

**ILLNESS AND MILK FEEDING LEVEL EFFECTS ON CALF  
BEHAVIOUR**

by

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## ABSTRACT

A consequence of the high rates of morbidity and mortality of calves on North American dairy farms is the necessity to develop tools for early identification of sickness. As the trend to group-housing increases, the use of automated feeding systems eases the study of feeding behaviour of individual calves within the group. Little is known about the behavioural changes associated with the onset of disease in dairy calves, especially changes in feeding and resting behaviour. The first study of this dissertation examined the effect of milk feeding level on the feeding and resting behaviour of group-housed dairy calves fed with an automated feeding system. In two separate experiments, calves allowed high levels of milk (ad libitum milk replacer and 12 L/d milk) showed reduced frequency of visits to the milk feeder with visits spread throughout the day and a low intake of concentrate until weaning. Low-fed calves (4 L/d milk or milk replacer) had a high frequency of visits to the milk feeder, however the majority of these visits (~ 90%) were unrewarded (i.e. no milk was served) and resulted in increased milk feeder occupancy times compared to high fed calves. Calves fed low levels of milk also spent less time lying down at 4 to 5 wks of age than high fed calves, probably due to the increased number of visits to the milk feeder. No differences in the incidence of illness were found between treatments. These results provide evidence that milk feeding level affects the expression of feeding behaviour, so it must be considered when assessing behavioural changes related to illness. A second study set out to explore the use of a supplemental heat source by sick calves on preference and lying behaviour. During their first 3 d of life calves fed high (12 L/d) or low (4 L/d) levels of milk did not differ in lying times nor did they show differences in preference for the use of the external source of heat. Although no calves were diagnosed with illness during the experiment, calves showed a marked preference for heat, spending more than 50% of their time under heat lamps regardless of environmental temperature fluctuations. In the third study, I set out to determine which behaviours were more susceptible to change in calves afflicted with mild sickness. Calves injected with a mild dose of endotoxin showed a decrease in the time spent at the hayrack, time spent self-grooming and ruminating. Moreover, when sickness was induced, calves showed an increase in the time spent lying and standing inactive. Lastly, the feeding behaviour of naturally sick calves and healthy counterparts fed high or low levels of milk from 4 previous studies was investigated. Sick calves fed high levels of milk showed a decrease in milk intake, visits to the milk feeder and duration of the visits during the day disease was diagnosed and during the subsequent 3 d. Sick low-fed calves only showed a reduction in the duration of the visits to the milk feeder. In conclusion, milk feeding level plays an important role in the understanding of the behavioural changes occurring at the onset of disease. Providing an external source of heat may increase the welfare of newborn calves and may also prove to be a useful tool for identifying sick animals, but further validation studies involving sick calves are needed. Monitoring reductions in milk intake and visits to the automatic feeder in high milk fed calves may be a useful measure in identifying sick calves. In contrast, other behavioural indicators of activity level, such as standing or lying down, may be more sensitive when identifying sick calves fed low levels of milk.

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## **CO-AUTHORSHIP STATEMENT**

I am the main author of all five chapters in this dissertation and prepared the entire manuscript including tables and figures. The following co-authors were involved in the design, analysis of data and preparation of the manuscripts included in this thesis: A.M.B. de Passillé and J. Rushen for the first four chapters, and they and M.A.G. von Keyserlingk for the fifth chapter.

# **CHAPTER 1. INTRODUCTION**

## **1.1 General introduction**

There has been an increasing public concern for the welfare of farm animals in the recent years (Harper and Henson, 2001). This concern is affecting the choices made by consumers of animal products and putting pressure on politicians to introduce animal welfare legislation. For example, the biggest North American fast food chains recently stated that their suppliers must follow welfare standards set by a panel of experts (Hewson, 2003). In the European Union (EU), the animal welfare legislation is one of the strongest in the world and some pressure is exerted to limit imports of animal products that do not follow EU legislation (World Trade Organisation, 2000).

Until recently, there has been little research on dairy calves, perhaps due to their low monetary value and the absence of a clear link with productivity in adult animals. There also appears to be little emphasis placed by dairy producers on obtaining accurate estimates of calf mortality. The USDA Dairy 2007 report (USDA, 2007) estimated an overall pre-weaned calf mortality of 7.8 % in the US, with most deaths occurring in the first two weeks of life. Although pre-weaned calf mortality has decreased slightly compared to figures in previous USDA reports (8.7 % in 2002 and 10.8 % in 1996), this mortality rate is still high compared to other farm species. These data, however, are collected by means of a survey circulated to producers, which can introduce an important bias if calf value is neglected. Dairy producers appear to routinely underestimate calf mortality rates and not surprisingly rank calf disease low in terms of economic value (Goodger and Theodore, 1986). Disease also affects calf welfare (Duncan and Dawkins,

1983) and prevention, early diagnoses and timely treatment of diseases, would minimise negative effects on the animal.

Dairy calves are commonly separated from their dam immediately after birth and fed either milk replacer or waste milk until they are weaned. Traditionally these milk fed calves were individually housed and fed a restricted amount of milk (Broom, 1991). One perceived advantage of individual housing of calves is the ease of disease detection. There is, however, a growing tendency to house calves in groups, where sickness detection may be more difficult (Chua et al., 2002). The move toward group housing combined with the need to reduce calf mortality rates suggests that there is a need to develop tools for the prodromal or early stage of disease diagnosis in young dairy calves to enable timely treatment. The challenge remains in identifying calves at risk for succumbing to disease prior to the clinical stages of the disease, particularly in group-housed situations. As automated milk feeders keep records of the feeding behaviour of all individuals in a group, one promising avenue is to identify behavioural changes associated with the onset of illness [sickness behaviour as defined by Hart (1988)].

This introduction provides a comprehensive review of the current literature on sickness behaviour in dairy calves. In the first part, I review the factors that increase the risk of sickness in dairy calves. Secondly, I describe how sickness behaviour is elicited and the mechanisms that generate and modulate its expression in the dairy calf. Finally, I identify those behaviours most promising in early diagnoses of ill health, including those provided by new technologies increasingly available in the dairy industry.

## **1.2 Factors affecting the health of dairy calves**

### **1.2.1 COLOSTRUM INTAKE AND THE IMMUNE SYSTEM OF THE CALF**

Colostrum is the first food secreted by the cow for the newborn calf during the first 24 to 36 h after giving birth (Pakkanen and Aalto, 1997). Besides the obvious effect of its nutritional content on health, there are other important factors that link an inadequate intake of colostrum with an increased risk of disease in calves.

In contrast to human and many other species, the type of placentation present in ruminants prevents transfer of any maternal immunity before birth (Weaver et al., 2000). Although the calf is born with a functional immune system and is able to react to certain antigen challenges, the system is considered to be naïve because it does not yet operate at an optimum level (Franklin et al., 2003).

Colostrum contains antibodies, known as immunoglobulins (Ig), which are a series of large glycoprotein molecules that constitute the main protection against diseases for the calf. The most abundant antibody is immunoglobulin G (IgG), which constitutes around 80 to 85% of all Ig present in colostrum (Pritchett, 1991). It is only by the Ig contained in colostrum that calves acquire immunocompetence. This mechanism for acquiring immunity is known as passive transfer and it protects the calf until its own immune system becomes fully functional at around 3 to 6 wks of age (Franklin et al., 2003).

Passive transfer has two main limitations: 1) the permeability of the small intestine of calves to the immunoglobulins present in colostrum is gradually lost within the first 24 h of life (Stott et al., 1979); and, 2) the quality of colostrum (Ig content) can



be highly variable, as it varies with age, parity, health, and other dam-related factors, including the nutritional management of the dry cow (Quigley and Drewry, 1998).

The quality of defence against diseases is thus directly related to the amount, quality, and timing of colostrum intake. The result of inadequate quantity, quality, or timing of colostrum intake, is a reduced concentration of circulating Ig in the blood of the calf. This condition is known as failure of passive transfer (FPT). It is generally accepted that FPT occurs when a calf's blood-serum concentration of IgG is less than 10.0 g/L (Gay, 1983). Although some risk factors have been identified, including mastitis and dystocic calving (Perino et al., 1995), FPT should be rare under good management conditions. Unfortunately there is a high prevalence of FPT in commercial farms (Godson et al., 2003). Filteau et al. (2003) found that 19% of beef calves sampled in Quebec presented FPT. Moreover, 26.5% of calves diagnosed as sick at blood-sampling time showed FTP compared to 17.7 % of healthy calves. FPT clearly increases the probability of a calf getting sick.

Colostrum also contains antimicrobial factors, such as lactoferrin, lysozyme, and lactoperoxidase, that complement the action of Ig (Pakkanen and Aalto, 1997). Other components include substances such as hormones and growth factors that aid in stimulating protein synthesis, cell division, and growth (Godson et al., 2003). Evidence has been found that colostrum enhances the development of the intestinal epithelium. Blättler et al. (2001) and Buhler et al. (1998) found that the villous circumference, area, height, and height/crypt depth ratio in the small intestine, particularly in the duodenum, were higher for calves fed colostrum compared with colostrum-deprived calves. Kelly and Coutts (2000) suggest that the increased development of the gut triggered by

colostrum, decreases disease susceptibility of the calf. A more developed epithelium would act as a barrier to the external environment, for example, by maintaining tight junctions between epithelial cells, enhancing its ability to secrete mucin and other antimicrobials. In addition, it has been demonstrated in humans that maternal milk enhances immune response through various of its components, although the exact mechanisms are still unknown (M'Rabet et al., 2008). Colostrum provides a good level of protection for some diseases like neonatal septicaemia and early pneumonia, but less so for diarrhoea, probably because affected calves are 3 to 6 wks of age, when passive immunity is declining (Donovan et al., 1998).

It can be concluded that one of the most important practices in calf rearing is the provision of adequate amounts of colostrum at birth. The combination of nutrients, antibodies, growth factors, and antimicrobials allows the neonatal calf to face the pathogens present in its new environment (Donovan, 1998). FTP, a very important threat to the welfare of dairy calves, can be minimised by observing good management practices in the maternity area that emphasize adequate quantity, quality and timing of colostrum intake. It should be noted that studies on the health of calves seldom take into account the calf's serum Ig levels. Although the experimental design should minimise this effect by randomly distributing FTP calves among treatments, it is a measurable variable that could be controlled.

## **1.2.2 TRADITIONAL FEEDING SYSTEM AND MILK RESTRICTION**

Under the current dairy farming practices, calves are separated from their mothers at a very early age (within 24 h after birth). The animals are then fed milk replacer or waste milk by bottle or bucket twice a day to reach a daily amount equivalent to 10% of their body weight (BW). This practice continues until the calf is weaned; depending on the farm, weaning takes place between 4 to 10 wks of age (Rushen et al., 2008).

However, this system has been put in doubt by demonstrating that current recommendations for nutrient requirements of the young calf are inadequate (Van Amburgh et al., 1998). In other domestic species such as lambs, pigs or even beef calves, young animals show milk intakes closer to ad libitum levels. When compared to these other species, dairy calves show considerably lower feed conversion efficiencies. Studies have shown that lower feed intakes lead to lower rates of gain and a smaller dilution of maintenance costs, resulting in lower feed efficiencies (Diaz et al., 2001).

### **1.2.2.1 MILK REQUIREMENT OF CALVES**

Calves left with their dam perform around 4 to 10 sucking bouts per day, each one lasting an average of 7 to 10 min (de Passillé, 2001). Because of this increased frequency and total intake of milk, weight gain in calves left with their dam can be several times that of calves raised under the traditional feeding method (Flower and Weary, 2001).

Calves separated from their dams achieve similar results by being fed milk ad libitum through a teat. This practice assures that the calf's nutritional requirements are met, improves their milk digestion (de Passillé et al., 1992), and allows the expression of natural sucking behaviour. Ad libitum teat feeding, which reduces the food intake

restriction imposed by the standard feeding system, can accommodate individual preferences for meal number and distribution during the day (Appleby et al., 2001). Calves fed higher (Khan et al., 2007) or ad libitum (Jasper and Weary, 2002) amounts of milk also have much better growth rates and spend more time resting (De Paula Vieira et al., 2008).

There is also evidence that the amount and frequency of milk intake affects the number and quality of calves' vocalisations, which can be an indicator of hunger. Thomas et al. (2001) found fewer and lower-frequency vocalisations from calves after separation from their dam and at weaning if milk intake was higher and more frequent.

#### **1.2.2.2 MALNUTRITION AND IMMUNITY**

Calves with a restricted intake not only risk hunger, but their developing immune system could also be disturbed by nutrient limitations. There is a clear link between malnutrition, infection and immunity. Studies in humans with chronic malnutrition have shown reduced performance of both the humoral and cellular immunity (Keith and Jeejeebhoy, 1997). Nutrient deficiencies impair cell-mediated immunity, phagocyte function, complement system, antibody concentrations and cytokine production (Chandra, 2002). A mechanism involving leptin has been proposed to explain the relation between nutrition and immunity. Low levels of serum leptin, which are associated with reduced body fat or nutritional deprivation, may increase susceptibility to infectious diseases by reducing the competence of T-helper cells and thymic function (Matarese et al., 2002).

Only the works by Nonnecke et al. (2000, and 2003) and more recently Foote et al. (2005) have directly studied the effect of nutrient intake on immunological functionality of dairy calves. Even though results show no major differences in immunity indicators between calves fed restricted or additional milk replacer, Nonnecke et al. (2003) demonstrated that increased milk intake might improve some functions of cell-mediated immunity in calves. The fact that the restricted fed calves were fed 140% of the recommended feeding rate (because of winter conditions and management practices) and limited animal numbers per treatment (9 and 10 for restricted and additionally-fed calves, respectively) may explain the lack of major differences between treatments in the previous study. In the work by Foote et al. (2005), restricted and additionally-fed calves were blood sampled to study in vitro proliferation and activation of lymphocytes. Results show a slight improvement of functional activity of T-cells in calves with enhanced nutritional status.

These results show that dairy calves are not only less efficient under restricted diets, but that their immunological status can be impaired when fed diets that provide low levels of nutrients. Nevertheless, some authors consider that the large amounts of milk consumed in ad libitum feeding may have two unwanted consequences: an increase in diarrhoea incidence and a reduced feeding efficiency after weaning.

### **1.2.2.3 AD LIBITUM FEEDING AND DIARRHOEA**

The relation between ad libitum milk consumption and diarrhoea might have originated on dairy farms using buckets to feed milk. Appleby et al. (2001) suggest that diarrhoea might be a problem in calves bucket-fed because part of the milk may enter the reticulum, while in teat-fed calves, the sucking reflex causes milk to pass directly to the abomasum through the oesophageal groove.

A recent study by Quigley et al. (2006) showed that calves fed additional amounts of milk replacer had longer duration of diarrhoea episodes. However, this finding should be viewed with caution, as calves that refused milk during the experiment were force fed the remaining milk (Borderas et al., 2007; see Appendix A). Force-feeding has been previously demonstrated to aggravate disease. Murray and Murray (1979) showed that force-fed mice experienced a 50% increase in mortality and shortened survival times compared to ad libitum fed mice.

Most studies show no link between diarrhoea and milk intake. Early works by Huber et al. (1984) and Nocek and Braun (1984) showed no difference in scour score between calves fed high or low quantities of milk. More recently, Appleby et al. (2001) and Jasper and Weary (2002) demonstrated that ad libitum teat-fed calves had the same or lower incidence of diarrhoea than limit-fed calves. Chua et al. (2002) fed ad libitum milk to calves assigned to pair- or individually housed treatments. There were no differences in scoring between the two groups, with calves scouring 3.1 to 3.7 d, respectively, in the 58 d trial. In another study by Diaz et al. (2001), high levels of milk feeding did not result in diarrhoea. In contrast to the Quigley et al (2006) study, Hammon et al. (2002) found more loose feces in restricted fed calves than in ad libitum fed calves.

In most studies, diarrhoea is assessed using a scours score on a scale of 1 (normal), 2 (soft), 3 (runny) and 4 (watery) (Kertz and Chester-Jones, 2004). Since scouring is directly scored, no repeatability measures between scorers are reported. Factors like increased milk intake could reduce the consistency of the faeces and increase the score value with no pathological association. Drackley (2004) states that calves fed large amounts of milk will have softer faeces and this effect is enhanced by providing milk replacer compared to whole milk. By simply increasing milk intake of calves to 18% BW, Bartlett et al. (2006) found that days with elevated fecal score were increased. Colour of faeces and other characteristics have been neglected as diagnostic features, although some evidence of colour change is mentioned for rotavirus and mucoid presence for *Cryptosporidium* (Blowey, 2004). The use of digital pictures of diarrheic faeces with a standard procedure (fixed distance, use of flashlight, etc.) could prove useful in establishing better criteria for scours scoring. This procedure would allow measures of scoring repeatability within and between scorers and the colour of faeces could also be assessed as an additional diagnostic tool.

Inconsistency in housing, management, and diagnoses may explain some of the variation in diarrhoea incidence among studies. Laboratory analyses are expensive and results can show a wide variety of pathogenic agents, even in non-diarrheic calves. In other cases, the isolation of a specific agent reflects more the stage of disease (for example virus in the primary and bacteria in the secondary stages). Nonetheless, the identification of pathogenic agents and their incidence in a sample of sick calves can guide treatment and management practices used to control the disease.

To illustrate this point we can look at results from Quigley et al. (2006) who found an increase in scours associated to enhanced milk replacer feeding. The authors induced illness in calves by exposing them to bedding previously infected by calves under a coronavirus challenge. When performing laboratory analysis for isolation of aetiology, the authors found *Salmonella*, *Cryptosporidium* and *E. coli* in addition to coronavirus.

In general, there appears to be no established link between ad libitum milk feeding and the incidence and severity of diarrhoea. Further, there is little mention in the literature as to a possible aetiology, thus differences could also be partly explained if each source of diarrhoea (mechanical, protozoal, bacterial or viral) affects in a different way the gastrointestinal tract and the resulting mechanism that elicits diarrhoea (osmotic, malabsorption, secretory or by impaired motility). For example, viral infections cause damage to the epithelial cells in the intestine thus impairing absorption, while bacteria like *E. coli* elicit a secretory mechanism to flush bacterial toxins out of the gut lumen.

A common practice in treating diarrhoea in milk-restricted calves is to further reduce the calf's intake. Garthwaite et al. (1994) showed that withholding milk actually slows recovery. However, the maximum quantity of milk offered in that study was the commercial recommendation (10% of BW). Furthermore, the aetiology of disease (*Cryptosporidium*) and the prompt clinical intervention could have also played an important role in the results of that study. Unfortunately, no studies have been conducted in order to establish whether milk withdrawal during diarrhoea episodes (or a restriction to 10% BW) minimise differences in scouring between restricted and ad libitum fed calves.



#### **1.2.2.4 EFFICIENCY AFTER WEANING**

Jasper and Weary (2002) demonstrated that calves could be fed milk ad libitum without deleterious consequences on concentrate or hay intake after weaning. They fed milk ad libitum, under a conventional system (10% BW), to dairy calves from birth to 63 d. Ad libitum fed calves had higher average daily gains compared to conventionally fed calves. This increased growth rate resulted in a 10.5 kg advantage at weaning time (36 d) and it persisted until the end of the experiment. Although calves were gradually weaned, the authors did not find differences in concentrate or hay intake between groups at weaning or post-weaning periods. Chua et al. (2002) found no difference in weight gain after weaning between paired and individually housed calves. Pair-housed calves continued to gain weight at pre-weaning levels during the week of weaning. In contrast, single-housed calves gained less weight. More recently, Khan et al. (2007) found that calves fed milk under an enhanced feeding system (20% BW) gained 36.25% more BW after weaning than calves fed under a conventional system.

#### **1.2.3 GROUP-HOUSING OF DAIRY CALVES**

Soon after birth, calves are normally separated from the dam and kept isolated from conspecifics. The preference for individual housing stems from the idea that it reduces disease transmission and the incidence of behavioural problems (particularly cross-sucking; Weary and Chua, 2000). Under natural conditions, calves spend much of their first week of life isolated from the herd, but afterwards calves spend more and more time together with other calves such that by their second week of age, the amount of time

spent in social interaction with other calves exceeds that spent with the dam. This behaviour was confirmed by Holm et al. (2002), who studied calves' motivation for social contact (head or full body contact) by operant conditioning. They found that calves' motivation to gain access to full social contact (measured by demand elasticity) was stronger than their motivation to gain access to head contact. These results suggest that group housing is preferred to individual housing. Group housing allows social behaviour and reduces the labour required to feed and house the calves (Broom and Leaver, 1978). Kung et al. (1997) claim that the time needed to manage an individual calf is 10 times that needed for a group housed calf. Babu et al. (2004) and Phillips (2004) also found that group-housed calves spend more time eating and show increased food intake compared with individually housed counterparts.

These apparent advantages for both calves and producers have resulted in an increasing tendency to house dairy calves in groups (Lidfors and Jensen, 2003). In Europe, animal welfare regulations state that calves can only be reared singly until 8 wks of age, but afterwards it is mandatory to house them in groups (Bøe and Færevik, 2003). In the case of organic farming, calves have to be group housed from 1 wk of age (Jensen and Holm, 2003). This global trend to convert calves to group-housing systems might also be facilitated by the appearance of automated milk feeders.

### **1.2.3.1 GROUP-HOUSING AND HEALTH**

It is claimed that group housing increases the incidence and prevalence of diseases (Tomkins, 1991; Hepola, 2003). Maatje et al. (1993) found that chronic and acute respiratory diseases and diarrhoea were more frequent in group-housed calves fed

using a feeding station than in calves individually housed with bucket feeding. These authors suggested that sick animals in group systems have to make a greater effort to get their daily rations than individually housed animals, resulting in lower average weight (- 7.3 kg) and daily gain (- 48 g/d) for grouped animals. In Sweden, the risk of respiratory disease was 2.8 times higher in calves housed in groups compared to calves housed individually (Hepola, 2003). The author concludes that group size and more specifically the space per calf seems to be one of the main risk factors. Svensson and Liberg (2006) found that calves in groups of 12 to 18 individuals showed higher incidence of respiratory illness when compared to calves in groups of 6 to 10 individuals.

Horizontal transmission of pathogens is frequently mentioned as the main preventive reason for isolating milk fed calves. Although diseases are primarily caused by pathogens that spread by direct contact, indirect contact (fomites, vectors, and aerosols) also plays a very important role in disease transmission, particularly in the case of virus. Hardman et al. (1991) have established that indirect transmission was more important than direct contact in *Salmonella* spreading among individually penned calves. Oral and nasal contact is also likely to occur in calves housed in individual crates (Friend et al., 1988). Another factor that can contribute to differences between housing systems is that individual housing permits a close observation of the calf by the caregiver, and may foster early detection of disease and prompt treatment (Chua et al., 2002). For example, Friend et al. (1988) suggest that once a calf develops scours, detection is easier in individually penned calves. These authors, however, also warned that early detection of common diseases could be more difficult if the individual housing restricts normal behaviour. A reduction in milk intake, one of the main signs used to diagnose illness, is

more easily spotted when calves are housed individually than in groups, unless automated feeders are used to record individual intake.

Not all studies report an increase in disease incidence for group-housed calves. Kung et al. (1997) reported that calves reared in groups and fed using a computer controlled feeding station required fewer days of medication than calves raised in hutches with bucket feeding twice a day (11 vs. 19 d, respectively). Webster et al. (1985) found no difference in disease incidence between group and individually housed calves. According to a Norwegian farm survey (Furuhaug, 1993; cited by Hepola, 2003), the installation of computer-controlled feeding systems have decreased the incidence of diarrhoea in calves but the number of pneumonia cases has not changed. Similarly, a Finnish and Swedish survey (Svensson et al., 2000), reported no difference in the incidence of diarrhoea between individual and group-housed calves fed by group-bucket or group-feeding station systems. Hänninen et al. (2003) found that diarrhoea incidence rates were lower for group-housed calves than for individually housed ones.

It is difficult to separate the effects of the grouping from other management variables. Although automated feeding systems can reduce labour and thus leave more time for observing calves, this spare time is normally assigned to other tasks in the barn and not for health observation. Besides, the implementation of new systems is always accompanied by a transition period where productivity and welfare indicators can actually be decreased (Rushen, 1994). Long-term epidemiological studies should be conducted to determine the impact of these systems on the risk of disease incidence. Although there is no evidence for long-term negative health effects of individual rearing

(Bøe and Færevik, 2003), group housing is generally encouraged as a 'good' welfare practice.

#### **1.2.4 GASTROENTERIC AND RESPIRATORY DISEASES**

After the perinatal period, the most economically important diseases of dairy calves on a worldwide basis are those affecting the respiratory and digestive systems (Perez et al., 1990). Results from the USDA Dairy 2007 report (USDA, 2007), show that digestive and respiratory disorders account for 56.5 and 22.5 % of the total heifers death respectively. There was essentially no change from 2002 to 2007 in the overall mortality of pre-weaned calves from gastroenteric disorders (62.1 % vs. 56.5, respectively) or from respiratory disorders (21.3 % vs. 22.5%, respectively).

