Welfare and performance of newborn and young dairy calves

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Widmung

Ich widme diese Thesis den beiden wichtigsten Menschen in meinem Leben – meinen Eltern. Eure Liebe, Euer Vertrauen in mich und Eure Unterstuetzung haben mir geholfen einen Traum zu erfuellen. Ich hab' Euch ganz doll lieb.

I dedicate this thesis to the two most important people in my life – my parents. Your love, trust and support helped me fulfil one of my dreams. I love you so very much.

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Approval from the Massey University Animal Ethics Committee has been obtained for the experiments described in this thesis.

Abstract

Physiological evaluation of newborn lambs at birth revealed four main causes of hypothermia and death: placental insufficiency, intrapartum hypoxaemia, inadequate heat production and starvation. No similar evidence seems to be available for calves and thus the present study measures parameters used in previous lamb studies to evaluate the physiological status of calves and the incidence of the four factors in newborn dairy calves.

The study was carried out in the Manawatu region during spring 2001. Multiparous and primiparous cows about to calve were observed continuously. All dystocias were assisted. Within 30 minutes of birth the rectal temperature of each calf and a jugular blood sample were taken. Time to stand on all four feet and birth weight were also measured. The packed cell volume and plasma concentrations of glucose, fructose and lactate were analysed as indices of prenatal and intrapartum status. A subset of calves was then followed up after pick-up to 4 days of age taking rectal temperature twice daily and a jugular blood sample at approximately 24, 48, 72 and 96 hours after birth. Plasma was analysed for glucose, beta-hydroxybutyrate, urea and gamma-glutamyl transferase concentrations to determine energy status of the calves for the first four days after birth and to determine whether calves had sufficient colostrum intake indicative of passive immunity.

The physiological status of calves at birth was fairly uniform. Calves born after dystocia had significantly higher plasma lactate concentrations, took significantly longer to stand and had significantly lower packed cell volumes than normally born calves. The higher plasma lactate concentrations and longer time to stand in these calves indicate hypoxia at birth and reduced vigour. As packed cell volume was not significantly elevated in calves with significantly elevated plasma lactate concentrations it is suggested that placental insufficiency was not a major problem. The majority of calves had relatively high rectal temperatures suggesting that thermogenesis was not impeded.

The majority of calves followed up to 4 days of age were in good energy balance. Starvation and hypothermia were not major issues as judged by relatively high plasma urea and beta-hydroxybutyrate concentrations and rectal temperatures. The majority of

calves had adequate gamma-glutamyl transferase concentrations suggesting effective passive uptake of immunoglobulins. However, all calves that died (n=8) had significantly lower concentrations than calves that became sick and subsequently recovered and those calves that remained healthy.

Overall, the physiological status of the calves of the present study between birth and 4 days of age was adequate. However, immune status plays an important role for the health and welfare of the newborn calves as judged by the fact that all calves that died failed to take in colostrum before pick-up.

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General Introduction

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General Introduction

1.1 What is animal welfare?

Over the last few decades, peoples' attitudes towards animals and their opinions on how animals should be treated have changed. More and more emphasis is now being placed on the well-being of animals in our care. Although a variety of ethical positions exist about acceptable and unacceptable ways to treat animals in farming, recreation and animal-based science (ANZCCART, 2000), the majority have the welfare of the animals concerned at heart.

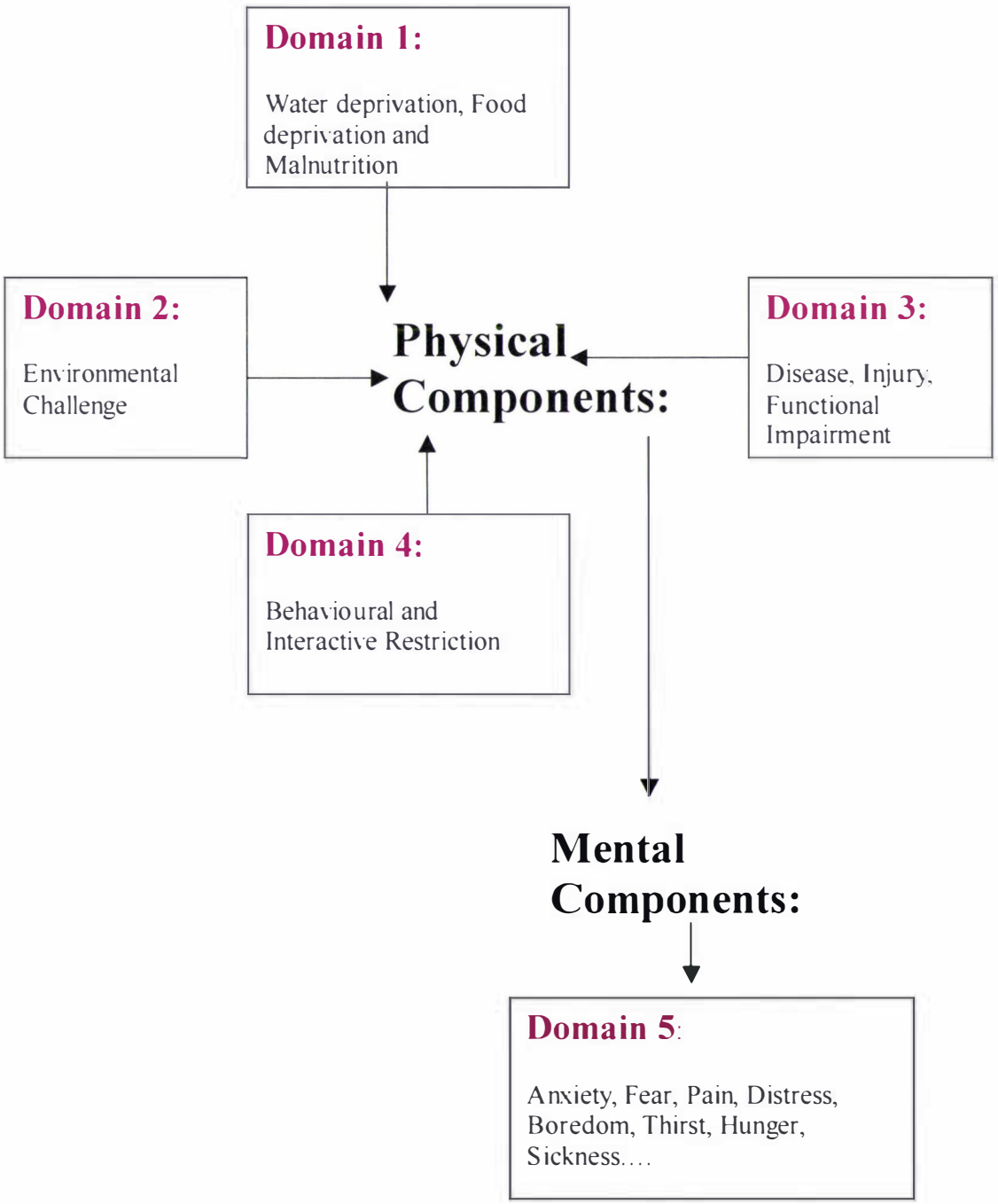
Animal welfare indicates how well an animal can adapt to and cope with its environment (Broom, 1988), both physiologically and psychologically, and how well it is managed by people (ANZCCART, 2000).

When environmental conditions are adverse, animals try to counteract the effects of such conditions on themselves. The animal has various regulatory and emergency systems available to cope with such environmental conditions (Broom, 1988). These systems may fail and reduce the fitness of the animal and where this occurs the individual is under stress and its welfare is poor (Broom, 1988).

A system which provides broad consideration of the various sources of welfare compromise is the system of the 'five domains of animal welfare compromise' (Figure 1.1) (Mellor & Stafford, 2001). The five domains draw attention to the nutritional, environmental, health, behavioural and mental needs of animals, which are important if the welfare of an animal is to be high (Mellor & Stafford, 2001). Welfare compromise in domains one to four is usually recognised by compromise in domain five, which

represents the negative mental dimensions of an animal's experience (Mellor & Stafford, 2001).

Figure 1.1: The five domains of animal welfare compromise [modified from Mellor & Stafford (2001)].



An animal will experience excellent welfare if its needs are met completely for all five domains, although this is not likely ever to be the case during the lifetime of an animal (Mellor & Stafford, 2001). Nevertheless the animal may experience good welfare during some or most of its life. Some welfare compromise is usual at some stage in an animal's life.

Mellor & Stafford (2001) list 6 points that help to clarify important features of such compromise.

1. Compromise is evident in one or more of the five domains of welfare.
2. It is not usual for an animal to experience welfare compromise in all five domains simultaneously.
3. Compromise in all five domains is not 'all or none'. There are different levels, which can be ranked as mild, moderate, marked, severe or very severe. This can be achieved by the use of diagnostic parameters, whose precision and efficacy have been established by scientific research and clinical experience.
4. All systems for maintaining animals, including on farms and in laboratories, have advantages and disadvantages for the welfare of the animal.
5. In order to minimise compromise in one domain, it is sometimes necessary to cause compromise in another domain. Thus, one might have to choose between two or more forms of compromise, trying to minimise the overall negative impact on the animal's welfare.
6. To minimise the extent of welfare compromise depends on our having a method of grading the extent of any welfare compromise.

The means by which problems and welfare compromise can be prevented or corrected are shown in Table 1.1 (Mellor & Stafford, 2001).

Table 1.1: Five domains of potential welfare compromise and how problems may be prevented or minimised (Mellor & Stafford, 2001).

Domain	Description	How prevented or minimised
1.	Water deprivation, food deprivation, malnutrition	Ready access to fresh water and an appropriate diet in sufficient quantities and with a composition that maintain full health and vigour.
2.	Environmental challenge	Providing suitable shelter and a comfortable resting area, whether outdoors or indoors.
3.	Disease, injury, functional impairment	Prevention and rapid diagnosis and treatment.
4.	Behavioural or interactive restriction	Providing sufficient space, proper facilities and the company of the animal's own kind.
5.	Mental (and physical) suffering	Minimising the conditions that produce unacceptable levels of anxiety, fear, distress, boredom, sickness, pain, thirst, hunger, and so on.

1.1.2 How do we measure animal welfare?

Suffering and happiness are important in the way we treat other human beings and using the same criteria for the evaluation of our treatment of animals seems natural (Duncan & Fraser, 1997). Even as early as the 18th century, concern about the treatment of animals and its effect on their subjective experiences, was awakened. Bentham (1789) proposed: 'The question is not, Can they reason? nor, Can they talk? but, Can they suffer?'. The capacity of animals to experience suffering and enjoyment seems to be central to the concern about animal welfare, as is agreed by the majority of critics of animal use (Duncan & Fraser, 1997). And, as Duncan & Fraser (1997) report, many scientists have emphasised the subjective feelings of animals as a key component in the scientific investigation of animal welfare. However, subjective feelings (i.e. emotions) are hard to measure, even in humans, and so far most research has used objective measures to evaluate the welfare of animals.

Nevertheless, there seems to be a notion that the evaluation of subjective experiences is possible. Wemelsfelder (2001) cites the philosopher Thomas Nagel to support this idea. Nagel, she writes, argues that there are two perspectives of understanding the world, the first-person perspective and the third-person perspective. The third-person perspective seems to correspond to our notion of objectivity and apparently leaves the individuals'

subjective perspectives behind. To explain consciousness and emotions from a third-person perspective seems incomplete, as subjectivity is a fact of life (Wemelsfelder, 2001). The first-person perspective on the other hand, provides a perspective of understanding that humans and animals are subjective centres of life and knowledge, and that the essence of subjective perspective is that there is something 'it is like to be ' us (Wemelsfelder, 2001). Wemelsfelder (2001) states that in a very straightforward sense, the first-person perspective is public, as one individual can say of another what the quality of the other's experience is. The first-person perspective is regarded by some philosophers in essentially dynamic terms, emphasizing that the whole, active, expressive organism provides a conceptual basis for understanding 'what it is like' to be that organism. Wemelsfelder (2001) concludes that such a perspective would complement the third-person perspective and that it could help answer questions of experience rather than physical organization. She argues that there does not seem to be a reason why the first-person descriptions of animals as expressive beings could not be reliable and repeatable. However, she sees the need to develop a dependable methodology, which provides concepts of animal behavioural expression with scientific context and credibility in their own right. Research in the first-person perspective would focus on the whole behaving animal rather than on fragments of physical movement and would thus describe behaviour as an integrated process expressive of experience. Nevertheless, the use of expressive language to describe animal behaviour is still regarded with distrust by many scientists, for fear of unwarranted anthropomorphism and the loss of scientific credibility (Wemelsfelder, 2001). However, Wemelsfelder (2001) explains that the belief that subjective experiences of animals are not open to empirical observation reflects the third-person perspective and that the very rationale of an independent, first-person approach is that in describing animals as expressive beings, their experience does become accessible to investigation. She also refers to some of her own work. She shows that description of animal behaviour as an expressive process is based on commonly perceived and systematically applied criteria and that the high internal validity of such descriptions indicates that they are not based on guesswork but on dependable empirical grounds. Although Wemelsfelder (2001) admits that observers may misinterpret or fail to notice certain behavioural expressions, she argues that this is likely to be the case if the observer is not sufficiently familiar with an individual animal or a certain species. She also states that understanding of what it is like to be the animals under investigation will grow with experience and although this understanding

may remain incomplete, she argues that misinterpretation is equally of concern for more conventional methods of measurement. Wemelsfelder (2001) argues that data gathered in this way may also be correlated with other behavioural and physiological data and that thus the biological relevance of these data will gradually emerge.

Despite the possibility that animal welfare may be assessed by reference to behavioural expression as described by Wemelsfelder (2001), the assessment of animal welfare nowadays still relies on an objective, multidisciplinary approach. Factors used to assess animal welfare include stress physiology, behaviour, immunology, injury, morbidity, mortality and productivity (Hemsworth & Coleman, 1998). Changes in these parameters may not give an absolute assessment of animal welfare, but they nevertheless seem to be useful indicators. A common way to measure animal welfare is to monitor the stress response of an animal. This type of research involves measuring the biological responses of an animal to environmental changes that may challenge their homeostasis (Hemsworth & Coleman, 1998). These challenges may be of environmental nature, animal based or stockperson related. Environmental factors may include housing, food and water supply, weather and changes in these factors. Animal based factors may include disease, injury, behaviour of other animals and possibly the use of certain drugs. Stockperson related factors seem to be based on the animal's fear of the stockperson.

The main biological responses available to the animal to cope with stressors are behavioural and physiological (Hemsworth & Coleman, 1998). However, these are not discussed in detail here as the present study used pathophysiology and physiological parameters related to energy status to determine the welfare of the calves involved.

Behaviour

A change in behaviour may be the first obvious response of an animal trying to cope with environmental change and may be an effective way of coping with stressors (Hemsworth & Coleman, 1998). For example, when exposed to cold, newborn animals may huddle with their mother or littermates in order to maintain thermal homeostasis.

Measuring behaviour may not be invasive and may not cause additional stress in the animal, unless the animal is stressed by being directly exposed to the observer.

Physiology

The physiological response to stressors includes changes in the autonomic nervous system and endocrine system. The earliest recognition of an animal's response to environmental change is the response of the autonomic nervous system, a response also referred to as the 'fight-or-flight' syndrome (Hemsworth & Coleman, 1998). This syndrome is characterised by a rapid and specific response of the autonomic nervous system and the secretion of catechomines from the adrenal medulla, which all serve to mobilise energy stores to cope with the challenge. The response includes increased heart rate and blood flow and increased glycolysis to increase circulating glucose concentrations (Hemsworth & Coleman, 1998). Should the stressor not be removed, a second series of events will occur (Hemsworth & Coleman, 1998). This is termed the acute stress response and includes the release of corticosteroids or glucocorticoids from the adrenal cortex (Hemsworth & Coleman, 1998). The major function of this response is to supply glucose from non-carbohydrate sources for the increased metabolic performance required (Hemsworth & Coleman, 1998). If the stressor remains, a chronic stress response develops, which is also corticosteroid dependent and comes at a physiological cost to the animal. How serious these costs are depends on the length of exposure to the stressor (Hemsworth & Coleman, 1998).

A common problem in assessing the physiological response to a stressor is that the techniques involved, such as blood sampling and confinement, are often stressful in themselves. Also, changes in heart rate or plasma cortisol levels may be due to pleasurable events such as exercise and in such circumstances it may be difficult to determine if welfare is compromised.

Other physiological factors that may be useful in determining the welfare of an animal may include those associated with energy and/or health status. These may include plasma metabolite concentrations, rectal temperature or indicators of immune function.

1.1.3 Why does neonatal calf welfare matter?

Calves are sentient beings and are thus able to experience negative subjective states such as pain or hunger, which reduce their welfare. In addition, the physiological systems of neonatal calves may not be fully mature and they may thus be less capable of coping with adverse environmental conditions and stressors such as pain. This may predispose them to more severe suffering than an older animal in the same situation. However, even if a neonatal calf at this stage of development is less aware of its environment and thus may suffer less than an older animal in the same situation, its welfare is of major importance for the longterm health and wellbeing of the animal. Exposing livestock to stressful situations is common in our husbandry systems. Dairy calves for instance are separated from their mothers soon after they are born. It may be possible that this early exposure to stress may influence the animal's development and thus may lead to welfare problems in later life. It seems that neonatal stress in livestock may cause an increase in subsequent emotionality and hypothalamic-pituitary-adrenal activity in response to novelty and stress (Lay, 2000). Thus, ensuring the welfare of the neonate may also improve the welfare of the animal later on in life when welfare compromise may be experienced more severely as the animal may be more aware of its environment than the neonate.

1.2 What do we know about neonatal calf welfare?

The change from the intrauterine to the extrauterine environment seems to represent one of the greatest challenges an animal will experience in its life (Mellor & Stafford, 2003). Neonatal debility and death are often concentrated in the first few days after birth, which seems to reflect the problems in the transition from the dependent intrauterine existence to the less dependent extrauterine life (Alexander, 1984). Neonatal calf mortality can be fairly high. In Canada calf mortality was found to be 8% indoors and 50% outdoors (Jordan *et al.*, 1969), while in the United States calf mortality ranged from 6-15% (Laster & Gregory, 1973). Although fundamental and applied research has clarified much about the causes and prevention of neonatal mortality in farm animals during the past 50 years and has provided practical means to minimise neonatal losses (Mellor & Stafford, 2003), there is only little information available on the possible welfare compromise the newborn calf may experience before death or during sickness due to different causes.

Although research has been undertaken to determine the welfare of calves during transport, at the slaughterhouse and during husbandry procedures, and the effect of colostrum on calf health and survival (Wesselink *et al.*, 1999; Mellor & Stafford, 2000; Stafford *et al.*, 2000; Todd *et al.*, 2000a; Stafford *et al.*, 2001), little information is available on the physiological, biochemical and pathophysiological status of the calf at birth in relation to factors involved in calf debility and death and how this could affect their welfare.

In contrast, the status of lambs at birth and the effects of various environmental variables have been well documented. In lambs it has been shown that hypothermia was one of the major causes of perinatal mortality (Eales & Small, 1980a; 1986b) and that it was caused by factors including placental insufficiency, maternal underfeeding during late pregnancy, premature birth, intrapartum hypoxaemia, cold exposure and starvation (Barlow *et al.*, 1987).

As our knowledge about the effect of environmental factors on calf welfare is limited, the next section will examine the possible welfare compromise that may be experienced by calves after birth and will also take into consideration the situation in newborn lambs, as our knowledge about lambs is quite extensive.

1.3 Animal welfare implications of neonatal ill-thrift and mortality in calves and lambs

1.3.1 Hypothermia

In non-tropical regions, hypothermia commonly precedes death in neonatal animals, but the reduced body temperature is not necessarily the cause of death, as hypothermia may have several causes in the newborn (Mellor & Stafford, 2003). Hypothermia may be caused by cold exposure and excessive heat loss, placental insufficiency, acute intrapartum hypoxaemia, immaturity at birth and inhibited heat production and starvation (Mellor & Stafford, 2003). For more detail on these factors please refer to the introductions in Chapters 2 and 3.

Possible welfare compromise caused by hypothermia

As calves and lambs are born during winter and early spring on most farms in New Zealand, they are likely to become cold-stressed immediately after birth, as the change from the intrauterine to the extrauterine environment includes exposure to low temperatures and possibly rain and wind. In lambs it has been shown that even when they are born into good weather conditions they will have to increase heat production after birth because their body temperature will usually be above the environmental temperature (Alexander, 1979). This is likely to be the case for calves as well. Exposure to cold stress in newborn animals may thus be normal and not inherently bad. Moreover, cold stress may be one of the stimuli contributing to arousal and onset of breathing and would therefore be of importance for the survival of the neonate. Although other factors such as tactile stimulation, exposure to gravity, and reduced O_2 and increased CO_2 tensions also contribute to arousal after birth, temperature may play an important role within the range of factors involved (Mellor & Gregory, 2003).

Lambs born into windy and rainy conditions are subjected to high heat loss and this is likely to be the case for calves as well. In most normal cases the resulting hypothermia is corrected by an increased thermogenic output during the first two hours after birth (Mellor & Cockburn, 1986) which offsets the effects of evaporation from the drying fleece or hair. The neonate is likely to regain normal rectal temperature if heat production can counter increased heat loss. Such neonates are likely to be strong enough to attempt to stand and suck colostrum, which will provide them with energy for further heat production. In those animals that have been compromised during pregnancy or birth and in lambs and calves that are multiples, heat loss may be higher than heat production, as energy reserves may be depleted, heat production may be inhibited or because these animals are lighter thus having an increased heat loss per unit of surface area (Randall, 1978; Alexander, 1979). These neonates are more likely to become and remain cold stressed and hypothermic after arousal has set in, and welfare compromise may ensue.

