, D.M., Huzzey, J.M. and von Keyserlingk, M.A.G., 2009. Using behaviour to predict and identify ill health in animals. *Journal of Animal Science*, 87, 770-777.

Weeks, C.A., Danbury, T.D., Davies, H.C., Hunt, P. and Kestin, S.C. 2000. The behaviour of broiler chickens and its modification by lameness. *Applied Animal Behaviour Science*, 67, 111-125.

Weir, G.K. and McNish, W.C. 1960. Displaced abomasum in cattle. *Canadian Veterinary Journal*, 1, 501-503.

West, B.J. and Goldberger, A.L. 1987. Physiology in fractal dimensions. *American Scientist*, 75, 354-365.

West, M.A. and Heagy, W. 2002. Endotoxin tolerance: A review. *Society of Critical Care Medicine*, 30, S64-S73.

White, H.S. 1980. Iron-hemoglobin. In: *Nutrition and the adult* (Ed. by R.B. Alfin-Slater and D. Kritchervsky). Plenum Press. University of Michigan, Michigan, US.

Williams, W.F., Yver, D.R. and Gross, T.S. 1981. Comparison of oestrus detection techniques in dairy heifers. *Journal of Diary Science*, 1738-1741.

Wright, C.E., Strike, P.C., Brydon, L. and Steptoe, A. 2005. Acute inflammation and negative mood: Mediation by cytokine activation. *Brain Behaviour and Immunity*, 19, 345-350.

Wyk van, J.A., Hoste, H., Kaplan, R.M. and Besier, R.B. 2006. Targeted selective treatment for worm management – How do we sell rational programs to farmers? *Veterinary Parasitology*, 139, 336-346.

Yang, Y. 1998. Rechnergestützte östrusüberwachung bei milchkühen unter anwendung der fuzzy-logic methode. As referenced from Firk et al., 2002.

Yeates, M. P., Tolkamp, B.J., Allcroft, D.J., Kyriazakis, I., 2001. The use of mixed distribution models to determine bout criteria for analysis of animal behaviour. *Journal of Theoretical Biology*, 213, 413-425.

Yeates, M.P., Tolkamp, B.J. and Kyriazakis, I. 2003. Consequences of variation in feeding behaviour for the probability of animals starting a meal as estimated from pooled data. *Animal Science*, 77, 471-484.

Yirmiya, R. 1995. Endotoxin produces a depressive-like episode in rats. *Brain Research*, 711, 163-174.

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G. and Kyriazakis, I. 2008. Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds. *Journal of Animal Science*, 86, 1891-1903.

Zorrilla, E.P., Inoue, K., Fekete, E.M., Tabarin, A., Valdaz, G.R. and Koob, G.F. 2005. Measuring meals: Structure of prandial food and water intake of rats. American Journal of Physiology – Regulatory Integrative and Comparative Physiology, 288, 1450-1467.