In most cases, gastroenteric and respiratory disorders are the product of different aetiologies. *E. coli*, *Salmonella*, *Cryptosporidium*, *Eimeria*, and a variety of viruses (mainly rotavirus and coronavirus) are related to gastrointestinal diseases. Fungi belonging to the *Candida* genre were also isolated in diarrhoeic calves during winter (Elad et al., 1998).

Pneumonia, recently termed as Bovine Respiratory Disease complex (BRD), signifies the multifactorial aetiology of the respiratory illness that affects cattle. The variant occurring in calves is called Enzootic Pneumonia. There are a range of infective agents, including respiratory syncytial virus (RSV), infectious bovine rinotracheitis (IBR), parainfluenza 3 (PI3), and bovine viral diarrhoea (BVD). Bacterial infections such as *Mycoplasma*, *Mannheimia (Pasteurella) haemolytica*, *P. multocida* and *Haemophilus*, can be secondary to viral damage or cause diseases in their own right. A viral-bacterial

synergy has been demonstrated by Hodgson et al. (2005) who found a primary bovine herpes virus -1 (BHV-1) infection followed by an opportunistic colonisation by *Mannheimia haemolytica*. They suggest that if stressful situations like social reorganization or transport are present, an increased susceptibility can result in high mortality of cattle, although mechanisms linking stress hormones and the viral-bacterial synergy are not fully understood. Some etiologic agents can also cause both respiratory and digestive disorders (e.g. bovine coronavirus (BCV)). The work by Storz et al. (2000) exemplifies the temporal sequence of shipping fever, with a respiratory bovine coronavirus primary infection and *Pasteurella* spp. as secondary infection agents.

For diagnostic purposes, the main problem with these diseases is that signs are general and related to the physiology of each system. No pathognomonic signs are evident in any of the possible aetiologies. Besides, laboratory analysis is expensive and often inconclusive, because several agents can be found depending of the stage of infection. As a result, it is common to offer unspecific and palliative treatments (e.g. broad-spectrum antibiotics, electrolytes, astringents). A detailed study of prodromal signs of these diseases could reduce mortality associated with late treatment, especially in the case of gastroenteric diseases where death results primarily from dehydration and electrolyte imbalances (Booth and Naylor, 1987).

Another important issue is the difficulty in establishing objective criteria for delimiting the course of disease. Duration of diseases is generally measured as days under treatment, leaving this variable to individual criteria used by the treating veterinarian. Most studies do not report a standard therapy or just mention which product (mainly antibiotics) was used as treatment. It is likely that treatments differ among experiments,

within studies, and among individual cases, especially for those animals that do not respond to initial standard therapy. These subtle changes are rarely reported, thus contributing to a deficient understanding of disease dynamics.

## **1.3 Sickness behaviour**

### **1.3.1 GENERAL DESCRIPTION**

This term refers to a pattern of behavioural changes that are frequently present when immune activation is elicited by systemic inflammation or infection. The behavioural symptoms represent just a part of a highly coordinated strategy of the organism to fight infection that includes the fever response and its associated physiological changes. These include hypophagia, hypodipsia, lethargy, hyperalgesia, hyper- or hypothermia, impaired learning and memory, decrease of body self-care activities like grooming, and reduced exploration of their physical and social environment including low or lack of libido (Dantzer, 2001; Cremeans-Smith and Newberry, 2003). All these measures serve to redirect the organism's efforts toward the physiological priorities for survival. The magnitude of redirection is proportional to the severity of the insult (Elsasser et al., 2004). These elements of sickness behaviour are recognised as clinical signs of disease and are commonly used by stock people and veterinarians for diagnosis of illness. However, their expression, which is context-dependent, competes with other motivational states (Millman, 2007). For example, a very recent study by Gregory et al. (2009) showed that sickness behaviour expression in hens reared in cages is reduced compared to hens reared in free-range facilities after induction of illness with equal doses of bacterial endotoxin.

It has been demonstrated that some substances known as proinflammatory cytokines are responsible of sickness behaviour (Dantzer, 2001). Cytokines are released by activated macrophages and monocytes as a response to infection due to pathogen associated molecular pattern receptors (PAMP) present on their membranes. Cytokines are generally classified in type I or pro-inflammatory cytokines and type II or anti-inflammatory cytokines. Tissue macrophages and Kupffer cells firstly attack infectious microorganisms that invade the body. These phagocytic cells express a variety of pattern-recognition receptors called Toll-like receptors (TLRs). TLR4 recognizes endotoxins from Gram-negative bacteria, while TLR2 recognizes Gram-positive bacteria. Both are present in the same phagocytic cell. The activation of TLR results in the production of pro-inflammatory cytokines; for example TLR4 activation results in the production of IL-1, which is able to induce its own synthesis and the synthesis of other cytokines that potentiate its effect (TNF- $\alpha$  and IL-6) (Konsman et al., 2002). These substances induce the presentation of non-specific symptoms of sickness known as the acute-phase reaction in both animals and human beings (Elmquist et al., 1997).

The first evidence of the existence of cytokines came from studies of slow-wave sleep in rabbits, where an endogenous pyrogenic protein produced in vitro by macrophages (later identified as IL-1) increased this phase of sleep after intravenous and central injections (Krueger et al., 1984).

Cytokines constitute the molecular signals that synchronize metabolic, physiological and behavioural components of the systemic response to infection and to the local inflammatory response. In mutant strains of animals where prevention of cytokine production by mononuclear phagocytes is observed, no signs of sickness



behaviour are present, although central injections of cytokines elicit a normal ill response (Johnson, 2002). The most known cytokines are interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor –  $\alpha$  (TNF-  $\alpha$ ), and the interferons (IFNs).

Several experiments have shown the relationship between cytokine levels and signs of disease. Evidence exists that these molecules are also involved in the onset of depression in humans, which widely overlaps with sickness behaviour in animals (Pöllmacher et al., 2002; Renault and Aubert, 2006; Dantzer et al., 2008). There is also evidence that cytokines produce spatial learning and memory deficits as a result of diminished hippocampal functioning (Sell et al., 2003).

### **1.3.1.2 MECHANISM OF ACTION OF CYTOKINES**

Since their discovery, cytokines have puzzled researchers as they consist of large proteins that cannot easily penetrate the blood-brain barrier (Elmquist et al., 1997). For this reason, physiologists had difficulties in identifying how the CNS was activated during physiological responses to systemic inflammation. In 1988, Sagar et al. (cited by Elmquist et al., 1997) reported that neuronal activation (by electrical stimulation or water deprivation) induced the expression of a gene by producing a measurable protein named Fos. The c-Fos gene is a widely studied oncogene linked to FBJ osteosarcoma viruses, hence its name (St-Arnaud and Quélio, 1998). Fos protein has provided a powerful method to map the patterns of neuronal activation following cytokines administration, despite the probable participation of cells or nuclear groups whose responses might not be associated with c-Fos expression (Elmquist et al., 1997).

It is believed that cytokines act on the brain in two successive waves. The first one is triggered by the activation of primary afferent neurons innervating the body site where the inflammatory reaction takes place, for example the site of infection. The second wave involves the slow diffusion of cytokines from the circumventricular organs and choroids plexus to brain targets such as the amygdaloid complex (Dantzer, 2001). The circumventricular organs and choroid plexus are neural regions that have no blood-brain barrier due to the presence of fenestrated capillaries (Konsman et al., 2002). In other words, peripheral cytokines, produced as a result of a local aggression (peripheral), induce the synthesis and release of cytokines in the brain (central).

The pathway involves the *vagus* nerve, which ends at the *nucleus tractus solitarius* (NTS) in the brain. This is demonstrated when severing the *vagus* nerve below the diaphragm resulted in blocking fever, sickness behaviour and induction of messenger RNA for IL-1 $\alpha$  in the brain (Elmqvist et al., 1997). Similarly, intraperitoneal injections of TNF- $\alpha$  induce non-rapid eye-movements sleep, but this effect is attenuated by vagotomy (Kelley et al., 2003). Marvel et al. (2004) have also shown a similar effect by injecting rats with 0.5  $\mu$ l of bupivacaine (an anaesthetic) into the NTS over a 5 min period, which causes a reversible inactivation of the dorsal vagal complex. However, several studies indicate that blood-borne immune stimuli can interact with vagal paraganglia in the thoracic cavity or the neck, activating the CNS response. Once they reach the *nucleus tractus*, neurons from this region project to the paraventricular and preoptic nuclei of the hypothalamus. The central amygdale is then reached via this pathway or via the parabrachial nuclei (Kelley et al., 2003). These pathways seem responsible for the

stimulation of the hypothalamic-pituitary-adrenal axis, most probably involved in the depressive effects on behaviour (Johnson, 2002).

### **1.3.2 SICKNESS AND TROPHIC BEHAVIOUR**

It is well known that a reduction of food and water intake is one of the first signs generally observed in sick animals. For example, Sowell et al. (1999) conducted two trials to study the feeding and drinking behaviour of group-housed steers identified as healthy or morbid. In the first trial, healthy steers spent more time at the feed bunk and had more feeding bouts than morbid steers. In the second trial, there were no differences in the time spent by either group of steers at the feed bunk, but healthy steers showed more daily feeding bouts than morbid animals. There was no difference in time spent at the water trough between healthy and morbid steers in either trial.

Many different cytokines are responsible for anorectic episodes during illness. The systemic administration of IL-1 $\alpha$  and TNF- $\alpha$  suppresses food intake within 1 h after treatment in diverse species (Kent et al., 1996). Anorexia is considered beneficial for survival in sick animals provided it does not persist too long. Experiments, with sick animals force-fed to levels of intake of healthy individuals, showed that mortality was near 100%, whereas only 50% of the infected animals fed to their own ad libitum intake died (Murray and Murray, 1979). More recently, Quigley et al. (2006) force-fed refusals to calves allowed larger amounts of milk replacer than the usual 10% BW and reported an increase in diarrhoea severity and duration when compared with restricted fed calves.

Chronic infusion of IL-1 $\alpha$  into the brain has caused chronic anorexia and the blockade of receptors to this cytokine was sufficient to inhibit anorexia caused by

peripheral inflammation (Johnson, 2002). The mechanism of another cytokine, TNF-  $\alpha$ , may be related to inducing lipolysis in the affected individual (Johnson, 1998).

Much of the anorexic effect of sickness is metabolic, although some authors have explored complementary mechanisms. For example, Aubert and Dantzer (2005) studied the role of taste in the reduction of intake by offering sweet (sucrose), bitter (quinine) or mixed-taste (saccharine) solutions to rats previously injected with bacterial endotoxin (LPS). These authors found no difference in water or sucrose intakes by control or ill animals, but treated animals increased their intake of quinine. When measuring the hedonic or aversive reactions to each solution, no differences were found between water, sucrose or quinine solutions, but sick animals reduced their hedonic reactions and increased their aversive reactions to saccharin. It is not clear whether LPS induces a reduced taste sensitivity or increased the perceived aversive properties of this product. Research on the role of taste as modulator of intake in sick calves has been ignored, although interesting relations could be explored, for example, by adding a sweetener with low caloric value, such as sucralose, to milk replacer in calves' diets. Another unexplored area is that of electrolyte solutions for scouring calves. Little is known about the modulation of voluntary intake of liquid in sick calves, and the role of electrolytes in the metabolism of pathogenic agents has not been studied to date.

### **1.3.3 FEVER**

One of the main signs of sickness in humans and animals is the presence of fever. This defence mechanism has a high metabolic cost, so it leaves less energy available for activities other than those related to heat production and conservation. The observed

reduction of activity levels in almost all vertebrate species is a direct consequence of this energy shift. The higher body temperature stimulates proliferation of immune cells and is unfavourable for the growth of many kinds of bacteria and viruses, not only by a thermal effect, but also decreasing the availability of zinc and iron for microbial replication (Dantzer, 2001).

It has been demonstrated that cytokines cause fever by inhibiting thermal-control neurons in the hypothalamus, increasing heat production by increasing metabolic rate and shivering, and reducing heat loss by thermoregulatory behaviours like fetal lying (Johnson, 2002). Other responses for heat conservation include piloerection and muzzle dryness (Dantzer, 2001).

#### **1.3.4 SICKNESS AND SLEEP BEHAVIOUR**

Cytokines also participate in the regulation of sleep. The presence of cytokine receptors throughout the brain, suggests that they play a physiological role as sleep-wake regulators in absence of immune challenge (Opp, 2005). It is now evident that cytokines play an important role in the regulation of the circadian rhythm of several systems (Clark et al., 2007). The two major cytokines involved are TNF-  $\alpha$  and IL-1- $\alpha$  although some evidence has been found for the role of other cytokines like type I interferons (IFN) in sleep or arousal regulation. During sleep, two major phases are identified: the rapid-eye movement phase (REM) and the non-rapid eye movement (NREM) (Majde and Krueger, 2005). In the later, thalamocortical neurons show slow oscillation patterns and indicate deep sleep. An infection, or the administration of small amounts of TNF and IL-1 cytokines, increases the duration of the slow wave activity throughout the NREM phase

(Majde and Krueger, 2005). More severe infections reduce REM sleep. Cytokines also seem to play an important role in the physiological events related to the influence of serotonin on sleep-wake behaviour (Manfridi et al., 2003). These findings suggest that sleep behaviour changes might be useful in the early diagnosis of disease. For instance, by continuous observation of resting behaviour, duration of sleep postures accompanied by ear flicks could be measured. Episodes of sickness in animals might show an increase in the duration and bout frequency of these sleeping behavioural patterns when compared to healthy periods.

### **1.3.5 MODELS FOR INDUCTION OF SICKNESS BEHAVIOUR**

#### **1.3.5.1 BACTERIAL LPS**

The same effects described for cytokines can be achieved by injections of molecules that induce their synthesis. The LPSs, are complex endotoxins from several bacteria. LPS and other substances have been termed as microbially derived activators of innate immunity or MDAs (Cremeans-Smith and Newberry, 2003) and have proven to be a valuable tool for modelling disease processes.

Lipopolysaccharides used in research are mainly obtained from *E. coli*, facultative anaerobic bacteria found in the gastrointestinal tract of mammals (Russell et al., 2000). Other types of bacterial LPS used in research arise mainly from *Salmonella*, *Pseudomonas* and other Gram-negative bacteria like *Klebsiella*. Due to its widespread use, the LPS obtained from *E. coli* is considered the standard endotoxin. However, the LPS from other bacteria elicit different proportions of diverse cytokines in vitro (Mathiak

et al., 2003) which could account for the different responses found in the immune response with each aetiology.

The response to LPS depends on the dose, the route of application, and the individual sensitivity, and is in part genetically determined. The primary target cells are the phagocytes of innate or natural immunity – peripheral monocytes, tissue macrophages and neutrophils (Alexander and Rietschel, 2001). Although LPS induce a broad spectrum of biological effects, fever, anorexia, and lethargy are the most common behavioural expressions.

Although LPS effects have been mainly studied in laboratory rodents, there is information concerning farm animals. For example, Johnson and von Borell (1994) reported food intake reduction, decreased activity and fever in pigs receiving LPS (0.5, 5 and 50 µg/kg). Briard et al. (1998) found that LPS administration in sheep (200 and 400 ng/kg) causes increased respiration, intermittent cough and diarrhoea, and a lack of alertness. High fever (41 to 43°C), lasting for 6h, was recorded in all animals. Mean plasma ACTH and cortisol levels increased 30 min after LPS administration and reached a peak 2 h post-injection, remaining high during 5 h (ACTH) and 6 h (cortisol). Arginin-vasopressin levels, which increased 30 min after endotoxin administration, peaked 45 min after LPS administration. This increase only lasted 2 h. More recently, Lavon et al. (2008) injected LPS to cows in oestrus and noted delayed ovulation due to a decrease in the LH surge.

Calves have been used in LPS challenges to assess immune response models and endotoxemia responses (Adams et al., 1990; Gerros et al., 1993; Elsasser et al., 1996; Bieniek et al., 1998). Unfortunately, due to the nature of these studies, authors have

focused on the immune response with very few of them reporting behavioural observations and none of them establishing a prodromal, or early sickness, behaviour model.

### **1.3.5.2 OTHER MODELS USED FOR INDUCTION OF SICKNESS BEHAVIOUR**

Apart from direct induction of disease by inoculation or exposure to the pathogen agent and bacterial LPS injections, other methods have proven useful to model sickness behaviour.

The occurrence of lipoid pneumonia by accidental aspiration of lipids in humans, has resulted in a pneumonia-induction model using instillation of mineral oil in the diaphragmatic lobe of the lung in several species including the calf. For example Bednarek et al. (1999) employed this method to induce pneumonia in calves and test the effects of steroidal anti-inflammatory drugs (SAID) and non-steroidal anti-inflammatory drugs (NSAID) on cellular immunity of calves. Animals developed the characteristic signs of pneumonia including fever, anorexia, and increase of circulating lymphocytes as a result of oil instillation.

Pulmonary inflammation has also been achieved as result of induced allergy, using ovalbumin sensitization by injection and posterior exposure to aerosols of the same substance. This model is widely used for research about allergies, especially asthma (Pastva et al., 2004).

The direct use of recombinant cytokines (mainly IL-1 $\alpha$  and TNF- $\alpha$ ) has also been widely utilized to model sickness behaviour, but probable multiple complex interactions limit the usefulness of this approach.



The principal limitation of all sickness-inducing methods is the immediateness of response. These models are ideal for understanding some of the complex relations of immune system with the rest of organic systems and the mechanism and function of therapeutic agents, but only once the signs of disease are fully expressed by the infected organism. Due to this rapid onset of disease, these models are of limited value in replicating the events occurring during the latency period, defined as the time between infection and the development of clinical signs. Animals in these models do not show a prodromal stage of disease, making these models less useful in the study of early detection of illness.

#### **1.4 BEHAVIOUR AS A TOOL FOR SICKNESS DIAGNOSES**

Producers and veterinarians have always used behaviour for diagnosis of sickness in animals, although the observation of first signs usually signifies that the pathogen has already done considerable damage to the host. Lethargy, depression, anorexia and sleep disorders, are among the most characteristic signs of the sickness behaviour. Some authors have tried to establish whether changes in continuously monitored ethograms could be used as diagnostic tools (Eberhardt et al., 2003) but results have been limited. Nowadays, technological advances such as position loggers, accelerometers, and record-keeping feeding stations, allow us to monitor these behaviours in a less costly and more efficient manner (Weary et al. 2009).

## **1.4.1 INTRODUCTION OF NEW TECHNOLOGIES FOR SICKNESS**

### **DIAGNOSES**

There is a global increase in the size of livestock farms. The Canadian 2006 Census of Agriculture showed that the number of livestock farms has continuously declined in recent years, although the number of cattle and pigs has increased. The census counted 229, 373 farms in 2006, a decrease of 7.1% since 2001. On the other hand, the number of animals has increased from 15.5 million in 2001 to 15.7 million in 2006. A more careful review shows that this trend is mostly due to an expansion in beef cattle numbers during this period. Although cows per farm increased, the number of dairy farms, and the number of dairy cattle have declined since 1991. Milk production has increased as result of improvements in nutrition, management practices and genetics (Beaulieu and Bédard, 2003).

These increases, combined with the difficulties in finding qualified stockpeople, have resulted in increased use of automated systems to replace time-consuming labour requirements arising from feeding and milking practices. Since these systems usually require animals to be group-housed, there is a worldwide tendency in dairy farming to house animals in groups.

Automated systems not only perform repetitive tasks, they also provide valuable information about the animals. It is nearly impossible to monitor continuously the behaviour and the physiological state of the animals by direct observation. Video recordings are limited in the details that can be observed especially when animals are group housed. Continuous or frequent measures of physiological data (i.e. temperature, blood metabolites, etc.) involve disturbing the subjects' natural behaviour and causing

distress when the observation is accompanied by an intrusive device or technique. The development of automated systems and telemetric devices has proved to be of substantial aid for recording behaviour and physiological measures.

The use of telemetric devices for measuring physiological constants like core temperature, heart rate, posture, etc., although still expensive for on-farm utilisation, are powerful research tools. Other technologies are also available and some examples illustrate their potential in sickness behaviour research.

Quimby et al. (2001) used radio frequency technology to record the total time that feedlot calves spent near the feedbunk. They found that ill calves spent less time near the feedbunk. This difference allowed them to predict morbidity 3 to 4 d before experienced feedlot pen riders.

Based on changes in daily walking activity measured by a pedometer, Edwards and Tozer (2004) found that dairy cows with ketosis, left displaced abomasum and digestive disorders had higher than average activity 8 d before the onset of clinical signs. If activity recording is combined with changes in milk yield, the authors suggest that these diseases could be detected between 5 and 6 d earlier than clinical diagnoses.

More recent studies showed that changes in feeding behaviour of dairy cows are useful as early predictors of metritis (Urton et al., 2005; Huzzey et al., 2007), mastitis (Lukas et al., 2008), as well as ketosis and lameness (González et al., 2008). These authors showed that ill cows change their feeding behaviour from weeks to days before being diagnosed by farm staff.

Since sleeping is disturbed by cytokines as a result of a pathogen insult, observations of sleep behaviour may be useful for early diagnoses, for example, by

measuring the duration of REM phase of sleep. This could be achieved by the observation of posture and the muscular twitching characteristic of this phase. Studies in cattle have shown that a lying posture with the head supported by the body or ground is characteristic of REM sleep (Ruckebusch, 1974). This was recently confirmed by Hänninen et al. (2008), who found that calves, resting with their head against the ground or body, were sleeping (measured by EEG) in 62% of cases.

Curran et al. (2001) correlated EEG and other physiological signs with behavioural observations of sleeping piglets. They found that pigs under REM phase displayed rapid-eye movements accompanied by ear and snout twitching. While under NREM phase, piglets had minimal body movement and closed eyes. Horne et al. (1989) correlated physiological with behavioural observations of lambs during sleep, characterizing REM phase when facial and ear twitchings were present. Similar results have been found in other species like dogs (Sullivan et al., 1978).

Furthermore, baby calves may be ideal for considering this behaviour. Their total sleep time averages 40% of the day (Hänninen et al., 2008), so changes in sleeping pattern could be more easily detected than in adults that average around 17% of time asleep. It is still unknown if changes in sleeping patterns precede the onset of diseases.

#### **1.4.2 MILK-FEEDING STATIONS**

Computer-controlled milk feeding systems were developed in the beginning of the 1980s in Germany (Hepola, 2003), mainly to facilitate calf rearing in groups. Group size can be up to 50 or 60 individuals according to the guidelines of some manufacturers

(DeLaval, 2009; Lely, 2009), although problems seem to increase with group size (Hepola, 2003).

There are several advantages of automated feeders over conventional bucket systems. The first is that calves have to suck a teat to get the milk. This partially satisfies the motivation for sucking, and allows calves to perform non-nutritive sucking after the meal is over. Another advantage is that in the case of powder milk replacer, the system reconstitutes milk when the calf arrives to the station, so milk is always fresh and warm (40°C). Another important advantage is that producers can distribute the total daily milk intake in small meals, mimicking the natural nursing of the cow that allows the calf to perform 4 to 10 sucking bouts. Day et al. (1987) reported that calves spend 52-64 min/d suckling from their dams. The total duration of ad libitum sucking per day, reported under an automated milk feeding system, is around 47-57 min (Jensen and Holm, 2003). As the calf grows older the feeding station may be adapted, so the calf takes fewer but larger meals as in natural conditions (Jensen and Holm, 2003). The main advantage from the early illness diagnosis point of view is that the system allows the producer to follow the daily intake of each individual (Hepola, 2003). Identification of anorexic animals can be done on a daily basis. Changes in feeding patterns do not necessarily include a reduction in milk intake; changes in the frequency and duration of feeder visits can also be monitored and may be useful in early diagnosis of disease. Studies have reported changes in feeding patterns when calves reared using automated milk feeders fell ill (Svensson and Jensen, 2007; Maatje et al., 1993). Weary et al. (2009) argue that changes in feeding patterns by ill calves (i.e. reduction of non-nutritive visits to the milk feeder) can be interpreted as a decline in sampling behaviour, defining the latter as the actions

taken by the animal to learn where and when food is likely to become available in the future. Bokkers and Koene (2001) compared activity and oral behaviour in veal calves under individual housing, group-housing and group-housing with an automated milk feeding system. The activity patterns show that individual and group housed calves have activity peaks during feeding time, while calves on the automated milk dispenser show an equally distributed pattern of activity over the day. The increase of the general activity during feeding time in non-automated systems, results from competition and social facilitation. The uniform pattern of activity observed in automated systems can be advantageous to early illness diagnoses, since subtle differences in feeding behaviour will not be masked by the competition elicited by milk servings.

If non-nutritive sucking is allowed at the milk feeder, visit duration time in the station doubles to 12 min on average and cross-sucking is reduced about 10-fold (de Passillé, 2001). In the experiment by Bøe and Havrevoll (1993) the number of ‘unrewarded’ (no milk provided) visits decreased from around 48 min/d at 6 wks of age to 30 min/d at 14 wks of age. Sick calves may decrease the frequency of unrewarded visits before reducing rewarded visits and intake (Svensson and Jensen, 2007).