Hypothermia immediately after birth

During the first moments after birth, it is possible that the newborn animal is aroused, but not fully conscious and aware (Mellor & Gregory, 2003). Its PO_2 levels are likely to be below those observed in conscious animals due to the transition from the intrauterine

to the extrauterine environment. PO_2 is low in the fetus to enable passive diffusion of oxygen down a concentration gradient from maternal to fetal blood. Thus, it may take some time after birth until blood oxygenation may become adequate for consciousness to develop. If the neonatal calf or lamb becomes hypothermic during this period, its welfare may not be compromised. Sentience and consciousness are major prerequisites for suffering and welfare compromise (Mellor & Reid, 1993). Calves and lambs are sentient beings, but without consciousness, even sentient beings cannot suffer, as the animal has to be aware of its own being and sensations in order to experience good or bad welfare (Mellor & Reid, 1993).

If hypothermia is severe enough, affected neonates may never gain full consciousness, as their brain metabolism may be slowed down so much that consciousness may be dulled even though brain oxygenation may be adequate for full consciousness to occur (Mellor & Gregory, 2003). In humans, unconsciousness was observed at low temperatures (Table 1.2). If temperatures are low enough, the lamb or calf may become unconscious and thus welfare compromise would not be encountered.

Premature animals may experience respiratory distress shortly after birth, which is a condition where pulmonary surfactant is insufficient for the proper expansion of the lungs (Eigenmann *et al.*, 1984). Here, low oxygenation of brain tissue will also be present and may prevent full consciousness and thus suffering from hypothermia. Also, there may be a combined effect of both hypoxaemia and hypothermia, which will affect the level of consciousness due to their inhibitory effect on brain metabolism.

If hypoxaemia is accompanied by hypercapnia, a strong drive to breathe is created (Mellor & Gregory, 2003). In conscious humans this is known to be associated with breathlessness noxious experiences. But in a newborn animal that is not conscious, breathlessness may not be an issue. On the contrary, a combination of hypoxaemia and hypercapnia may be needed for the animal to start breathing at all (Mellor & Gregory, 2003). But even if breathing is initiated and brain oxygenation is increased, the animal may still not become conscious and aware due to the effect of hypothermia on brain metabolism. Welfare compromise due to hypothermia in lambs and calves experiencing respiratory distress may thus not be encountered.

Table 1.2: Symptoms of hypothermia observed in humans (Schimmelpfenig & Lindsey, 1991).

Mild hypothermia (35-32°C)	Moderate hypothermia	Severe hypothermia (31.9°C and lower)
- The person feels chilled - Minor impairment of muscular performance - Shivering - Mental deterioration - Responses are slow and/or improper - Shivering reaches maximum when body temperature as low as 35-33.5°C	- Muscular coordination deteriorates - The person may be stumbling, walking slowly, lacking energy, becoming apathetic and lethargic - Responses to questions may be inappropriate - Slurred speech - Confusion about time and space - As body temperature approaches 31°C gross muscular incoordination becomes obvious - The person may become forgetful and display inappropriate behaviour - Pulse rate and blood pressure fall as body becomes cooler	- Shivering stops at temperatures below 31°C - Energy reserves are depleted - Obvious metal deterioration - Incoherence, disorientation, irritability - Exposed skin is cold and very blue - Between 29 and 26°C severe muscular rigidity may occur and the person may become unconscious - Pulse may be undetectable - Respiration will cease at temperatures around 25°C

Exposure to cold and hypothermia after consciousness is established

Animals that are cold stressed or become hypothermic after consciousness has set in may experience welfare compromise, as they may be fully aware of their situation.

Hoffman & Pozos (1989) found that rapid cooling had a profound effect on the human perception of cold, which they referred to as the 'overshoot phenomenon', where sudden drops of temperature would be perceived as more pronounced cold sensations as if reflecting much colder temperature differences. Thus, calves and lambs that are exposed to a fast cooling medium may be more aware of the temperature differences and thus feel less comfortable than animals exposed to more subtle temperature changes.

Cold exposure and possibly mild hypothermia may be unpleasant, but they are likely to lead to behavioural and physiological responses, which will restore the animal to a more pleasant state of being. Feeling cold may thus be of major importance to avoid

hypothermia by employing behavioural means, and welfare compromise due to initial cold stress and possibly mild hypothermia may not be experienced. The animal may seek shelter, huddle with its mother or littermates and employ physiological means such as vasoconstriction, if mature enough, and heat production will be increased.

But even if more severe hypothermia occurs despite behavioural and physiological means to prevent it, welfare may not be compromised. In humans, mental deterioration was observed even during mild hypothermia (Table 1.2), which may be due to decreased resting potentials and decreased amplitude and increased duration of action potentials, as well as reduced nerve conduction velocity and impairment of synaptic transmission following reduced neurotransmitter release (Kochs, 1995). Hypothermia was also associated with decreased mental functions such as memory registration (Coleshaw *et al.*, 1983) and unawareness of being hypothermic (Kaufman, 1983). Although mental deterioration was found to set in during mild hypothermia, changes in EEG and evoked potentials were minimal down to 33.5°C (Kochs, 1995). Fitzgibbon *et al.* (1984) concluded though, that minor electrical changes might be associated with detectable impairment of cognitive processes. In neonatal pigs it was found that a reduction of rectal temperature to 34-35°C decreased cerebral blood flow and cerebral metabolic rate by 40-50% (Busija & Leffler, 1987). Thus, animals that are mildly or moderately hypothermic may not be aware of becoming hypothermic due to reduced cerebral metabolism. Suffering may not be present as altered brain function may reduce the neonate's awareness of its situation and thus would preclude welfare compromise

In cases where calves and lambs are aware of their situation, suffering may be due to an increased unpleasantness of feeling cold, which may be noxious enough to cause welfare compromise. But the period of suffering may only be short if severe hypothermia ensues soon after the onset of hypothermia, as severe hypothermia was associated with obvious mental deterioration and eventual unconsciousness in humans (Schimmelpfenig & Lindsey, 1991).

Welfare compromise may be encountered where the animal experiences cold-stress beyond that needed to induce behavioural and physiological measures, but where hypothermia is not yet an issue. In human subjects it was observed that the colder the stimulus temperature (20°C to 0°C), the greater the cold pain perceived (Chery-Croze,

1983). Kunkle (1949) reported that pain would be induced by prolonged and intensive cooling of distal parts, associated with deep cooling and enhanced by ischaemia. These findings may have implications in calves and lambs that are conscious and exposed to cold water or air. In the study by Kunkle (1949), subjects immersed a finger into water of 0-10°C and experienced pain for over one hour. It may thus be possible that calves and lambs may also experience cold pain in their extremities, when exposed to rain and air temperatures around 0°C or when lying in a puddle of water or mud. As pain is a very unpleasant subjective experience, welfare compromise would result when pain is severe enough. It may only be subtle, increasing the chance that the animal may seek shelter or move from the puddle of water to stop the painful experience, thus avoiding hypothermia.

In humans, the skin temperature threshold for cold-pain was found to be at 15°C (Hoffman & Pozos, 1989). Studies in lambs show that at or near thermoneutral ambient temperature, lamb skin temperatures of the trunk and extremities are approximately the same order. At low air temperatures (below 10°C) though, skin temperatures of the extremities fall considerably below trunk skin temperatures and those of the pasterns, tips of the ears and tail are only a few degrees above air temperature (Haughey, 1973). If the threshold for cold pain is similar as that in humans, pain may develop in the extremities at air temperatures below 10°C. This may also be the case in calves. But it may also be possible that animals are adapted to these kind of temperatures as they are likely to encounter them more often than humans and thus their cold pain threshold may be lower, reducing the chance that they may experience pain and welfare compromise, unless very cold ambient temperatures are encountered (see frostbite below). Even if the cold pain threshold is similar to that of humans, welfare may not be compromised if the animal becomes hypothermic before skin temperature drops to the pain threshold. Suffering may be reduced or not experienced at all, provided that the animal is not conscious or consciousness is reduced far enough for the animal not to be able to perceive pain. If this were the case, hypothermia may even prevent welfare compromise in calves and lambs exposed to painful and other negative subjective experiences.

More severe problems such as frostbite (frozen tissues) may not be prevalent in calves and lambs in New Zealand as the weather conditions are not usually extreme enough. It may be a more common problem in livestock in Canada and regions where severe

winters are experienced. In humans, frostbite was observed to cause symptoms such as burning or stinging followed by numbness (McDade, 1962). Pain thus may not be an issue initially due to numbness, but may be common during rewarming and thawing (McDade, 1962). Although frostbite as such may not be common in New Zealand, cold injury in neonates may be present. A study by Haughey (1973) in Australia showed that peripheral oedema was related to low environmental temperatures and was frequently observed in the hind limbs and tail of lambs. If this were painful or impair function, welfare compromise may be experienced depending on the degree of noxiousness of the pain experienced and if impaired function interferes with feeding and other vital behaviour. But hypothermia may precede oedema formation and the animal may thus not be aware of the damage and suffering may not be an issue.

Rewarming

If rewarming is associated with the so-called 'afterdrop', where core temperature declines during rewarming (Green Larach, 1995), calves and lambs may become even more hypothermic and their survival may be jeopardised. In humans, this can be observed when external rewarming methods are employed, causing cold blood from the previously vasoconstricted extremities to return to the body core or by conductive heat loss from the core to the cold periphery (Green Larach, 1995). It is assumed that external methods of rewarming can cause fatal arrhythmias through the afterdrop. But as the hypothermic neonate is likely to be either unconscious or has a reduced mental capacity due to slowed brain metabolism, this may not be a problem for its welfare.

Also, rewarming may be associated with pain. In humans, Kunkle (1949) found that recurring pains due to local chilling were mainly due to partial rewarming of the tissues and vasodilatation. The pain observed might be associated with rapid rewarming of previously cold and ischaemic and thus damaged tissue (Kunkle, 1949). If calves or lambs are rewarmed fast, pain may thus develop when consciousness returns or increases. Welfare compromise would be likely in this event, depending on the degree of noxiousness.

Another issue that may be of importance in rewarming hypothermic neonates is the possible increase of plasma lipopolysaccaride concentrations. In a study by Gaffin *et al.* (2000) on humans, it was shown that rewarming human patients was probably

associated with a risk of endotoxaemia. They proposed that hypothermia reduced splanchnic blood flow causing ischaemic damage to the gut wall and translocation of lipopolysaccarides from the gut into the vascular space. Upon rewarming, splanchnic blood flow would resume, translocating lipopolysaccarides from the splanchnic to the systemic circulation as a bolus. If this were the case in newborn calves and lambs, survival may be threatened, especially if the animal has consumed little or no colostrum and is thus a- or hypogammaglobulinaemic and therefore more susceptible to such an insult. If endotoxaemia should result, welfare compromise may be experienced (see section 1.3.4).

1.3.2 Starvation

Starvation has several pathophysiological consequences. First, it will lead to hypoglycaemia in the newborn animal as its energy reserves are depleted, which will lead to cerebral compromise in warm conditions and hypothermia in cold conditions (Mellor & Cockburn, 1986; Mellor & Stafford, 2003). Second, the gut of starved newborn animals will not exhibit accelerated cellular maturation, enhanced functional capacity and increased growth normally seen in the gut of fed animals during the first 1 to 3 days after birth (Mellor & Stafford, 2003). Third, starvation deprives the newborn calf and lamb of their only significant source of immunoglobulins. See also the introduction of Chapter 3 for further details.

Potential for welfare compromise during starvation

As the neonate is born with energy reserves and sufficient high blood glucose to fuel basal metabolism and enable it to increase heat production immediately after birth (Mellor & Cockburn, 1986), hunger may become a welfare issue only later in the immediate neonatal period. Naturally, neonates with reduced energy reserves such as premature calves and lambs or those that experienced maternal underfeeding during late pregnancy may face depletion of energy reserves and hunger earlier during starvation. This will also be the case for neonates exposed to adverse weather conditions, where energy reserves are consumed more quickly (Mellor & Cockburn, 1986).

But calves and lambs can be observed to seek the teats of their mother as soon as they are able to stand and walk (Lynch *et al.*, 1992). Could this be a sign of hunger or an innate drive to suck soon after birth to ensure energy supply? If it is an innate drive the

idea above, that the animal will not experience hunger in the immediate neonatal period, may be valid. But what if the neonate does experience hunger? Would this lead to welfare compromise?

First, some degree of hunger is necessary for animals to engage in feeding. Therefore, mild and probably moderate degrees of hunger may not present a compromise to the newborn's welfare, but would enable it to balance energy deficits by ingesting food. Marked and severe starvation though may cause welfare compromise. In animals that become hypothermic before hunger is severe enough to cause compromise, suffering due to severe starvation may be nonexistent, as hypothermia may reduce awareness of hunger and urgency feelings about the animal's situation. But if the animal is fully conscious it will be able to experience hunger sensations and, therefore, welfare compromise. Prolonged starvation in normothermic lambs was observed to increase teat-seeking behaviour initially, which may be an indication that hunger was experienced (Alexander, 1962c). This was followed by severe hypoglycaemia and a period during which the lambs were asleep most of the time or standing with back hunched, uttering feeble cries (Alexander, 1962c), which may be an indicator of distress due to hunger and the effects of hypoglycaemia. But if the lambs are asleep or in a coma, they cannot suffer from welfare compromise, as sleep is a form of reversible unconsciousness. Thus, suffering may be relieved for the period the animal is asleep. Lambs in the study by Alexander (1962c) died between 16 hours and 5 days after the onset of starvation, and lambs survived for longer in warmer weather conditions and/or when they had higher birth weights. Thus the period over which the animal is suffering from hunger may be different for individual lambs, depending on the weather conditions they were born into and/or their birth weights. Similar results may be expected in starving calves.

Hypothermia may be a common feature in starved neonates, as heat production cannot counter heat loss due to reduced or depleted energy reserves. It was observed by Alexander & Williams (1966) that lambs lose their sucking drive at body temperatures below 37°C. This may be due to the inhibitory effects of hypothermia on brain metabolism, which may create a sense of reduced urgency for food intake, implying that hunger may be reduced. If this were the case the animal is not likely to suffer from starvation when combined with hypothermia severe enough to cause mental

deterioration. But if teat seeking is reduced by the effect of hypothermia on the brain causing inability to perform certain motor functions, such as sucking, hunger may possibly still be present. But welfare compromise in this case may be restricted to a short period, as the progressive hypothermia would lead to mental deterioration and thus the possibility that suffering from hunger is not experienced anymore.

Starvation of newborn animals may be accompanied by dehydration and thirst as the milk that is normally taken in also provides the animal with necessary fluids. Thirst may be a very noxious stimulus although, as with hunger, mild thirst will be necessary for the animal to initiate drinking. Moderate or severe thirst may be likely to cause welfare compromise if the neonate is aware of its situation. But Alexander (1962c) observed that lambs that were given water every hour during his experiment did not survive for longer during a starvation period than lambs not given water. Thus, it was assumed that these lambs died of starvation. Therefore, dehydration might not be an issue for neonatal welfare as starvation is likely to kill the animal before the effects of dehydration will. But the effects of dehydration, and therefore thirst, may still be present before death from starvation and thus thirst might be an issue in neonatal animals starved but not given water. However, Savory & Kyriazakis (1997) cite a paper in which water intake by experimental animals was shown to be reduced by about 70-80% without leading to any physiological adverse effects. Also, hypovolaemia due to dehydration is reported to lead to decreased tissue metabolism, cellular deterioration, muscle weakness and depressed mental function in humans (Guyton and Hall, 1996). Thus, it may be assumed that dehydration that is severe enough may lead to a reduced awareness of the situation and possibly to a reduced awareness of thirst. Therefore, the period over which an animal can suffer from severe thirst might be reduced and welfare compromise may only exist over a short period.

If the neonate survives starvation and dehydration, another factor of importance for its long-term welfare is the reduced absorption of immunoglobulins due to starvation. If the calf and lamb receive colostrum later in the neonatal period, when the immunoglobulin concentration of milk and the absorptive capacity of the intestine are reduced, the risk of those neonates being hypogammaglobulinaemic will be increased and so will be the susceptibility to contract diseases. Diseases are a major source for poor welfare and welfare compromise (see section 1.3.4). Also, the reduced exposure to growth factors

due to starvation may negatively affect future gut function and the calf's and lamb's welfare (Mellor, 1988).

1.3.3 Maternal factors

Maternal factors include maternal underfeeding during late pregnancy (see the Introduction Chapter 2), mismothering and impaired bonding. Mismothering and impaired bonding may be the result from inexperience, debilitating maternal undernutrition during late pregnancy, dystocia-induced maternal exhaustion, separation by misadventure, interference by other females or by stockhandlers or the inability of the mother to recognise her litter size (Mellor & Stafford, 2003)

Potential for welfare compromise

Maternal underfeeding may lead to a reduction in fetal growth (Mellor & Murray, 1982b) and thus the newborn will enter the extrauterine environment with reduced weight and possibly reduced energy stores. Also, udder development may be negatively affected by maternal underfeeding (Mellor & Murray, 1985). Thus, the newborn animal may not only find itself compromised by reduced energy stores and increased susceptibility to heat loss due to low weight, but also by reduced food supply by the dam. In addition, underfed ewes have been found to be more likely to abandon their young or exhibit poorer maternal care (Mellor, 1988). Thus, newborn animals of these mothers may suffer from hypothermia and starvation (see sections 1.3.1 and 1.3.2).

Maternal licking, among other things, stimulates the newborn animal to breathe (Mellor & Gregory, 2003). Maternal absence may therefore lead to a delay in the onset of breathing. This may lead to hypoxaemia as well as hypercapnia, which, together, are a powerful drive to breathe and were observed to cause breathlessness in humans. Although breathlessness is an unpleasant experience, the newborn lamb and calf may not experience its unpleasantness, as they are not likely to be fully conscious at this stage (Mellor & Gregory, 2003). In addition a strong drive to breathe may be vital for the newborn lamb and calf to start breathing at all (see section 1.3.1). Therefore welfare compromise would not be present.

Also, it may be argued that the neonate may suffer from impaired bonding with its mother and anxiety and distress if abandoned by the mother. But this was not observed

in calves that were removed from their mothers after suckling. Calves did not call when separated from their mothers until 9-18 hours after separation, when hunger probably became an issue (Weary & Chua, 2000; Flower & Weary, 2001). On the other hand, lambs are followers in contrast to calves. Calves are hiders, spend most of their time by themselves and only suck 3 to 5 times daily (Hafez, 1964). Thus, calves may be adapted to be separated from their mother, while lambs follow their mothers (Arnold and Dudzinski, 1978) and suck frequently (6 to 20 times daily) (Hafez, 1964). Thus emphasis on the mother-young bond may be stronger in sheep and thwarted bonding drive may lead to welfare compromise due to distress and anxiety. But lambs were found to bond with alien ewes when separated from their mother 20 to 27 hours after birth and after successful maternal bonding (Mellor *et al.*, 1993). This may support the assumption that a non-established or impaired bond with the mother is not of major importance for the neonate's welfare early in life. However, this may be different in older animals, where a strong bond between mother and young has been established. Welfare compromise may be encountered when these animals are separated from their mothers.

1.3.4 Infections and injuries

At birth the newborn is thrust from a sterile into an infective environment and any factors that impede colostrum intake and hinder the uptake of immunoglobulins thus increase the newborn's susceptibility to infectious disease (Mellor & Stafford, 2003). Infectious diseases may include diarrhoea, pneumonia, septicaemia, peritonitis, pleurisy, septic arthritis and other respiratory, enteric or general infections (Mellor & Stafford, 2003) (see also the Introduction Chapter 3).

Injuries may occur during birth, accidents, in cases of predation and during husbandry procedures. Injuries during birth include brain damage, liver damage, fractures, abrasions and bruises, which may be due to feto-pelvic disproportion, malpresentation and human intervention during dystocia (Mellor & Stafford, 2003). Accidental injuries postnatally may be due to maternal kicking, pushing, butting, standing and lying on, biting and pawing at the neonate (Mellor & Stafford, 2003). Also, accidental injuries may occur during handling of neonatal animals by farm staff. Husbandry procedures, which cause tissue damage include castrations, ear notching or ear tagging, tail docking and dehorning.

Potential for welfare compromise

Pain and sickness are major issues in infectious diseases, and may lead to severe welfare compromise and often death. The issues involved with injuries, predation and husbandry procedures that may cause possible welfare compromise, include anxiety, pain and the resulting distress.

Sickness

Signs of feeling ill in infected animals include listlessness, fatigue, reduced social interaction, inappetance, discomfort, apparent mental confusion, impaired memory, learning difficulties and in some cases, fever (Gregory, 1998). These sickness behaviours have survival value as they help to promote recovery (Gregory, 1998). Inappetance, for example, is one behaviour that is increased during disease and is likely to promote proteolysis and shunting of blood supply from the gastrointestinal tract to areas needed for the body's defence reaction (Gregory, 1998). However, significant suffering may occur during the fulminating and recovery phases of non-fatal infections and during the fulminating and terminal phases of fatal infections (Mellor & Stafford, 2003). This though may only be the case if the infected animal is conscious. However, sleep and drowsiness are also observed during disease and one could argue that the increased incidence of sleep may reduce the period for which the animal may be distressed by feeling ill. Although, sickness behaviours associated with infectious diseases seem to be an integral part of the mechanisms that help animals to recover from the disease (Gregory, 1998), sickness may be very distressing especially if pain is involved and effective prevention and treatment of infections are major ways of reducing the animal's suffering (Mellor & Stafford, 2003).