Automated milk feeding machines are improving and it is likely that further capabilities will be incorporated into future models. More research is needed on how to best manage these feeding stations, especially regarding group size and the resulting interactions between members of the same group (group dynamics). If early diagnosis relies mainly on the frequency and distribution of the different kind of visits (rewarded or non rewarded), group size and group dynamics are factors that would affect visiting patterns. For example, subordinate calves in big groups could restrict their visits in

number and distribution. Changes in visiting patterns because of sickness could be very subtle in these calves, making it difficult to use visiting patterns as a diagnostic tool.

## **1.5 Objectives**

This review gives evidence that sickness is one of the major threats to calf welfare and that its incidence is affected by multiple factors related to current rearing methods. Since sickness behaviour appears to reduce behavioural activity to conserve energy resources and divert them to the immune system, assessment of changes in feeding, drinking, sleeping, thermoregulation and daily activity patterns of calves, fed with an automated milk feeding system, could be a powerful tool to detect the onset of illness before other clinical symptoms become evident. This would be especially valuable for calves kept in group housing systems, where illness detection is less efficient than in individual housing systems.

Consequently, the overall objective of my research was to examine the usefulness of behavioural tools to predict or identify sickness in pre-weaned group-housed dairy calves fed by an automated milk feeder. I addressed my research objective by studying the principal behaviours that regulate the energy intake and expenditure of the dairy calf: feeding, thermoregulation, and resting.

My particular objectives were: to identify which behaviours were more prone to change during the onset of disease in pre-weaned dairy calves; to determine if automatically generated records could identify these behavioural changes, and to identify the factors that influence the expression of these behavioural changes, specifically the feeding behaviour as affected by the feeding level.

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## **CHAPTER 2. FEEDING BEHAVIOUR OF CALVES FED SMALL OR LARGE AMOUNTS OF MILK<sup>1</sup>**

### **2.1 Introduction**

There is a growing interest in group housing of unweaned dairy calves using automated milk feeding systems (Hepola, 2003), that substantially reduces labor requirements (Kung, 1997), calves' distress at regrouping (Bøe and Færevik, 2003), and allows for more flexibility in feeding schedules (Hepola, 2003). However, there is growing evidence that the advantages of automated feeders may depend upon the amount of milk or replacer fed to the calves.

Conventionally, milk-fed calves in North America are fed milk or milk replacer twice a day for a total intake of 8 to 15% of body weight. However, the nutritional requirements of young calves have been underestimated (Bartlett et al., 2006) and calves fed higher volumes (Khan et al., 2007) or ad libitum (Jasper and Weary, 2002) amounts of milk or milk replacer have much higher growth rates. Ad libitum fed calves spend more time resting (De Paula Vieira et al., 2008) which may further improve their energy balance since calves have higher energy expenditure when standing than when lying down (Schrama et al., 1995). Increased levels of milk feeding (up to 8 L/d) have been found to improve the efficiency of use of automated milk feeding systems by reducing the number

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<sup>1</sup> A version of this chapter has been published. Borderas T.F., A.M. de Passillé, and J. Rushen. 2009. Feeding behavior of calves fed small or large amounts of milk. *J. Dairy Sci.* 92: 2843-2852.

of visits the calves make to the feeders (Jensen, 2006). However, to my knowledge there is no work addressing even larger milk allowances on automatic milk feeder use.

Some report a negative effect of increased milk allowance on health of dairy calves (Quigley et al., 2006). However, other studies show no increase or even a decrease in illness with high milk allowances (Appleby et al., 2001; Jasper and Weary, 2002; Khan et al., 2007). In two experiments, we examined how the milk feeding level of pre-weaned calves affects their behavior on the automated feeder, their health, and their growth. We hypothesized that larger volumes of milk or milk replacer would increase calf growth, improve the efficiency of use of the feeder, and have no negative effects on calf health.

## **2.2 Methods**

The institutional animal care committees (monitored by the Canadian Council for Animal Care) approved all procedures described in this study.

### **2.2.1 EXPERIMENT 1**

Thirty eight female and 12 male Holstein calves (BW =  $45.16 \pm 6.1$  kg [mean  $\pm$  S.D.]) were separated from their mother within the first 24 h after birth, weighed, fed at least 4 L of colostrum, and allocated to individual pens (2.15 m x 1.7 m) before being moved to a wood-shavings bedded group pen (6.45 m x 3.4 m) at  $4.62 \pm 1.16$  d of life. To reduce competition between young and older calves, animals were moved after 21 d to a second wood-shavings bedded group pen (6.45 m x 3.4 m) where only older calves were kept for another 29 d. Dynamic groups were used with calves being added to the first pen as they entered the experiment and moving to a new group as they aged. Group size

varied in both pens during the experiment within a range of 3 to 16 and a mean ( $\pm$  S.D.) of  $9.85 \pm 1.78$  calves housed in the same pen at the same time.

Calves were fed a milk replacer (Violac, Coopérative Fédérée de Québec, Montreal, Canada) distributed at a nipple-feeding station by an automated milk feeder (Lely© CALM). Milk replacer nutrient content was 18.5% crude protein, 18% crude fat, 0.5% crude fiber, 0.75% Ca and 0.65% P. Gradual weaning was started at d 44 of trial using the weaning program included in the automated feeder. Calves did not receive further milk replacer after d 48. A 22% crude protein concentrate (Goliath XLR, Coopérative Agricole des Cantons, Québec, Canada) was available ad libitum from the first day in group-pens through a feeding bowl at a feeding station controlled by the automated milk feeder system. Hay was available ad libitum from the first day in hayracks (2 per pen) which were freshly filled twice a day (0800 h and 1600 h).

Calves were randomly assigned to either a limited (LIMIT) milk allowance (4 L/d) or an ad libitum (ADLIB) milk allowance (24 L/d). In the ADLIB group, calves could receive meals of 0.5 L to 6 L, with an accumulation of allowance at the rate of 1 L/h (i.e. total daily accumulation of 24 L). For LIMIT calves, the meal size ranged from 0.5 to 2 L. For both groups, a new allotment of milk was given at 1000 h and 2300 h.

We recorded the daily intake of milk replacer and grain as well as the number of rewarded and unrewarded visits at the milk and grain feeder. Body weight (kg) of all calves was measured on d 1, 21 and 50.

Daily health checks were performed by a qualified veterinarian on all calves during the whole trial. During the first 3 wk of life, the health check included rectal temperature, cardiac and respiratory rates and sounds, presence of diarrhoea (firm, soft, liquid),



presence of nasal and ocular discharges (none, clear, turbid), general state of the coat and dehydration including tent test and muzzle humidity (dry, humid, damp). During the rest of the trial (wk 4 to wk 7) calves were visually assessed for presence of diarrhoea, presence of nasal and ocular discharges, general state of the coat, and muzzle humidity. All medical treatments were recorded.

### **2.2.2 EXPERIMENT 2**

Twenty-three female and 5 male Holstein calves (BW =  $43.4 \pm 4.9$  kg [mean  $\pm$  S.D.]) were separated from their mother within the first 24 h after birth, weighed, fed at least 4 L of colostrum, and allocated to wood-shavings bedded individual pens (1.3 m x 1.8 m) before being moved to a wood-shavings bedded group pen (2.6 m x 6.5 m) at  $5.19 \pm 1.27$  d of life. Group size varied during the experiment within a range of 4 to 13 and a mean ( $\pm$  S.D.) of  $10.78 \pm 1.91$  calves housed in the same pen at the same time.

All calves were fed whole milk from an automated milk feeder (Lely© CALM) equipped with a weigh scale under the feeding stall to record body weight. A 21.6% crude protein concentrate (Unifeed Ltd., Chilliwack, Canada) was available ad libitum from the first day on group-pens through a feeding bowl at a feeding station belonging to the automated milk feeder system. Hay was available ad libitum from the first day through hayracks, which were freshly filled twice a day (0900 h and 1500 h).

For the purposes of another experiment, calves were blocked for coat color and then randomly allocated to be offered either 4 L (N = 14) (LOW) or 12 L (N = 14) (HIGH) of whole milk/d (taken from the bulk tank of the farm with  $\sim$  150 cows in lactation) from d 5 to 43 of age. The HIGH group meals size could vary from 0.5 L to 6 L, with a

distribution rate of 0.5 L/h (i.e. maximum accumulation of 12 L/d). In the LOW group, the meal size range was from 0.5 to 2 L. For both groups, a new allotment of milk was given at 0900 h and 1900 h.

We recorded the daily intake of milk and grain as well as the number of rewarded and unrewarded visits to the milk and grain feeder. Body weight was recorded every day. In addition, the number of lying bouts and the total time spent lying were recorded during 7 d on calves in both treatments at two different age ranges (from 7 to 14 d of age and from 28 to 35 d of age) using Tinytag data loggers (Gemini Dataloggers Ltd., Chichester, UK) previously validated by O’Driscoll et al. (2008). Health checks were performed daily on all calves by a qualified veterinarian. These included respiratory and cardiac rates and sounds, rectal temperature, general appearance (active, dullness), presence of diarrhoea (firm, soft, or liquid faeces) and presence of ocular discharges (none, clear, turbid). All medical treatments were recorded.

Boluses of neomycin and sulphamethazine (Neo Sulfa-E®, Vetoquinol Inc., Canada) were used in 2 calves in experiment 1. A kaolin-pectate suspension (Kaopectate®, Pfizer Inc., Canada) was used on 4 calves in experiment 1. Rehydrating solutions (Electrolytes Plus®, Vetoquinol Inc., in experiment 1 and Hydrafeed®, EXL Laboratories, in experiment 2) were used for the remaining sick calves in both experiments. Medical treatments lasted an average ( $\pm$  S.D.) of 2.42 ( $\pm$  0.8) d with a range of 1 to 4 d.

### **2.2.3 STATISTICS**

For both experiments, the amount (L) of served milk or milk replacer and concentrate, and the number of total visits (rewarded and unrewarded) and the duration

(min) of each visit to the milk and concentrate feeders were automatically recorded by the feeding system. Visits were defined using the frequency distribution of natural log intervals between times when the receiver on the feeder gained or lost contact with the RFID transmitter on the calf, following the methods described by von Keyserlingk et al. (2004). Occupancy time of the feeders was calculated as the sum of the durations of all visits for each calf each day. The interval between visits was calculated as the time (min) elapsed between a visit and the subsequent one. Milk or milk replacer refusal (L) was measured by an independent scale where a container collected the refuse discarded by the milk feeder right after each visit. Milk or milk replacer intake was calculated as the difference between milk served by the milk feeder and the refusal.

In experiment 1, we analyzed the data in 4 periods: period 1 included d 1 to 21 of trial; period 2 included d 22 to 43, period 3 covered the period of gradual weaning (d 44 to d 48), and period 4 covered the period after weaning (d 49 and 50). In experiment 2, we only took observations during periods 1 and 2 before weaning began. Results from both experiments were analyzed using a repeated measures mixed model (PROC MIXED) of SAS (SAS Institute, 1999) including calf as random component of the model and treatment, period of the trial, and their interaction as fixed components. Feeding behaviours (milk or milk replacer intake, concentrate intake, number of visits to the milk and concentrate feeders, duration of the visits, and interval between visits) were the response variables. In experiment 2, lying behaviour was also included with the frequency and duration of lying bouts as response variables.

In experiment 1, differences between average daily weight gains were calculated with a linear model (PROC GLM) where body weight at d 1 was used as a covariable. In

experiment 2, body weights recorded at every visit to the feeder by the feeding station scale were sorted by calf and the daily median was calculated as the daily body weight. The median daily weight gains were used to calculate an average daily weight gain per week.

## 2.3 Results

In both experiments, there were significant ( $P < 0.01$ ) effects of treatment, period of the trial and their interaction for all variables, with the exception of the duration of the visits to the concentrate feeder, for which there was only a tendency ( $P = 0.09$ ) for the effect of treatment in experiment 1, and no effect of the interaction of treatment and period of the trial in experiment 2 ( $P = 0.43$ ).

Figure 2.7.1 shows the average daily intake of milk or milk replacer by calves in experiment 1 (Figure 2.7.1A) and 2 (Figure 2.7.1B) and the mean daily intakes of milk replacer or milk for each period of the trial are shown in Tables 2.6.1 and 2.6.2 respectively. In both experiments the calves fed large amounts of milk replacer or milk (ADLIB and HIGH) increased their consumption during the first 2 weeks, reaching a maximum intake that plateaued during the following 4 wk. The calves in experiment 1 drank considerably more milk replacer than the whole milk drank by the calves in experiment 2. In experiment 1 the average (mean  $\pm$  S.D.) meal size was higher ( $P < 0.001$ ) for ADLIB calves ( $2.68 \pm 1.07$  L) than LIMIT calves ( $1.72 \pm 0.42$  L), while in experiment 2 the average meal size did not differ ( $P = 0.35$ ) (HIGH mean  $\pm$  S.D. =  $2.00 \pm 0.63$  L; LOW =  $1.93 \pm 0.45$  L).

Figure 2.7.2 shows the average daily intake of concentrate by calves in experiment 1 (2.7.2A) and 2 (2.7.2B), and Tables 2.6.1 and 2.6.2 show the concentrate intake during each period of the trial. Concentrate intake for all calves was negligible during the first 14 d. LIMIT and LOW calves showed an increase ( $P < 0.001$ ) of concentrate consumption during the second period (d 22 to 43). Concentrate intake by the high fed calves remained low until weaning. In experiment 1, concentrate intake by ADLIB calves increased markedly during and after weaning but remained below that of LIMIT calves.

The total frequency of visits to the milk feeder was higher in LIMIT and LOW calves than ADLIB and HIGH calves during the first 42 d (Tables 2.6.1 and 2.6.2). Most of the visits performed by low fed calves in both experiments were unrewarded (90.6 % for LIMIT calves in experiment 1 and 89.07% for LOW calves in experiment 2). This resulted in a higher occupancy time of the milk feeder during the first 21 d for LOW calves in experiment 2 and during the first and second period (43 d) for LIMIT calves in experiment 1 when compared to their higher fed counterparts (Tables 2.6.1 and 2.6.2). During the weaning period in experiment 1 (d 44 to 48) there was no difference in occupancy time of the milk feeder between treatments ( $P = 0.26$ ). However, there was a tendency ( $P = 0.07$ ) for LIMIT calves to again spend more time in the milk feeder after weaning (Table 2.6.1).

Figure 2.7.3 shows the frequency of visits to the milk feeder in both experiments for each hour of the day. High-fed (ADLIB and HIGH) distributed their visits throughout the day while low-fed calves (LIMIT and LOW) showed an increase in the number of visits around the time when milk became available to them at the feeder (1000 h and 2300 h for experiment 1; 0900 h and 1900 h for experiment 2). In experiment 1, LIMIT calves had

25.32% of their total rewarded visits between 1000 h and 1200 h and 20.33% between 2300 h and 0100 h. In experiment 2, LOW calves made 16.01% of the total rewarded visits between 0900 h and 1100 h and 30.21% between 1900 h and 2100 h.

In both experiments, the total frequency of visits to the concentrate feeder was always higher for the LIMIT and LOW calves when compared to ADLIB and HIGH calves (Tables 2.6.1 and 2.6.2) and they increased throughout both trials for all calves. The duration of these visits tended ( $P = 0.09$ ) to be higher in LIMIT calves compared to ADLIB calves in experiment 1 (Table 2.6.1), and was always higher ( $P = 0.003$ ) for LOW calves when compared to HIGH calves in experiment 2 (Table 2.6.2). Occupancy time of the concentrate feeder by LIMIT and LOW calves was always higher than in ADLIB and HIGH calves during the first two periods (Tables 2.6.1 and 2.6.2 respectively). During weaning in experiment 1 (Period 3) ADLIB calves tripled the occupancy time of the concentrate feeder compared to the previous period, but it was still significantly lower than the occupation time of the LIMIT calves. After weaning was completed, calves in both treatments spent similar amounts of time at the concentrate feeder ( $P = 0.29$ ).

In experiment 1 ADLIB calves showed a higher daily weight gain ( $P < 0.001$ ) during the first 21 d ( $1.05 \pm 0.06$  kg/d) than LIMIT calves ( $0.48 \pm 0.04$  kg/d) but LIMIT calves had higher gains ( $P = 0.01$ ) between d 22 and d 50 ( $0.80 \pm 0.04$  kg/d) than ADLIB calves ( $0.62 \pm 0.05$  kg/d). However, the overall average daily weight gain (from d 0 to d 50) was higher ( $P = 0.03$ ) for ADLIB calves ( $0.80 \pm 0.05$  kg/d) than LIMIT calves ( $0.66 \pm 0.04$  kg/d). In experiment 2, HIGH calves had a higher weight gain than LOW calves for the

first 4 wks of trial (Figure 2.7.4, Table 2.6.3). There were no differences between HIGH and LOW calves in weight gain during wk 5 and 6 in experiment 2 (Table 2.6.3).

There were no differences between the high and low fed calves in the incidence of gastrointestinal and respiratory disease in either experiment (Table 2.6.4).

Lying time in experiment 2 was affected by treatment but only when calves were older (4 to 5 wks). At 2 wk of age, no differences were found in lying time (h/d) between HIGH calves ( $18.7 \pm 0.14$  h/d) and LOW calves ( $18.6 \pm 0.14$  h/d). When calves reached 4 to 5 wks of age both groups were spending less time lying, with HIGH calves lying  $17.28 \pm 0.14$  h/d and LOW calves lying  $16.92 \pm 0.14$  h/d ( $P = 0.024$ ).

## **2.4 Discussion**

To the best of my knowledge, this is the first dissertation to compare the effects of providing ad-libitum milk feeding levels to conventional restricted milk feeding levels to dairy calves fed using an automated milk feeder. Results from this study confirm that dairy calves fed by an automatic feeding system can ingest large volumes of milk or milk replacer from a very young age (Appleby et al. 2001; Hammon et al., 2002; Jasper and Weary, 2002). The amounts of milk consumed in experiment 2 were similar to the amounts drunk when calves of this age are allowed to suckle from their dams (de Passillé and Rushen, 2006). The increased amount of milk replacer consumed by calves in experiment 1 as compared to whole milk intake in experiment 2 most likely reflects the lower nutrient content of the milk replacer (e.g. 18.5% vs. 27% CP respectively). Although calves in the HIGH or ADLIB treatments showed higher weight gains than LOW or LIMIT fed calves during this period, the low quality of the milk replacer could

also explain the lower weight gains observed in calves fed HIGH levels during the second period in experiment 1 compared to LOW fed calves. Differences in the milk meal size between treatments were apparent in experiment 1 but not in experiment 2, and this may also be due to the poor nutritional quality of the milk replacer. However, the objective of the experiment was to compare different levels of intake regardless of the nutritional value of the feed and both treatments were fed the same milk replacer. The fact that the same behavioural patterns were found in both experiments in despite of the low nutritional level of milk replacer, strengthens rather than weakens the conclusions. Contrary to some findings (Quigley et al., 2006) but in agreement with other authors (Appleby et al. 2001; Jasper and Weary, 2002; Khan et al., 2007) we did not find any increase in the number of sick calves when high or ad libitum amounts of milk or milk replacer were fed.

As reported previously (Appleby et al., 2001; Chua et al., 2002; Hepola, 2003), the intake of concentrate was negligible during the first 2-3 wks of age, suggesting that at this age calves have difficulties compensating for reduced milk intake by increasing their concentrate intake (De Paula et al., 2008). That this results in hunger for low fed calves is shown by the large increase in the frequency of visits to the milk-feeder, the great majority of which were unrewarded.

These results support suggestions (e.g. Appleby et al., 2001) that limiting milk intake during the first 3 wks of life leaves the calves hungry and so is detrimental to their welfare during this period.

Calves fed HIGH or ADLIB levels of milk showed a low number of visits to the milk feeder that were evenly distributed throughout the day. On the contrary LIMIT and LOW



calves showed a large number of visits to the milk feeder, most of them unrewarded, as previously reported by Jensen (2006). Furthermore, rewarded visits were concentrated just prior to the twice daily provision of the milk allowance. This suggests that calves appeared to be capable of anticipating the timing of milk availability. This concentration of visits close to the time of renewed milk availability most likely would increase competition between the calves. The time that the milk feeder was occupied was higher for LIMIT or LOW calves in both experiments, indicating that low levels of milk reduce the efficiency of the milk feeder.

During the second period (d 22 to 43), the LOW fed calves began to eat larger amounts of concentrate. During this period, there was a reduction in the number of visits to the milk feeder and in the resulting occupancy time for LIMIT and LOW calves. This has also been reported by Jensen (2006). ADLIB and HIGH calves also showed an increase in concentrate intake and concentrate feeder utilization during period 2 but the concentrate intake levels were still very low when compared to LIMIT or LOW calves. Thus the advantages of a higher allowance of milk or milk replacer may be lower during this period than during the first few weeks of life, since the calves are more able to compensate by eating grain. In experiment 1, the highest occupancy time of the milk feeder in ADLIB calves took place during weaning (period 3). The increased time of occupancy was due to an increased number of visits by the ADLIB calves, suggesting that the reduction of available milk replacer and the low intake of concentrate elicited food searching similar to the observed in LIMIT calves during the first period.

Weight gains in both experiments were within the range reported in similar conditions (Appleby et al., 2001). ADLIB and HIGH calves had increased gains during

the first 3 to 4 wks but this advantage disappeared when LIMIT and LOW calves increased their concentrate intake. However, similar to the work reported by Jasper and Weary (2002) body weight of ADLIB and HIGH calves was superior to that of LIMIT and LOW calves at the end of each trial due to their initial advantage in gain as a result of their early increased milk consumption. Although concentrate intake increased dramatically at weaning in ADLIB calves in experiment 1, it remained well below the intake level of LIMIT calves, suggesting that a better weaning strategy is needed when feeding calves high levels of milk or milk replacer.

Claims are often made that milk feeding systems with a single teat can feed up to 45 calves. Our findings confirm previous reports (Hammon et al., 2002; Jensen, 2003; Nielsen et al., 2008) that when calves are fed a limited milk allowance, feeder occupancy time increases, mainly by an increase in the frequency of unrewarded visits. Mean daily occupancy time was as high as 77 min/calf for LIMIT calves in experiment 1, and 54 min/calf for LOW calves in experiment 2, which suggests that only a limited number of calves can be assigned per feeder when milk or milk replacer is allowed at low levels. The occupancy time of ADLIB or HIGH calves is similar to that reported by other authors (Appleby et al., 2001; Jensen, 2006). These results suggest that the automated feeder cannot feed more than 20 calves when milk feeding levels are low. However, it is possible that the duration or frequency of unrewarded visits by each calf may be reduced as group size increases due to increased competition between the calves.

## **2.5 Conclusions**

Unweaned calves will drink considerably more milk or milk replacer than is traditionally provided to them, without any negative effects on their health. During the first 3 wks of life, calves failed to compensate for low milk intakes by increasing grain intake. Consequently, feeding higher amounts of milk improves weight gain. Low fed calves make many more visits to the milk feeder which reduces the efficiency of the automated feeding equipment and diminishes the advantages of such system by limiting the number of calves that the system can host. Due to increased hunger, restricting milk or milk replacer allowance to dairy calves during the first 3 wks of life is likely to be detrimental to their welfare. After 3 wks of age, the ability of calves to eat grain increases and the advantages of feeding larger amounts of milk are reduced.

## 2.6 Tables

**Table 2.6.1.** Experiment 1: Least square means ( $\pm$  S.E.) of milk replacer (L/d) and concentrate (kg/d) intake, total visits, visit duration (min), and occupancy time of milk and concentrate feeder (min/d) of ad libitum (ADLIB) and limit-fed (LIMIT) calves by period of the trial (n = 25 calves per treatment).

	Period 1 (d 1 to 21)		Period 2 (d 22 to 43)		Period 3 (d 44 to 48)**		Period 4 (d 49 to 50)	
	ADLIB	LIMIT	ADLIB	LIMIT	ADLIB	LIMIT	ADLIB	LIMIT
<b>Milk replacer intake (kg/d)</b>	13.45 $\pm$ 0.28*	4.11 $\pm$ 0.28 *	14.67 $\pm$ 0.28 *	4.11 $\pm$ 0.28 *	5.43 $\pm$ 0.37 *	1.88 $\pm$ 0.36 *	0	0
<b>Total Visits to feeder</b>	8.74 $\pm$ 0.80 *	23.74 $\pm$ 0.78 *	7.28 $\pm$ 0.80 *	17.16 $\pm$ 0.78 *	17.94 $\pm$ 0.96	17.14 $\pm$ 0.94	11.34 $\pm$ 1.23 *	15.93 $\pm$ 1.24 *
<b>Visit duration (min)</b>	5.22 $\pm$ 0.24 *	3.73 $\pm$ 0.23 *	5.37 $\pm$ 0.23 *	2.89 $\pm$ 0.23 *	2.92 $\pm$ 0.27	2.32 $\pm$ 0.26	1.75 $\pm$ 0.33	1.85 $\pm$ 0.33
<b>Occupancy time (min/d)</b>	40.48 $\pm$ 3.06 *	77.17 $\pm$ 3.00 *	34.69 $\pm$ 3.05 *	47.87 $\pm$ 2.99 *	43.89 $\pm$ 3.57	38.21 $\pm$ 3.50	19.97 $\pm$ 4.45	31.30 $\pm$ 4.47
<b>Concentrate Intake (kg/d)</b>	0.001 $\pm$ 0.05	0.10 $\pm$ 0.05	0.02 $\pm$ 0.05 *	0.97 $\pm$ 0.05 *	0.19 $\pm$ 0.06 *	2.01 $\pm$ 0.06 *	0.56 $\pm$ 0.07 *	1.84 $\pm$ 0.07 *
<b>Total Visits to feeder</b>	4.50 $\pm$ 1.29 *	18.24 $\pm$ 1.20 *	8.07 $\pm$ 1.17 *	27.94 $\pm$ 1.14 *	18.83 $\pm$ 1.33 *	33.83 $\pm$ 1.31 *	26.15 $\pm$ 1.64 *	32.36 $\pm$ 1.67 *
<b>Visit duration (min)</b>	1.87 $\pm$ 0.20	2.32 $\pm$ 0.19	1.42 $\pm$ 0.19	2.30 $\pm$ 0.18	1.91 $\pm$ 0.20	2.62 $\pm$ 0.20	2.67 $\pm$ 0.22	2.48 $\pm$ 0.22
<b>Occupancy time (min/d)</b>	2.49 $\pm$ 3.17 *	19.39 $\pm$ 3.11 *	11.47 $\pm$ 3.16 *	56.25 $\pm$ 3.10 *	36.16 $\pm$ 3.69 *	76.66 $\pm$ 3.61 *	62.79 $\pm$ 4.59	69.77 $\pm$ 4.62

\* Significant differences ( $P < 0.05$ ) between treatments within period

\*\* Period 3 is the weaning period: Milk replacer was gradually reduced using the weaning program from the automated feeder

**Table 2.6.2.** Experiment 2: Least square means ( $\pm$  S.E.) of milk (L/d) and concentrate (kg/d) intake, total visits, visit duration and occupancy time of the milk and concentrate feeder of calves with High or Low allowance of whole milk by period of the trial (n = 14 calves per treatment).

	<b>Period 1</b>		<b>Period 2</b>	
	<b>(d 1 to 21)</b>		<b>(d 22 to 43)</b>	
	<b>HIGH</b>	<b>LOW</b>	<b>HIGH</b>	<b>LOW</b>
Milk Intake (L)	8.10 $\pm$ 0.16 *	3.74 $\pm$ 0.16 *	9.99 $\pm$ 0.16 *	3.89 $\pm$ 0.16 *
Total Visits to the feeder (n)	11.85 $\pm$ 1.19*	23.05 $\pm$ 1.19 *	10.82 $\pm$ 1.20 *	19.40 $\pm$ 1.20 *
Visit duration (min)	3.23 $\pm$ 0.31	2.58 $\pm$ 0.31	2.80 $\pm$ 0.32 *	1.72 $\pm$ 0.32 *
Occupancy time (min/calf/d)	32.21 $\pm$ 3.02 *	53.65 $\pm$ 3.02 *	26.25 $\pm$ 3.06	30.89 $\pm$ 3.07
Concentrate Intake (kg)	0.04 $\pm$ 0.07	0.16 $\pm$ 0.07	0.12 $\pm$ 0.07 *	0.66 $\pm$ 0.07 *
Total visits to the feeder (n)	8.53 $\pm$ 1.55 *	17.92 $\pm$ 1.55 *	11.70 $\pm$ 1.58 *	28.90 $\pm$ 1.57 *
Visit duration (min)	0.92 $\pm$ 0.09	1.14 $\pm$ 0.09	0.99 $\pm$ 0.09 *	1.70 $\pm$ 0.09 *
Occupancy time (min/calf/d)	8.72 $\pm$ 1.89 *	22.01 $\pm$ 1.89 *	11.40 $\pm$ 1.91 *	43.61 $\pm$ 1.91 *

\*Significant differences ( $P < 0.05$ ) between treatments within period

**Table 2.6.3.** Least square means ( $\pm$  S.E.) of daily weight gain (kg/d) per week of trial for calves fed a HIGH or LOW milk allowance in experiment 2.

Week	Treatment	
	HIGH	LOW
1	0.648 $\pm$ 0.10 *	0.329 $\pm$ 0.10 *
2	0.548 $\pm$ 0.10 *	0.243 $\pm$ 0.10 *
3	0.940 $\pm$ 0.10 *	0.405 $\pm$ 0.10 *
4	0.876 $\pm$ 0.09 *	0.482 $\pm$ 0.11 *
5	0.794 $\pm$ 0.10	0.635 $\pm$ 0.11
6	0.583 $\pm$ 0.15	0.489 $\pm$ 0.16

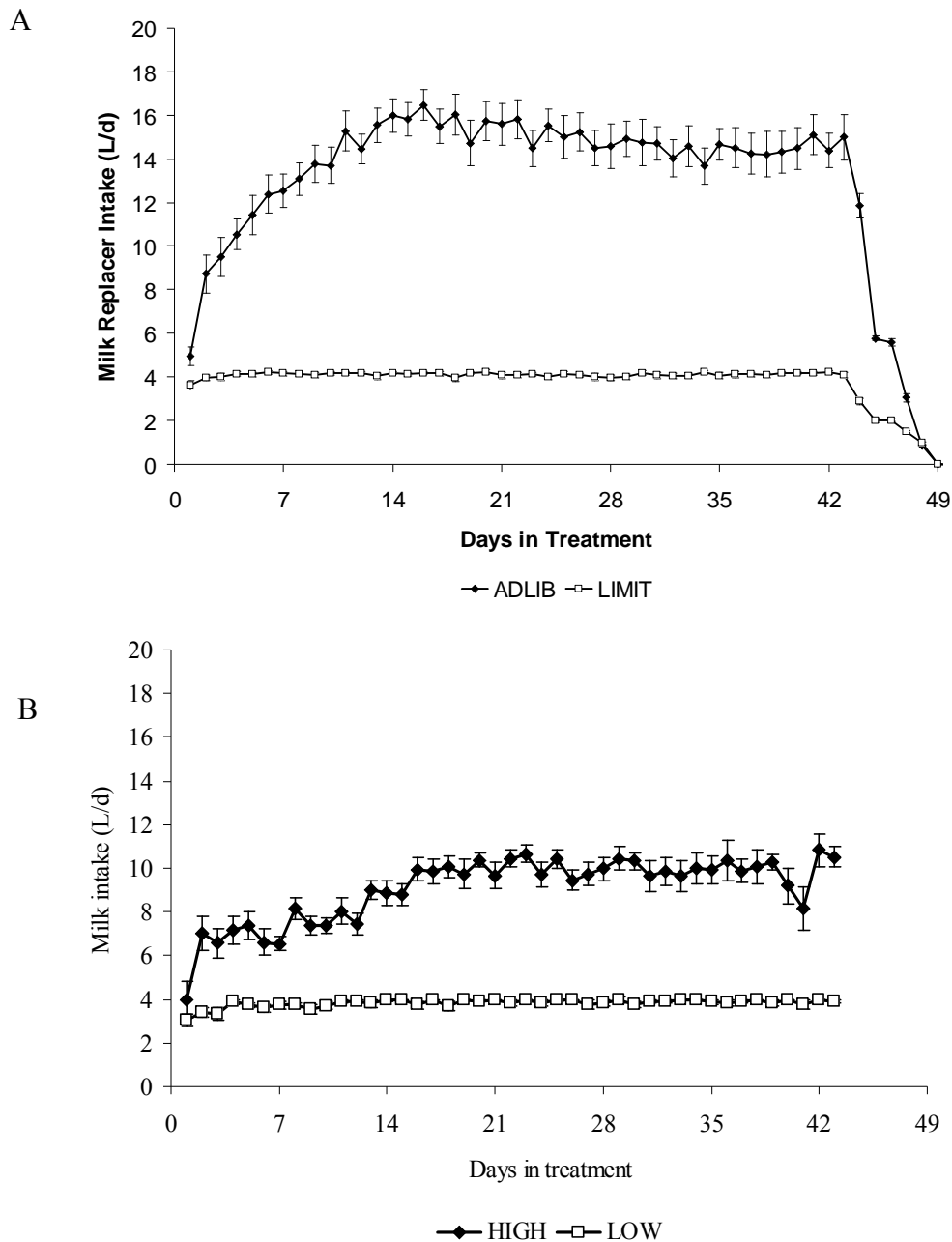
\* Significant differences between treatments ( $P < 0.05$ )

**Table 2.6.4.** Number of calves suffering from gastrointestinal illness, respiratory illness or both in both treatments in experiments 1 and 2.

<b>Illness</b>	<b>Experiment 1</b>		<b>Experiment 2</b>	
	<b>ADLIB</b>	<b>LIMIT</b>	<b>HIGH</b>	<b>LOW</b>
Only gastrointestinal	6	7	3	2
Only respiratory	2	7	0	0
Gastrointestinal + Respiratory	7	1	1	2
<b>TOTAL SICK</b>	15	15	4	4
Total number of calves	25	25	14	14

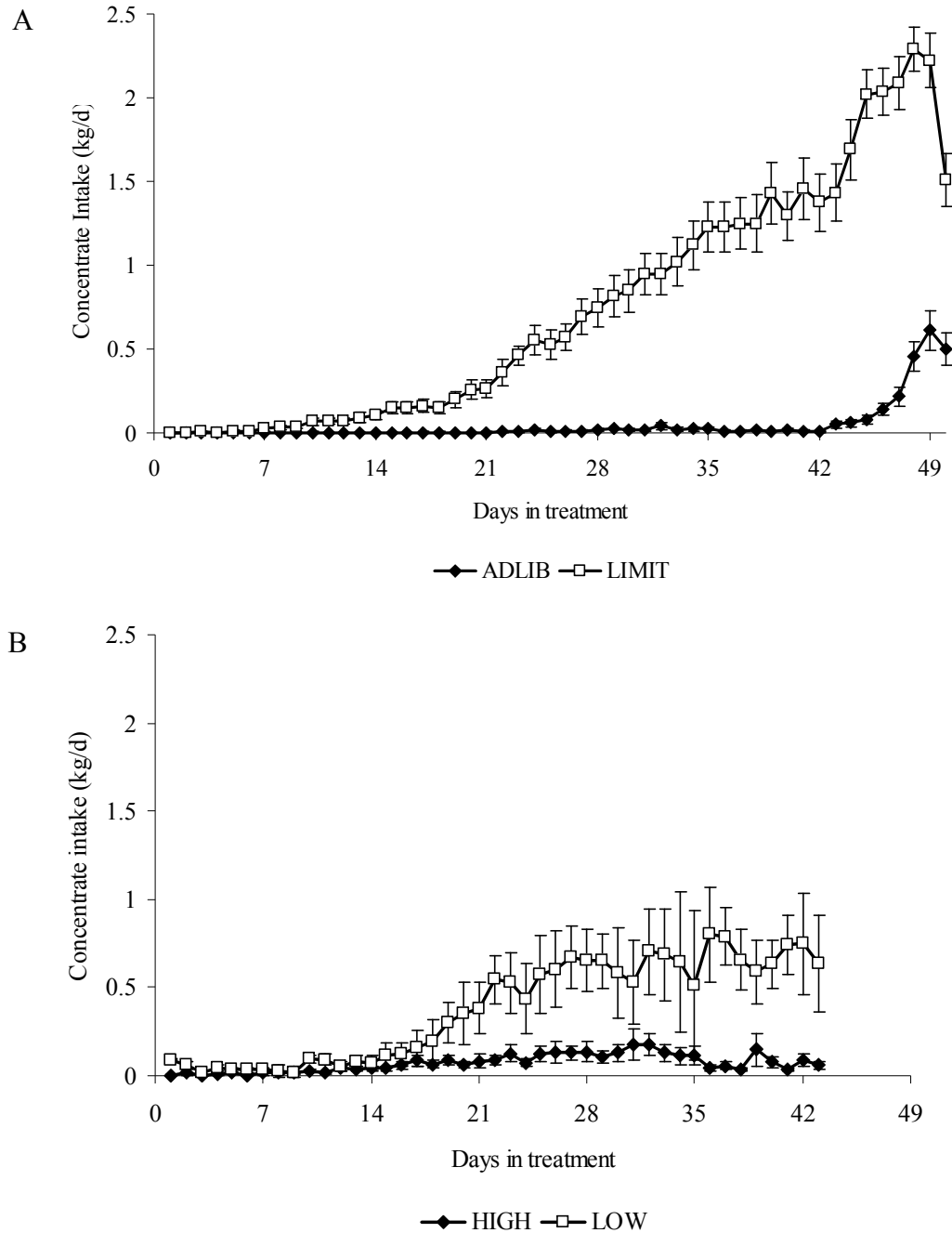
## 2.7 Figures.

**Figure 2.7.1.** Mean ( $\pm$  S.E.) daily intake (kg) of milk replacer of calves fed either ad libitum (ADLIB) or limited (LIMIT) milk replacer in experiment 1 (A), or high (HIGH) and low (LOW) levels of milk in experiment 2 (B). Calves in A were weaned from d 44 to d 48. No milk replacer was offered on d 49 and d 50

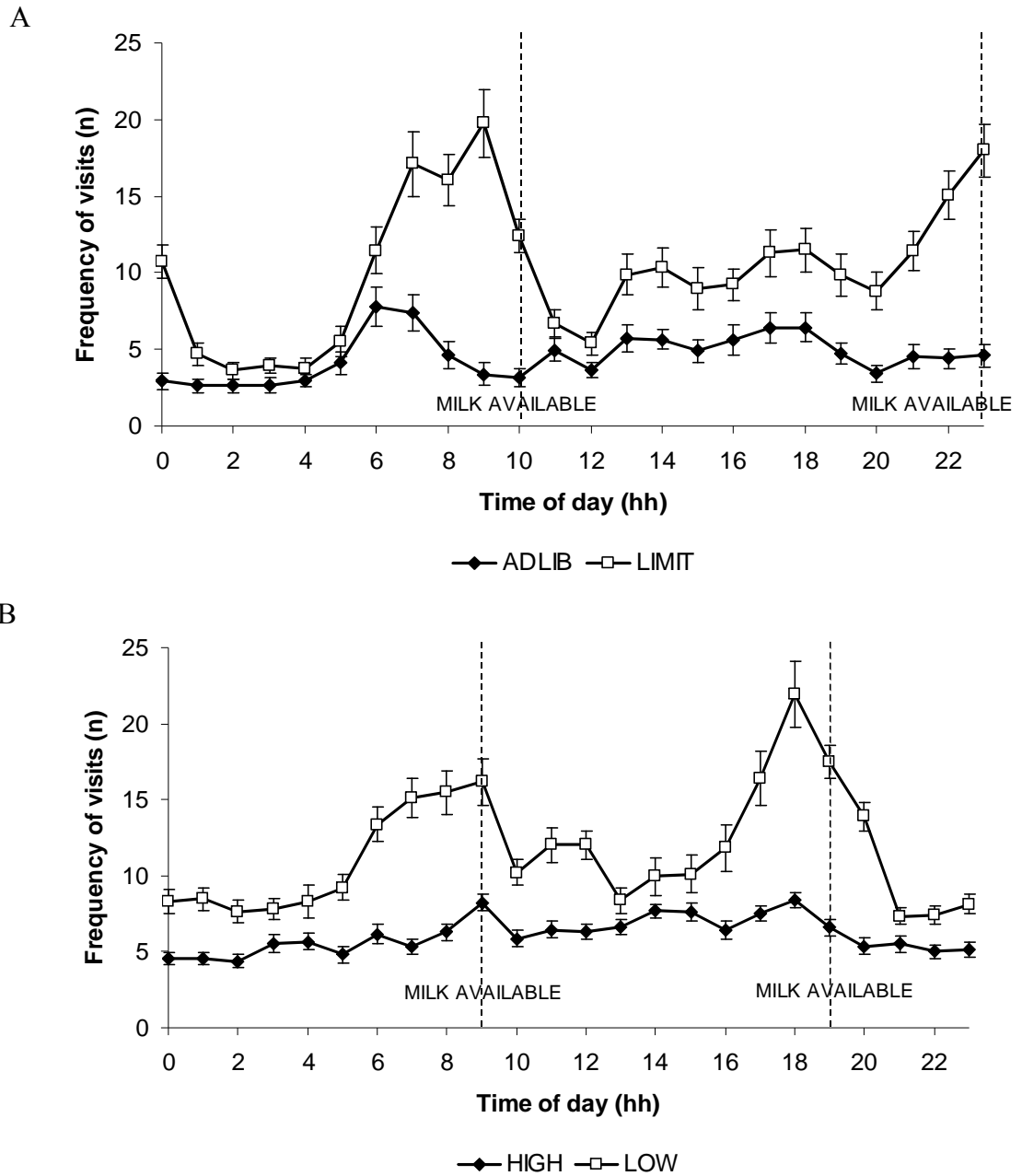




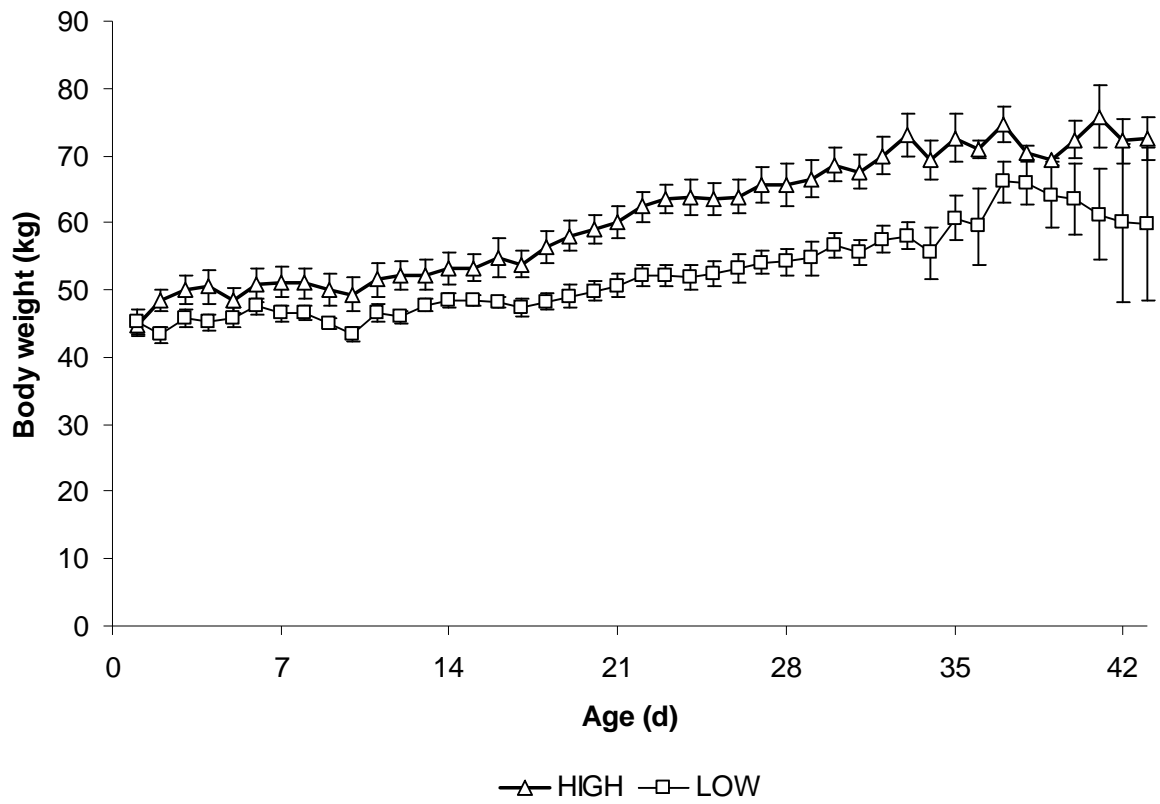
**Figure 2.7.2.** Mean ( $\pm$  S.E.) daily intake (kg) of concentrate of calves fed either ad libitum (ADLIB) or limited (LIMIT) milk replacer in experiment 1 (A) (n=50), or high (HIGH) and low (LOW) levels of milk in experiment 2 (B) (n=28). Calves in A were weaned from d 44 to d 48.



**Figure 2.7.3.** Mean ( $\pm$  S.E.) frequency of visits at each hour of the day in experiment 1 (A) (n=50) and experiment 2 (B) (n=28) [Mean of 10 calves/d/pen]. The vertical dashed lines indicate the time that the automated milk feeder gave the calves a new allotment of milk.



**Figure 2.7.4.** Mean ( $\pm$  S.E.) body weight (kg) of calves fed either high (HIGH) or low (LOW) levels of milk in experiment 2 (n=28).



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## **CHAPTER 3. TEMPERATURE PREFERENCES OF THE NEWBORN DAIRY CALVES FED DIFFERENT LEVELS OF MILK<sup>2</sup>**

### **3.1 Introduction**

Dairy calves are often exposed to cold temperatures and separating the calf from its dam deprives it of an important source of heat. A lack of rhythmicity in body temperature during the first days of life suggests that young calves have difficulties coping with sudden changes in temperature (Piccione et al., 2003). Cold temperatures have been associated with increased calf mortality (Svensson et al., 2006; Johanson and Berger, 2003) especially during the first weeks of age (Azzam et al., 1993), a generalized increase of protein degradation (Scott et al., 1993), impaired absorption of immunoglobulins from colostrum (Norheim and Simensen, 1985; Olson et al., 1980), and increased pneumonic lung lesions (Reinhold and Elmer, 2002). Heat lamps are commonly used as supplemental heat sources for piglets and chicks; supplemental heating is not normally used for dairy calves except for resuscitation during the first 24 h of life (Uysteprust et al., 2002).

Attempts to increase the cold resistance of calves have focused on providing more ingested calories (e.g. Scibila et al., 1987) but Richard et al (1988) found no evidence that milk-fed calves increase intake in response to cold temperatures. Dairy calves can thermoregulate by choosing a warmer microclimate (Hänninen et al. 2003), and by

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modifying their behaviour in cold temperatures by resting more, especially in postures which reduced the area of skin exposed to the air (Hänninen et al. 2003). However, few studies have looked at dairy calves' thermal preferences. The aim of this study was to examine calves' preferences for a supplemental heat source, their use of resting positions that may help thermoregulation and the effect of milk feeding levels.

## **3.2 Methods**

### **3.2.1 ANIMALS AND TREATMENTS**

The institutional animal care committee (monitored by the Canadian Council for Animal Care) approved all procedures described in this study. The experiment was carried out in Agassiz, British Columbia, Canada, between November 2006 and February 2007, when minimum, maximum and mean ( $\pm$  SD) temperatures were  $-9.20$  °C,  $12.70$  °C, and  $3.71 \pm 0.54$  °C respectively. Two temperature loggers (model U23-001, HOBO Pro V2 Temp/RH Data logger, Onset computer corporation, Bourne, MA) were placed in opposite corners of the naturally-ventilated open barn where the calves were housed to continuously record barn temperature (BARNTMP), relative humidity (BARNRH), and dew point (BARNDEWP). Mean ( $\pm$  SD) and ranges [minimum to maximum] for these three variables were  $6.27$  °C ( $\pm 3.12$  °C) [ $-5.46$  °C to  $16.63$  °C],  $71.61\%$  ( $\pm 14.17$ ) [ $34.69\%$  to  $85.38\%$ ], and  $1.34$  °C ( $\pm 5.05$  °C) [ $-15.90$  °C to  $8.90$  °C].

Holstein calves (13 females, 14 males, BW =  $43.07 \pm 1.11$  kg, mean  $\pm$  S.D.) were housed individually for the first 3 d after birth in wood-shavings bedded pens (3.81 x 1.96 m). We used four pens that were in a single row but which were separated by a solid wall so the calves could not see each other when they were lying down in the pen. The



experimental calves were visually isolated from other calves that were not in the experiment. In order to avoid a pen bias, we assigned the experimental calves to these four pens balancing for milk level and colour of the coat. Calves entered the experiment as they were born in the dairy farm. Consequently, each calf was treated independently. The calves were moved to the pens on d 1 after separation from the dam and their first intake of colostrum ( $4.11 \pm 0.55$  L/d, mean  $\pm$  S.D.). A blood sample was collected by jugular venipuncture within 24 to 48 h after the feeding of colostrum to measure the degree of passive transfer by refractometry of total serum protein (Reichert AR200 Digital handheld refractometer, Reichert, Depew, New York). The average serum protein level for the calves was  $6.23 \pm 0.73$  g/dl (mean  $\pm$  SD). No calves showed serum protein levels below 5.0 g/dL.