Pain

Welfare compromise in infected calves and lambs may also be due to pain and the resulting distress. Although pain is of evolutionary value as it may prevent further damage to already damaged tissues, moderate and severe pain are likely to lead to welfare compromise. Pain may prevent feeding and may lead to starvation-induced hypothermia, which may dull the pain and reduce welfare compromise (Mellor & Stafford, 2003). On the other hand, animals in pain may be alert, restless and continuously distressed, which supports the need for prevention and/or treatment of pain (Mellor & Stafford, 2003). The duration and intensity of pain are likely to influence the

extent of distress and welfare compromise experienced. Mild pain might be very distressing if prolonged, while a very short moderate pain may not be. Severe pain may cause welfare compromise if acute and prolonged.

Birth injury has the potential to cause serious welfare compromise after the onset of consciousness. If the neonate's pain pathways are functional, pain is a likely outcome. On the other hand it may be argued that the animal, not knowing an existence without pain, may find it less aversive and welfare compromise may therefore be less. However, pain has an evolutionary value in alerting the animal to tissue damage that has occurred or might occur. Thus, being able to experience pain very soon after birth would be advantageous for the animal to prevent further tissue damage. Indeed, it is known that lambs exhibit behavioural and physiological responses to painful stimuli 4 hours after birth (Mellor & Murray, 1989). Thus, it is likely that such newborn animals might be able to perceive birth injuries as painful and, therefore, welfare compromise may be present after awareness has set in. If the birth injury includes CNS damage, however, the neonatal animal may not be conscious or brain areas responsible for pain perception and processing may be damaged. CNS damage may also lead to hypothermia (Mellor, 1988). If CNS damage is not severe enough to impair mental function but hypothermia is severe enough to cause mental deterioration or unconsciousness, painful injury acquired at birth and any associated welfare compromise may not be experienced.

Injuries, regardless of their origin, may impair function, which may lead to starvation due to the inability of neonates to suck and possibly will result in reduced immunoglobulin levels in their blood. This, as mentioned in previous sections, is likely to increase the animals' susceptibility to infectious diseases and welfare compromise.

Anxiety

An issue associated with predation is anxiety, which may occur when the animal is hunted or approached by a predator. This is likely to cause distress and thus welfare compromise. But it is likely that newborn ruminants are not very anxious, as up to a certain age newborn animals seem to allow humans to approach without trying to escape (personal observation in calves). Although they might be observed to follow their mother during a predator attack, this may be a function of trying to maintain the mother–young bond (Arnold and Dudzinski, 1978) rather than a function of trying to

escape the predator. If this were the case, suffering would be unlikely. If anxiety were an issue even during the early hours after birth, welfare compromise may be present. This may also be true for calves and lambs that are older and capable of a fear response. However, in the New Zealand situation predatory attacks may be rare.

Husbandry procedures have been observed to cause pain (Mellor, 1991; Molony & Kent, 1993; McMeekan *et al.*, 1999; Stafford *et al.*, 2000; Mellor & Stafford, 2000) and, if the animal is old enough, may cause anxiety due to their exposure to humans and human handling.

1.4 Physiological and physical status of calves between birth and 4 days of age

The physiological and physical status of lambs at birth and its effects on the newborn lamb are well documented. This knowledge allowed an analysis of the possible welfare compromise newborn lambs experience during and after birth. In contrast, only little information is available on the similar status of calves at birth and following birth. Thus, the aim of the present study was to collect preliminary data on the physiological and physical status of calves at birth and following birth, by adopting indices used in the study of newborn lambs by Barlow *et al.* (1987) (Table 1.3, page 29). These indices included packed cell volume (PCV), plasma lactate concentrations, plasma fructose concentrations, birth weight, rectal temperature within 30 minutes of birth, and the age at death. Additional indices used in the present study included the plasma concentrations of glucose, urea, beta-hydroxybutyrate and gamma-glutamyl transferase (GGT).

1.4.1 Physiological parameters used in the present study

Parameters previously used in lambs

Packed cell volume

Packed cell volume, or haematocrit, is the fraction of blood composed of red blood cells as determined by the centrifugation of blood. Elevated packed cell volume (PCV) in the newborn lamb has been shown to be associated with chronic hypoxaemia due to placental insufficiency (Mellor, 1988). During hypoxaemia, the kidney is stimulated to produce the hormone erythropoietin, which in turn enhances red blood cell production

until the hypoxaemia is relieved (Zanjani *et al.*, 1973). Thus, increased production of red blood cells due to prenatal hypoxaemia-induced increase in erythropoietin production is the likely reason for increased PCV in newborn animals that have previously experienced placental insufficiency *in utero*.

Lactate

Lactate is the product of anaerobic metabolism and elevated plasma lactate concentrations in the newborn animal may indicate that the animal has experienced chronic oxygen shortage *in utero* due to placental insufficiency (Mellor, 1988) or acute oxygen shortage during birth (Eales & Small, 1985).

Walser & Maurer-Schweizer (1979) describe how, under normoxic conditions, glucose as a main energy source is reduced to pyruvate via the citric-acid cycle, and how pyruvate in its turn is metabolized further aerobically to CO_2 and H_2O. During oxygen shortage glucose can only be metabolized anaerobically to pyruvate, which is then reduced to lactic acid, as oxidation of pyruvate to CO_2 and H_2O is not possible without oxygen. However, anaerobic metabolism has disadvantages. Not only is the energy output small, but carbohydrate reserves are rapidly exhausted and metabolic acidosis develops by accumulation of lactic acid (Walser & Maurer-Schweizer, 1979).

Fructose

Plasma fructose concentrations in the newborn give an indication of the energy status of the calf before birth. That is because fetal plasma fructose is produced by the placenta from glucose derived from the fetal circulation, and its concentrations change in parallel with fetal plasma glucose levels [DJ Mellor, unpublished data related to Mellor (1988)]. Measuring plasma fructose levels after birth will give a more accurate estimate of fetal glucose concentrations and energy status of the fetal calf before birth, as plasma glucose concentrations are less stable during birth than plasma fructose concentrations [DJ Mellor, unpublished data related to Mellor (1988)].

Birth weight

One of the most obvious consequences of inadequate placental development is fetal growth retardation (Mellor, 1988). Thus, low-weight single calves and lambs are likely to have experienced placental insufficiency or maternal undernutrition. However,

Barlow *et al.* (1987) found that it was not birth weight itself that predisposed lambs to die, rather the presence of disease and physiological incapacities associated with the low birth weight were important contributory causes.

Rectal temperature

Measuring rectal temperature enables the detection of hypothermic animals when they are in the early stages of hypothermia and do not necessarily appear to be hypothermic (Eales *et al.*, 1982a). In addition, young animals seem to respond to infectious diseases with an increase in rectal temperature (Todd *et al.*, 2000a) and thus could be detected by means of measuring rectal temperature. Rectal temperature may also be a useful tool to detect whether a calf or lamb has taken in food. Starving newborn animals will become hypothermic due to the depletion of energy stores and a resulting low rate of heat production (Eales *et al.*, 1982a). However, a newborn animal that has taken in sufficient food will be able to sustain heat production and is likely to have a close to normal rectal temperature (Mellor and Cockburn, 1986).

Age at death

Eales *et al.* (1982a) observed that there were two ages at which hypothermia was more likely to occur in newborn lambs. Most lambs were found to die of hypothermia either between birth and five hours of age or somewhere between 12 and 36 hours of age. The major causes of hypothermia in the first period were excessive heat loss and depressed heat production. However depletion of energy reserves was not characteristic for lambs becoming hypothermic during this period. Hypothermia during the second period was associated with starvation, depletion of energy reserves and a low rate of heat production (Eales *et al.*, 1982a).

Additional parameters used in the present study

Glucose

Carbohydrate reserves will be mobilised to provide energy for the newborn calf before colostrum is taken in. Plasma glucose concentrations after birth thus give an indication of the energy status of the newborn calf after birth. They may also aid in the determination of whether calves have taken in food or not (Todd *et al.*, 2000b), especially when combined with other parameters such as rectal temperature and plasma GGT concentrations (Wesselink *et al.*, 1999). Measuring plasma glucose concentration

in calves aged 1 to 4 days and in cows thus gives an indication of the energy status in these animals.

Beta-hydroxybutyrate and urea

Tissues preferentially use glucose for energy, but the quantities of glucose normally stored in the entire body of the newborn are small and can supply the energy required for body function for perhaps half a day (Mellor & Cockburn, 1986). Thus, the major effects of starvation are progressive depletion of tissue fats and proteins (Alexander, 1962c).

Beta-hydroxybutyrate is a ketone body, which is produced when fat is mobilised for energy production. Liver ketone body synthesis occurs when beta-oxidation proceeds at a rate which exceeds that at which acetyl-CoA enters the citric acid cycle (White and Baxter, 1994). Beta-hydroxybutyrate concentrations in plasma would therefore give an indication on the energy status of the newborn calf (Todd *et al.*, 2000a). A combination of low plasma glucose concentrations in the hypoglycaemic range and elevated plasma concentrations of beta-hydroxybutyrate indicate utilisation or depletion of liver glycogen reserves and increased lipid mobilisation (Todd *et al.*, 2000a). This indicates a reduction of energy intake by the calf or possibly increased energy need such as during cold exposure or during disease where the immune system is very active and requires increased energy input.

Urea is a by-product of protein catabolism. Plasma concentrations of urea thus also are an indicator of the energy status of an animal (Todd *et al.*, 2000a). Protein may be mobilised during starvation to provide a source of energy or during disease to provide amino acids to support protein synthesis as part of immune responses.

Table 1.3: Criteria to help determine the cause of death in lambs, based on altered blood or plasma composition, birth weight, rectal temperature and age at death (adapted from Barlow *et al.*, 1987; Mellor, 1988).

Category	Diagnostic variable	Comment
A Placental insufficiency	High packed cell volume High plasma lactate	Chronic fetal hypoxaemia
	Low plasma fructose Low birth weight	Fetal undernutrition and growth retardation
	Low rectal temperature	Inhibited heat production
	Age at death <12 hours	
B Acute intrapartum hypoxaemia	Packed cell volume not high High plasma lactate	Acute fetal hypoxaemia
	Low rectal temperature	Inhibited heat production
	Age at death <12 hours	
C Inadequate thermogenesis	Packed cell volume not high Plasma lactate not high	Fetal normoxia
	Low rectal temperature	Excessive heat loss or inhibited heat production
	Age at death < 12hours	
D Starvation	Normal packed cell volume Normal plasma composition Normal birth weight	No prenatal or intrapartum predisposing factors
	Normal rectal temperature	
	Age at death > 12 hours	
E No diagnosis		

Gamma glutamyl-transferase (GGT)

GGT is a membrane-associated protein involved in amino acid transport and GGT activity in milk and colostrum is high (Thompson & Pauli, 1981). Low total plasma protein and gamma globulin levels were concurrent with low serum GGT, which suggested a relationship between colostrum ingestion and serum GGT levels

(Thompson & Pauli, 1981). Braun *et al.* (1982) reported that blood plasma GGT activity was an easy and inexpensive way of testing colostrum intake. Perino *et al.* (1993) showed that GGT activity was high in calves that had sucked colostrum. They also found that, although GGT did not reflect the amount of colostrum consumed, a degree of activity greater than 200 IU/L suggested that the calf had consumed colostrum.

1.5 Outline of thesis

The purposes of this thesis were to determine the impact of pre-, intra- and postpartum factors on the physiological and physical status of calves at birth and their subsequent performance for the first 4 days after birth.

Chapter 2 examines the effects of pre-, intra- and postpartum factors on the physiological and physical status of calves at birth, while Chapter 3 concentrates on the effect these factors may have on the subsequent performance of calves over the first 4 days after birth and their survival. The findings of the present thesis are compared with the results for lambs. Chapter 4 includes a general discussion of the results of Chapters 2 and 3, and also provides concluding suggestions for stockhandlers and ideas for future research. The experimental limitations of the present study are also presented in Chapter 4.

Chapter Two:

The Physiological and Physical Status of Calves at Birth

Abstract

In newborn lambs it was observed previously that hypothermia was one of the major causes of perinatal mortality. Hypothermia was not only caused by excessive heat loss but also by the suppression of heat production. Factors involved included placental insufficiency brought about by maternal underfeeding or stress during early pregnancy, premature birth, intrapartum hypoxaemia, cold exposure and starvation.

In the present study we examined the incidence of pre-, intra- and post-partum factors on the physiological status of dairy calves. The results have shown that the physiological status of calves at birth is fairly uniform. Prepartum factors, namely placental insufficiency and maternal underfeeding during late pregnancy were not a major problem in the calves of this study. Assistance was provided for the present cows when birth did not apparently progress for over one hour. Intrapartum hypoxaemia was subsequently observed in assisted calves and was accompanied by elevated plasma lactate concentrations and reduced calf vigour. Assisted calves were not observed to become hypothermic in response to intrapartum hypoxaemia as was observed previously in newborn lambs, which might have been an effect of early assistance. However, a prolonged time to stand in assisted calves may have delayed colostrum intake and may have interfered with the intake and absorption of immunoglobulins, which are important for the animals' protection against infectious diseases. Environmental variables such as weather, air temperature and time of day at birth did have an influence on rectal temperature, plasma glucose concentration and time to stand. There was a significant difference in plasma lactate concentrations and calf birth weight between calves that became sick and healthy calves, however, there were no significant differences between these groups in any of the other parameters measured. Calves that died had significantly higher birth weights and plasma lactate concentrations than calves that remained healthy. From these findings it can be concluded that calves seem to have a good chance of survival when they are alive at birth and that factors to which calves are exposed later in life may have a greater influence on morality rates. In assisted calves, however, this may only be true for calves that are assisted early during difficult labour. Early assistance might reduce the effects of intrapartum hypoxaemia, such as inhibition of heat production, which the calf is likely to experience.

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2

The Physiological and Physical Status of Calves at Birth

2.1 Introduction

Calf debility and death may constitute a serious economic loss to the dairy industry in New Zealand. Calves are important to the dairy farmer as potential replacement heifers for milk production. Calves that are not wanted are sold for slaughter or for beef production. Reducing calf debility and death allows the farmer a greater number of heifers from which to choose replacements. This will help to ensure that calves of the next season are born to strong and healthy animals. In addition the welfare of newborn calves will be improved directly by stockhandlers knowing about the causes of mortality and ill thrift and by having available to them remedies to restore the animals to good health and welfare.

There is little information on the physiological, biochemical and pathophysiological status of the calf at birth in relation to factors involved in calf debility and death. In contrast, the status of lambs at birth and the effects of various environmental variables have been well documented (Alexander, 1962a; b; Alexander & Williams, 1966; Halliday *et al.*, 1969; Alexander, 1970; Alexander *et al.*, 1972; Eales & Small, 1980a ; b; Eales *et al.*, 1982a; Eales *et al.*, 1983; McBride & Christopherson, 1984; Eales & Small, 1985; Eales & Small, 1986a; Mellor & Cockburn, 1986; Barlow *et al.*, 1987; Olson *et al.*, 1987; Mellor, 1988). Hypothermia was found to be one of the major causes of perinatal mortality in lambs (Eales & Small, 1980a; 1986a), and Barlow *et al.* (1987) demonstrated the physiological background to hypothermia. Hypothermia was not only caused by excessive heat loss, but also by the suppression of heat production. Factors involved included placental insufficiency, maternal underfeeding during late pregnancy, premature birth, intrapartum hypoxaemia, cold exposure and starvation.

2.1.1 Perinatal debility and death

Prenatal factors predisposing to perinatal debility and death

Placental insufficiency

Placental weight and size increase after implantation of the embryo and continue to grow until 13 weeks of gestation in sheep (Mellor, 1987) and 20 weeks of gestation in cattle (Cooper *et al.*, 1998). In sheep a wide range of placental sizes has been observed (Mellor, 1983), which may be explained by the effect of moderate or severe maternal undernutrition or stress during the period of placental development and growth. In ewes with poor placental development, fetal lamb growth is retarded (Mellor, 1988). In low-weight fetuses of this type chronic hypoxaemia and hypoglycaemia are common during late pregnancy, accompanied by hyperlactataemia, elevations in packed cell volume and low plasma fructose and insulin levels. Cortisol and catecholamine levels are also likely to be elevated and other endocrine disturbances can be associated with placental insufficiency (Mellor, 1988).

If the lamb survives birth, its postnatal survival may be jeopardised by the effects of the chronic prenatal hypoxaemia inhibiting spontaneous respiration at birth and heat production postnatally (Mellor, 1987). In addition, chronic hypoglycaemia will reduce energy store deposition, or may deplete energy stores, which are vital to fuel heat production after birth (Mellor, 1987). This may be due to a deficiency of nutrient supply, but also to the presence of catecholamines, which are glycogenolytic (Mellor, 1983).

Although mild metabolic and respiratory acidosis and hyperlactataemia generally develop in calves during birth (Szenci, 1983; Vermorel *et al.*, 1983; Adams *et al.*, 1995), newborn calves that are compromised by poor placental development would have a more severe degree of acidosis and hyperlactataemia. Thus they may take longer to correct the acidosis and remove lactate from their blood after birth.

It follows that the same criteria developed by Mellor (1988) for use with lambs could be applied to calves. Thus, calves with low birth weights, elevated packed cell volumes,

low plasma fructose levels, high plasma lactate concentrations and low rectal temperatures soon after birth are likely to have had a small placenta.

Maternal underfeeding during late pregnancy

Maternal underfeeding during late pregnancy may compromise the wellbeing of the newborn animal by reducing fetal growth. In sheep it was observed that severe underfeeding of ewes for 16 days in the last third of pregnancy, led to a reduction in fetal growth after which refeeding was associated with an immediate increase in fetal growth rate (Mellor & Murray, 1982b). In ewes where severe underfeeding continued for over 21 days, subsequent refeeding did not restore fetal growth rate (Mellor & Murray, 1982b). Thus lambs born to ewes poorly fed throughout late gestation will be lighter at birth and will have reduced energy stores: the first of these may result in a proportionally increased heat loss due to a greater surface area to body weight ratio in low-weight newborns (Alexander, 1979), and the second may lead to inadequate heat production and hypothermia due to early depletion of reduced energy reserves (Mellor & Cockburn, 1986).

Udder development in sheep was also negatively affected by underfeeding in late gestation (Mellor & Murray, 1985). At day 105 of gestation udder development was retarded within 3 days of the onset of underfeeding, which reduced total yield of colostrum during the first 18 hours after birth by decreasing the prenatal accumulation of colostrum and its subsequent secretion rates (Mellor & Murray, 1985).

Thus, the newborn animal may not only find itself compromised by reduced energy stores and increased susceptibility to heat loss but also by reduced availability of colostrum and milk. In addition, underfed ewes may be more likely to abandon their lambs or exhibit poorer maternal care (Mellor, 1988).

Premature birth

Premature birth may be caused by an increase in fetal plasma levels of cortisol due to reduced oxygen and/or nutrient supply (Mellor, 1988) and placental insufficiency therefore often leads to premature birth. In lambs with reduced placental size due to premating excision of 100 uterine caruncles, premature birth occurred (Mellor & Pearson, 1977). Mellor & Pearson (1977) suggested that the chronic fetal hypoxaemia,

which increased the packed cell volume (PCV) by 6%, was able to increase fetal plasma levels of corticosteroids to levels sufficient to cause premature birth.
Maternal undernutrition and stress during late pregnancy may also cause premature birth due to transplacental transfer of maternal cortisol (Mellor, 1988).

Premature lambs may have an increased susceptibility to hypothermia due to immature shivering and non-shivering thermogenic mechanisms or due to the small size of energy reserves available for heat production (Mellor, 1983). Also, these animals are small and have an increased rate of heat loss due to a high surface area to body weight ratio. Immaturity of other organ systems may also adversely affect the survival of the premature newborn animal.

Natal factors predisposing to perinatal debility and death

Intrapartum hypoxaemia

During normal parturition, calves experience a mild degree of hypoxia, which leads to a mild respiratory and metabolic acidosis and elevated plasma lactate concentrations. In healthy calves the respiratory acidosis is corrected soon after birth when effective respiratory and cardio-vascular functions are established (Vermorel *et al.*, 1989; Adams *et al.*, 1995), although it was observed by Szenci (1985) that respiratory acidosis may persist until at least 48 hours after birth. Metabolic acidosis was observed to increase shortly after birth, probably due to diffusion of lactate into the blood (Vermorel *et al.*, 1989), but mild forms were reported to be corrected within a few hours of birth (Szenci, 1985). Intrapartum hypoxaemia, on the other hand, may be experienced during difficult and protracted births or due to umbilical cord occlusion or rupture. The resulting high concentrations of plasma lactate and hydrogen ions and metabolic and respiratory acidosis have been shown to inhibit neonatal heat production sufficiently to become a risk to survival (Vermorel *et al.*, 1983; Vermorel *et al.*, 1989; Adams *et al.*, 1995). Inhibition of heat production due to intrapartum hypoxaemia is likely to last for a shorter time period, about 30 minutes in lambs (Eales & Small, 1985), than the longer lasting effects, several hours, of hypoxaemia due to placental insufficiency (Eales & Small, 1980b).

Power (1989) noted that adequate arterial oxygenation was important for the attainment of full thermogenic capacity by the neonates of a variety of species. This may be due to the fact that brown adipose tissue metabolism requires the presence of oxygen (Anderson & Bates, 1984). Also, high levels of circulating adrenaline secreted during hypoxia may reduce blood flow to thermogenic tissues thus reducing heat production (Alexander, 1970). It may also be possible that metabolic acidosis and hypoxaemia suppress sympathetic nervous system activity and thus reduce heat production by reducing both shivering and non-shivering thermogenesis (Eales & Small, 1980b).