At one end of the pen, two infrared heat lamps (250 W/ R40/HR, Satco products, Brentwood, New York) were fixed from a support so that the head of the bulb stood at 1.1 m from the ground, 0.61 m from the wall, and 0.66 m between them (Figure 3.7.1). We considered the pen area to be divided into three equal sized thermal zones with decreasing temperature gradient (HOT = Zone under the heat lamps; WARM = Central zone; COLD = Farthest zone from the heat lamps). The temperature at 5 fixed points within each zone was measured with a black globe thermometer (model 210-4417, Novalynx Corporation, Grass Valley, California) placed 0.3 m above the floor, and left at each point for 20 min when no calves were in the pen (Figure 1). The outside temperature range when the black globe thermometer measurements were taken was between 7.90 °C and 12.80 °C.

Calves were semi-randomly assigned to two treatment groups balancing for sex, birth weight and coat colour. Coat colour was evaluated visually and the resulting categories were predominantly black (more than 50% of coat black), predominantly white (more than 50% of coat white) and mixed (approximately 50% black and 50% white). The calves were individually fed pasteurized whole milk at either a high (Treatment HIGH: 30 % BW; N = 15) or low (Treatment LOW: 8% BW; N = 12) daily allowance; the latter reflected current rearing approaches in North American commercial farms. Milk was provided at a temperature of  $27.67 \pm 2.54$  °C (mean  $\pm$  S.D.) twice a day for 2 h (8:00 a.m. to 10:00 a.m. and 4:00 p.m. to 6:00 p.m.) in 20 L plastic buckets connected by a hose to a nipple attached to the midpoint of the front fence of the pen, in the Warm zone. The amounts (kg) of milk offered and consumed were recorded for each meal for each calf. No water or concentrate were provided during the 3 d trial to avoid the presence of the calf in the warm zone for other reasons than thermal preference.

Calves were weighed daily before the afternoon meal after a health check was performed by trained personal. The health check included rectal temperature, respiratory and cardiac rates and sounds, presence of diarrhoea, presence of nasal and ocular discharges, general state of the coat and dehydration including tent test and muzzle humidity. None of the calves were found to suffer digestive or respiratory problems during these first 3 d of life and no rectal temperature above 39.5 °C was recorded.

A temperature logger (model U23-001, HOBO Pro V2 Temp/RH Data logger, Onset computer corporation, Bourne, MA) was placed in a black cotton bag that was fixed with Velcro<sup>®</sup> strips to the lumbar zone of each calf during the entire trial. The resultant temperature (CALFTEMP) was recorded every minute and used to estimate the microclimate in the areas that the calf chose to occupy.

Video cameras (model WV-BP334, Panasonic, Suzhou, China), were placed 2.74 m above and 1 m in front of the pens' wall to record the position and the posture of each animal for 24 h/d during the 3 d trial. The position and posture of the calves during the morning and afternoon feeding periods (2 h each) was not included in the analysis. Scan samplings at 5, 10, 15 and 20 min were compared for 72 h videos of 10 calves. Initial analyses found no significant differences in proportions of total time spent in each posture between sampling intervals. Therefore, scan sampling at 20 min intervals was done by a single observer. At each time sample, the position of the calf's head and rump was categorized as being in the Hot, Warm or Cold zones. The calf's posture (standing or lying down) was also scored. In addition, when the calf was lying down, the position of the front and back legs was recorded as contracted (extremities folded making contact with the body) or extended (unfolded extremities separated from the body).

### **3.2.2 STATISTICAL ANALYSIS**

Each day was divided into 4 periods: morning (6:01 a.m. to 12:00 p.m.), afternoon (12:01 p.m. to 18:00 p.m.), evening (18:01 p.m. to 12:00 a.m.) and night (12:01 a.m. to 6:00 a.m.). The mean ( $\pm$  S.D.) daily temperatures for each period were: Morning:  $5.47 \pm 3.15$  °C; Afternoon:  $7.51 \pm 3.05$  °C; Evening:  $5.85 \pm 3.09$  °C; Night:  $5.13 \pm 3.16$  °C.

The effect of treatment on the average daily milk intake (kg) and weight gain (kg) was tested using the mixed procedure (PROC MIXED) of SAS (SAS Institute, 1999). The model used included treatment (HIGH or LOW milk allowance), day of trial (1, 2, or 3) and their interaction.

The calves' position relative to the heat lamps was calculated in the following way. For each period of the day, we calculated the percent of observations (out of 18 for each period) in which the calf's head was in each of the three temperature zones. These data were analyzed using the mixed procedure (PROC MIXED) of SAS (SAS Institute, 1999), with a model that included treatment (HIGH or LOW milk allowance), zone (Hot, Warm or Cold), and the interaction between zone and treatment, period (Morning, Afternoon, Evening and Night), sex of the animal, and day of trial, and a three way interaction between zone, period and day, with calf nested within treatment. No effects of sex were found and so this was removed from the analysis. Since we found no differences in actual milk intake on d 1, we did a second analysis using data from d 3 only, without the day factor in the model.

The effect of treatment on resting behaviour (lying down) of all calves was analyzed using the original mixed model that included day of trial except that we also included the position of the calf as a factor and the interactions between treatment, day of trial and position of the calf. Since the initial analysis found no differences between the Warm and the Cold zone in the amount of time the calves spent in each zone, we combined these two zones and compared them with the Hot zone. The position of the calf was categorized as 2 = Either the head or the rump or the whole body of the calf is in the Hot zone, or 1 = All parts of the body of the calf are in the Warm or Cold Zone. Variables in this model included percentage of daily time spent either standing or lying down, and percent of lying time spent with the front and back legs extended or contracted. Temperature difference between the temperature logger on the calf and the temperature logger on the barn (TEMPDIFF) was analyzed using the same model.

Spearman correlation coefficients were calculated between the time spent by the calves in or out of the Hot zone (POSITION), the barn temperature and the temperature difference between loggers on the calf and in the barn (TEMPDIFF), using data that were ranked within calf, in order to remove between-calf variability.

### 3.3 Results

Table 3.6.1 shows the least square means of daily weight gains and amounts of milk consumed by each group of calves using data from all 3 d. We found overall effects of treatment, day, and their interaction both in daily milk intake and in daily gain of weight ( $P < 0.001$ ). No differences between treatments were found in milk intake or weight gain on d 1 with both groups of calves losing weight. HIGH calves increased their milk intake on d 2 and d 3 and these intakes were greater than those of LOW calves. LOW calves lost weight on d 2 and gained nothing on d 3 while HIGH calves gained weight on the subsequent two days. No illness was detected in any of the calves.

There was a significant effect of Zone on the percentage of time that the calves spent in each of the three temperature zones ( $P < 0.001$ ) with calves being observed significantly more often in the Hot Zone than in the other two zones (Fig. 3.7.2). There was no significant interaction between Zone and Treatment ( $P = 0.17$ ), but there was a significant interaction between Zone and Day ( $P = 0.005$ ; Fig. 3.7.3), with calves increasingly found in the Hot Zone rather than the Warm zone as they aged. There was a trend for an interaction between Zone and Period ( $P = 0.06$ ) with calves generally being in the Hot zone more during the Morning and Afternoon. When only the results for d 3

were analyzed, there were no significant effects ( $P > 0.10$ ) of treatment on the percentage of time that the calves spent in each zone, nor any significant interaction between treatment and period.

Using the data from all 3 d, BARNTEMP and CALFTEMP were significantly correlated ( $r = 0.49$ ,  $n = 71$ ,  $P < 0.001$ ). There were no significant effects of treatment on the difference between the temperatures recorded by the loggers in the barn and on the calf (TEMPDIFF) and no interactions between treatment and day or period ( $P > 0.10$ ). However, there was a significant effect of Period on TEMPDIFF ( $P = 0.01$ ). TEMPDIFF (CALFTEMP-BARNTEMP) was highest at night ( $12.79 \pm 0.38$ , mean  $\pm$  S.E.) and lowest in the afternoon ( $12.09 \pm 0.38$ , mean  $\pm$  S.E.). There was no significant correlation ( $r = 0.07$ ,  $n = 66$ ,  $P = 0.55$ ) between BARNTEMP and the calf POSITION, meaning that the proportion of observations during a period in which the calf was in the Hot Zone rather than the Cold Zone was not related to barn temperature. The difference between the temperatures recorded in the barn and on the calf (TEMPDIFF) was positively correlated with the Position of the calf ( $r = 0.32$ ,  $n = 66$ ,  $P = 0.007$ ) meaning that the more often the calf was in the Hot Zone, the greater the difference between CALFTEMP and BARNTEMP.

Calves in HIGH and LOW treatments spent respectively  $85.60 \pm 0.9$  % and  $83.27 \pm 1.0$  % of their daily time lying down. There was no effect of treatment or day (both  $P = 0.11$ ) but Period of the day had an effect ( $P < 0.001$ ), with calves lying down more time in the evenings ( $87.07 \pm 1.0$  %) or nights ( $88.55 \pm 1.0$  %) than in mornings ( $80.02 \pm 1.0$  %) and afternoons ( $82.08 \pm 1.0$  %). No differences were found between the Hot zone and the Cold or Warm zone in the percentage of time in each zone that the calves spent lying

down (Cold or Warm zones =  $84.16 \pm 1.0$  %; Hot zone =  $84.63 \pm 1.0$  %). No effect of treatment, day or period was found when comparing the percentage of daily time spent by calves lying down with their front or back legs extended or contracted ( $P > 0.10$ ).

### **3.4 Discussion**

During the first 3 d of life, calves showed a strong preference to be in the area of the pen in which the heat lamps were placed, spending more than half of their time in the Hot zone even though this was only one third of the available pen area. This zone had a temperature 4 to 6 °C higher than the other two zones. The calves' preference for the Hot zone increased as the calves aged but we found no evidence that this temperature preference was affected by the temperature in the barn, even though this varied considerably during the course of the experiment. The lower critical temperature for calves of this age is 15 °C (NRC, 2001) and the barn temperature was often lower than this, reaching a minimum of -5°C. Nor did we find any evidence that increasing the amount of milk fed to the calves reduced their thermal preferences. Schrama et al. (1992) reported that calves fed below maintenance level exhibited a decreased heat production, probably due to a reduction of thyroid hormone level and the exhaustion of body energy reserves. We hypothesized that calves fed a lower amount of milk would show a larger preference for the warmer areas of the barn. However, we found no evidence that the amount of milk fed affected calf temperature preferences, even when effects on growth were apparent on d 3.

The lack of an effect of barn temperature and milk feeding level on calves' thermal preferences may reflect the limited ability of very young calves to thermoregulate both behaviourally and physiologically, as reported by Stanko et al. (1991). In natural

environments, the cow provides the calf with its main external source of heat, and during the first days of life, the cow initiates most of the contact rather than the calf (Jensen, 2001). Consequently, the very young calf may have little ability to thermoregulate by choosing a warm environment. Although we showed that young calves do choose a warmer environment, they may have limited ability to fine-tune their preferences based on external temperatures or the amount of metabolic heat produced. Indeed, the increased preference for the heat lamps as the calves aged may be evidence of a gradual improvement in their ability to thermoregulate by choosing a warmer environment.

During the first 3 d of life, the calves ingested large amounts of milk, i.e. up to 8 L in HIGH calves. Intakes on d 1 were low, but this may reflect the 4 L of colostrum provided earlier the same day. Even though feeding milk at 8% BW/d is common commercial practice in North America, low fed calves lost weight during the three days of trial, which has been reported previously in calves fed at 10% BW/d (Jasper and Weary, 2002). The absence of any kind of illness indicate that neonatal calves can be fed high amounts of milk from their earliest days with no deleterious consequences, as previous studies (De Paula Vieira et al., 2008; Appleby et al., 2001; Jasper and Weary, 2002) have shown in older calves.

Calves spent most time lying down, which has been previously reported (Hänninen et al., 2008). In older calves, lying down may be a form of thermoregulation. Schrama et al. (1993) found that 6 d to 13 d old calves kept under low ambient temperatures attain their lower critical limit at 17 °C while standing, while calves lying down attain their lower critical limit at 13.5 °C. Resting on the sternum or with the legs contracted may also be a form of thermoregulation. However, we found no effect of



feeding level on resting postures. This suggests that the lack of an effect of milk feeding level on thermal preferences was not due to the fact that the calves were thermo-regulating by adopting these resting postures.

We attached temperature loggers to the calf in an attempt to directly measure the ambient temperatures as felt by the calf, rather than relying on observations of the calf's position in the pen. The temperatures recorded on the calf were correlated with barn temperatures, showing that these loggers were reflecting overall ambient temperatures rather than just the calves' body temperature. However, they were consistently higher than barn temperatures, suggesting that they may have been affected by the calves' body temperature. However, we found some evidence that the temperatures recorded on the calf were being affected by the micro-environment selected by the calf; the difference between the temperature on the calf and the barn temperature was greater when the calves were under the heat lamps than when they were far from the heat lamps. Thus, these loggers have some potential to measure more directly the ambient temperatures chosen by the calf.

Although calves are precocial animals with capacity to maintain their own body temperature (Broad et al., 2006) they are born with limited body energy reserves and only modest insulation afforded by their hair coat and body fat. Despite the calves' difficulties with thermoregulation, it is not common practice to provide indoor-housed dairy calves with a supplementary heat. Uysteprust et al. (2002) found that calves under an infrared heat lamp maintain a higher rectal temperature without increasing energy mobilization and have improved pulmonary ventilation. Our results show that young calves do show a preference for warmer environments even when external temperatures are mild, and that

feeding them higher amounts of milk may not reduce their need for external sources of heat at this young age. Consequently, there may be some advantages in providing them with an external source of heat.

### **3.5 Conclusions**

Our results suggest that the use of heat lamps during the first days of life is a good management practice since even well-fed calves show a strong preference for this external source of heat.

### 3.6 Tables

**Table 3.6.1.** Least square means ( $\pm$  S.E.) of daily weight gain and milk intake during the first 3 d after birth of calves fed either a HIGH (n = 15) or LOW (n = 12) allowance of milk.

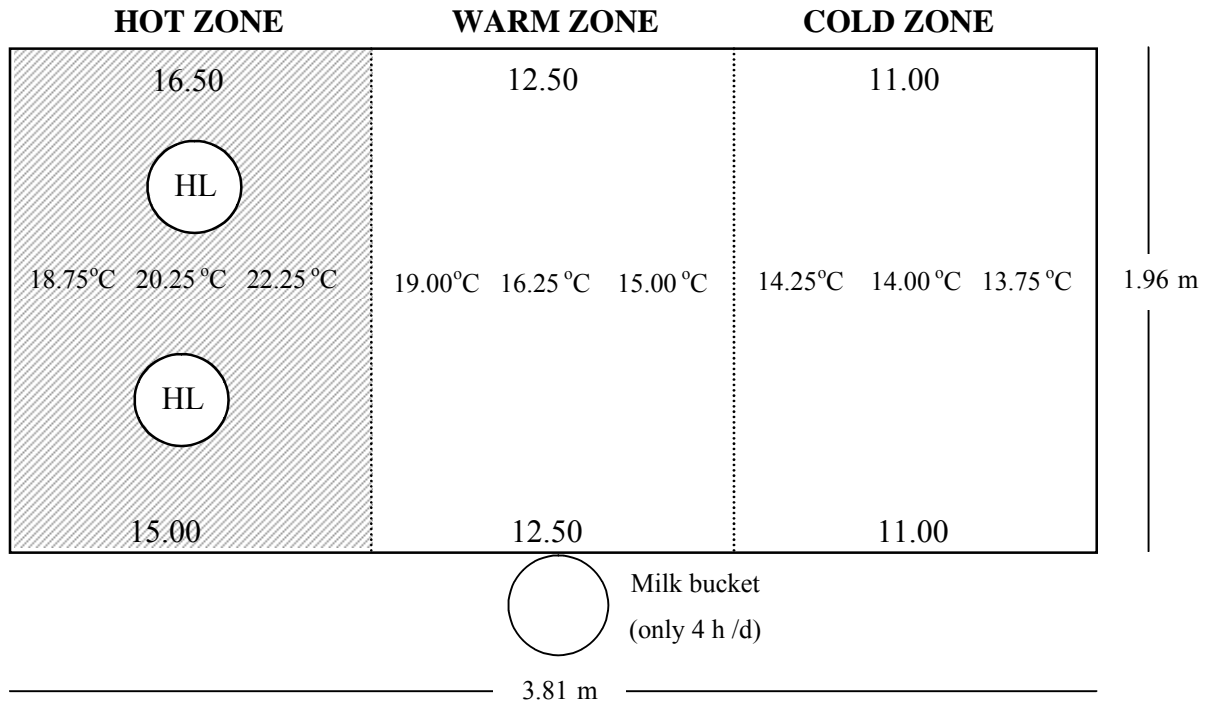
Treatment	HIGH			LOW		
Day	1	2	3	1	2	3
Daily weight gain (kg/d)	$-0.48 \pm 0.20^{1, a}$	$0.65 \pm 0.20^{2, b}$	$1.09 \pm 0.23^{2, b}$	$-0.58 \pm 0.22^{1, a}$	$-0.73 \pm 0.22^{1, a}$	$0.01 \pm 0.24^{1, b}$
Milk intake (kg/d)	$3.59 \pm 0.39^{1, a}$	$6.28 \pm 0.39^{2, b}$	$7.92 \pm 0.39^{3, c}$	$2.76 \pm 0.44^{1, a}$	$3.23 \pm 0.44^{1, a}$	$2.98 \pm 0.44^{1, a}$

<sup>1, 2</sup> Different numbers indicate significant differences ( $P < 0.01$ ) **between** treatments at each day.

<sup>a, b</sup> Different letters indicate significant differences ( $P < 0.01$ ) between days **within** treatment.

### 3.7 Figures

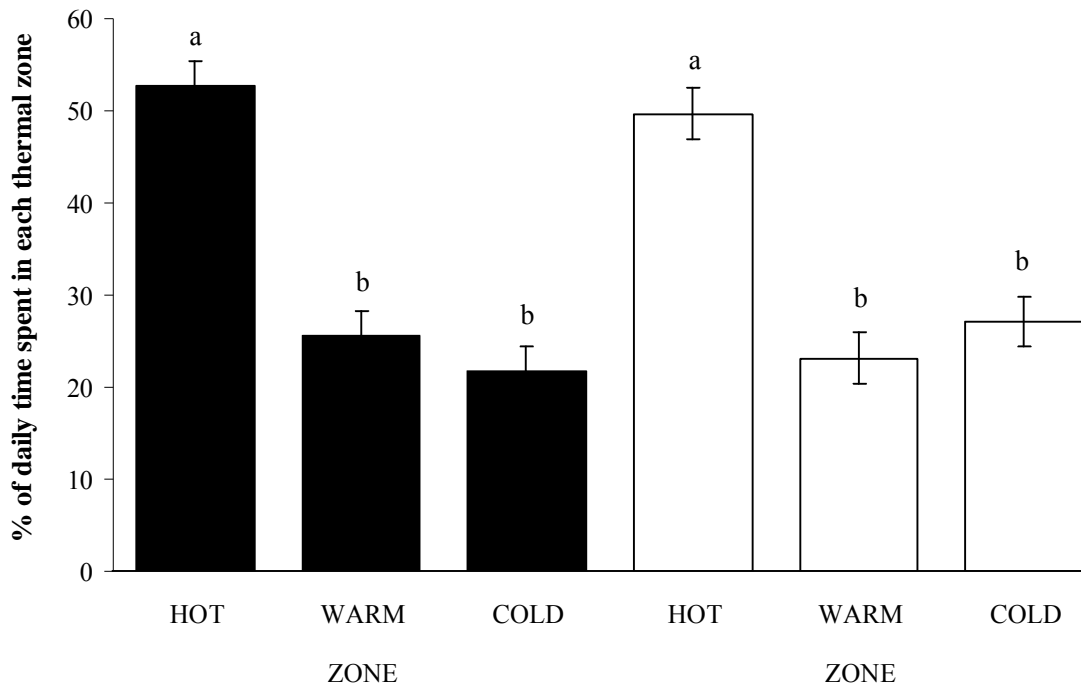
**Figure 3.7.1.** Layout of the experimental pen with each thermal zone (HOT, WARM, COLD) and temperature gradient measured with a black globe thermometer\*.



HL = 250 W IR Heat lamp

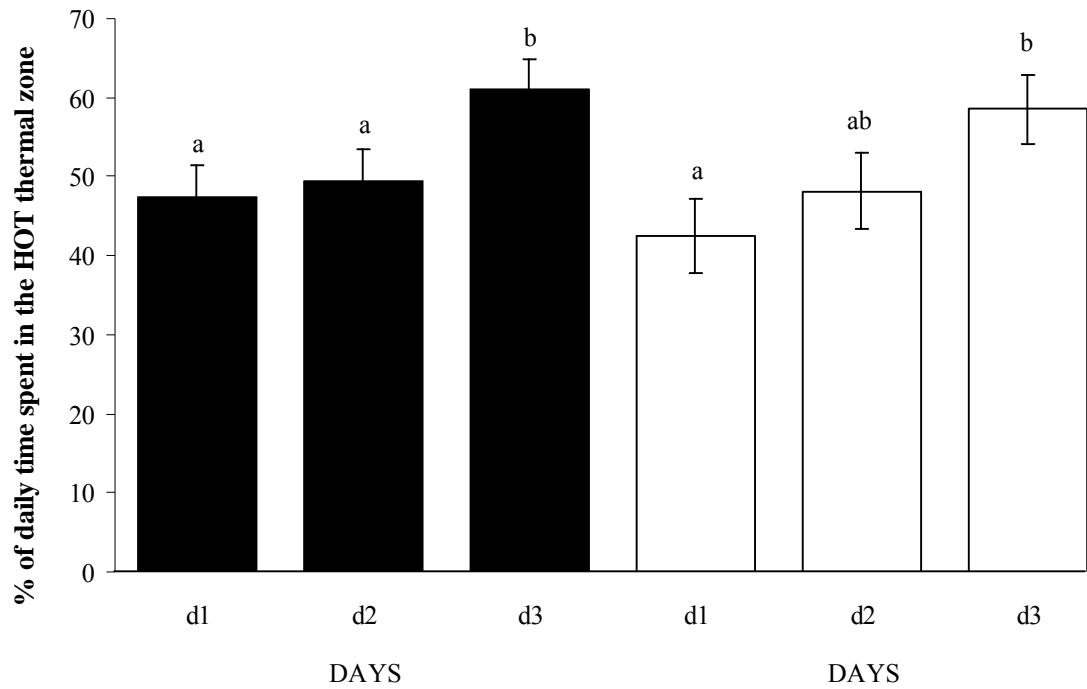
\* Data from an independent sample

**Figure 3.7.2.** Mean ( $\pm$  S.E.) percent of time that the calves under HIGH (black bars) (n = 15) or LOW (white bars) (n = 12) milk allowance were observed under each thermal zone (HOT zone is directly under heat lamps).



<sup>a, b</sup> Different letters indicate differences **within** treatment ( $P < 0.001$ ).

**Figure 3.7.3** Mean ( $\pm$  S.E.) percent of time that the calves under HIGH (black bars) (n = 15) or LOW (white bars) (n = 12) milk allowance were observed in the Hot zone on each day of trial.



<sup>a, b</sup> Different letters indicate differences **within** treatment ( $P < 0.001$ ).

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# **CHAPTER 4. BEHAVIOUR OF DAIRY CALVES FOLLOWING A LOW DOSE OF BACTERIAL ENDOTOXIN<sup>3</sup>**

## **4.1 Introduction**

Rates of illness remain high among dairy calves (Losinger and Heinrichs, 1997; USDA, 2002; Svensson et al., 2003). Treatment is more effective if done early and so there is a need for better early detection of illness (González et al., 2008).

Animals respond to illness with a consistent pattern of behavioural changes, including reduced feeding and social behaviour and increased rest (Johnson, 2002; Dantzer, 2007). These behavioural changes occur simultaneously with physiological changes and are adaptive responses helping the animals cope with illness (Owen-Ashley et al., 2006). Veterinary diagnosis involves some assessment of these behaviours (Broom, 2006). A better understanding of which behaviours change as illness develops may help improve the early detection of illness. Research on laboratory animals shows that behavioural responses to illness are stimulated by injections of bacterial lipopolysaccharide (LPS; Wen and Prendergast, 2007), operating through cytokines (Johnson, 1998; Dantzer, 2001; Larson and Dunn, 2001). Some research has examined behavioural responses to LPS in swine (Johnson and von Borell, 1994; Wright et al., 2000). Immune and physiological responses of calves to LPS have been described

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<sup>3</sup> A version of this chapter has been published. Borderas T.F., A.M. de Passillé, and J. Rushen. 2008. Behavior of dairy calves after a low dose of bacterial endotoxin. *J. Anim. Sci.* 86:2920-2927.

(Kinsbergen et al. 1994; Elsasser et al. 1996; Deluyker et al. 2004), but no studies have documented behavioural responses to induced sickness in cattle.

To produce an immunological response, high doses of LPS are often used. The effect of low doses of LPS, which might be more typical of the early onset of illness, has been studied only in primates (Krabbe et al., 2005; Willette et al., 2007).