The acidosis may not only reduce the thermoregulatory capability of the newborn animal, but it apparently also has adverse effects on vigour, strength of the sucking reflex and absorption of immunoglobulins (Grove-White, 2000).

Using the criteria developed for investigating lambs (Mellor, 1988), calves with high plasma lactate concentrations as well as low rectal temperatures soon after birth, but a packed cell volume that is not elevated, are likely to have experienced intrapartum hypoxaemia.

Environmental factors predisposing to perinatal debility and death

Cold exposure

a) Routes of heat loss

Heat loss in the newborn calf, as in any other animal, can occur via conduction, convection, radiation and evaporation.

Conductive heat loss, the transfer of heat from the body to objects in contact with it, may occur when the temperature of the calf is higher than the ground it lies on, the water that it may lie in or the air that surrounds it. Moving air or objects with high heat capacity such as concrete floors may lead to high conductive heat loss.

Convection occurs when heat is lost due to the movement of lower temperature air that is in contact with the body surface. Insulating benefits due to air trapped by the coat (wool or hair) of an animal can be reduced substantially by convective air movement (Carstens, 1994).

Radiation is the transfer of heat via infrared heat rays. If skin temperature is higher than the temperature of its surroundings, radiation from the body is higher than radiation to the body and heat loss will occur.

Evaporation is the change from liquid into vapour and is accomplished by input of heat. In the newborn calf evaporation of the amniotic fluid from its coat can cause a substantial loss of heat, which is exaggerated by cold ambient temperatures and wind. Rain further increases heat loss in cold conditions.

Heat retaining mechanisms include insulation by the coat and subcutaneous fat, vasoconstriction, piloerection and postural changes. The insulatory effects of the coat and piloerection will be offset by amniotic fluid in the coat and there is usually little subcutaneous fat in the newborn ruminant (Alexander, 1979). Vasoconstriction reduces heat loss from the extremities, if the necessary mechanisms are sufficiently developed (Anderson & Bates, 1984). This can be offset if the small size of the animal increases heat loss due to a high surface area to body weight ratio (Randall, 1978).

Adverse weather conditions such as wind, rain and cold temperatures will increase heat loss from the animal and will increase the energy requirements for heat production (Vermorel *et al.*, 1983).

Although the calf may be able to use behavioural means such as huddling with its mother, lying in long grass and searching for shelter later on in life, immediately after birth it may not be able to use these behaviours to preserve heat. Thus, heat loss is likely to be high in newborn calves, even in moderate weather conditions, and heat production will have to be increased in order to maintain core body temperature at a constant level. Thompson & Clough (1970) reported that newborn calves held at 20°C reached their summit metabolic rate 15 minutes after birth and maintained that at the same level for 3 hours after which it decreased. This coincided with the period of evaporation of amniotic fluid and drying of the calves' coats (Vermorel *et al.*, 1983).

b) Thermal zones

The relationship between an animal's metabolic heat production and the ambient temperature is used when assessing its thermoregulatory ability (Carstens, 1994). The

thermoneutral zone of an animal is a range in ambient temperatures in which the animal's heat production is minimal and independent of changes in the ambient temperature. The lower border of the thermoneutral zone is called the lower critical temperature and is defined as the ambient temperature below which the animal must increase its metabolic heat production to maintain thermal balance (Carstens, 1994). The lower critical temperature of an animal depends on its ability to withstand heat loss and on its resting metabolic heat production (Carstens, 1994). Therefore, increased insulatory efficiency decreases the lower critical temperature and improves the cold tolerance of the animal. Gonzalez-Jimenez & Blaxter (1962) found that the lower critical temperature of 2-day-old fed Ayrshire calves was 13°C in dry and still-air. In lambs the lower critical temperature immediately after birth was found to range between 37°C and 32°C for light and heavy lambs, respectively (Carstens, 1994). With increasing age the lower critical temperature decreases due to increased efficiency of thermoregulatory mechanisms (Carstens, 1994). When the newborn animal encounters situations where heat loss exceeds summit metabolic rate (maximum rate of heat production), the cold lethal limit is reached, when hypothermia begins (Carstens, 1994).

c) Thermogenesis

Temperature regulation is carried out by neural feedback mechanisms involving the temperature regulating centres in the hypothalamus and thermal receptors in the hypothalamus, skin and spinal cord. The increase in metabolic rate in response to cold exposure can be achieved by shivering and non-shivering thermogenesis (Randall, 1978;Alexander, 1979) and exercise may also play an important role. In the lamb and calf all of these mechanisms are employed to increase heat production when heat loss is high.

Shivering thermogenesis and physical activity

Shivering consist of involuntary, periodic contractions of skeletal muscle. It appears soon after birth in calves held at 10°C and apparently stops when their hair coat is almost dry. Heat production due to shivering increases soon after the onset of shivering and the increment can range from 33% to more than 100% (Vermorel *et al.*, 1983). In lambs shivering thermogenesis is a major contributor to increased heat production and this is also the case in newborn calves (Alexander, 1979; Vermorel *et al.*, 1983). Physical activity may also contribute to heat production. A calf that attempts to or is

standing increases its heat production by 30-100% (Vermorel *et al.*, 1983), but this may in part be due to increased non-shivering heat production caused by the increased convective heat loss from the calf's body while moving and standing.

Energy that is converted into heat is mainly supplied from liver and muscle glycogen stores and lipids before colostrum is consumed (Mellor & Cockburn, 1986). Liver glycogen is the major source of circulating glucose in the unfed newborn. Muscle glycogen is used exclusively for shivering heat production and physical activity and only unmetabolised lactate is released into the blood (Mellor & Cockburn, 1986). After successful sucking, colostral lactose and lipids become the major sources of energy used for heat production (Mellor & Cockburn, 1986).

Non-shivering thermogenesis

The major site of non-shivering thermogenesis is brown adipose tissue (Anderson & Bates, 1984), which is innervated by the sympathetic nervous system (Benito, 1985) and is predominantly found in perirenal adipose tissue depots (Carstens, 1994). Other areas of BAT accumulation include the pericardiac, peritoneal and intramuscular tissue (Carstens, 1994). The ability of BAT to generate heat depends on the rate of substrate oxidation in the mitochondria. BAT adipocytes have a higher concentration of mitochondria and blood supply than white adipocytes (Carstens, 1994) and are thus well adapted for heat production. Activation of cold-sensitive receptors in the hypothalamus and spinal cord leads to a release of noradrenaline in BAT (Benito, 1985). It binds to adrenergic beta-receptors and causes an increase in blood flow to brown adipocytes and an increase in oxygen consumption. Noradrenaline injections in calves increased heat production for 30 min by 73% on average but may lead to an increase of 200% (Vermorel *et al.*, 1983). Other hormones such as thyroid hormones, glucagon and glucocorticoids have also been shown to affect cold thermogenesis in animals (Carstens, 1994). Glucocorticoids may play an indirect role in cold thermogenesis through their effect on glycogen and lipid mobilisation (Carstens, 1994).

Although non-shivering heat production is likely to contribute a large proportion of the overall heat production in newborn calves, BAT is rapidly converted into white adipose tissue during the first month of life (Vermorel *et al.*, 1983).

Factors leading to hypothermia

Weight plays an important role in a newborn's susceptibility to hypothermia. Low weight newborns have a high surface area per unit of body weight and their rate of heat loss is therefore proportionally increased. But also, summit metabolism per unit of surface area is reduced in low weight neonates (Carstens, 1994). Low-weight neonates are therefore less able to maintain thermal balance during cold exposure and are more likely than normal or heavy weight neonates to become hypothermic.

As mentioned above, chronic prenatal hypoxaemia due to placental insufficiency and intrapartum hypoxaemia may lead to impaired heat production in the neonate. Hypoxaemia causes stimulation of the sympathetic nervous system, which increases the release of catecholamines. This leads to fast mobilisation and break-down of body energy stores (Vermorel *et al.*, 1983), especially carbohydrates. Lactate levels increase due to anaerobic metabolism and the result is a metabolic acidosis. This may lead to a suppression of the sympathetic nervous system after birth, which could inhibit shivering thermogenesis. Also, the oxygen deficit probably reduces metabolic rate and cellular metabolic activity may be reduced by acidosis and hyperlactataemia, both of which would adversely affect BAT metabolism and non-shivering thermogenesis (Vermorel *et al.*, 1983). The lower energy stores found in premature lambs (Mellor, 1983; Mellor & Cockburn, 1986) will reduce the period during which the lamb can produce enough heat to prevent hypothermia. This may also be the case in premature calves.

Using the criteria developed for investigating lambs (Mellor, 1988), calves with low rectal temperatures soon after birth, but packed cell volumes and plasma lactate concentrations that are not elevated, are likely to have experienced increased heat loss and/or inadequate heat production after birth.

Starvation

Although starvation is also a major postnatal factor that may predispose the newborn calf to debility and death, it is not discussed here, as the present chapter deals with factors of relevance to the immediate postnatal period. Starvation is not likely to be an issue until several hours after birth as the animal will be able to use body energy stores such as liver glycogen and labile lipids to fuel metabolism and heat production.

Therefore, the issue of starvation is discussed in Chapter 3, which examines calves from 1 to 4 days of age.

2.1.2 Aims of the present experiment

The aims of the present study were as follows:

- To establish basic biochemical and physical profiles of newborn calves to support future research;
- To determine the perinatal factors that predispose newborn calves to debility and death;
- To compare predisposing factors in calves with those established for lambs.

2.2 Materials and Methods

2.2.1 Animals

Cows

The body condition scores of 99 Friesian heifers and 397 Friesian and Jersey mixed-aged cows at Massey University Dairy Farm No.4 were determined during July 2001, two weeks before the peak of the calving season. The heifers had been mated by Angus bulls and their calves were Angus crosses (hereafter referred to as Angus calves), while mixed aged Friesian cows were mated by Friesian bulls thus giving birth to Friesian calves. Eight Jersey cows were mated by Jersey bulls, but only three Jersey cows gave birth during this study and Jersey calves were therefore included in the Friesian group as calves of mixed-aged cows. Of the 496 cows and heifers eight could not be identified and 19 were not scored. The body condition scoring system used a scale from 0 to 10, assessing hip and backbone fat distribution, where 0 indicated very poor condition and 10 an overweight condition (Edmonson *et al.*, 1989).

Cows and heifers that were close to giving birth, as judged by vulvar swelling and udder size, were separated from the rest of the herd into a calving mob. Cows in the calving mob were observed in the field for 24 hours a day by two observers during each of six 4-hour shifts for three weeks during the peak of the calving season.

The peak of the calving season was the period during which the highest calving rate occurred and was the period from the 6th to 27th August 2001.

Calves

At calving the dam's livestock number, gender of the calf, time of birth and weather conditions on a four-point scale (1= still/dry, 2= windy/dry, 3= still/wet, 4= windy/wet) were recorded. Air temperature at the time of birth was noted retrospectively from temperatures recorded nearby at AgResearch, Palmerston North. Newborn calves were blood sampled by jugular venepuncture (Photograph a) using sterile 0.9x25mm [20Gl"] needles (Becton Dickinson vacutainers systems, Belliver Industrial Estate, Plymouth, UK; ref no. 360214, lot 00B06, expiry 2005-02), mounted on a vacutainer system using sodium-heparin vacutainers (10ml, Becton Dickinson, Franklin Lakes, NJ, USA; Ref

no. 366480, Lot 0263678, Expiry 2002-09) at a mean of 13 min (± 8.33 min standard deviation) after birth and the blood samples were put on ice immediately. The rectal temperature was measured before, at the same time or immediately after the blood sample was taken at a mean time of 12 min (± 8.2 min standard deviation) after birth, using a digital thermometer (Photograph b) and the calves were ear-tagged (Photograph c). The time taken to stand on all four feet for at least 30 seconds was recorded and after standing, but before sucking, the calf was weighed (Photograph d) using a Tru-Test AG500 weighing indicator with an 80kg suspension loadcell (Tru-Test Ltd, Auckland, NZ) and a sling suspended from a metal bar, into which the calf was placed. After weighing, the calf's navel was disinfected using iodine spray.

Blood samples were centrifuged at 3000rpm for 15 min in a Labofuge 200 (Heraeus Sepatech, Biolab Scientific, NZ). A capillary tube (Chase Instruments, plain capillary tubes cat. No. 2502, Biolab Scientific, NZ) of mixed whole blood was removed from the sample before spinning and was used to determine packed cell volume (PCV) by the capillary tube centrifugation method using a micro-capillary centrifuge (Model MB, International Equipment Company, Needham HTS, Mass.USA) and associated reader (Patent 2750671, International Equipment Company, Needham HTS, Mass.USA). The plasma of the centrifuged samples was pipetted, using disposable plastic pipettes (Samco ® transfer pipettes; cat No. 225, purchase ID. *+H56822533*; Samco Scientific Corp., San Fernando, Ca., USA), into two 1.5ml Eppendorff tubes (RayLab NZ Ltd., Auckland, NZ; code P4010-03, item code: 10301013, Batch 0 28 88). About 0.5ml of plasma was put into each tube. The remainder of the plasma was placed into a 5 ml screw top vial (Sarstedt, SA, Australia; No. 60.9921.532, CH-B/lot No.00976, Exp 11-2003, sterile). The samples were stored on ice in an upright position until the end of each shift (maximum of 4 hours). They were then placed into a deep freeze at -20°C overnight and were then transferred into another deep freeze at -50°C, where they were stored until required for analysis.

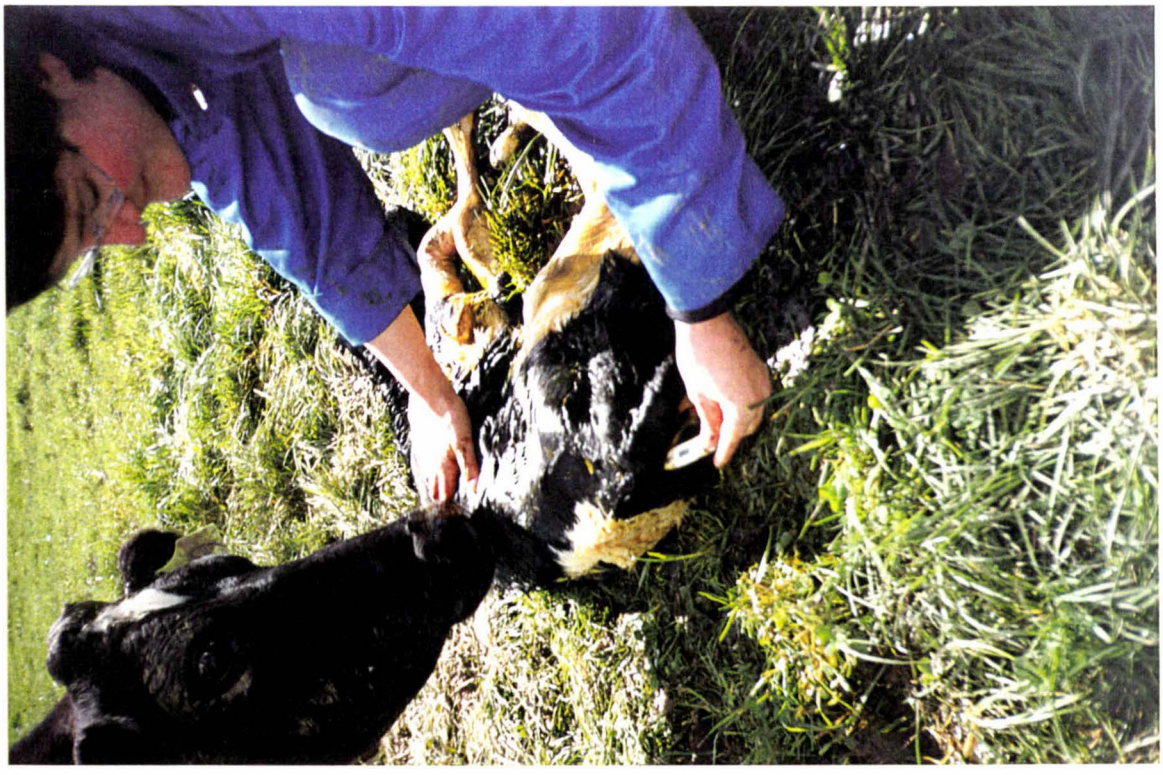
Sodium-heparin vacutainers do not contain enzyme inhibitors that prevent a rise in lactate concentrations in blood samples due to glycolysis, whereas fluoride-oxalate vacutainers do. These were not used due to cost. In order to account for possible differences between blood taken with sodium-heparin tubes and that taken in fluoride-oxalate tubes, additional blood samples were taken in 30 animals after the initial birth

sample was taken. As high lactate levels can be found in animals with high PCV due to placental insufficiency (Mellor, 1988), the 60 blood samples were taken from 30 calves that had low, medium or high PCVs in the initial birth sample, in order to cover a range of lactate levels.

Two samples were taken from the same animal, one into a sodium-heparin vacutainer (10ml) and the other into a fluoride-oxalate vacutainer (5 ml, Becton Dickinson, BD vacutainer cat. FX367713, expiry date March 2002, Biolab Scientific, NZ). These were centrifuged at the same time after taking the sample, and at the same time as the initial birth sample to ensure consistency. Therefore, the blood samples taken from a calf whose initial blood sample was centrifuged 5 minutes after taking the sample were also centrifuged 5 minutes after taking that sample.

When cows had difficulty during the birth process, farm staff were informed after labour did not apparently progress for over one hour or if the cow was in obvious distress. Some cows were assisted in the field while others had to be taken to yards so that farm staff could pull the calf out. Assisted calves had all the necessary samples taken but, in most cases, the time taken to stand was not recorded.

Photograph 1a and b: Taking a jugular venous blood sample and rectal temperature from a newborn calf.



Photograph 1c and d: Ear tagging and weighing a newborn calf.

2.2.2 Plasma analyses

Blood samples were analysed for plasma lactate, fructose and glucose concentrations using a Cobas Fara 2 analyser after being well mixed following thawing. For the lactate analysis a Roche/Hitachi kit No. 1822837 was used (Noll, 1974). Glucose and fructose were determined by the UV method using the Boehringer Mannheim kit no.139106 (Kunst *et al.*, 1984). The plasma of the samples taken in addition to the birth samples (30 in sodium-heparin vacutainers, 30 in fluoride-oxalate vacutainers) were analysed for lactate only, using the method noted above.

2.2.3 Statistics

To determine if lactate levels rise significantly in sodium-heparin tubes compared to fluoride-oxalate tubes, a correlation analysis and linear regression were undertaken. The equation of the slope of the regression line was determined to assess whether lactate values in heparin tubes had to be adjusted to take into account increased lactate levels due to enzyme activity.

The overall data collected were separated into two groups according to breed (Friesian or Angus) and a correlation analysis was undertaken for all birth variables, giving the Pearson correlation coefficients and significance levels (p-values). A non-parametric one-way ANOVA was done for the two groups, as some of the data were not normally distributed. Care was taken to ensure that the ranked data were homogenous by assessing the residual versus predicted value plots.

The analysis included comparing calves that were assisted with those not assisted; whether ambient temperatures were below 10°C or above 10°C at birth; male and female calves; calves born to a mother with high (5.0-5.8), medium (4.1-4.9) or low (3.1-4.0) body condition score (BCS); calves born during different times of day (2-6 am, 6-10am, 10-2pm, 2-6pm, 6-10pm, 10-2am); and calves born during different weather conditions [1(still/dry), 2 (windy/dry), 3(still/wet), 4 (windy/wet)]. A non-parametric one-way ANOVA was also undertaken to compare calves with and without meconium staining at birth, those that had mothers with or without milk fever, and animals that became sick (14 Friesian calves included in analysis) or died (8 Friesian calves included in analysis)

between birth and 1.5 months of age with those that remained healthy. Healthy Friesian calves only included those not sold between birth and 1.5 months of age (in total 53 calves). The comparison between groups of different health status was only undertaken with Friesian calves due to the small number of Angus calves in the three groups.

For groups with only two components (eg: assisted vs non-assisted) the Wilcoxon Two-Sample test was used and provided two-way p-values. Here, comparing the means of the two groups allowed identification of the components that were higher or lower. In the groups with more than two components (eg: body condition score), the Kruskal-Wallis chi-squared test was used. Post-hoc tests involved ranking of the variables in question and using ranked variables to perform a one-way ANOVA and mean separation tests, in this case, the Tukey test. Simple statistics were calculated for creating graphs with means and standard errors.

A covariate analysis (ANCOVA) was used to determine if correlations between certain variables were affected by other parameters. A chi-squared test was undertaken to determine significant differences between groups of animals.

Multivariate analysis was also undertaken on the birth data. A Canonical Discriminant Analysis (CDA) was done for the separate grouping 'assisted versus non-assisted calves', 'weather groupings 1-4', 'body condition score groupings 1-3', 'air temperature below and above 10°C', 'time of birth 1-6', 'meconium staining', 'milk fever' and 'health status' of calves of both breed groups (if possible).

2.3 Results

During the three weeks of observations, 234 calves were born. Of those, eight calves were twins and seven calves died before, during or shortly after birth. Four of the seven dead calves were assisted Angus calves. An additional 10 calves died between 12 hours to 1.5 months of age. Thus there was an overall mortality rate of 7.3% (17/234). Twins have not been included in the statistical analysis in this chapter.

Of the single calves that did not die around birth, 169 were Friesian calves and 50 Angus calves. A significantly higher proportion of Angus than Friesian calves was assisted (chi-square p = <0.0001). A significantly higher proportion of males than females was assisted (chi-square p = 0.05). Further results are presented in Table 2.3.1.

Table 2.3.1: Summary of calf data.

	Friesian (n=169)	**Angus (n=50)**
Males (53.4% of all calves)	50.3% (85/169)	64% (32/50)
Females (46.6% of all calves)	49.7% (84/169)	36% (18/50)
Assisted calves (16% of all calves)	8.9% (15/169)	40% (20/50)
Assisted males (60% of assisted calves)	40% (6/15)	75% (15/20)
Assisted females (40% of assisted calves)	60% (9/15)	25% (5/20)
Sick calves (birth to 1.5 months of age (7.8% of all calves))	8.3% (14/169)	6% (3/50)
Dead calves (12 hours to 1.5 months of age (4.6% of all calves))	5.3% (9/169)	2% (1/50)
Calves born with meconium staining (7.8% of all calves)	8.9% (15/169)	4% (2/50)
Calves born to mothers that had milk fever	3.2% (7/169)	0% (0/50)

2.3.1 Lactate correction

There was a significant positive correlation ($r=0.986$; $p < 0.0001$) between the lactate concentrations in the sodium-heparin and the fluoride-oxalate vacutainers (Figure 2.3.1). The slope of the equation for the straight line was close to one, revealing a difference of about 7% between the groups. Therefore, it was decided not to correct lactate data, as the correction would not be biologically significant in relation to the full range of lactate concentrations observed.

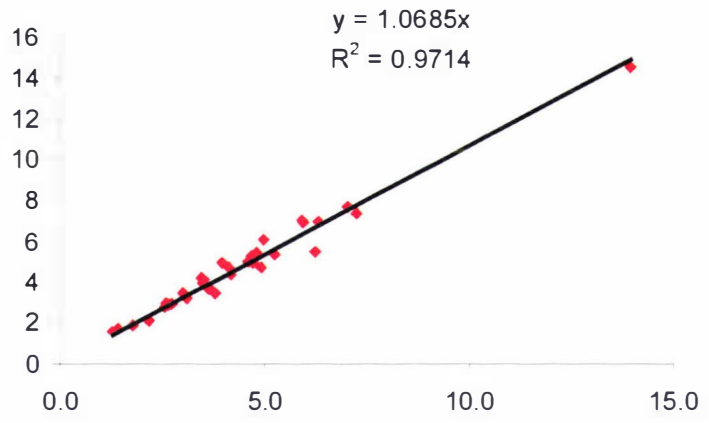


Figure 2.3.1: Correlation of lactate levels between sodium-heparin tubes (Y) and fluoride-oxalate tubes (X).

2.3.2 Calf birth variables

Table 2.3.7 (page 79) shows a summary of the results of the non-parametric one-way ANOVA for the different groups. There were no significant differences in any of the birth parameters in Friesian calves born to mothers that had milk fever and those that did not. Also, only two Angus calves had meconium staining and an ANOVA was not done for these animals. As only six Angus calves were not sold before 1.5 months of age, no ANOVA was undertaken to determine the differences between healthy, sick and dying calves in this breed. Any ANOVA results that did not show significant differences between groups have not been included in the results below, but all of those have been considered in the summary in Table 2.3.7 (page 79).

2.3.2.1 Time to stand

The mean time to stand in Friesian calves was 55 ± 5 min (range 3 to 480 min), while the mean time to stand of Angus calves was 59 ± 19 min (range 5 to 480 min). There was no significant difference between the two breeds in time to stand (Table 2.3.6; page 63). This is apparently supported by Figure 2.3.2, which shows that the percentages of calves over the range of times to stand were distributed quite evenly.

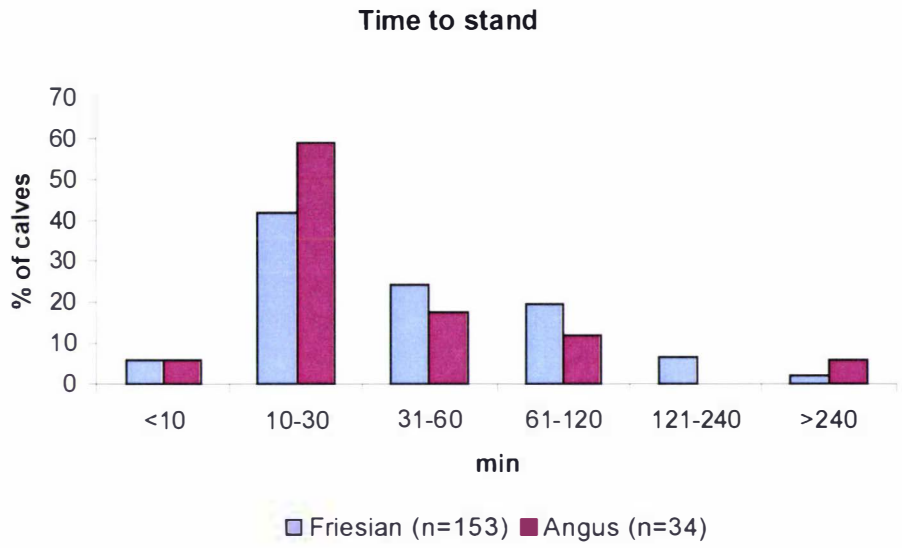


Figure 2.3.2: Percentage of Friesian (blue) and Angus (red) calves in each time to stand bracket (Friesian n=153, Angus n=34).

The time to stand of Friesian calves showed significant correlations with weather (positive, the worse the weather the longer the time taken to stand), rectal temperature (negative), air temperature (negative), calf lactate levels (positive) and calf glucose levels (positive) (Table 2.3.2; page 59). In Angus calves time to stand showed a significant and positive correlation with calf lactate levels only (Table 2.3.4; page 61). There were a large number of missing values in this category, due to many calves being assisted in the yards (see above).

Assisted versus unassisted calves

There was a significant difference between the assisted and unassisted Friesian calves in the time taken to stand (Table 2.3.3; page 60). The post-hoc test showed that assisted

calves took longer to stand (96 ± 24.6min) than unassisted calves (53 ± 5.0 min) (Figure 2.3.3). In Angus calves, there were no significant differences between assisted and unassisted calves in time to stand. This may be due to the large standard error that was observed for assisted Angus calves (Figure 2.3.3).

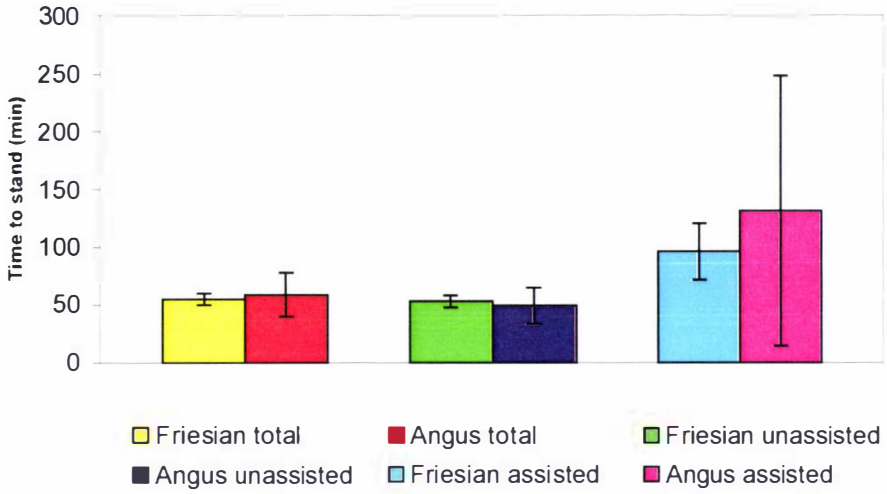


Figure 2.3.3: Mean time to stand (± standard error) of Friesian and Angus calves.

Air temperature at birth

There were significant differences in the time to stand between the two temperature groups (below 10 °C and above 10 °C) (Table 2.3.3; page 60). The post-hoc tests showed that time to stand was longer in Friesian calves born during cold temperatures (below 10 °C, 66 ± 8.4min; above 10°C, 46 ± 5.5min). There were no significant differences between the two temperature groups in time to stand for Angus calves.

Weather

There were significant differences in the time to stand for Friesian calves between the four weather categories (Table 2.3.3; page 60). Post-hoc tests showed that calves born into weather categories 1 (still/dry) (40 ± 3.2min) and 2 (windy/dry) (59 ± 15.8 min) stood significantly earlier than calves born into weather category 4 (windy/wet) (105 ± 18.1 min). There were no significant differences in the time to stand for Angus calves of the four weather categories.

Time of day at birth

There were significant differences in the time to stand for Friesian calves between some of the time of birth categories (Table 2.3.3; page 60). The post-hoc tests showed calves born during 2-6am took significantly longer to stand (77 ± 12.2 min) than calves born during 2-6pm (35 ± 5.4 min). Angus calves of the six time of birth categories did not show any significant differences in time to stand.

2.3.2.2 Rectal temperature

The mean rectal temperature of Friesian calves was 38.8 ± 0.05 °C (range 35.3 to 39.9°C). The mean rectal temperature for Angus calves was 38.9 ± 0.11°C (range 36.8 to 40°C). There were no significant differences in rectal temperature between breeds (Table 2.3.6; page 63), which is apparently also supported by Figure 2.3.4 below.

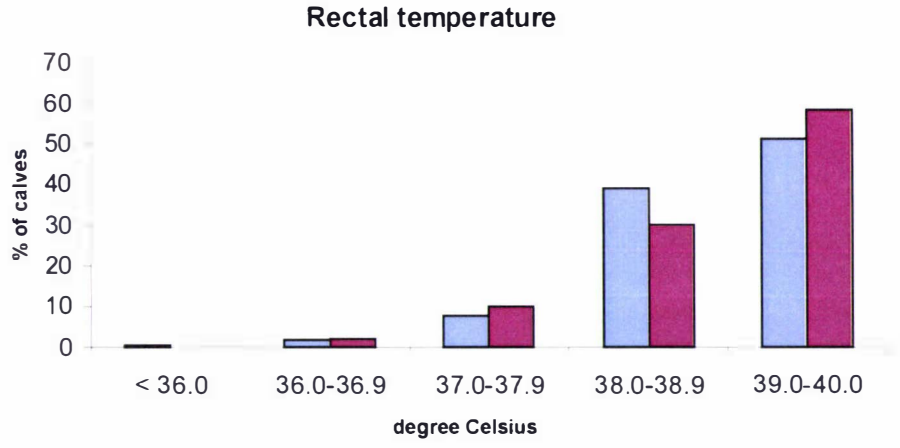


Figure 2.3.4: Percentage of Friesian (blue) and Angus (red) calves in each rectal temperature bracket (Friesian n=167, Angus n=50).

The rectal temperature of Friesian calves showed significant correlations with weather (negative, in bad weather conditions rectal temperatures were low), time to stand (negative), air temperature (positive), calf lactate levels (negative) and calf glucose levels (negative) (Table 2.3.2; page 59). In Angus calves, rectal temperature also showed a significant positive correlation with air temperature (Table 2.3.4; page 61).

Assisted versus unassisted calves

There were no significant differences in rectal temperature between assisted and unassisted Friesian and Angus calves.

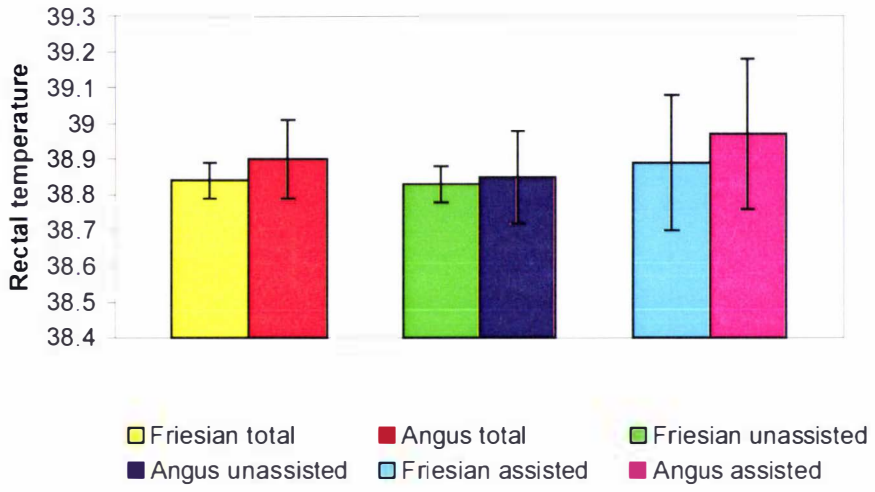


Figure 2.3.5: Mean rectal temperature (± standard error) of Friesian and Angus calves.

Meconium staining versus no staining

There were significant differences in rectal temperature of Friesian calves between the two groups (Table 2.3.3; page 60). However, the post-hoc tests showed that calves with meconium staining had significantly higher rectal temperatures (39.3 ± 0.08°C) than did those without staining (38.8 ± 0.06°C).

Gender

There were no significant differences between rectal temperatures of Friesian male and female calves, but Angus males had significantly lower rectal temperatures (38.7 ± 0.15°C) than Angus females (39.2 ± 0.14°C) (Table 2.3.5; page 62).

Air temperature at birth

There were significant differences between the two temperature groups (below 10 °C and above 10 °C) in the rectal temperature of Friesian and Angus calves (Table 2.3.3 and 2.3.5; pages 60 and 62). The post-hoc tests showed that rectal temperature was lower in Friesian calves born during colder temperatures (below 10 °C, 38.6 ± 0.09°C; above 10°C, 39 ± 0.05°C). The post-hoc tests showed that Angus calves born into colder

temperatures also had lower rectal temperatures (below 10 °C, 38.5 ± 0.18°C; above 10°C, 39.2 ± 0.13°C).

Time of day at birth

There were significant differences in rectal temperature of Friesian calves between some of the time of birth categories (Table 2.3.3; page 60). The post-hoc tests showed that calves born during 2-6am (38.6 ± 0.15°C) and 10pm-2am (38.5 ± 0.19°C) had lower rectal temperatures than did calves born during 2-6pm (39.2 ± 0.07°C). Angus calves in the six time of birth categories did not show any significant differences in rectal temperature.

Table 2.3.2: Correlations between different variables at birth in Friesian calves. (Correlation coefficient (x), p-value (y), number of animals (z)).

Parameters	Gender	Weather	BCS	Rectal temperature	Weight	PCV	Time to stand	Air temperature	Lactate	Glucose	Fructose
BCS	0.074x 0.354y 158z	-0.089 0.266 158	1								
Rectal temperature	0.043 0.581 167	-0.213 0.006 167	0.127 0.115 156	1							
Weight	0.269 0.0006 160	0.020 0.802 160	0.021 0.804 149	0.054 0.501 158	1						
PCV	-0.091 0.248 162	-0.001 0.988 162	0.139 0.090 151	0.035 0.659 160	0.138 0.088 153	1					
Time to stand	-0.007 0.936 153	0.337 <0.0001 153	0 1 142	-0.592 <0.0001 153	-0.063 0.444 148	-0.005 0.952 146	1				
Air temperature	0.086 0.264 169	0.027 0.727 169	0.029 0.717 158	0.288 0.0002 167	0.053 0.507 160	-0.093 0.239 162	-0.182 0.025 153	1			
Lactate	0.023 0.764 169	0.132 0.087 169	0.068 0.398 158	-0.182 0.019 167	-0.002 0.982 160	-0 011 0.891 162	0.2489 0.002 153	-0.035 0.65 169	1		
Glucose	-0.031 0.685 169	0.129 0.096 169	-0.173 0.030 158	-0.293 0.0001 167	0.116 0.143 160	-0.050 0.531 162	0.371 <0.0001 153	-0.247 0.001 169	0.229 0.003 169	1	
Fructose	0.079 0.309 169	0.093 0.230 169	0.054 0.497 158	-0.052 0.504 167	0.102 0.200 160	-0.033 0.681 162	0.016 0.848 153	-0.118 0.126 169	0.043 0.577 169	-0.029 0.710 169	1

Table 2.3.3: Results of non-parametric one-way ANOVA for Friesian calves (p-values).

Group	BCS	Weight	Rectal temperature	PCV	Time to stand	Lactate	Glucose	Fructose
Assisted versus unassisted	0.941	0.142	0.604	0.034	0.033	0.006	0.421	0.224
Milk fever versus no milk fever	0.805	0.481	0.933	0.707	0.747	0.419	0.093	0.377
Healthy, sick and dead calves	0.7934	0.026	0.459	0.342	0.175	0.032	0.178	0.372
Meconium staining versus no staining	0.201	0.352	0.0008	0.674	0.630	0.154	0.900	0.294
BCS comparison		0.871	0.444	0.156	0.392	0.903	0.032	0.400
Air temperature comparison	0.821	0.695	0.002	0.094	0.005	0.479	0.0002	0.184
Male-female comparison	0.4851	0.0003	0.835	0.163	0.704	0.871	0.467	0.292
Weather comparison	0.187	0.780	0.079	0.056	0.003	0.635	0.020	0.421
Time of birth comparison	0.329	0.305	0.008	0.037	0.045	0.004	0.127	0.673

Table 2.3.4: Correlations between different variables at birth in Angus calves (correlation coefficient (x), p-value (y), number (z)).

Parameters	Gender	Weather	BCS	Rectal temperature	Weight	PCV	Time to stand	Air temperature	Lactate	Glucose	Fructose
BCS	0.169x 0.250y 48z	-0.192 0.192 48	1								
Rectal temperature	-0.301 0.034 50	-0.134 0.352 50	-0.048 0.748 48	1							
Weight	0.245 0.101 46	0.048 0.750 46	-0.193 0.203 45	0.101 0.503 46	1						
PCV	-0.162 0.271 48	0.034 0.817 48	0.055 0.715 46	0.198 0.177 48	0.090 0.563 44	1					
Time to stand	-0.073 0.680 34	0.125 0.481 34	-0.234 0.191 33	-0.353 0.406 34	0.047 0.793 34	0.108 0.557 32	1				
Air temperature	-0.051 0.727 50	0.083 0.568 50	0.050 0.736 48	0.283 0.047 50	0.231 0.123 46	-0.090 0.542 48	-0.138 0.438 34	1			
Lactate	0.0081 0.951 50	-0.184 0.201 50	0.128 0.3845 48	0.166 0.250 50	-0.120 0.428 46	-0.066 0.657 48	0.525 0.001 34	-0.023 0.875 50	1		
Glucose	0.231 0.107 50	-0.169 0.240 50	0.297 0.040 48	-0.190 0.186 50	-0.156 0.302 46	-0.107 0.471 48	-0.088 0.623 34	-0.285 0.045 50	0.372 0.008 50	1	
Fructose	0.084 0.561 50	-0.176 0.223 50	0.030 0.841 48	-0.035 0.811 50	-0.051 0.737 46	-0.059 0.688 48	0.134 0.449 34	0.192 0.181 50	0.008 0.959 50	-0.069 0.636 50	1

Table 2.3.5: Results of non-parametric one-way ANOVA for Angus calves (p-values).

Group	BCS	Weight	Rectal temperature	PCV	Time to stand	Lactate	Glucose	Fructose
Assisted versus unassisted	0.079	0.05	0.285	0.324	0.503	0.018	0.294	0.122
BCS comparison		0.982	0.967	0.884	0.206	0.380	0.051	0.537
Air temperature comparison	0.726	0.100	0.002	0.582	0.931	0.430	0.108	0.478
Male-female comparison	0.148	0.05	0.037	0.381	0.594	0.856	0.113	0.461
Weather comparison	0.654	0.946	0.083	0.864	0.820	0.758	0.129	0.389
Time of birth comparison	0.258	0.601	0.06	0.074	0.111	0.742	0.913	0.043

Table 2.3.6: Results of non-parametric one-way ANOVA for breed comparison (Friesian versus Angus calves) (p-values).

Parameter	BCS	Rectal temperature	Weight	PCV	Time to stand	Glucose	Lactate	Fructose
Breed	<0.0001	0.380	0.169	0.124	0.124	0.104	<0.0001	0.015

2.3.2.3 *Plasma glucose concentrations*

The mean plasma glucose concentration of Friesian calves was 5.59 ± 0.17 mmol/L (range 1.8 to 12.8 mmol/L), while that of Angus calves was 6.12 ± 0.31 mmol/L (range 2.4 to 9.0 mmol/L). There were no significant differences in calf glucose concentrations between calves of the two breeds (Table 2.3.6; page 63), which is apparently supported by Figure 2.3.6 below.

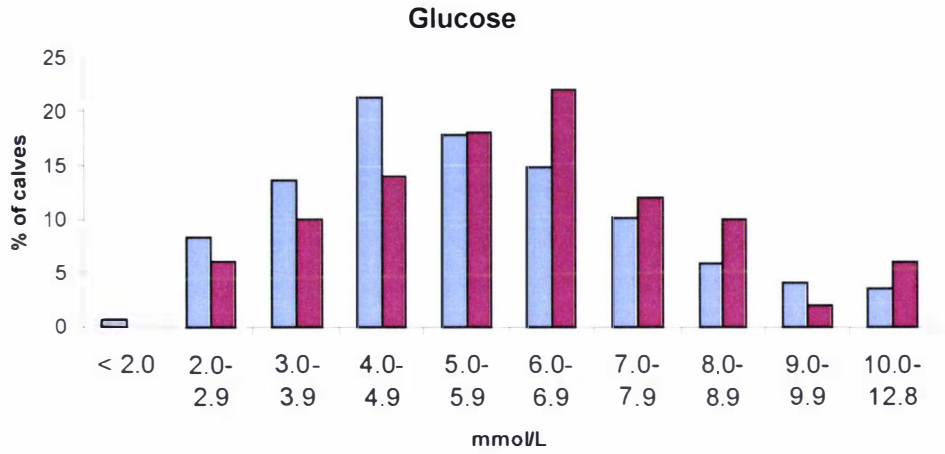


Figure 2.3.6: Percentage of Friesian (blue) and Angus (red) calves in each plasma glucose concentration bracket (Friesian n=169, Angus n=50).