To help understand the early behavioural response of dairy calves to a mild immunological challenge, we examined the effect of low doses of LPS on heart rate, respiratory frequency, and body temperature of calves and examined the behavioural changes that accompanied the presence of fever, which was used as the main indicator of the response.

## **4.2 Methods**

This study was conducted at the Dairy and Swine Research and Development Centre at Lennoxville, Quebec, Canada. The institutional animal care committee (monitored by the Canadian Council for Animal Care) approved all procedures described in this study.

Seven young [(mean  $\pm$  SD) age:  $22.83 \pm 3.6$  d; BW =  $51.92 \pm 4.5$  kg] and 8 older ( $153.92 \pm 8.1$  d;  $181.08 \pm 14.8$  kg) Holstein calves (2 males, both young, and 13 females: 5 young and 8 old) were housed in individual pens (2.15 m x 1.7 m) with free access to water through an automatic water bowl. All animals were kept in the same heated room with an average temperature of 18°C and 12 h/d light (0700 to 1900). Normal feeding management consisted of younger calves being bucket fed 4 kg of milk replacer (CP = 18.5 %; Violac. Coopérative Fédérée de Québec, Montreal, Canada) and 0.250 kg of

concentrate (CP = 22.0%; Goliath XLR. Coopérative Fédérée de Québec, Montreal, Canada) twice a day (at 0800 and 1500 h) and older calves bucket fed 0.5 kg of concentrate at the same times. All animals had free access to hay in a hayrack freshly filled twice a day shortly after concentrate was offered. Since the aim of the project was to examine behavioural and physiological changes associated with induced fever in otherwise healthy calves, we used only calves that showed no signs of illness during the experiment. The health check performed by a qualified veterinarian of all calves included assessment of body temperature, respiratory and cardiac rates and sounds, presence of diarrhoea, presence of nasal and ocular discharges, general state of the coat and dehydration (tent test and muzzle humidity).

All calves of both ages were fitted with an indwelling jugular catheter 3 d before the start of the experiment, and half the calves of each age group were assigned randomly to receive either a low (0.025 µg/kg, iv) or high (0.05 µg/kg, iv) dose of bacterial LPS (*E. coli* O55:B5. L6529; Sigma, St. Louis, MO) in 50 ml volume. These doses were established through a previous pilot study, which showed that both of these doses induced fever in young and old calves but were mild enough to not cause prostration, severe pathological consequences, or pre-shock state.

A cross-over design was used in which each calf was used as its own control. Treatment consisted of injections of LPS with injections of physiological saline (**SAL**) administered as a control on a separate day. One half of the calves received LPS on d 1 through the jugular catheter just before to the morning feeding at 0800 h, while the other one half received SAL by the same procedure. The volume infused in both treatments was 50 ml per calf. After 7 d, the treatments were reversed and calves that had received

LPS previously were injected with SAL while calves that had received SAL previously were injected with LPS. The order of treatment and control days were balanced across age groups and doses.

A health check that included rectal body temperature (RT), heart rate (HR) and respiratory frequency (RF), as well as eyes and nose secretions, state of coat, and humidity of the muzzle, was performed on each calf before the initial injection. Calves were observed directly for the following 60 min to ensure no septic shock resulted as consequence of LPS injection.

To determine the effectiveness of the low doses of endotoxin, RT, HR and RF were recorded by a qualified veterinarian before injections, at 1-h intervals during the first 4 h, and at 6, 8, 10, 12 and 24 h after first injection. To measure RT, a digital thermometer was fully inserted into the rectum and temperature was recorded to the nearest 0.1°C. Heart rate was measured by applying a stethoscope to the seventh left intercostal space near the sternum and counting heartbeats during a 20-s period. After assessing HR, RF was measured by counting the number of expansions of the thoracic wall during a 30-s period.

Video cameras were placed 2 m in front of and 2.5 m above the pen walls to record the behaviour of each animal (see Table 4.6.1 for description of behaviours). Recordings began 24 h before injection of LPS or SAL and ended 24 h after. Differences in body temperature between treatment and control days were observed 2 to 6 h after the start of the experiment for both age groups. Because the maximum rectal body temperature occurred 4 h after LPS injection, the behaviour of the calves was observed during the 2 h before and 2 h after the maximum rectal body temperature was reached

**(FEVER PEAK)** and during the same period of time during the control days. A single observer blind to treatment scored the calves following the behavioural definitions in Table 4.6.1 and using the Observer program (Noldus Information Technology, Wageningen, 2003). These behaviours were identified based on key behaviours associated with sickness behaviour (Dantzer 2001). Milk and concentrate intake were monitored by weighing refusals in the milk or concentrate buckets during the experimental day at 8 and 12 h after the initial injection.

#### **4.2.1 STATISTICAL ANALYSIS**

The effect of LPS on RT, HR, and RF of all calves was analyzed using the mixed procedure of SAS (SAS Institute, Cary, NC). The model included treatment (LPS or SAL), age (young or old), dose (high or low), and time as factors, with calf nested within age and dose. The interactions between treatment, age, and dose were also included. Physiological variables were analyzed for the 24-h period following treatments with baseline values (0 h) as covariates. Behavioural data from calves during the period of peak fever were analyzed using the mixed procedure with a model that included the same factors and interactions previously described for the physiological variables. Variables in this model included total duration, bout frequency, and mean bout duration of behaviours. Since bouts of self-grooming were very short, we analysed only the frequency of the bouts. Natural logarithm transformations ( $\ln x + 1$ ) were used when variables did not show normal distribution. Transformed variables included time spent eating hay and time spent ruminating.

### 4.3 Results

Figures 4.7.1 to 4.7.3 show the time course of changes in RT, HR, and RF following LPS and SAL injections. Table 4.6.2 shows the mean ( $\pm$  S.E.) values ( $n = 15$ ) of the physiological variables. Administration of LPS increased RT and RF in all calves. During the 4 h period of FEVER PEAK, the average maximum RT for the calves was  $40.55 \pm 0.13^{\circ}\text{C}$  during the treatment day. This temperature was reached  $4.80 \pm 0.32$  h after the LPS injection. The average temperature at this time on the control day was  $39.16 \pm 0.05^{\circ}\text{C}$ . Heart rate was not affected by LPS treatment. An effect of age ( $P = 0.001$ ) on RF was observed, with older calves showing a higher RF ( $44.60 \pm 0.88$  bouts/min for all calves) than younger calves ( $37.87 \pm 0.69$  bouts/min for all calves). No significant effects of dose ( $P = 0.78$ ) or interactions between age and dose ( $P = 0.48$ ) were reported.

No effect of treatment was observed in the amounts of milk and concentrate consumed by younger or older calves. No refusals were recorded for milk or concentrate during the experimental day at 8 h and 12 h after the initial injection of LPS or SAL.

Tables 4.6.3 and 4.6.4 show the effect of treatment on behavioural variables and postures of calves, during the 4-h period of FEVER PEAK. Treatment with LPS resulted in a decreased duration of rumination, which was due to a reduction in both the frequency of bouts ( $P = 0.002$ ) and the mean bout duration ( $P = 0.01$ ); LPS also reduced the total duration of time spent eating hay, and increased the total duration of lying inactive. There was a trend for longer bouts for lying inactive with LPS. Although calves tended to spend more time standing inactive following LPS, this effect was not statistically significant due to the large standard error following LPS. However, LPS increased both the frequency of bouts and the mean bout duration of standing inactive. No differences



between treatments were found for time spent eating concentrate, drinking water, and time spent standing up or lying down on the side or sternum. Treatment with LPS decreased the frequency of bouts of self-grooming (LPS vs. SAL:  $13.47 \pm 1.75$  vs.  $24.07 \pm 3.12$ ,  $P = 0.008$ ).

There was no effect of dose or interaction between treatment and dose or the age of the calves except for a significant interaction between age and treatment for time spent inactive (young calves: LPS  $179.95 \pm 10.46$  min SAL  $160.99 \pm 10.46$  min; older calves: LPS  $139.42 \pm 9.83$  min SAL  $71.38 \pm 9.83$  min,  $P = 0.03$ ). An effect of age was found on time spent drinking water (younger calves:  $1.67 \pm 1.30$  min; older calves:  $6.99 \pm 1.22$  min,  $P = 0.001$ ), eating hay (younger calves:  $2.27 \pm 1.26$  min; older calves:  $31.50 \pm 1.25$  min,  $P = 0.001$ ), and time lying inactive (younger calves:  $161.46 \pm 7.81$  min; older calves:  $85.10 \pm 6.91$  min,  $P = 0.001$ ).

#### **4.4 Discussion**

Following a mild dose of LPS, calves showed increased RT and RF, indicative of a fever response. During the 4-h period that preceded and followed the peak in body temperature, LPS injections also reduced the time spent ruminating, grooming, eating hay, and increased the time spent lying and standing inactive. These behavioural changes may be early indicators of developing illness in calves. However, there was no change in the overall time spent standing or lying, or in the amount of milk or concentrate eaten.

Most studies report a decrease in food intake following an endotoxin challenge (Wright et al., 2000; Elander et al., 2007; Kim et al., 2007). Steiger et al. (1999) found a significant decrease in food intake 4 h after infusing Holstein x Jersey heifers with 2

$\mu\text{g}/\text{kg}$  of LPS during 100 min, with the difference in food intake between control and treated animals still evident 24 h after the infusion. In our experiment, milk and concentrate intake were not affected during FEVER PEAK. Although we did not measure actual hay intake, the time spent by the calves at the hayrack was decreased during FEVER PEAK. The lack of an effect on milk and concentrate intake may be due to the mild doses used. Johnson and von Borell (1994) reported that the reduction of food intake by LPS administration was dose-dependent and short-lived. Pigs receiving the lowest dose in their experiment ( $0.5 \mu\text{g}/\text{kg BW}$ ) even showed a compensatory increase in feed intake, masking the anorexic effect of LPS shown by male pigs challenged with larger doses ( $50 \mu\text{g}/\text{kg BW}$ ). Alternatively, the high feeding motivation of the calves may have limited the effect of LPS on milk intake, given that the commercial levels of milk fed to calves, which we used, are well below ad libitum intake levels (Jasper and Weary, 2002). The small amount of time needed by the calves to consume their portion of milk and concentrate may also have masked any effect. In contrast, hay intake might have been affected due to the low nutritional value of this component of the diet compared with the high nutritional value of the milk and concentrate. Aubert and Dantzer (2005) reported that rats challenged with LPS showed no difference in the frequency of hedonic responses to a sucrose solution compared with controls injected with a saline solution. Those results showed that sickness does not interfere with the hedonic value of sucrose, suggesting that sick animals can still ingest highly nutritious food.

We noticed a marked reduction in the total duration of rumination during the period of peak fever due to both a reduced bout frequency and a reduced bout duration. The observed reduction of rumination could be caused by decrease in hay intake,

depression of the gastric centers causing stasis (Leek, 2001), or a combination of both factors. Takeuchi et al. (1995) also found a reduction in rumination in goats challenged with LPS. A reduction in rumination time is considered as an indicator of anxiety in cattle. For example, Bristow and Holmes (2007) reported reduction in rumination time associated with increases in cortisol under stressful situations.

Previous research has indicated an increased time spent lying down in other species following LPS (Johnson and von Borell, 1994). However, some studies report a decrease in lying time (Tuchscherer et al., 2006). We found no change in overall time spent standing or lying during the period of peak fever. These results are consistent with a lack of an effect reported in goats (Takeuchi et al., 1995). Lying on the side is thought to be a form of thermoregulatory behaviour increasing heat loss, while resting with the neck relaxed has been proposed as an indicator of REM sleep (Hänninen 2007; Hänninen et al., 2008). We did not observe any change in the amount of time that calves spent resting in these positions. However, the calves spent more time lying inactive when challenged with LPS. The LPS also reduced the frequency and duration of bouts of standing inactive. Owen-Ashley et al. (2006) reported reductions in activity of LPS challenged sparrows. Reduced activity linked to fever may be an energy-conserving mechanism, but it may also be related to the depression that is reported to occur after challenge with LPS (Konigsson et al., 2002). It is now accepted that a link exists between depression and the activation of immune system by means of cytokine action in the brain (Dantzer and Kelley, 2007). Immobility has been reported in mice challenged with LPS in highly stressful situations, such as forced swimming tests, and this is blocked by use of an anti-depressant (Renault and Aubert, 2006). Harden et al. (2006) found that the reduction of

voluntary wheel running in rats challenged with LPS was reversed by administration of specific antibodies to an LPS-elicited cytokine (IL-6). Injections of LPS also reduced the frequency of self-grooming events, as has been previously reported in LPS-challenged rats (Yirmiya et al, 1994), mice (Hollis et al., 2006), and goats (Takeuchi et al., 1995).

The physiological measures were recorded to assess whether or not the very low doses of LPS used were stimulating a fever response. The effects of LPS on body temperature and RF agree with those reported previously for calves injected with LPS (Hüsler and Blum, 2001) and calves experimentally infected with bovine viral diarrhoea virus and *Mannheimia haemolytica* (Ganheim et al., 2003). Steiger et al. (1999) found a very mild increase in RT (to 39.4°C) after an endotoxin dose of 2 µg/kg infused during 100 min into Holstein x Jersey heifers with a BW of 311 kg. This dose is 4 times greater than the dose used in our study, but we found a similar increase in body temperature. No differences were found between high and low doses of LPS. This could be related to the fact that both doses are already very mild for calves. Heart rate did not increase in our study, although increased HR following LPS has been observed previously for calves (Königsson et al., 2002) and other species (Albertini et al., 2002). Possibly the responses of the calves to the handling involved in measuring HR may have masked any effects of LPS.

Injections of LPS have often been used to study sickness behaviour (Dantzer, 2004), but there are limitations with using LPS to model behavioural responses to illness. Most obviously, injections of LPS help us understand the behavioural correlates of the activation of the immune system, rather than behavioural consequences of a full infection. A major problem in studying the initial behavioural responses to spontaneously

occurring illness is the difficulty in establishing the time at which the illness begins. One of the main advantages of using mild doses of LPS to model early sickness behaviour is that it does provide a clear starting point. However, it is unlikely that the time course of the changes in LPS concentrations in the blood following injection would model the time course following naturally-occurring illness. Thus, LPS injections may not be a good model for study the full time course of the behavioural responses to illness. However, this disadvantage is reduced when using low doses of LPS to mimic the beginning of illness. At the doses used in this experiment, the main disadvantage of the LPS model for early signs of sickness is the immediate presence of clinical signs after LPS injection and its short duration compared with naturally acquired diseases. In general, there is a lack of knowledge of how the behaviour is affected during the incubation period of a viral or bacterial disease. The utilization of even lower doses could be explored, because it has been reported that very low doses of LPS do not elicit fever or other major sickness signs, but affect cognitive process in chickens (Sell et al., 2001) and cognitive and emotional parameters in humans (Krabbe et al., 2005). Further research is needed on the effect of non-febrigenic doses and their effects on behaviour in cattle. Despite the limitations with the procedure, we maintain that injections of low doses of LPS can help us understand the behavioural correlates of the beginning of illness.

## **4.5 Conclusions**

In summary, low doses of LPS may mimic low concentrations of circulating bacterial endotoxin during the beginning of acute gram-negative infections. Therefore, behavioural changes of calves after being exposed to mild doses of LPS could be matched to the changes associated with the beginning of some infectious diseases.

Behaviours such as self-grooming, rumination, and ingestion of hay are reduced, while time spent lying and standing inactive is increased. In spite of fever, ingestion of milk and concentrate remained unchanged. However, the lack of an incubation period, the short duration of the effect, and individual differences between calves in sensitivity to LPS are factors that must be considered as limitations of this model for early detection of illness.

## 4.6 Tables

**Table 4.6.1.** Definition of behaviours assessed during the observation period.

Behavior/Posture	Description
Standing	Animal is standing on its four feet or walking
Lying sternal	Animal is lying on its sternum
Lying lateral	Animal is lying on its side
Lying with head supported by the neck	Animal is lying on sternal or lateral position with its head in an upright position supported by the neck muscles
Lying with neck relaxed	Animal is lying on sternal or lateral position with its head resting on either the floor or any part of its own body
Standing inactive	Standing still with no obvious movement or behaviour being performed
Lying inactive	Lying still with no obvious movement or behaviour being performed
Eating concentrate	Animal has muzzle in the concentrate bucket
Eating hay	Animal has muzzle in the hayrack or directed towards and less than 30 cm from the hayrack and performing feeding movements
Drinking water	Animal has muzzle on or in the water bowl
Rumination	Animal showing regular chewing bouts initiated by a regurgitation movement while standing or lying away from the concentrate or hay feeders
Self-grooming	All events where the animal performed licking or scratching of any part of its own body. Each individual licking movement was recorded.

**Table 4.6.2.** Mean ( $\pm$  S.E.) values of rectal temperature (RT), heart rate (HR) and respiratory frequency (RF) on control days with injection of saline<sup>+</sup> (SAL) and treatment days with injection of Lipopolysaccharide<sup>+</sup> (LPS) for the 24 h period following injections (n = 15).

Treatment	4 h Fever Peak	20 h	24 h	
	Mean $\pm$ SE			
RT, °C	LPS	40.03 $\pm$ 0.07*	39.30 $\pm$ 0.04	39.58 $\pm$ 0.05*
	SAL	39.10 $\pm$ 0.03*	39.14 $\pm$ 0.03	39.12 $\pm$ 0.02*
RF, bouts/min	LPS	46.50 $\pm$ 2.45*	42.86 $\pm$ 1.31	44.34 $\pm$ 1.26*
	SAL	36.11 $\pm$ 0.98*	39.91 $\pm$ 0.89	38.36 $\pm$ 0.68*
HR, beats/min	LPS	111.56 $\pm$ 1.86	112.35 $\pm$ 1.95	112.01 $\pm$ 1.38
	SAL	106.25 $\pm$ 1.98	110.89 $\pm$ 1.84	109.00 $\pm$ 1.37

\* indicates a significant difference ( $P < 0.05$ ) between LPS and SAL.

<sup>+</sup> low LPS dose = 0.025  $\mu\text{g kg}^{-1}$ ; high LPS dose=0.05  $\mu\text{g kg}^{-1}$ ; 50 ml of saline or LPS solution was injected.

No statistical differences found due to the dose ( $P > 0.10$ ) therefore the results from the two doses were combined.



**Table 4.6.3.** Mean ( $\pm$  S.E.) total time (min) spent performing behaviours during the 2 h before and after peak rectal temperature following saline (SAL)<sup>+</sup> and lipopolysaccharide (LPS)<sup>+</sup> injections (n = 15).

Behavior	Treatment	Duration (min)	<i>P</i> -value
Standing	LPS	90.15 $\pm$ 12.58	0.13
	SAL	102.76 $\pm$ 12.85	
Lying sternal	LPS	146.26 $\pm$ 11.96	0.11
	SAL	133.92 $\pm$ 12.12	
Lying lateral	LPS	3.62 $\pm$ 1.75	0.91
	SAL	3.32 $\pm$ 2.48	
Lying with head supported by	LPS	107.74 $\pm$ 7.87	0.99
	SAL	107.58 $\pm$ 7.86	
Lying with neck relaxed	LPS	42.13 $\pm$ 12.21	0.19
	SAL	29.67 $\pm$ 7.87	
Standing inactive	LPS	26.27 $\pm$ 5.05	0.11
	SAL	7.35 $\pm$ 1.34	
Lying inactive	LPS	132.63 $\pm$ 10.60	0.02
	SAL	104.39 $\pm$ 12.63	
Eating concentrate	LPS	2.03 $\pm$ 1.32	0.28
	SAL	4.83 $\pm$ 2.02	
Eating hay	LPS	23.11 $\pm$ 6.93	0.04
	SAL	31.52 $\pm$ 7.54	
Drinking water	LPS	5.10 $\pm$ 1.78	0.57
	SAL	3.93 $\pm$ 0.73	
Ruminating	LPS	6.42 $\pm$ 3.69	0.001
	SAL	24.57 $\pm$ 6.64	

<sup>+</sup>low LPS dose = 0.025  $\mu\text{g kg}^{-1}$ ; high LPS dose=0.05  $\mu\text{g kg}^{-1}$ ; 50 ml of saline or LPS solution was injected. No statistical differences found due to the dose ( $P > 0.10$ ) therefore the results from the two doses were combined.

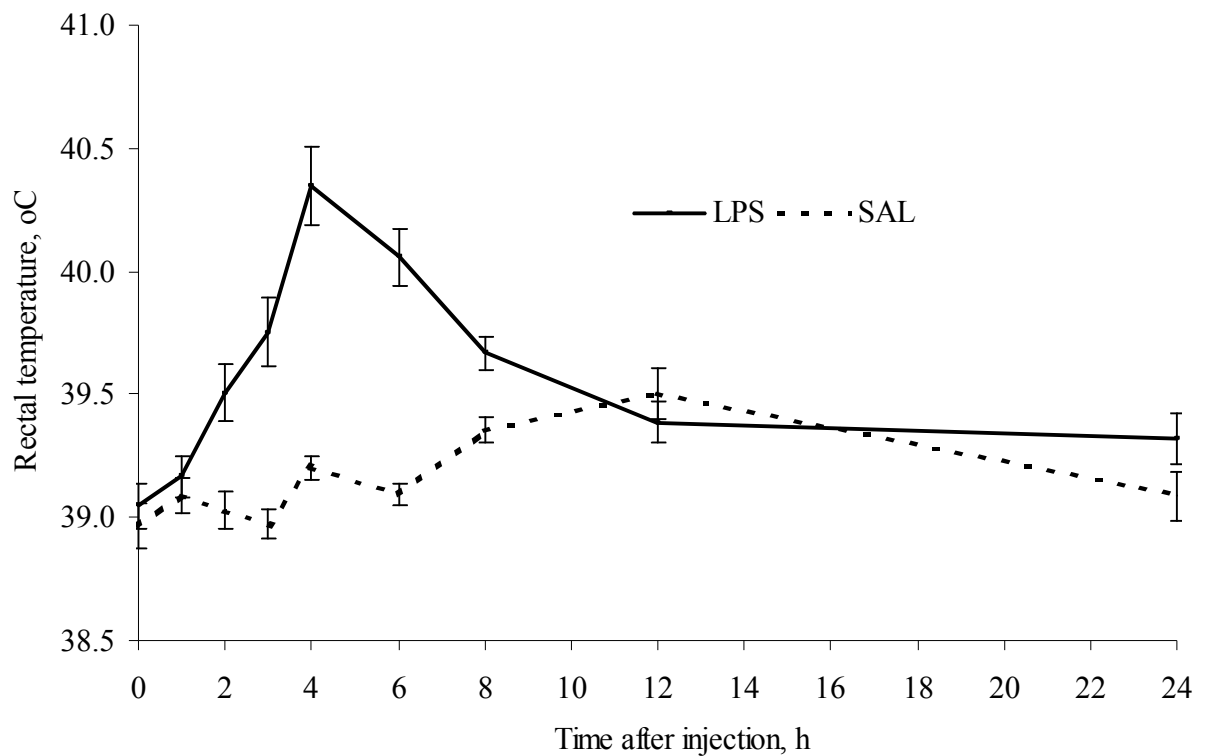
**Table 4.6.4.** Mean ( $\pm$  S.E.) bout frequency and bout duration of behaviours during the 2 h before and after peak rectal temperature following saline (SAL)<sup>+</sup> and lipopolysaccharide (LPS)<sup>+</sup> injections (n = 15).