Glucose concentrations showed significant correlations with body condition score of cows (BCS) (negative), rectal temperature (negative), time to stand (positive), air temperature (negative) and calf lactate levels (positive) in Friesian calves (Table 2.3.2; page 59). In Angus calves glucose levels showed significant correlations with BCS (positive), air temperature (negative) and calf lactate levels (positive) (Table 2.3.4; page 61).

Assisted versus unassisted calves

There were no significant differences in plasma glucose concentrations between assisted and unassisted Friesian and Angus calves (Figure 2.3.7).

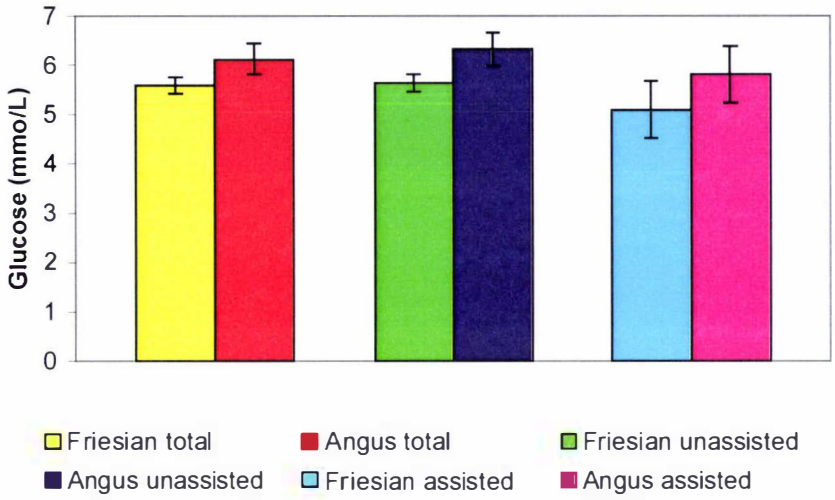


Figure 2.3.7: Mean glucose levels (±standard error) of Friesian and Angus calves.

Maternal body condition score

There were significant differences in plasma glucose concentrations of Friesian, but not of Angus, calves between the three ranges of BCS (Table 2.3.3; page 60). The post-hoc test showed that calves born to mothers with the low BCS had higher plasma glucose concentrations (6.63 ± 0.53mmol/L) than calves born to mother with the high BCS (4.80 ± 0.40 mmol/L).

Air temperature at birth

There were significant differences between the two temperature groups (below 10 °C and above 10 °C) in plasma glucose concentrations of Friesian (Table 2.3.3; page 60), but not Angus calves. The post-hoc tests showed that plasma glucose concentrations were higher in calves born into low air temperatures than those born into high air temperatures (below 10 °C, 6.25 ± 0.25mmo/L; above 10°C, 5.04 ± 0.20 mmol/L).

Weather conditions

There were significant differences in plasma glucose concentrations of Friesian (Table 2.3.3; page 60), but not Angus calves between the four weather categories. Post-hoc tests showed that calves born into category 2 (windy/dry) had significantly lower glucose levels (5.03 ± 0.34 mmol/L) than those born into category 4 (windy/wet) (6.88 ± 0.52mmol/L).

2.3.2.4 Plasma lactate concentrations

The mean plasma lactate concentration of Friesian calves was 4.84 ± 0.26 mmol/L (range 1.3 to 23.9 mmol/L) and of Angus calves 7.52 ± 0.54 mmol/L (range 2.8 to 18.0mmol/L). There were significant differences in lactate levels between the two breeds. The post-hoc test showed that Friesian calves had significantly lower lactate levels than Angus calves (Table 2.3.6; page 63). A higher percentage of Friesian calves had lactate levels of 3.9mmol/L or less, while a higher percentage of Angus calves had lactate levels of 10mmol/L or more (Figure 2.3.8).

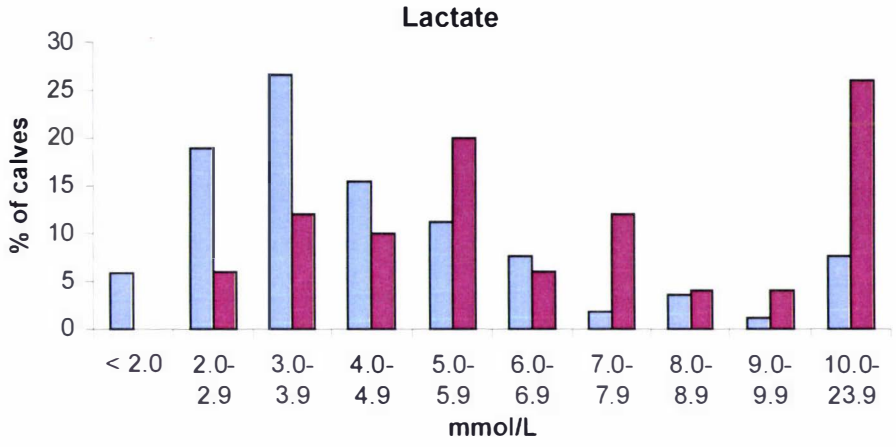


Figure 2.3.8: Percentage of Friesian (blue) and Angus (red) calves in each plasma lactate concentration bracket (Friesian n=169, Angus n=50).

Lactate levels of Friesian calves showed significant correlations with rectal temperature (negative), time to stand (positive) and calf plasma glucose levels (positive) (Table 2.3.2; page 59). Lactate levels of Angus calves showed significant correlations with time to stand (positive) and calf plasma glucose levels (positive) (Table 2.3.4; page 61).

Assisted versus unassisted calves

Figure 2.3.9 shows that lactate levels were higher in Angus than in Friesian calves. There were significant differences in plasma lactate concentrations between assisted and unassisted Friesian and Angus calves (Table 2.3.3 and 2.3.5; pages 60 and 62). The post-hoc tests showed that assisted Friesian calves had significantly higher lactate levels (6.31± 0.76mmol/L) than unassisted Friesian calves (4.7 ± 0.27mmol/L). Assisted

Angus calves also had significantly higher lactate levels (9.05 ± 0.87 mmol/L) than unassisted Angus calves (6.49 ± 0.62mmol/L) (Figure 2.3.9).

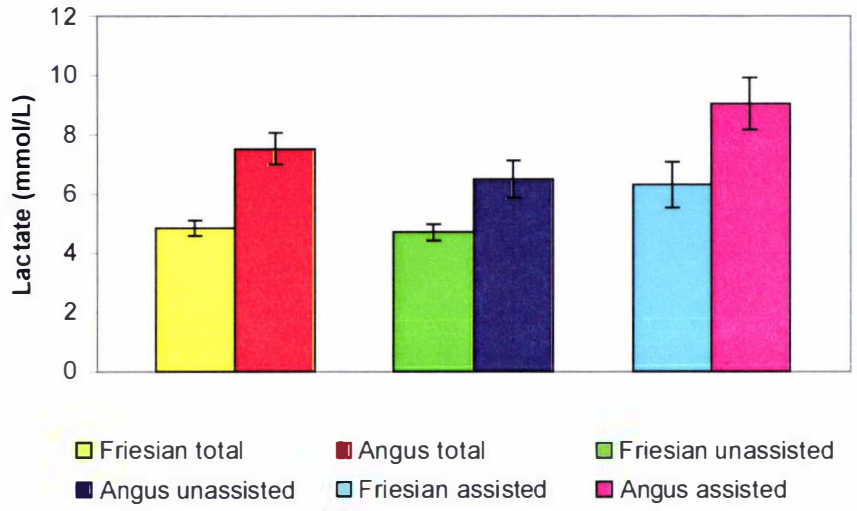


Figure 2.3.9: Mean lactate levels (± standard error) of Friesian and Angus calves.

Time of day at birth

There were significant differences in plasma lactate concentrations of Friesian (Table 2.3.3; page 60), but not Angus calves, between the time of birth categories. Friesian calves born during 2-6am (6.03±0.82mmol/L) and 2-6pm (5.27±0.43mmol/l) had significantly higher lactate levels than those calves born during 6-10pm (4.10±0.68 mmol/L).

Healthy, sick and dying calves

There was a significant difference in plasma lactate concentrations of Friesian calves at birth, between calves that remained healthy and those that died between 12 hours and 1.5 months of age (Table 2.3.3, page 60). Friesian calves that remained healthy had lower birth plasma lactate concentrations (4.74±0.54mmol/L) than calves that died (8.16±2.40mmol/L). However, this difference might have been influenced by two calves in the group of dying calves that had high plasma lactate levels (calf 207= 23.9 mmol/L, calf 284= 11.1mmol/L).

2.3.2.5 Birth Weight

Mean birth weight was 37.4 ± 0.3 kg in Friesians (range 28.1 to 47.2 kg) and 36.42 ± 0.58 kg in Angus calves (range 27.8 to 45.1 kg). There were no significant differences in weight between the calves of the two breeds (Table 2.3.6; page 63) (Figure 2.3.10).

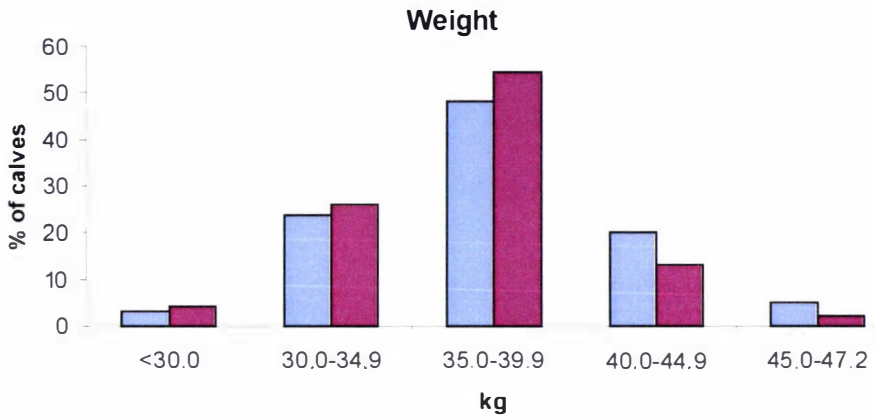


Figure 2.3.10: Percentage of Friesian (blue) and Angus (red) calves in each weight bracket (Friesian n=160, Angus n= 46).

Weight was significantly and positively correlated with gender in Friesian calves but no correlations with weight were found in Angus calves (Table 2.3.2 and 2.3.4; pages 59 and 61).

Assisted versus unassisted calves

Figure 2.3.11 shows that assisted calves of both breeds generally had higher birth weights than unassisted calves. There were significant differences in birth weight between assisted and unassisted in Angus calves (Table 2.3.5; page 62), but not in Friesian calves. The post-hoc tests showed that assisted Angus calves were significantly heavier (38.1 ± 1.04 kg) than unassisted Angus calves (35.5 ± 0.66 kg).

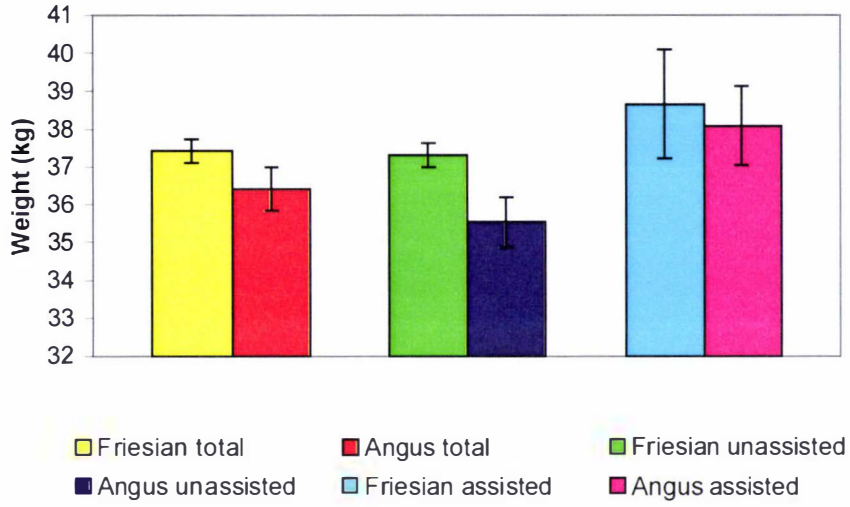


Figure 2.3.11: Mean weight (± standard error) of Friesian and Angus calves.

Gender

There were significant differences between Friesian and Angus males and females in birth weight (Table 2.3.3 and 2.3.5; pages 60 and 62). Friesian males were heavier (38.5 ± 0.4kg) than Friesian females (36.4 ± 0.4 kg). The same was found for Angus calves, where males were heavier (37.1 ± 0.7kg) than females (35.1 ± 0.9kg).

Healthy, sick and dying calves

There was a significant difference in birth weight between Friesian calves that remained healthy and those that died between 12 hours and 1.5 months of age. Calves that remained healthy up to 1.5 months of age were significantly lighter at birth (36.0 ± 0.5kg) than calves that died (39.8 ± 1.66kg).

2.3.2.6 Packed Cell Volume

The mean packed cell volume (PCV) was 41.3 ± 0.5 % (range 25 to 56 %) in Friesian calves and 39.9 ± 1.1 % (range 22 to 58 %) for Angus calves. There were no significant differences in packed cell volume between breeds (Table 2.3.6; page 63). PCV was not very different between the two groups (Figure 2.3.12). The exceptions were PCVs between 30 to 40%. More Angus calves seemed to have had PCVs between 30-34%, while more Friesian calves had PCVs between 35 and 39%.

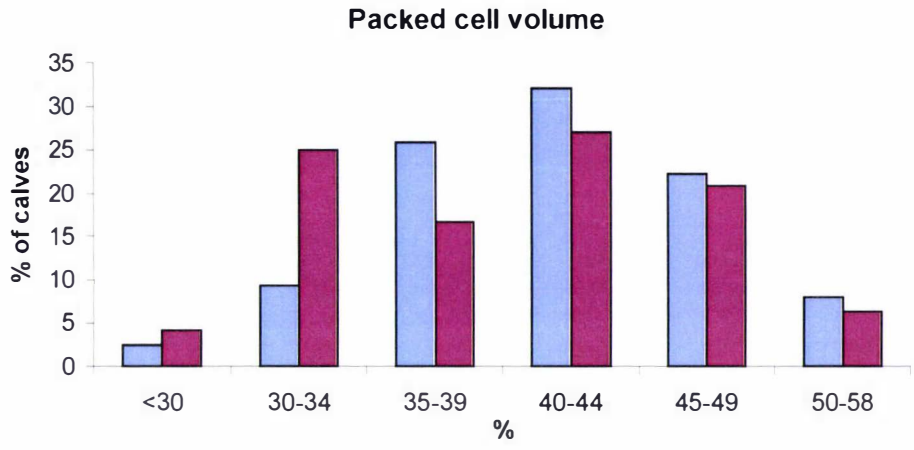


Figure 2.3.12: Percentage of Friesian (blue) and Angus (red) calves in each PCV bracket (Friesian n=162, Angus n=48).

Packed cell volume did not show any significant correlations with other birth variables in calves of both breeds (Table 2.3.2 and 2.3.4; pages 59 and 61).

Assisted versus unassisted calves

Packed cell volume seemed to be lower in assisted calves than unassisted calves. This is supported by the significant differences in PCV between assisted and unassisted Friesian (Table 2.3.3; page 60), but not Angus calves. The post-hoc tests showed that assisted Friesian calves had significantly lower PCV (38.5 ± 1.2%) than unassisted Friesian calves (41.6 ± 0.49%) (Figure 2.3.13).

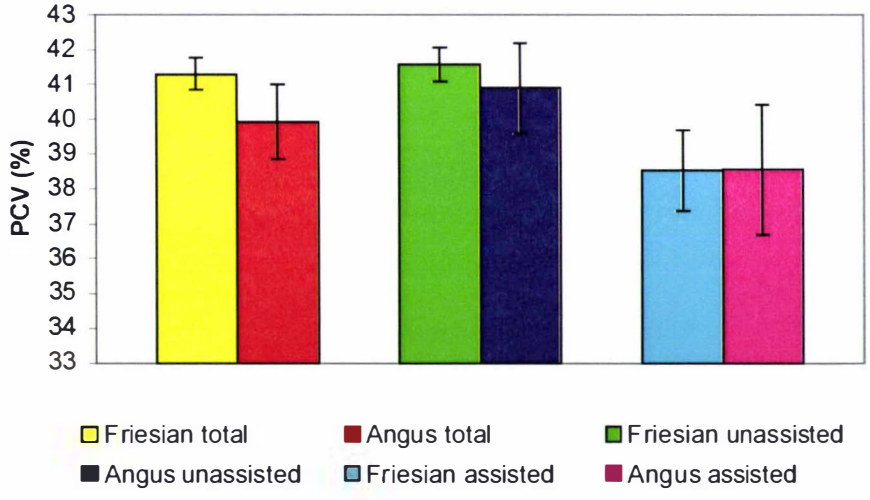


Figure 2.3.13: Mean packed cell volume (± standard error) of Friesian and Angus calves.

Time of day at birth

There were significant differences in PCV of Friesian (Table 2.3.3; page 60), but not Angus calves, between the time of birth categories. Friesian calves born during 6-10pm had significantly lower PCV (38.7 ± 1.2 %) than calves born during time 6-10am (43.8 ± 0.8%).

2.3.2.7 Plasma fructose concentration

Mean plasma fructose concentration for Friesian calves was 4.10 ± 0.11 mmol/L (range 1.2 to 7.8 mmol/L), while mean plasma fructose concentration for Angus calves was 4.73 ± 0.22 mmol/L (range 1.4 to 9.0 mmol/L). There were significant differences in plasma fructose levels between the two breeds (Table 2.3.6; page 63). Plasma fructose concentrations were significantly higher in Angus than in Friesian calves. This seemed to be mainly due to fructose levels between 4.0-4.9 mmol/L and fructose levels above 6.0 mmol/L (Figure 2.3.14). A higher percentage of Angus calves seemed to have fructose levels above 4.0mmol/L compared with Friesian calves (Figure 2.3.14).

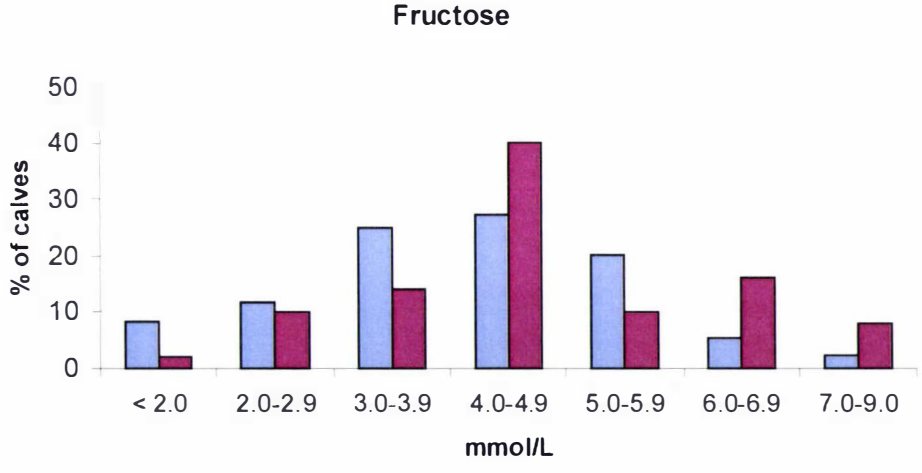


Figure 2.3.14: Percentage of Friesian (blue) and Angus (red) calves in each plasma fructose concentration bracket (Friesian n= 169, Angus n= 50).

There were no significant correlations between plasma fructose levels and any of the other birth variables in Friesian or Angus calves (Table 2.3.2 and 2.3.4; pages 59 and 61).

Assisted versus unassisted calves

Assisted Friesian and Angus calves seemed to have higher fructose levels than unassisted calves, but the difference was small (Figure 2.3.15). There were no significant differences in plasma fructose concentrations between assisted and unassisted calves in Friesian or Angus calves.

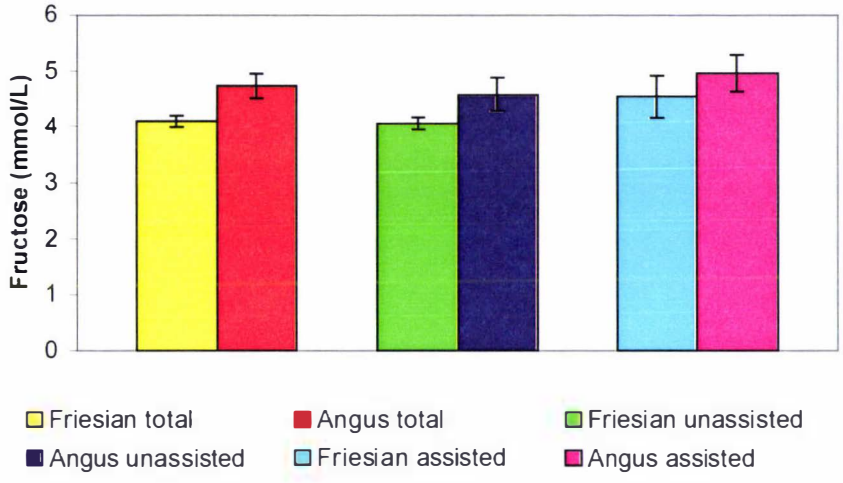


Figure 2.3.15: Mean fructose levels (± standard error) of Friesian and Angus calves.

Time of day at birth

There were significant differences in plasma fructose concentrations in Angus (Table 2.3.5; page 62), but not Friesian calves, between the time of birth categories in plasma fructose concentrations. Post-hoc tests showed that Angus calves born during 2-6pm had significantly higher plasma fructose levels (5.43 ± 0.46 mmol/L) than Angus calves born during 6-10pm (4.26 ± 0.27 mmol/L).

2.3.3 Cow variables

2.3.3.1 Maternal body condition score

Mean body condition score was 4.5 ± 0.04 (range 3.1 to 5.8) for the dams of Friesian calves and 5.0 ± 0.46 (4.2 to 5.8) for the dams of Angus calves. There were significant differences between maternal body condition score for the calves of the two breeds (Table 2.3.6; page 63). Angus calves were born to mothers with significantly higher body condition score than Friesian calves (Figure 2.3.16).

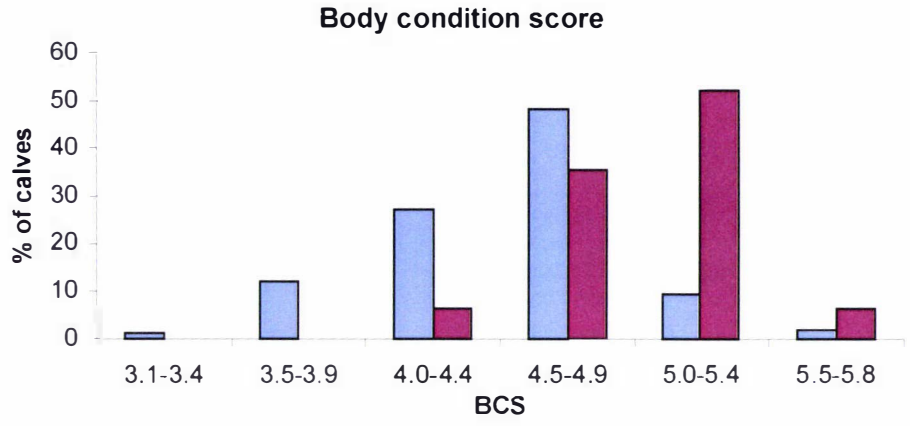


Figure 2.3.16: Percentage of Friesian (blue) and Angus (red) calves in each maternal BSC bracket (Friesian n=158, Angus n= 48).

In Friesian calves maternal BCS showed a significant negative correlation with calf plasma glucose concentration only (Table 2.3.2; page 59), while in Angus calves maternal BCS was significantly and positively correlated with calf plasma glucose levels (Table 2.3.4; page 61).

There were no significant differences in Friesian or Angus calves in any of the other comparisons that were undertaken, including assisted versus unassisted calves (assisted versus non-assisted see Figure 2.3.17)

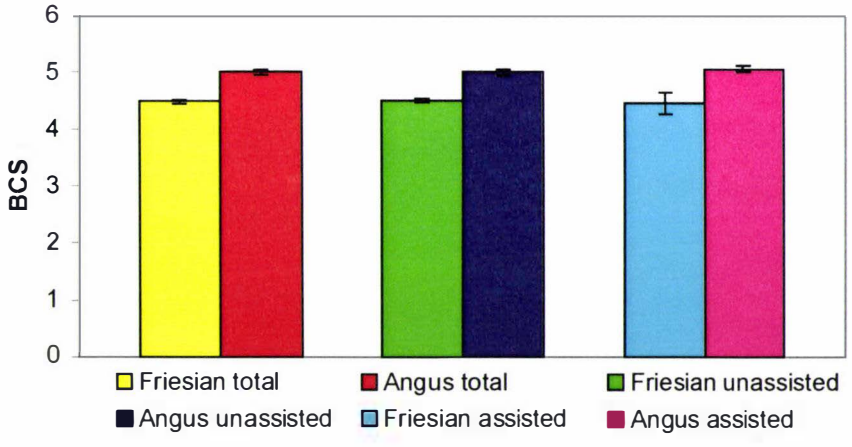


Figure 2.3.17: Mean maternal body condition score (± standard error) for the dams of Friesian and Angus calves.

2.3.4 Environmental factors

The environmental factors, which were measured included air temperature, weather conditions and time of day at birth. Performing a chi-squared test showed that there were no significant differences between Friesian and Angus calves in time of day at birth. Figure 2.3.18 shows that the percentage of calves in the different weather categories seems to have been similar in Friesian and Angus calves. The same can be said for air temperature at birth. Angus calves were born more commonly during the period from 2pm to 2am, while Friesian calves were born evenly throughout the day (Figure 2.3.18).

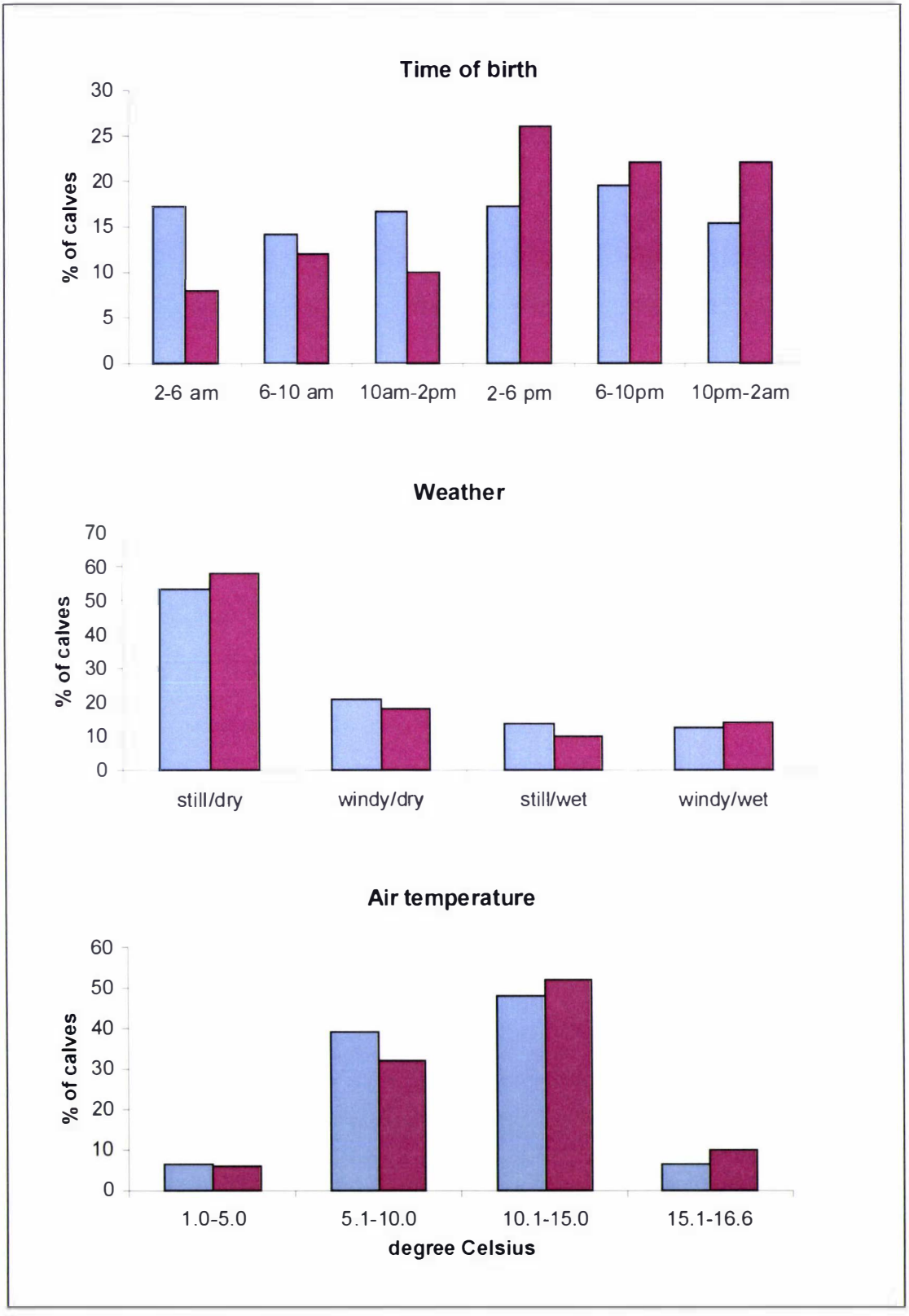


Figure 2.3.18: Percentage of Friesian (blue) and Angus (red) calves born in the six time of day at birth categories, the four weather categories, and the percentage of calves born during different air temperatures (Friesian n=169, Angus n=50).

2.3.5 Summary of major similarities and differences between breeds

A summary of correlations between the different birth variables in both breeds is shown in Figure 2.3.19 (page 78). Calves of both breeds showed significant correlations between time to stand and plasma lactate concentration (positive), while only Friesian calves had significant correlations between time to stand and rectal temperature (negative), air temperature (negative), weather (positive) and plasma glucose concentrations (positive). In both breeds rectal temperature and air temperature showed a significant positive correlation. Rectal temperature was also correlated with weather (negative), plasma glucose and lactate concentrations (both negative) and time to stand (negative) in Friesian, but not Angus calves. As both plasma glucose and plasma lactate concentrations were correlated with rectal temperature, an ANCOVA was undertaken to determine if the correlation was still present if rectal temperature was removed from the model. The partial Spearman correlation coefficient (0.210) of the ANCOVA had a p-value of 0.007 for the correlation between glucose and lactate in Friesian calves. This indicates that the two variables were correlated even after their correlation with rectal temperature was removed from the model.

In Friesian as well as Angus calves there was a correlation between maternal BCS and calf plasma glucose levels, but this relationship was negative in Friesian calves and positive in Angus calves. Plasma glucose levels were positively correlated with plasma lactate levels in calves of both breeds. In both breeds, air temperature and plasma glucose levels were negatively correlated. Gender and weight were positively correlated in Friesian, but not Angus calves.

The birth variables, which exhibited significant differences between the different groups, are shown in Table 2.3.7 (page 79). In Friesian as well as Angus calves, assisted calves had higher lactate levels than unassisted calves. In Friesian, but not Angus calves, assisted calves had significantly lower PCV and took significantly longer to stand than unassisted calves. In Angus, but not Friesian calves, assisted calves were heavier than unassisted calves. An ANOVA comparing calves with meconium staining and those without was only performed in Friesian calves and rectal temperature was significantly higher in meconium-stained calves compared to unstained calves. Friesian

calves born to mothers with low BCS had significantly higher plasma glucose levels than those calves born to mothers with high body condition score. This was not found in Angus calves. In Friesian as well as Angus calves, rectal temperature was significantly lower in calves born at air temperatures below 10°C. In Friesian, but not Angus calves, calves that were born at air temperatures below 10°C were found to take significantly longer to stand and had significantly higher plasma glucose levels than calves born at temperatures above 10°C. In both breeds, males were significantly heavier than females. Also, Angus females, but not Friesian females, had higher rectal temperatures than male calves. In Friesian, but not Angus calves, there were significant differences between calves of the four weather categories in time to stand and plasma glucose concentrations. In Friesian, but not Angus calves, there were significant differences between calves of the six 'time of birth' categories in time to stand, rectal temperature, plasma lactate concentrations and packed cell volume. In Friesian calves significant differences in plasma lactate concentrations and birth weight were found between calves that remained healthy and calves that died between 12hours and 1.5 months of age.

Figure 2.3.19: Summary of significant correlations between different birth variables in Friesian and Angus calves (+ positive correlation, - negative correlation).

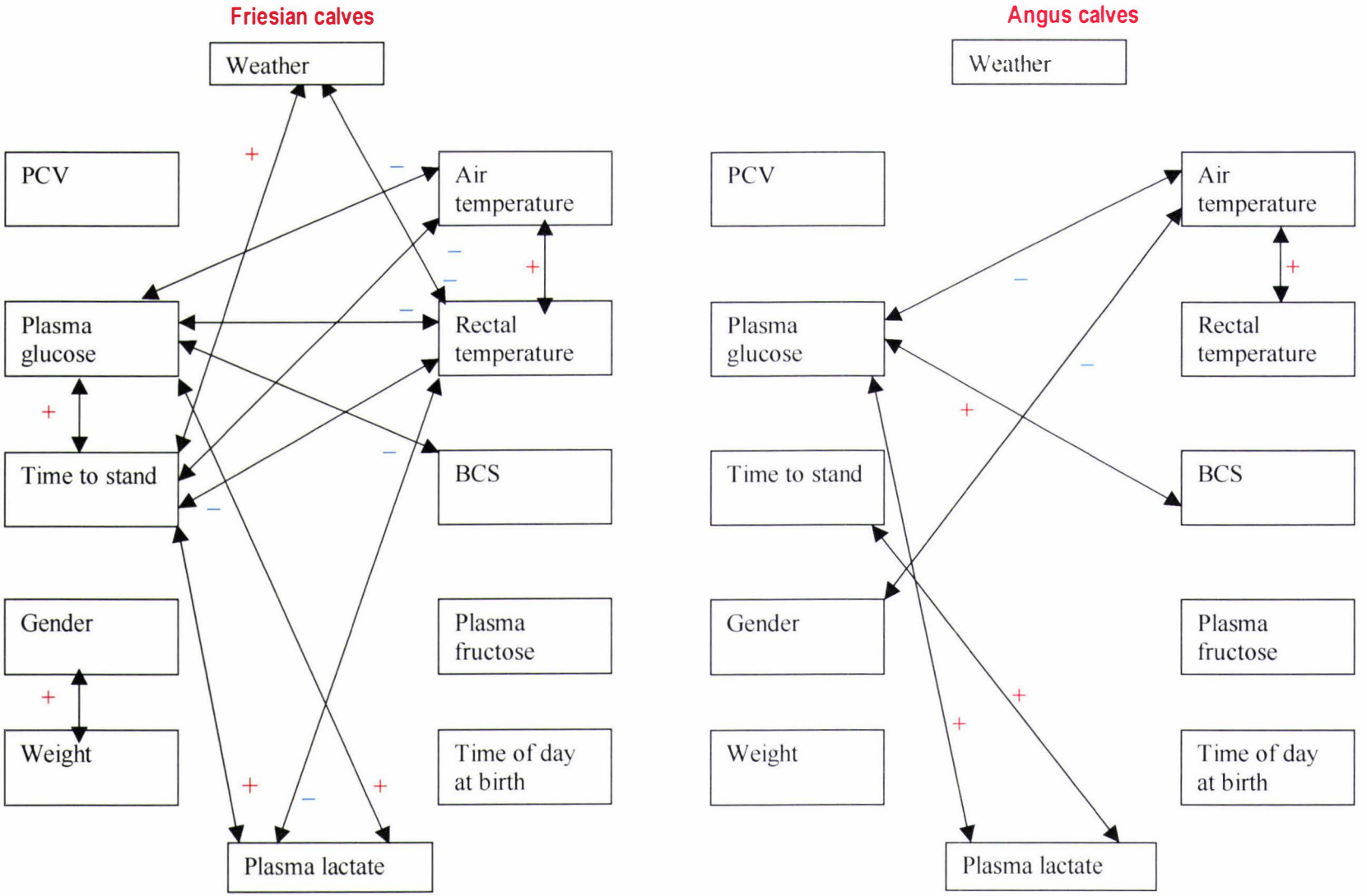


Table 2.3.7: Summary of ANOVA results for significant differences between groups in Friesian (F/+) and Angus (A/*) calves.

Parameters	BCS		Weight		PCV		Rectal temperature		Time to stand		Plasma glucose		Plasma lactate		Plasma fructose	
Breed	**F**	**A**	**F**	**A**	**F**	**A**	**F**	**A**	**F**	**A**	**F**	**A**	**F**	**A**	**F**	**A**
Assisted versus unassisted				*	+				+				+	*		
Meconium versus no staining							+									
Milk fever versus no milkfever																
Sick, dead and healthy calves			+										+			
BCS											+					
Air temperature							+	*	+		+					
Gender			+	*				*								
Weather									+		+					
Time of day at birth					+		+		+				+			*

2.4 Discussion

The major findings and conclusions from this research were as follows:

- Placental insufficiency and maternal underfeeding during late pregnancy did not seem to play a major role in calves of this study.
- The majority of calves that died before, during or immediately after birth were assisted Angus calves.
- There were significant differences in birth weight and plasma lactate concentrations between calves that remained healthy, became sick and died between 12 hours and 1.5 months of age.
- Meconium stained calves did not have significantly higher plasma lactate levels, but did have significantly higher temperatures than calves without meconium staining.
- Primiparous cows required significantly more assistance than multiparous cows. The majority of assisted calves were males, which was mainly due to the high percentage of Angus males being assisted. Males of both breeds were found to be significantly heavier than females, which could have contributed to an increased requirement for assistance in males due to feto-pelvic disproportion. Only assisted Angus calves tended to be heavier than unassisted Angus calves. Multiparous cows may therefore have been assisted due to other reasons, such as mal-presentations or weakness.
- Surviving assisted calves (Friesian and Angus) had significantly higher plasma lactate concentrations than unassisted calves, indicating that these calves experienced intrapartum hypoxaemia.
- Assisted Friesian calves took significantly longer to stand and had lower packed cell volumes than unassisted Friesian calves.
- Air temperature, weather and time of day at birth had an influence on rectal temperature, plasma glucose concentrations and time to stand, but this was more pronounced in Friesian than in Angus calves.

- Overall, variability of the data was small and the status of calves at birth was relatively uniform. Mortality rates were low, which could have been influenced by the experimental protocol to call for assistance when there had been no apparent progress with labour for one hour.

The conclusions of this study are discussed in detail in the following sections.

2.4.1 Prenatal Factors

Detrimental prenatal factors apparently only played a small role in the calves of this study, as indicated by the small occurrence of placental insufficiency and maternal undernutrition during late pregnancy.

In sheep, placental insufficiency was associated with low plasma fructose level, low birth weight, low rectal temperatures and increased plasma lactate levels and packed cell volume (Barlow *et al.*, 1987; Mellor, 1988). Maternal underfeeding during late pregnancy in ewes was associated with low birth weights and low fructose levels (Mellor, 1988). Newborn calves, which had a small placenta, would thus be expected to have a high PCV and plasma lactate concentration, low birth weight, rectal temperature and plasma fructose concentrations. Also, calves from mothers that were underfed during late pregnancy would be expected to have low birth weights and low plasma fructose concentrations.

The following observations support the notion that placental insufficiency and maternal underfeeding during late pregnancy were not a significant problem in the calves in this study.

1. There was no positive correlation between plasma lactate concentration and packed cell volume (Table 2.3.2 and 2.3.4). Although some animals had PCVs over 50% (Figure 2.3.12), plasma lactate concentrations in these animals were not elevated.
2. There were no negative correlations between plasma lactate concentrations and plasma fructose levels and weight (Table 2.3.2 and 2.3.4). Only one or two calves had low plasma fructose levels as well as relatively high plasma lactate

levels. Only a few calves had low weights in combination with relatively high lactate levels. There was a negative correlation between plasma lactate concentrations and rectal temperature in Friesian calves, which was probably due to five calves that had rectal temperatures below 38°C and lactate levels above 10mmol/L. However, this would be more likely to be an effect of intrapartum hypoxaemia, as there was no other evidence for placental insufficiency.

3. There were no negative correlations between PCV and plasma fructose concentrations, weight and rectal temperature (Table 2.3.2 and 2.3.4). A few calves had low fructose levels in combination with high PCV (above 50%). However, the highest PCVs were not associated with low rectal temperatures.
4. There were no positive correlations between plasma fructose concentrations and weight or rectal temperature (Table 2.3.2 and 2.3.4). Only a few calves showed low fructose levels in combination with rectal temperatures below 38°C. Only one calf below 30kg had low plasma fructose concentration.
5. There was no positive correlation between weight and rectal temperature (Table 2.3.2 and 2.3.4). Only one calf weighing below 30kg had a rectal temperature below 38°C.
6. Maternal body condition score was not correlated positively with birth weight or plasma fructose levels (Table 2.3.2 and 2.3.4).
7. Although maternal body condition scores were not recorded at the beginning of pregnancy, we can assume that maternal undernutrition during placental growth and development was not an issue, as the cows overall had medium condition scores (range of 3.1 to 5.8 on a scale from 1 to 10) at the end of pregnancy. If any of the cows had been underfed during early pregnancy it is not likely that they would have improved their condition during late pregnancy.

Although the spread of PCV in this study is very high, suggesting the possibility that some measurements were not done properly (blood samples not mixed well before PCV was determined), it is not likely that only those samples from calves with placental insufficiency were underestimated. A study by Dalton (1967) also shows a wide spread of PCV from 33 to 51% for newborn calves. Thus, the PCV measurements of the

present study may have been correct. Nevertheless, the PCVs below 30% may be unreliable due to poor mixing of the blood samples.

Unexpectedly, calves with meconium staining did not have higher lactate levels than calves without meconium staining, but had significantly higher rectal temperatures than non-stained calves. Meconium staining, due to the anal sphincter relaxing and meconium being released into the amniotic fluid, may be a sign of hypoxic stress during parturition (Kasari, 1989). If so, these calves would have had high lactate levels due to hypoxia. It may be possible that the hypoxic stress of these calves was not sufficient to cause high lactate levels, but strong enough to cause meconium release into the amniotic fluid. Also, hypoxic stress might have been transient and occurred a sufficient time before birth for its metabolic effects to have passed by the time blood samples were taken after birth. Dufty & Sloss (1977) reported that there was little or no relationship between the intensity of meconium staining and the duration of anoxia. Thus, a short period of hypoxaemia that may not elevate lactate levels might still cause meconium staining or transient hypoxia may have caused staining before birth. The higher rectal temperatures observed in the calves in the present study are hard to explain.