Behavior	Treatment	Bout	<i>P</i>	Bout Duration	<i>P</i>
Standing	LPS	3.76 $\pm$ 0.40	0.11	25.60 $\pm$ 2.15	0.38
	SAL	4.69 $\pm$ 0.39		22.93 $\pm$ 2.13	
Lying sternal	LPS	4.64 $\pm$ 0.46	0.80	38.29 $\pm$ 4.37	0.55
	SAL	4.48 $\pm$ 0.46		34.56 $\pm$ 4.34	
Lying lateral	LPS	1.44 $\pm$ 0.32	0.51	4.91 $\pm$ 5.92	0.33
	SAL	1.03 $\pm$ 0.47		16.62 $\pm$ 8.72	
Lying with head supported by neck	LPS	13.47 $\pm$ 0.99	0.13	10.79 $\pm$ 1.70	0.53
	SAL	11.28 $\pm$ 0.98		12.29 $\pm$ 1.69	
Lying with neck relaxed	LPS	9.88 $\pm$ 0.94	0.09	4.26 $\pm$ 0.64	0.61
	SAL	7.45 $\pm$ 1.01		3.77 $\pm$ 0.69	
Standing inactive	LPS	9.96 $\pm$ 1.05	0.002	2.48 $\pm$ 0.29	0.005*
	SAL	4.71 $\pm$ 1.06		1.23 $\pm$ 0.29	
Lying inactive	LPS	4.48 $\pm$ 0.48	0.18	34.31 $\pm$ 3.98	0.07
	SAL	5.43 $\pm$ 0.49		23.65 $\pm$ 4.04	
Eating concentrate	LPS	1.12 $\pm$ 0.90	0.48	0.44 $\pm$ 0.35	0.34
	SAL	2.04 $\pm$ 0.90		0.91 $\pm$ 0.35	
Eating hay	LPS	6.37 $\pm$ 1.31	0.16	2.03 $\pm$ 0.38	0.14
	SAL	9.05 $\pm$ 1.31		2.85 $\pm$ 0.38	
Drinking water	LPS	4.09 $\pm$ 1.2	0.60	1.12 $\pm$ 0.15	0.13
	SAL	5.00 $\pm$ 1.2		0.80 $\pm$ 0.15	
Ruminating	LPS	0.65 $\pm$ 0.33	0.002	2.88 $\pm$ 1.70	0.01*
	SAL	2.27 $\pm$ 0.33		9.18 $\pm$ 1.70	

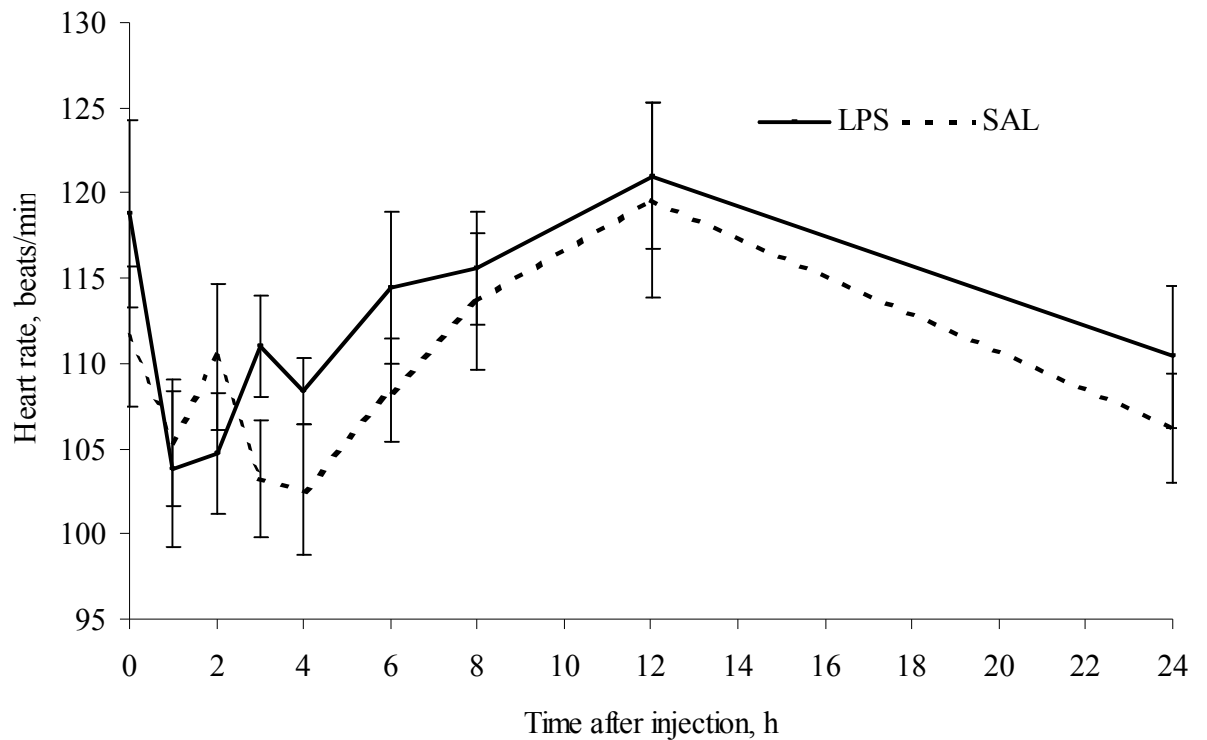
<sup>+</sup> low LPS dose = 0.025  $\mu\text{g kg}^{-1}$ ; high LPS dose = 0.05  $\mu\text{g kg}^{-1}$ ; 50 ml of saline or LPS solution was injected. No statistical differences found due to the dose ( $P > 0.10$ ) therefore the results from the two doses were combined.

## 4.7 Figures

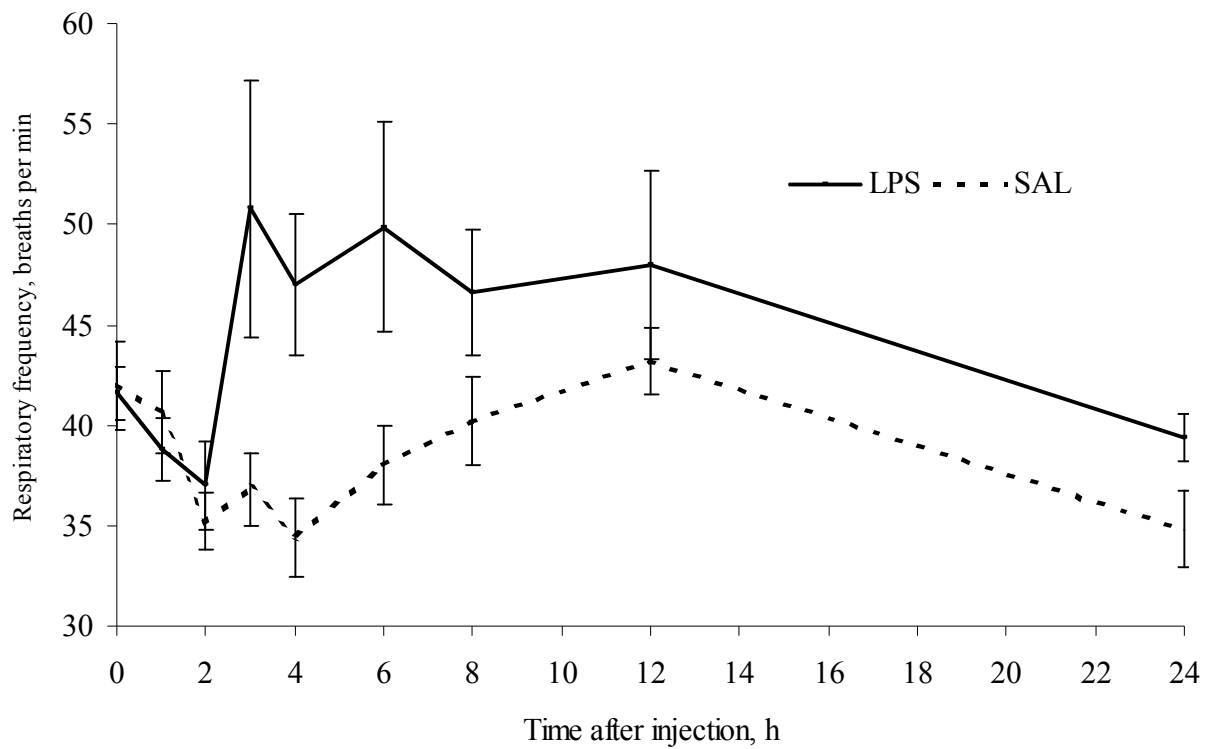
**Figure 4.7.1.** Mean ( $\pm$  S.E.) rectal temperature of calves at each sampling time following injections of either lipopolysaccharide (LPS) or saline (SAL) ( $n = 15$ ). A low dose ( $0.025 \mu\text{g kg}^{-1}$ ) and a high dose ( $0.05 \mu\text{g kg}^{-1}$ ) of LPS were used. No statistical differences found due to the dose ( $P > 0.10$ ) therefore the results from the two doses were combined. A volume of 50 ml of saline or LPS solution was injected.



**Figure 4.7.2.** Mean ( $\pm$  S.E.) heart rate of calves at each sampling time following injections of either lipopolysaccharide (LPS) or saline (SAL) ( $n = 15$ ). A low dose ( $0.025 \mu\text{g kg}^{-1}$ ) and a high dose ( $0.05 \mu\text{g kg}^{-1}$ ) of LPS were used. No statistical differences found due to the dose ( $P > 0.10$ ) therefore the results from the two doses were combined. A volume of 50 ml of saline or LPS solution was injected.



**Figure 4.7.3.** Mean ( $\pm$  S.E.) respiratory frequency of calves at each sampling time following injections of either lipopolysaccharide (LPS) or saline (SAL) ( $n = 15$ ). A low dose ( $0.025 \mu\text{g kg}^{-1}$ ) and a high dose ( $0.05 \mu\text{g kg}^{-1}$ ) of LPS were used. No statistical differences found due to the dose ( $P > 0.10$ ) therefore the results from the two doses were combined. A volume of 50 ml of saline or LPS solution was injected.



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## **CHAPTER 5. AUTOMATED MEASUREMENT OF FEEDING BEHAVIOUR TO DETECT ILLNESS IN MILK-FED CALVES<sup>4</sup>**

### **5.1 Introduction**

The use of automated feeders has facilitated group-housing of milk fed calves due to increased labour efficiency (Kung et al., 1997; Bøe and Færevik, 2003; Hepola, 2003). However, concern remains that detection of illness is more difficult (Svensson and Jensen, 2007). Therefore, there is a need for tools that identify sick calves or those at risk for disease.

One response to disease is reduced feed intake (Johnson, 2002) and this is often the first sign that an animal is ill. Measures of feeding behaviour may, therefore, be useful in detecting illness. Changes in feeding behaviour preceding illness of animals fed with automated feeders have been reported in dairy cows (Urton et al., 2005; Huzzey et al., 2007; Lukas et al., 2008) and beef cattle (Sowell et al., 1998; Quimby et al., 2001). In calves, Maatje et al. (1993) and Svensson and Jensen (2007) have reported effects of illness on feeding behaviour of calves fed by automated milk feeding systems, but the effects were dependent on levels of milk intake of the calves. In veal calves fed large amounts of milk, Maatje et al. (1993) found that the rate of milk intake and total

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<sup>4</sup> A version of this chapter has been accepted for publication. Borderas, T.F., J. Rushen, M.A.G. von Keyserlingk, and A.M.B. de Passillé. 2009. Automated measurement of changes in feeding behavior of milk-fed calves associated with illness. *J. Dairy Sci.* doi:10.3168/jds.2009-2109

consumption were the best indicators of disease. In contrast when calves were fed restricted amounts of milk, Svensson and Jensen (2007) reported that the measures identifying calves at risk for disease were limited to frequency of unrewarded visits to the milk feeder and not on actual milk intake. Borderas et al. (2008) also noted that illness induced by injections of bacterial endotoxin in young milk fed calves fed restricted amounts of milk decreased time spent eating hay but not milk intake. Therefore, the aim of this study was to assess if behavioural changes occurring before and during the onset of spontaneously occurring illness in dairy calves could be detected by automated milk feeding equipment, and whether these changes were affected by of the amount of milk or milk replacer provided.

## **5.2 Methods**

The institutional animal care committees (monitored by the Canadian Council for Animal Care) approved all procedures described in this study.

Using data collected from 4 previous experiments (experiments 1 to 4) we examined the feeding behaviour of sick and healthy unweaned, group-housed dairy calves up to 21 d of age fed either low (n=13) or high (n=19) milk or milk replacer.

In these experiments, Holstein calves (birth weight =  $44.99 \pm 6.04$  kg [mean  $\pm$  S.D.]) were separated from their mother within the first 24 h of life, weighed, fed at least 4 L of colostrum, and allocated to individual pens until 4 or 5 d of life, when they were transferred to group-pens. In all experiments calves were always kept at a density of at least  $1.4 \text{ m}^2/\text{head}$ . Group size ranged from 3 to 16 in experiment 1, from 4 to 13 in experiment 2 and was fixed at 4 and 5 calves per pen in experiment 3 and experiment 4

respectively. In all experiments, the calves were semi-randomly allocated to milk feeding levels while balancing for birth weight. Table 5.6.1 shows the feeding treatments for each experiment. In all experiments, calves were allowed ad libitum access to hay and water. Concentrate was available ad libitum from the first day after moving to the group-pens through a feeding bowl at a feeding station adjacent to the automated milk feeder system. For more detailed methods on rearing conditions for experiments 1, 2 and 3 as well as the growth and performance data please refer to Chapter 2 (experiment 1) and Chapter 3 (experiment 2) of this thesis, as well as Sweeney (2007) and Sweeney et al. 2008 (experiment 3). Rearing conditions in experiment 4 were similar to those in experiment 3 except for the length of the feeding regime.

In experiments 1, 2 and 4, daily health checks that included rectal temperature, respiratory and cardiac rates and sounds, presence of diarrhoea, presence of nasal and ocular discharges, general state of the coat and dehydration including tent test and muzzle humidity were performed daily by a trained veterinarian. In experiment 3, calf health was checked daily by the barn staff following a standard operating procedure developed in conjunction with a veterinarian. Table 5.6.2 shows the number of calves in each experiment on each feeding level diagnosed with gastrointestinal or respiratory disease or both. The age frequency distribution for when illness was detected is shown in Figure 5.7.1. Medical treatment was recorded for every sick calf. Boluses of neomycin and sulphamethazine (Neo Sulfa-E®, Vetoquinol Inc., Canada) were administered to 2 calves in experiments 1 and 2 calves in experiment 3. A kaolin-pectate suspension (Kaopectate®, Pfizer Inc., Canada) was administered to 4 calves in experiment 1. Rehydrating solutions (Electrolytes Plus®, Vetoquinol Inc., Canada) in experiment 1 and

Hydrafeed®, EXL Laboratories, Minneapolis, MN, in experiments 2, 3 and 4) were provided to the remaining sick calves. Treatments lasted on average ( $\pm$  SD) 2.37 ( $\pm$  0.8) d with a range of 1 to 4 d.

### **5.2.1 STATISTICAL ANALYSIS**

Data from calves with 2 or more episodes of illness were eliminated from the analysis. For each sick calf ( $n = 32$ ), we identified a healthy calf that was in the same experiment, on the same feed level, of the same age, and of similar birth weight (Birth weight: mean  $\pm$  S.D.; sick calves:  $45.68 \pm 6.2$ ; healthy calves:  $44.31 \pm 5.9$ ) as its matched pair (Total  $n = 64$ ). Day 0 was assigned as the day at which illness was first detected in the sick calves and the equivalent day for the healthy matched controls. Data collected for each calf (and its matched pair) included average daily milk intake (kg), the total number of daily visits to the milk feeder and the average duration of visits to the milk feeder. To remove instances where the receiver on the milk feeder briefly lost contact with the transmitter on the calf, a visit to the milk feeder was defined using the frequency distribution of natural log intervals between visits recorded by the milk feeder station following the methods described by von Keyserlingk et al. (2004). Since we had missing data from some animals prior to illness, we tested the effect of illness on these variables separately for the period before and up to illness detection (d -2 to 0) and after illness detection (d 1 to 7). The presence of marked effects of milk feeding level on feeding behaviour (e.g. Chapters 2 and 3), resulted in our testing the effects separately for calves fed high or low rations of milk using the mixed procedure (PROC MIXED) of SAS. To test for differences between experiments we initially included experiment as a



factor and the interaction of experiment with the feeding behaviour measures. However, no effect of experiment (or interaction) was noted so this term was removed from subsequent analyses. The resulting final model included the matched pair of calves (High = 19 pairs; Low = 13 pairs), the health status (sick or healthy), day of sickness (-2 to 0 or 1 to 7), and the interaction of health status and day of sickness, with calf nested within health status. Where a significant overall effect ( $P < 0.05$ ) (or a trend  $P > 0.05$  but  $< 0.10$ ) of health status was found, we used least square means to compare the effect of health status by day. As the majority of sick calves identified suffered from a combination of gastro-intestinal and respiratory diseases (Table 5.6.2), we were unable to test for the effect of different illnesses. In preliminary analyses we also tested the effects of antimicrobial agents (i.e. neomycin, sulphamethazine), as there is some indication that these agents may affect intake behaviour (Merck, 2006). However, results showed no differences in feeding behaviour between treated and untreated sick animals and thus the data collected from treated calves was retained.

### **5.3 Results**

The degrees of freedom for the F values and probability values of the mixed models for the effects of health status, day of sickness, and the interaction between health status and day of sickness on milk intake, total visits to the milk feeder and duration of the visits to the milk feeder for calves fed high or low levels of milk are provided in Table 5.6.3. Daily milk intake, the frequency of visits to the milk feeder and the mean duration of visits to the milk feeder for sick and healthy calves at both feed levels are shown in Figures 5.7.2A, 5.7.2B and 5.7.2C, respectively.

Sick calves fed a high level of milk showed a reduction in the frequency of visits to the milk feeder, and a trend for reduced milk intake and mean duration of visits, in the days preceding and on the day that illness was detected (d -2 to 0) ( $P < 0.05$ ). There was no interaction between health status and day of sickness. However, differences between sick and healthy calves before the onset of illness in these variables were limited to d 0 (Figure 5.7.2). In the days following the detection of illness, sick calves fed a high level of milk showed a decrease in milk intake, frequency of visits to the feeder and the mean duration of visits ( $P < 0.05$ ) (Table 5.6.3). We also observed an interaction between health status and day of illness for milk intake ( $P < 0.05$ ). However, daily comparisons indicated differences in the mentioned variables (or non-significant trends) between healthy and sick calves only on d 1, 2 and 3 (Figure 5.7.2).

Sick calves fed low levels of milk or milk replacer showed no differences in milk intake or frequency of visits to the feeder, either before or after illness was detected (Table 5.6.3), but had a reduced duration of the visits to the milk feeder on the day that illness was detected and the following 3 d (Figure 5.7.2C).

## **5.4 Discussion**

Illness in calves fed high milk volumes was associated with a reduced milk intake, a reduced number of visits to the milk feeder and an increased duration of visits to the feeder. These differences were most apparent on the day of diagnosis and the 3 subsequent days. In contrast, sick calves that were fed a low level of milk or milk replacer maintained milk intake but showed a reduced duration of visits on the days following illness. Detailed feeding behaviour measures such as those generated by

automated milk feeders may be useful in detecting illness in group-housed dairy calves, supporting the suggestions of Maatje et al. (1993) and Svensson and Jensen (2007). However, the precise information from automated feeders that is best able to detect calves at risk of illness or indeed ill will depend on the level of milk being fed to the calves.

Although other studies have reported changes in feeding behaviour preceding the onset of disease (Lukas et al., 2008; Svensson and Jensen, 2007) we did not find any changes in the days before the detection of illness. However, this failure to observe any changes in feeding behaviour before illness may have been a consequence of the lack of severity of the disease. First, no calves died and second, calves responded quickly to treatment (no differences were found in any variable between sick and healthy calves after d 4 indicating that the calves were likely suffering from only mild forms of disease). Furthermore, almost all the daily health checks were performed by a qualified veterinarian and were likely more rigorous than would normally be used on commercial dairy farms. This likely resulted in more opportune medical treatment that prevented the aggravation of their condition.

It is very unlikely that the effects were due to the veterinary treatment of the calves. Animals showing mild gastrointestinal illness were treated with only 2 L/d of an electrolyte solution and 100 ml/d of a kaolin-pectate solution. Milk intake of sick animals was not affected by medical treatment. Medicated sick animals showed a slight non-significant increase in milk intake ( $0.38 \pm 0.2$  kg/d) compared to sick animals receiving no medication. Thus, we are confident that the observed behavioural changes were indeed in response to illness and not to the treatment.

Similar to the results reported by Maatje et al. (1993), milk intake was the most responsive variable to changes in health status for high milk-fed calves. Daily frequency of visits to the milk feeder also decreased in sick calves fed high levels of milk and this corresponds to the observed decrease in milk intake. The average duration of the visits to the milk feeder was increased in sick calves fed high levels of milk, which could also be an indicator of illness. Sick calves visit the milk feeder less frequently and it takes them more time to consume a smaller meal as compared to healthy calves. Maatje et al. (1993) reported that a decrease in drinking rate (which corresponds to a longer duration of the visit) was a reliable indicator of disease.

Svensson and Jensen (2007) reported that calves fed only 6 L/d showed no reduction in milk intake but did reduce the number of visits to the milk feeder. Similarly, we found no reduction in milk intake among calves fed low amounts of milk (4 L/d). However, we did not find any reduction in the number of visits to the feeder either. The failure to see any change in milk intake in low fed calves may have been a result of the limited milk available to them. For instance, despite being sick the calves provided access to high milk volumes still maintained intakes of approximately 8 L/d, which was double the milk volume provided to the low milk fed calves. Interestingly, the only change in feeding behaviour detected in calves fed low levels of milk was a decreased duration of the visits to the milk feeder. Previously, Borderas et al. (2008) reported that injections of bacterial endotoxin reduced the time calves spent feeding on hay but did not reduce milk intake, when the milk allowance was 4 L/d. Together, these results show that the level of hunger of low-fed calves masked the effect of disease on their feeding behaviour. The feeding level is clearly an important factor influencing the expression of sickness behaviour and

should be included among the elements modulating sickness in a context-dependent frame (Millman, 2007).

## **5.5 Conclusions**

Milk feeding level clearly affects which aspects of milk feeding behaviour change in relation to sickness and thus must be taken into consideration when using changes in feeding behaviour for identifying sick pre-weaned calves. Sick calves fed high levels of milk showed decreased milk intake and total visits to the milk feeder, and an increased duration of each visit as compared to healthy calves with the same feeding level. Sick calves fed low levels of milk only showed a decreased duration of each visit as compared to healthy low-fed calves.

## 5.6 Tables

**Table 5.6.1.** Feeding conditions for experiments 1 to 4.

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Milk or milk replacer	Milk replacer*	Milk	Milk	Milk
Milk allowance (L/d) for HIGH calves	Ad libitum	12 L/d	12 L/d	12 L/d
Milk allowance (L/d) for LOW calves	4 L/d	4 L/d	n.a.	4 L/d
Concentrate CP (%)	22†	21.6‡	21.6‡	21.6‡
Feeder brand	Calm®. Lely Inc.	Calm®. Lely Inc.	CF1000 CS COMBI®. De Laval Inc.	CF1000 CS COMBI®. De Laval Inc.

\* Violac®, Coopérative Fédérée de Québec, Montreal, Canada

† Goliath XLR®, Coopérative Agricole des Cantons, Québec, Canada

‡ Unifeed Ltd., Chilliwack, Canada

**Table 5.6.2.** Number of calves recorded as suffering from gastrointestinal and respiratory illness in each feeding treatment (high (H) or low (L) levels of milk or milk replacer) in each experiment. Only 32 sick calves were included in the present study.

Illness	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	H	L	H	L	H	H	L	
	<b>Only gastrointestinal</b>	6	7	3	2	5	4	2
<b>Only respiratory</b>	2	7	0	0	0	0	0	
<b>Gastrointestinal + Respiratory</b>	7	1	1	2	0	0	0	
<b>TOTAL</b>	15	15	4	4	5	4	2	

**Table 5.6.3.** F value (F) and probability level (P) for the effect of health status, day of sickness and the interaction of health status and day of sickness on milk intake (Milk), total visits to the milk feeder (Visits) and duration of the visits to the milk feeder (Duration) of calves fed high (HIGH) and low (LOW) levels of milk.

	Health Status		Day of sickness		Health Status x Day of sickness	
	F	P	F	P	F	P
			Sick day -2 to 0			
HIGH <sup>1</sup>						
Milk	3.30	0.07	0.85	0.43	1.69	0.19
Visits	5.52	0.02	3.69	0.03	0.77	0.47
Duration	3.61	0.06	0.74	0.48	0.08	0.92
LOW <sup>2</sup>						
Milk	1.09	0.30	0.22	0.80	0.16	0.85
Visits	0.53	0.47	1.24	0.30	0.01	0.99
Duration	4.51	0.04	0.03	0.97	0.97	0.38
			Sick day 1 to 7			
HIGH <sup>3</sup>						
Milk	12.89	< 0.001	7.43	<	2.15	0.04
Visits	15.87	< 0.001	0.11	0.99	0.28	0.94
Duration	17.06	< 0.001	0.24	0.96	0.98	0.44
LOW <sup>4</sup>						
Milk	0.23	0.63	1.18	0.32	1.05	0.39
Visits	1.55	0.21	3.80	0.001	0.68	0.67
Duration	23.5	< 0.001	0.99	0.43	1.08	0.37

<sup>1</sup>Error DF = 88

<sup>2</sup>Error DF = 60

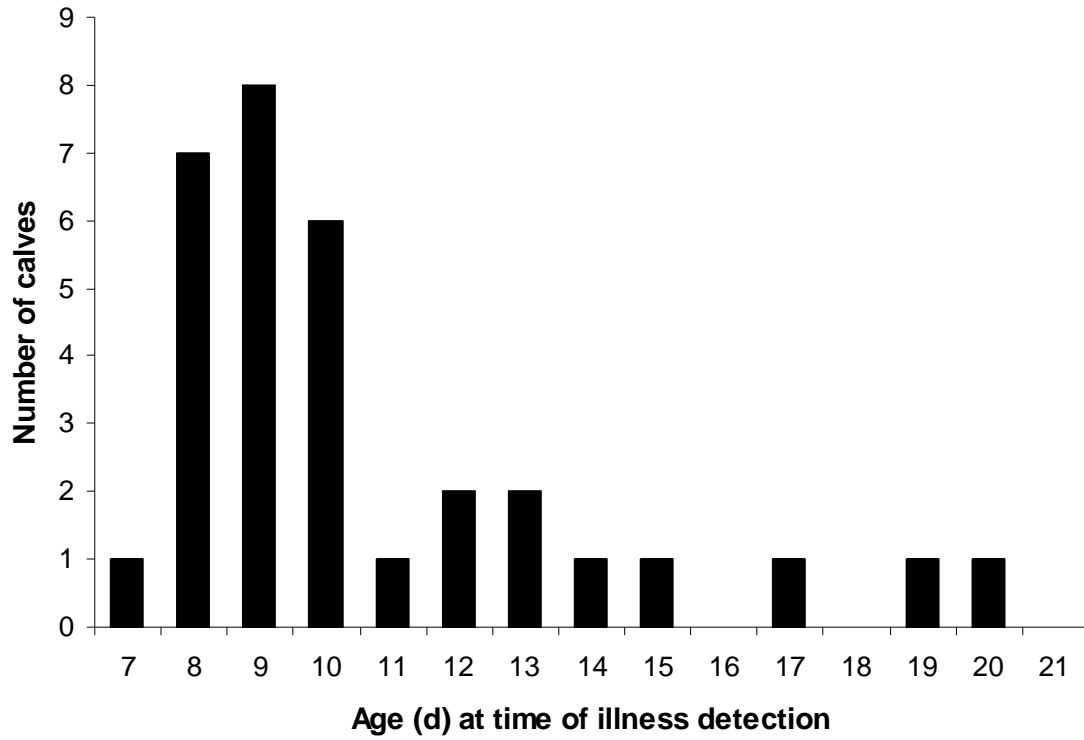
<sup>3</sup>Error DF = 239

<sup>4</sup>Error DF = 156

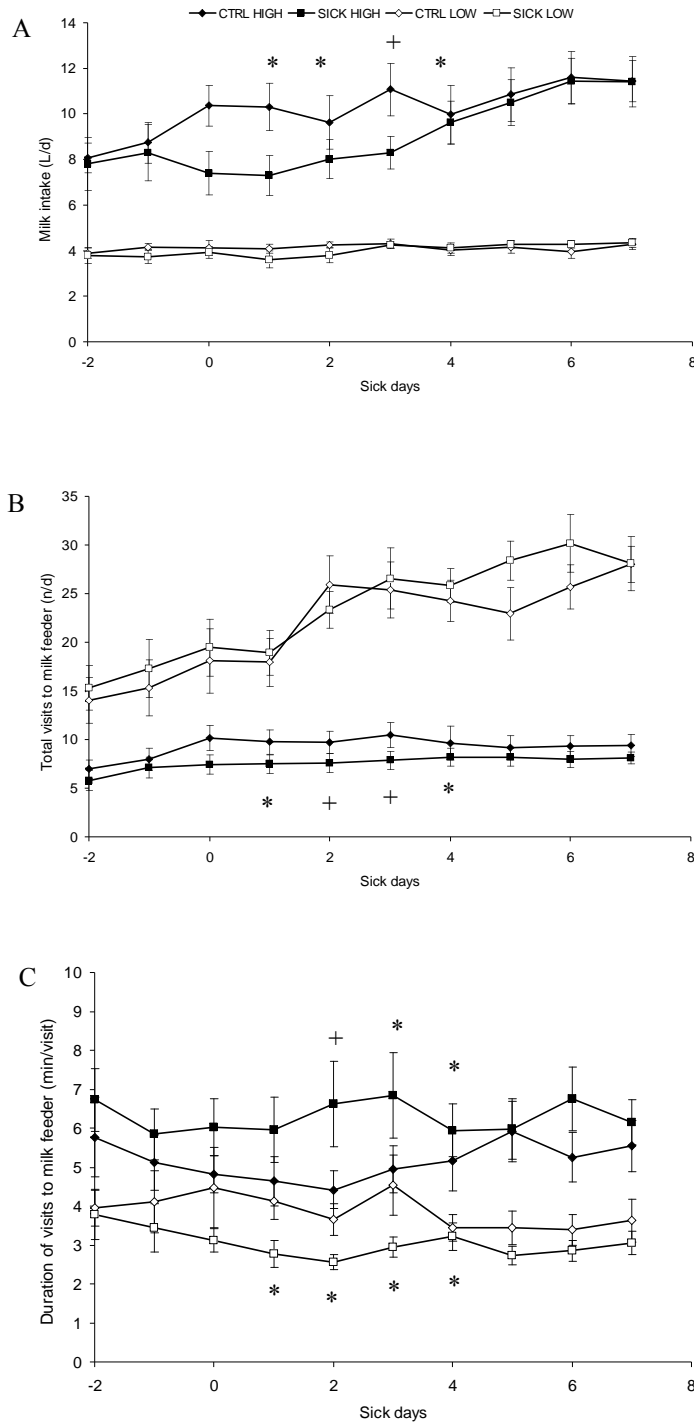


## 5.7 Figures

**Figure 5.7.1.** Number of calves that became ill at various ages.



**Figure 5.7.2.** Mean ( $\pm$  S.E.) daily milk intake (A), daily total visits to the milk feeder (B), and duration of visits to the milk feeder (C) of sick (n=32) and healthy calves (n=32) fed either high and low fed amounts of milk or milk replacer relative to day of illness (0 = day at which illness first detected).



Differences between sick and healthy calves within each level of milk fed \* P < 0.05, + P < 0.10

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## **CHAPTER 6. GENERAL DISCUSSION**

The overall objective of my research was to examine the usefulness of behavioural tools to predict or identify sickness in pre-weaned group-housed dairy calves fed by an automated milk feeder.

Given that sickness behaviour is a response of the organism to divert energy from non-maintenance functions to the immune system (Dantzer, 2001), I addressed my research objective by studying the principal behaviours that regulate the energy intake and expenditure of the dairy calf: feeding (Chapters 2, 4, and 5), thermoregulation (Chapters 3 and 4) and resting (Chapters 2, 3 and 4).

### **6.1 Feeding behaviour and feeding level**

The first step of my research was to assess the influence of feeding levels on the behaviour of healthy dairy calves, as there is evidence that feeding behaviour is influenced by the amount of milk offered to these animals regardless of their health status (Jensen, 2006). Additionally, comparing the studies by Maatje et al. (1993) and Svensson and Jensen (2007) there is circumstantial evidence that feeding level may influence the behavioural responses to illness. In fact, in North America, calves are raised using 2 different feeding strategies: a low milk level vs. a high milk level (more than double of the low level) (Drackley, 2008). However, it is a common belief among producers and dairy scientists that high levels of milk increase the incidence of scours in dairy calves.

The most important finding of this first study is that the feeding level has effects on calves when healthy, not only on the expression of feeding behaviour, but on their resting

behaviour as well. The frequency of visits to the milk feeder and to some extent occupancy time differed between calves fed high and low levels of milk. Low milk-fed calves showed a high frequency of visits to the milk feeder, very likely motivated by hunger (De Paula et al., 2008), and also showed a decrease in resting time. Other interesting finding is that morbidity was similar in calves fed high or low milk levels. The fact that I found the same result in two experiments conducted at different geographic locations and with different management practices, confirms the findings of other studies (Appleby et al. 2001; Jasper and Weary, 2002; Khan et al., 2007) that high levels of milk intake do not induce sickness in dairy calves. Calves that fell ill during this study and their healthy counterparts provided data used to examine the behavioural correlates of illness in Chapter 5.

Due to the operating conditions at the farm, I carried out this study by using dynamic groups (calves being added as they entered the experiment and moving to a new group as they aged). This condition prevented me from measuring how the feeding and resting behaviour would be affected by different fixed group sizes. There is evidence that competition also affects the visiting pattern to the milk feeder (Jensen, 2004). Frequency and duration of visits could be affected by increased competition, masking changes in feeding behaviour that ill calves would express under less competitive circumstances. Probably increased competition would reduce the high frequency of unrewarded visits that were observed in low-fed calves, but more research is required to test this prediction.

Small differences in levels of milk feeding (4.8 L/d vs. 2.8 L/d) did not show differences in feeding behaviour of calves (Jensen and Holm, 2003), but large differences in milk allowance (4.8 L/d vs. 9.2 L/d) did affect the feeding behaviour (Nielsen et al.,

2008). Consequently, I pushed the boundaries of the feeding levels by allowing calves in the enhanced nutrition treatment to consume milk or milk replacer at ad libitum or near ad libitum levels (high), while calves in the traditional approach treatment (low) were fed only 4 L of milk replacer per day, which is a common practice on commercial farms. However, it could also have been interesting to assess the effect of an intermediate milk allowance (i.e. 7 to 8 L/d). Feeding intermediate levels of milk to dairy calves is increasingly common on commercial farms (Drackley, 2008). I expect that this intermediate milk feeding level would result in behaviour similar to that observed at the high milk level early in the milk feeding period, but that the behaviour would change to a feeding pattern similar to that expressed by the low-fed calves. Such a pattern would provide further supporting evidence that hunger (expected in the milk-grain transition period) triggers the increase of unrewarded visits to the milk feeder. Considering the results from a welfare perspective, it seems likely that the traditional approach of feeding calves slightly above maintenance level leaves calves in a permanent state of hunger, especially during the first 14-21 d of life. Dairy calves younger than 21 d of age are unable to compensate for low intakes of milk by eating concentrate, and hence often show reduced gains of weight. Natural weaning in cattle is a long term process that allows a gradual shift from milk to solid food. The use of higher milk allowances, especially during the first weeks of life, followed by extended weaning periods where milk is gradually reduced until the calf is physiologically capable to digest solid foods (i.e. Khan et al., 2007), could probably be the best strategy to avoid hunger and take advantage of the growth potential of young calves.



I also found an elevated number of visits to the milk feeder performed by calves fed low volumes of milk. This finding confirms previous reports (Hammon et al., 2002; Jensen, 2003; Nielsen et al., 2008). Moreover, the increased occupancy time is primarily due to an increase in the frequency of unrewarded visits. This implies that, as a simple factor of occupancy time, group size cannot exceed 20 to 25 calves per feeder when low levels of milk are offered to calves, and contradicts the claims made by manufacturers that this equipment can feed up to 45 calves with a single teat. An additional problem to such large groups is the possible effect of group size on the incidence of other undesirable behaviours, such as cross sucking, that could further decrease the recommended number of animals per group fed from a single feeder.

## **6.2 Thermoregulatory behaviour and feeding level**

Since fever has a high metabolic cost (Kluger et al., 1998), I hypothesized that the presence of an external source of heat would save energy for a fever response to infection. The ultimate goal was to evaluate the potential of an external source of heat as a diagnostic tool for the detection of ill calves, as sick animals would spend more time closer to the source of heat. Thus, the first step was to assess, by means of a preference test, if calves could identify and use an external source of heat (infra-red lamps) as an aid for conserving energy (Chapter 3). This study sets the base of an experimental model to test the thermal preferences of the calf in further research of sickness behaviour. As thermoregulatory behaviour is also affected by the level of intake (Schrama et al., 1992) the response of calves fed high and low levels of milk was compared, hypothesizing that

calves with less caloric intake would be more motivated to use the external source of heat.

My results show that calves are attracted to heat and will choose to lie in a warmer environment independently of feeding level provided. This indicates that, like piglets and lambs, the use of an external source of heat for dairy calves during the first days of their life has merit. This preference for a warmer environment demonstrates that calves can recognize a source of heat. It also shows that this setup can be used in further research to examine the thermal preferences of the calf by its position in a thermal gradient and by the temperature recorded in the temperature logger attached to the back of the calf.

In this study, calves were kept on trial for the first 3 d of life as this is a critical period for survival after birth (USDA, 2007). However, the fact that calves increased the time spent under the heat lamps as they got older, suggests that I should have observed the calves for a minimum of 7 d, when thermoregulatory physiology of the calves seem to stabilize (Piccione et al., 2003). Prolonging the trial duration would have also increased the likelihood of calves being identified as having disease and/or fever.

To enhance the effect of environmental temperature on thermoregulation, I performed this study in a naturally ventilated barn during wintertime, with a temperature range below the lower critical limit of Holstein dairy calves. Although the preference for heat sources seemed to be independent of environmental temperature, a trend for an increased use of the heat lamps was observed in low-fed calves during the night, which was the coldest period of the day. Piccione et al. (2003) report that body temperature of calves shows a marked decrease at dusk, but this observation was confounded by a concurrent drop in environmental temperature during that period of the day. These

authors explain the drop in body temperature as part of the natural rhythmicity of the species, minimizing the effect of the difference environmental temperature. For these reasons it would be interesting to repeat a similar experiment during the summer months, when environmental temperatures are well above the lower critical limit of Holstein dairy calves. I hypothesise that heat is one of the attractive stimulus that help the calf find the udder of their dam during this critical period. This could be one of the factors that make the calves' preference for a heat source independent from environmental temperatures.

My results support the use of an external source of heat in new-born dairy calves as a management practice similar to that used for piglets. Even though the zone of thermal neutrality is colder for dairy calves compared to pigs, calves in naturally-ventilated barns in most parts of North America may spend almost the entire winter season with average temperatures below the Holsteins' lower critical limit.

Another interesting finding is that calves fed *ad libitum* can drink up to 8 L of milk from their first day of life without any adverse effect to their health. This demonstrates that high amounts of milk can be fed to calves from birth, and contrary to belief by many industry professionals and scientists (i.e. Quigley et al., 2006) does not provoke illness. This finding also suggests that, even from the first days of life, feeding lower quantities of milk could result in hunger and compromise the welfare of the calf. Based on these results I would strongly recommend that heat lamps or a similar heat source with a thermostatic control, and a high level of milk feeding, should be implemented in pens housing calves immediately after birth. It would be interesting to carry a long-term study on the effect of the use of an external source of heat (i.e. heat lamps) on productivity, morbidity, and mortality rates of dairy calves, as to better understand their comfort needs.

### **6.3 Resting behaviour and feeding level**

Resting is another behaviour greatly affected by illness, as reduced activity is one of the best ways to save energy and avoid predators (Dantzer et al., 2008). Changes in resting behaviour could act as complementary information to detect sick calves when changes in other behaviours are inconclusive or inexistent. For example, calves fed low levels of milk show few, if any, changes in feeding behaviour but some changes in resting behaviour occurred when administered with mild doses of LPS (Chapter 4). Although I used position loggers to record resting behaviour, devices such as accelerometers show promise, particularly as these technologies improve our ability to collect data on commercial facilities, allowing not only the automated measure of standing and lying episodes, but the time that calves spend in activity and at rest. When low doses of LPS were administered to calves (Chapter 4), time spent standing inactive increased, but these data were challenging to obtain from video recordings. Accelerometers could automatically record activity.

### **6.4 Modelling the onset of disease**

Since it is difficult to identify the precise moment when disease begins (Quimby et al., 1991), the third step of my dissertation was to test a model of disease onset using low dose injections of a bacterial endotoxin (LPS) to assess if feeding and resting behaviours change at the beginning of an illness episode (Chapter 4).

This study showed that a mild dose of endotoxin does not affect the intake of milk and concentrate when calves are fed at low levels, but decreases the time spent at the hayrack. It would have been interesting to include a high level of milk feeding and ad libitum access to grain feeding to quantitatively assess the drop in appetite induced by the endotoxin.

Other findings in this study included an increase in the time spent standing inactive and a decrease in time spent self-grooming and ruminating. These behavioural changes are less susceptible of automated measuring to detect changes during illness. However, the miniaturization of accelerometers or other devices such as myoelectrical activity recording systems could prove useful in the future.

Although this endotoxin model may be difficult to use when studying the changes of behaviour throughout the whole course of disease, it has potential to answer questions on the expression of behavioural changes occurring at the very early onset of disease.

## **6.5 Behaviour of healthy and sick calves**

Having established that feeding and resting behaviour changed with different feeding levels, and that low-fed calves induced to illness by LPS did not reduce milk and grain intake, I assessed how feeding behaviours changed in spontaneously sick calves compared to healthy counterparts when they were fed high or low levels of milk (Chapter 5). Results confirm that milk feeding level has a major impact on how calves change their behaviour when they become sick. One of the most important findings was that when calves spontaneously become sick, calves fed high levels of milk showed a reduction in

appetite while calves on low levels of milk feeding did not. Interestingly low fed calves also did not reduce their appetite when illness was induced with LPS (Chapter 4). The low feeding levels used may have prevented the animals from showing any reduction in consumption.

In high and low fed calves, differences in feeding behaviour between sick calves and their healthy counterparts were modest making it difficult to establish a quantitative criterion to diagnose calves as sick based on these behavioural differences. These small differences may have been a consequence of the lack of severity of the disease and a very close health monitoring. In commercial facilities, differences could be more evident as less frequent health checks could result in more calves succumbing to severe illness.

## 6.6 Future research

The milk intake pattern observed in Figure 2.7.1, suggests that calves could be reared by emulating the lactation curve that cows naturally show, although shortened in its duration. In the experiments described in Chapter 2, milk intake plateaued at 3 wks of age, so this could be considered as the peak of the lactation curve which is normally attained at 8 to 11 wks by the dam. From this point, calves could be brought to weaning with a smooth reduction of milk allowance similar to the natural weaning process imposed by the involution of the udder after the peak yield. The duration of this lactation curve could take 90 d instead of the 210 to 300 d that is shown by the dam. The use of automated milk feeders makes this approach feasible and could result in a more natural switch from milk to solid feed. I encourage future work in this area to test this prediction.

As demonstrated in Chapter 3, calves are capable of recognizing and using an external source of heat. Therefore, it would be interesting to examine if sick calves would elect to use the heat source during the build up of fever, but unfortunately no calves became sick during my experiment so I could not test this. A more direct test for the hypothesis that fever increases motivation of calves to use the external source of heat, would have been the induction of fever by injection of mild doses of endotoxin similar to the study reported in Chapter 4. I strongly encourage future research in this area.

As well, further studies could be carried out with lower sub-pyrogenic doses of LPS (i.e. doses that do not induce fever) to assess if detectable behavioural changes are present when no other clinical signs of disease are. Another promising approach would be the long term administration of very low doses of LPS by the use of portable infusion pumps. Recently, inexpensive disposable infusion pumps have become available in the

market, improving the simulation of long-term endotoxin release by bacteria as seen in naturally occurring infection.

Studies in laboratory animals have also shown individual differences in sickness behaviour expression linked to gender, genetic lines, social and maternal isolation and even brain lateralization (Neveu et al., 1998). Future research in calf sickness behaviour must put an emphasis on individual differences to identify and assess other factors modulating the expression of sickness behaviour. A detailed recording of early lifetime events that could potentially affect the expression of the sickness behaviour should be considered to explain observed inter-individual differences. For example, calves issued from dystocic calvings are at increased risk of mortality and morbidity, even when dystocia is classified as mild (Lombard et al., 2007). Increased morbidity seems to be related to a decrease in the quantity and absorption of colostrum immunoglobulins, but it is also believed that high levels of cortisol at dystocic calvings weaken the calfs' immune system, hence a reduced expression of sickness behaviour could be expected.

## **6.7 Concluding remarks**

The aim of the animal sickness behaviour repertoire is to maximize its survival probability when challenged by a pathogen infection. Since the activation of the immune defences against infections implies a high metabolic expense, individuals might modulate the expression of sickness behaviour in an adaptive way to maximize survival. Weary et al. (2009) suggest that only behaviours that offer long-term fitness (i.e. play, grooming) will increase or decrease during illness, as resources are diverted to critical short-term fitness behaviours like temperature maintenance. The accumulated evidence shows that



the expression of sickness behaviour is context-dependent, with individuals modulating the expression of signs of disease according to their circumstances. Season of the year, gender, presence of conspecifics or predators, and many other factors contextualize the degree in which sickness behaviour is expressed. The challenge in farm animals is to identify these factors and determine to what degree they modulate the expression of measurable behaviours of the animal during sickness episodes.

In this dissertation, I studied feeding level as one of the main factors that control measurable changes in feeding behaviour, a key measurable component of the sickness response in dairy calves. I conclude that some behavioural changes are useful to identify sickness in pre-weaned group-housed calves. Changes in milk intake, frequency of visits to the milk feeder, and resting time, seem to be the most promising identifiers of illness, but the level of milk provided must be taken into account as an important factor modulating the expression of these behaviours in dairy calves.

In an era of increasing automation of tasks in the dairy industry, animals are given the opportunity to express their natural behaviours. Automates make possible to the animal to choose when and how much to eat or even when to be milked. Additionally, automates allow the producer to monitor the behaviours expressed by the animal on a daily basis. The analysis of the different recorded behaviours (i.e. feeding, drinking, resting) could prove useful to the producer for detection of early clinical signs in dairy calves. , unequivocally improving the overall welfare.

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## APPENDICES

### **Appendix A. The effects of force-feeding sick dairy calves: A comment on Quigley et al. (2006)<sup>5</sup>**

In a recent article, Quigley et al. (2006) claim that feeding increased amounts of commercial milk replacer to calves increases the incidence of disease. The aim of this letter is to provide what we consider to be a more accurate interpretation of the data presented.

Calves were fed either on a constant low plane of nutrition (454 g/d of milk replacer from d 0 to 28) or at increasing amounts (454, 681, and 908 g/d on d 0 to 7, 8 to 14, and 15 to 31, respectively), and then reduced to 454 g/d from d 31 to 41. Calves that failed to drink their entire ration were administered the remainder using an esophageal feeder. Calves on the low plane of nutrition had a lower incidence of diarrhoea, required fewer veterinary treatments and tended to have lower mortality rates than calves fed more milk. The authors conclude that feeding higher levels of milk replacer “should be done with caution in highly stressed calves”.

In contrast, we suggest that the increased incidence of illness observed in the calves fed the higher levels of milk replacer was due to sick calves being force-fed. Previous research has shown that reduced appetite is an adaptive response to disease and that force feeding further debilitates sick animals and increases the risk that animals

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<sup>5</sup> A version of this appendix has been published. Borderas, F., M.A.G. von Keyserlingk, D.M. Weary, J. Rushen, A.M. de Passillé, and M.E. Van Amburgh. 2007. Letter to the Editor: The effects of force-feeding sick dairy calves. A comment on Quigley et al. (2006). *J. Dairy Sci.* 90: 3567-3568.

succumb to infection. Murray and Murray (1979) reported some of the first evidence showing the detrimental effects of force feeding sick animals: mice infected with *Listeria monocytogenes* and force fed had 50% greater mortality compared to infected mice provided food ad libitum. Quigley et al. (2006) did not report the number of calves that were fed with the esophageal feeder, but the likelihood of force feeding was greater for the calves receiving the higher quantities of milk, due to both increased intakes and the longer milk-feeding period (41 versus 28 d).

Reduced appetite is part of a coordinated set of behavioural changes that develop in animals during infection (Dantzer 2001) that are part of the adaptive response to pro-inflammatory cytokines controlled through neurohormonal mechanisms (Hart 1988). This ‘sickness behaviour’ is not a sickness-induced debilitation, but rather a coping mechanism that enables the individual to better counteract the infection (Hart 1988; Aubert 1999). From this perspective, force-feeding (rather than the access to milk per se) would undermine the calf’s attempt to mount this adaptive response to infection (Johnson 1998) and put the calf at increased risk of morbidity and mortality.

The study by Quigley et al. (2006) suffers from a number of other methodological problems. The quantity of milk replacer fed to the calves was increased abruptly, rather than gradually. As acknowledged by the authors (p 209), this abrupt change may itself have increased the risk of diarrhoea. The diagnosis of illness in the calves was initially based on the occurrence of diarrhoea. However, the fecal scoring system was a subjective 1 to 4 scale based solely on fecal consistency. The authors provide no evidence that this measure was repeatable or a valid indicator of any underlying pathology. This aspect is critical in the present study as calves consuming greater amounts of milk replacer were

also consuming greater quantities of liquid; this increased intake of liquids likely decreased fecal consistency in a way that was not related to pathology. Thus, the fecal scores presented do not necessarily indicate a high incidence of illness. Furthermore, body temperatures were only measured in calves that showed high fecal scores rather than being done systematically for all calves. Thus, the method of detecting fever was likely biased towards calves fed higher amounts of milk replacer.

Finally, there was no mention of blinding of the observers to the treatment group of the calves, which is an additional important potential source of bias in the results. The calves used were exposed to bedding that was considered to contain coronavirus but this was done in an uncontrolled way by using a small quantity of bedding from a previous experiment. No measures were taken of actual coronavirus exposure and there was no evidence that viral contamination was equally distributed between the two treatment groups.

In summary, we argue that the effects reported by Quigley et al. (2006) were misinterpreted. Clearly, force-feeding sick animals should not be (and never has been) recommended. Also, new and validated methods of assessing diarrhea that truly indicate the presence of illness are required for calves fed increased milk rations. More integrated, systematic and thorough research is now required to properly understand how access to more milk affects the young calf's ability to devote energy to maintenance, growth, and immune function.



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