Placental insufficiency is one of the major prenatal factors that predispose lambs to debility and death. Factors involved with placental insufficiency in sheep include maternal stress and exercise, ambient temperatures and maternal undernutrition during the period of placental development (Mellor, 1983). Twin or triplet pregnancies where the number of placentomes per fetus is reduced and a restriction on implantation sites due to the death of littermates soon after implantation (Mellor, 1983) may also be involved. A study undertaken by Cooper *et al.* (1998) showed that, in two groups of cattle that had a liveweight difference of 43.7kg due to differing nutrition during the first half of gestation, early pregnancy nutrition did not appear to influence overall fetal growth as measured by calf birth weight. However, the calves in the study by Cooper *et al.* (1998) were not tested for signs of placental insufficiency in late pregnancy such as plasma fructose and lactate levels and packed cell volume.

Another prenatal factor that may adversely influence neonatal survival and performance is maternal underfeeding during late pregnancy. In sheep, severe underfeeding during late pregnancy was shown to decrease fetal growth rate within 3 days by about 45% (Mellor, 1983). This may be due to fetal hypoglycaemia as well as fetal endocrine changes (decrease in insulin and increase in growth hormone and corticosteroid concentrations) in response to reduced nutrient supply (Mellor, 1983), which may lead to mobilisation of energy reserves or inhibition of energy store deposition. Fetal glycogen and fat reserves are mobilised and/or depleted during long periods of underfeeding (Mellor, 1983). Although postnatal heat production is not inhibited in these animals, the reduced energy reserves will reduce the period for which thermogenesis can be maintained in the absence of colostrum intake (Mellor & Cockburn, 1986; Mellor, 1988). Maternal underfeeding during late pregnancy in sheep was also associated with reduced accumulation of colostrum prenatally, delayed onset of lactogenesis and a reduced total production of colostrum and milk for the first 18 hours after birth (Mellor *et al.*, 1987) and poorer maternal care (Alexander, 1960). These factors might have been due to the delayed prepartum decrease in plasma progesterone levels in underfed ewes (Mellor *et al.*, 1987), due to the possibly inhibitory action of progesterone on oestrogen and prolactin action, which are required for eliciting maternal behaviour and lactogenic actions respectively (Mellor, 1988). As the early intake of adequate amounts of colostrum is important for the newborn ruminant to establish passive immunity, provide fuel for thermogenesis and growth factors for gut development (see Chapter 3), maternal underfeeding may thus have adverse effects on the survival of the newborn.

In a study by Hight (1968) maternal undernutrition during late pregnancy was associated with lower calf birth weights and increased mortality rates, when a low plane of nutrition was maintained until 3 weeks before calving. Also, Kroker & Cummins (1979) found that nutritional restriction in late pregnancy caused reduced pelvic growth, the effort put into labour was reduced, dystocia rates increased, calf survival during the first 2 weeks after birth decreased and calf growth rates to weaning were reduced in heifers, which lost approximately 0.5kg per day during late pregnancy.

As the body condition score range of cows in the present study was compact and only covered a range of 3.1 to 5.8 on a scale from 1 to 10, the small variation in birth features of the present calves and the low incidence of placental insufficiency can be explained. In situations where very low body conditions scores may be encountered in cows, placental insufficiency and maternal undernutrition during late pregnancy may well become a threat to calf well-being and survival.

2.4.2 Natal factors

Of the total of 219 calves, 35 (16%) were assisted, as labour in their cases did not progress after one hour of observation. Assisted calves of both breeds were found to have higher plasma lactate levels than unassisted calves (Table 2.3.3 and 2.3.5; Figure 2.3.9). As lactate is a main product of anaerobic glycolysis (Walser & Maurer-Schweizer, 1979), which is experienced during periods of oxygen deprivation, assisted calves in the present study seemed to have experienced intrapartum hypoxaemia. Therefore, the visual assessment of delayed labour was probably accurate.

Assisted Friesian calves in the present study took longer to stand than unassisted calves (Figure 2.3.3). The time to stand was not available for most of the Angus calves. Plasma lactate levels and rectal temperature were significantly and negatively correlated in Friesian calves, while plasma glucose and lactate concentrations were positively correlated in both breeds (Table 2.3.2 and 2.3.4).

Intrapartum hypoxaemia in sheep is associated with elevated plasma lactate, hydrogen ion, glucose, catecholamine and cortisol concentrations (Eales & Small, 1985; Mellor, 1988) and may be the result of umbilical cord compression, protracted labour and birth difficulties (Mellor, 1988). It has been found in lambs that intrapartum hypoxaemia inhibits postnatal heat production sufficiently to jeopardize survival (Mellor, 1988). Eales & Small (1980a and b) report that severe hypoxia during birth was implicated as a major factor leading to hypothermia in lambs aged 12 hours or less, which had high plasma lactate concentrations. Exposure to hypoxia in newborn lambs resulted in a 66% depression of summit metabolic rate (maximum thermogenic response to cold stress) in newborn lambs that were immediately exposed to cooling after the period of hypoxia (Eales & Small, 1985). The deleterious effects of hypoxaemia on heat production

capacity were only observed in lambs with severe acidaemia (Eales & Small, 1985), and mild acidaemia, commonly found after birth, was not observed to cause adverse effects (Eales & Small, 1980b).

As in lambs, newborn calves were found to have lower blood pH, higher cortisol levels and pCO_2 after difficult births than after normal parturition (Massip, 1980). Higher plasma lactate concentrations were also found in calves made hypoxic for 24 hours postpartum (Tyler & Ramsey, 1991). Vermorel *et al.* (1989) reports that calves born after very difficult and prolonged parturition had severe and long-lasting acidosis with high lactate levels during the first hours of life and that their heat production was about 22% lower than in unassisted calves 2 hours after birth and 14% lower from 13 hours onwards. Adams *et al.* (1995) review that models mimicking the effects of severe dystocia usually resulted in metabolic acidosis, elevated circulating lactate and glucose concentrations, hypothermia and death. In their own study, plasma lactate concentrations remained higher for at least 4 hours after birth in the calves that needed the most assistance, but the difference between these calves and those requiring less assistance subsided by 12 hours of age, indicating effective clearance of plasma lactate in calves with more severe birth effects. Bellows & Lammoglia (2000) also found that severe dystocia resulted in lower calf rectal temperature and increased serum glucose levels. This supports the findings of the present study that rectal temperature and glucose were both correlated with lactate. In the present calf study, plasma glucose concentrations were positively correlated with lactate concentrations (Table 2.3.2a/c). As lactate concentrations were higher in assisted calves, it can be assumed that glucose levels, as positively correlated with lactate, were also increased, although no significant differences were found in plasma glucose levels between assisted and unassisted calves. Bellows & Lammoglia (2000) assumed that the elevated glucose levels might have been a reflection of mobilisation of liver glycogen induced by adrenaline and cortisol as a result of hypoxia-induced glycolysis. Alexander (1979) surmised that adrenaline, which is released in response to cold stress, increases blood glucose in the short-term by inhibiting insulin secretion, stimulating an increase in glucagon secretion and an increase in glycogenolysis in the liver.

The correlation between lactate and glucose in the present study was not affected by rectal temperature. However, glucose levels were positively correlated with air temperature and there was a significant difference between glucose levels in calves born during good and bad weather, with those born into bad weather having higher glucose levels. It seems that some calves might show elevated plasma glucose levels in combination with elevated plasma lactate levels, due to hypoxia-induced glycolysis mediated possibly by catecholamines and cortisol, while other calves show an increase in plasma glucose levels in the absence of elevated plasma lactate levels, in response to colder weather, an effect which is also due to catecholamine and cortisol action. In some calves both causes might have contributed to increased plasma glucose levels.

Time to stand was found to be delayed in assisted Friesian calves of the present study as reported before (Dufty & Sloss, 1977; Edwards, 1982; Kasari, 1989). The delay in standing might have been due to weakness resulting from prolonged birth. Edwards (1982) observed that time to stand was strongly correlated with duration of birth in unassisted calves and suggested that the degree of hypoxia experienced by the calf may determine its early vigour.

Of the calves that died around birth, the majority were assisted. Significantly more primiparous cows required assistance compared to multiparous cows. Only assisted Angus calves were heavier than unassisted calves (Table 2.3.5, Figure 2.3.11) and the majority of those were males (75%). This may indicate that assistance in primiparous cows might have been mainly due to dystocia as a result of feto-pelvic disproportion, but factors such as unstretched pelvic tissues and reduced straining (observed during this study) may also have been involved. In multiparous cows, there was no difference in weight between assisted and unassisted calves (Table 2.3.3, Figure 2.3.11) and the numbers of males and females that were assisted were similar (6 males versus 9 females). Intrapartum hypoxaemia in assisted Friesian calves thus might have been due to protracted labour, abnormal presentation of the calf, umbilical cord occlusion or premature rupture as well as to muscular weakness from metabolic disorders such as milk fever (Meijering, 1984).

In sheep, dystocia occurs in 4 to 36% of ewes depending on breed and is mostly caused by fetal malpresentation or feto-pelvic disproportion (Mellor, 1988). Meijering (1984) states that in cattle, feto-pelvic disproportion seems to be the most common cause of dystocia, especially in first calf heifers. This supports the finding of this study, that assisted calves born to heifers were heavier than unassisted calves. Fetal malpresentation was found to be less common than feto-pelvic disproportion, and was mainly observed in older cows than heifers (Meijering, 1984), which also supports the present suggestion that the older cows examined here did not seem to need assistance due to feto-pelvic disproportion, but rather malpresentations or other reasons. Weak labour, for example, may not only be a consequence of difficult calving, but may also be its cause (Meijering, 1984). Weak labour was reported to be most frequent in older cows, and was often associated with milk fever (Meijering, 1984). Sex of the calf has been found to be a major source of variance in levels of dystocia, with male calves being affected more commonly (Meijering, 1984; Morris *et al.*, 1986). This seems to be mainly due to the differences in size between genders (Morris *et al.*, 1986), which was also observed in the present study, with males being heavier than females.

In the present study, two calves that had very high lactate levels (>15mmol/L) were found to become hypothermic soon after birth. One of the calves died, while the other was covered with a blanket and transferred into the calf shed where it recovered. This seems to indicate the possible effect of acidaemia on the heat production capacity of newborn calves, but also that, like lambs (Eales *et al.*, 1982b), calves are able to recover from severe hypothermia (< 36°C) by providing shelter and additional support such as blankets or food.

2.4.3 Environmental factors

The effects of environmental conditions were more pronounced on Friesian than Angus calves. This could be possibly attributed to the lower number of Angus calves leading to statistical insensitivity. Therefore, this section mainly deals with Friesian calves, extrapolating to Angus calves.

Newborn calves of the present study had higher rectal temperatures and stood faster in warm conditions, during good weather and during the afternoon, but had lower rectal

temperature and took longer to stand in cold conditions, during bad weather and during the night and early morning (Table 2.3.2, Figure 2.3.19). Calves that took longer to stand thus had lower rectal temperatures, but also higher glucose levels compared to calves that stood sooner after birth (Table 2.3.2, Figure 2.3.19). Calves with lower rectal temperatures had higher plasma glucose levels than those with higher rectal temperatures. Glucose levels were higher in calves born at lower air temperatures and in bad weather conditions, than in calves born at higher air temperatures and good weather conditions.

Inadequate heat production has been found to affect survival in newborn lambs (Barlow *et al.*, 1987; Mellor, 1988). Lambs that die from this cause were observed to have normal packed cell volume and lactate levels that were not elevated, but rectal temperatures were low (Barlow *et al.*, 1987). Excessive heat loss or inhibited heat production may be the cause for hypothermia in these lambs and environmental factors may play an important role. Alexander (1970) noted that the environmental demands made upon the ability of the newborn animal to metabolise energy immediately after birth may well exceed the demands made at any other stage of life and that the survival of the young depends upon its ability to achieve a high metabolic rate. Adverse weather conditions such as wind, rain and cold air temperatures will exaggerate the demand placed upon the animal, as heat will be lost from the body at a considerable rate (Alexander, 1970). This will be due to a birth coat soaked in fetal fluids causing evaporative heat loss, as well as a high surface area to body weight ratio, which increases heat loss in relation to body weight (Alexander, 1970). If summit metabolism is reached, no further increase in heat production is possible, and if the rate of heat loss exceeds heat production, the animal will become hypothermic (Alexander, 1970). In lambs, high rates of heat production were found to be maintained for about 2 hours after birth to balance the high rates of heat loss resulting from poor insulation by the wet fleece and evaporation from drying of the fleece (Mellor & Cockburn, 1986). In lambs that became hypothermic, plasma lactate and glucose concentrations were elevated in the first few hours after birth but decreased thereafter (Eales *et al.*, 1982a).

In newborn calves, a drop in rectal temperature over the first few hours after birth has been observed and is probably a result of the greater heat loss during evaporation of amniotic fluid from the coat (Thompson & Clough, 1970). Thompson & Clough (1970) estimated that in calves the average heat loss through evaporation in the first hour after birth was around 180kcal/h, while the average resting heat production was about 95kcal/h. But it is not known how much maternal licking would reduce heat loss from evaporation during this time (Thompson & Clough, 1970). Associated with this, Daniels *et al.* (1979) found that blood glucose rose immediately after birth, which may have resulted from the action of the adrenal glands in response to the stresses of the birth process or from increased sympathetic efferent activity due to the cold environmental temperature.

Low rectal temperatures were linked to low environmental temperatures and other adverse weather conditions as well as increased glucose levels in some of the calves of the present study. This seems to support the notion that glucose levels are elevated during cold stress. Although a positive correlation was also found between plasma lactate and glucose concentrations in some of the calves of this study, not all calves with elevated glucose concentrations also had elevated lactate concentrations. Thus, although some calves might have had elevated plasma glucose concentrations due to hypoxia-induced catecholamine action, other calves are likely to have had elevated plasma glucose concentrations due to the thermogenic response to cold, also mediated by catecholamines. This is supported by Olson *et al.* (1981), who observed that plasma catecholamine concentrations increased in the response to cold stress in calves and that animals, which did not experience intrapartum hypoxaemia, also showed an increase in plasma glucose levels due to catecholamine action in response to cold.

In a study by Gonzalez-Jimenez & Blaxter (1962) calves reacted to an environmental temperature that was sufficiently low to induce piloerection, by standing up. Exercise was shown to increase heat production (Vermorel *et al.*, 1983) so that standing and walking may be more useful in adverse weather conditions than lying.

Calves of the present study took longer to stand at lower air temperatures and in adverse weather conditions. The difference between the findings of the present study and those of Gonzalez-Jimenez & Blaxter (1962) may be due to the fact that their animals were between 2 days and 4 weeks of age, while the calves in the present study were newly born. The fact that animals in the present study showed a delay in time to stand during adverse weather conditions may be a heat conserving behaviour by the calf as lying would reduce the surface area exposed to wind and rain and thus heat loss. Although lying may increase conductive heat loss to the ground in the short term, it may lead to reduced heat loss in the long term. This could be the case if the area the calf is lying on warms up adequately for heat transfer from the calf to the ground to become reduced. Also, newly born calves that have reduced rectal temperatures may not be vigorous enough to stand earlier to increase heat production by exercise. A study by Schrama *et al.* (1993) showed that 6-day-old male calves had a higher thermal requirement during standing than lying. Perhaps this is also the case in newborn calves, so that a delay in the time to stand in adverse weather could improve the thermal status of calves that remain lying down compared with those that stand.

2.4.3 Healthy, sick and dying calves

Friesian calves that died had significantly higher birth weight and significantly higher plasma lactate concentrations than Friesian calves that remained healthy. However, there were no significant differences between these groups in any of the other birth parameters examined. Elevated birth weight compared with other calves might have predisposed dying calves to death due to birth injuries or prolonged time to stand associated with their larger weight. However, only two calves in this group took an hour or longer to stand (60 and 150 minutes). It thus seems that calves that died were more likely to have experienced intrapartum hypoxaemia. As only one of the calves that died was assisted, it is possible that the higher weight observed in these calves could have contributed in some way to the oxygen shortage during the birth process. On the other hand, only two calves that subsequently died had very high plasma lactate concentrations while the other six calves had levels comparable to those of sick and healthy calves (below 6.6 mmol/L). However, it is possible that for some calves plasma lactate concentrations of this magnitude could have had a detrimental effect on their survival. As shown in Chapter 3, calves that died had low plasma GGT levels indicative of failure of immunoglobulin transfer. Calves might have been weakened due to

elevated plasma lactate concentrations and the birth process, which might have been more difficult due to their higher weight. Although they stood relatively early they might have been too weak to suck.

2.5 General Discussion

The problems that were anticipated from studies in sheep were not found in the present study. The physiological and physical status of calves at birth was fairly uniform. This however may not be surprising, as in comparison to ewes, cows of the present herd were intensively managed and thus well fed with medium overall body condition scores. In sheep, body conditions scores may be expected to vary more widely due to less intensive management and food intake control, which may lead to a less uniform status of lambs at birth. However, it has to be acknowledged that the status of single lambs is also fairly good and thus, that the findings of the present study support those from single lambs. Mortality rates in newborn single lambs were lower than those of twins and triplets in a study by Barlow *et al.* (1987). In addition, Eales *et al.* (1982a) found that the majority of lambs that became hypothermic in their study were twins or triplets.

The facts that the physiological and physical status of calves was fairly uniform and that there were only a small number of significant differences in the birth parameters measured between calves that remained healthy and those that subsequently became sick or died, suggests that calves have a good chance of survival if they do not experience placental insufficiency or intrapartum hypoxaemia. This seems also to be supported by the fact that calves that died had significantly elevated plasma lactate concentrations compared with healthy calves and thus were likely to have experienced intrapartum hypoxaemia. Although intrapartum hypoxaemia did not have any adverse effect on calf thermogenesis in the present experiment, this might have been due to the early assistance given here to dystocic cows. But assisted calves were found to take longer to stand which might have delayed colostrum intake leading to hypogammaglobulinaemia and an increased susceptibility of such calves to infectious disease (Chapter 3). Also, the fact that weather conditions were fairly mild would have influenced the calves' ability to maintain rectal temperatures and their ability to avoid hypothermia.

Early assistance seemed to have some benefits, as the majority of the perinatal deaths occurred where assistance was required and where assistance was late. Also, calves seemed less likely to become hypothermic if their dams were assisted early, as none of the assisted calves in this study were found to become hypothermic. Thus, early assistance in difficult births is advised to reduce the incidence of hypothermia in newborn calves as well as the incidence of weak calves that may take longer to stand and therefore might have delayed colostrum intake.

2.5.1 Problems with experimental set-up

- Rectal temperature measurements were made about 12 minutes after birth, so that the detection of the effectiveness of initial thermogenesis may have been less sensitive with the present calves than if temperature measurements had been taken 30 minutes after birth, as occurred when lambs were studied by Barlow *et al.* (1987).
- Assistance was given quite early to cows showing signs of dystocia and this might have minimised compromise both during and shortly after birth, thus contributing to the overall uniformity of calf physiological status.
- The number of calves for the comparison between sick, healthy and dying calves was fairly low. In addition, information on health status was only available on calves that remained on the farm (possibly replacement heifers). Thus, the incidence of disease and death may be higher or lower than reported in this chapter and there might have been different significant differences in birth parameters between sick, dying and healthy calves if all calves of this study could have been followed up.
- There might have been an observer effect on the cows as well as the newborn calves that might have influenced the results of the present study. Human observers were present 24 hours a day and their presence might have influenced the incidence of dams that had to be assisted, the time to stand in newborn calves and other factors. For example, calves might have stood earlier in the presence of humans than they would normally. Also, the presence of humans, especially at night, in addition to use of torches, might have led to changes in the timing of calving.

2.5.2 Future research

Future research regarding the impact of pre-, intra- and post-partum factors on the physiological and physical status of newborn dairy calves and their subsequent performance could include the following:

- The use of cows with a wider range of body condition scores. This would allow the impact of nutritional state, and if it occurred, placental insufficiency, on calf viability and performance to be assessed in more detail.
- Measurement of rectal temperature for several hours after birth. This would allow the success of thermoregulation in newborn calves to be followed for a longer period, in particular the success of initial thermoregulation.
- The use of various protocols for assistance of dystocic cows in order to assess the impact of various degrees of intrapartum hypoxaemia on the calves' ability to produce heat to maintain body temperature, and the effect of intrapartum hypoxaemia on colostrum and milk intake.
- Measurements in neonatal calves born during adverse weather conditions to determine, in comparison with lambs, how well calves cope with environmental stressors.
- Studies of other factors calves are exposed to after birth such as milk intake, housing situations, mixing of calves and dominance hierarchies, to determine whether these factors predispose calves to become sick and/or die during the days, weeks and months after birth.
- It seems important to follow the calves up for possibly several months to determine significant differences in birth parameters between calves that remain healthy and those that become unhealthy and those that die. This would give a more accurate account on whether calves have a good start in life and whether it really is factors later on that predispose them to morbidity and death.

Chapter Three:

The Physiological and Physical Status of Calves between 1 to 4 Days of Age

Abstract

In the previous chapter it was established that the physiological status of calves in a dairy herd was fairly uniform at birth and that those calves had a good start in life. A proportion of disease and ill thrift in calves might therefore be due to factors that occur after the immediate postnatal period. In lambs, a major postnatal factor contributing to debility and death was starvation and its effect on thermogenesis and passive immunity. In the present study we examined the physiological and physical status of calves between 1 and 4 days of age. Starvation and hypothermia were not major problems up to 4 days of age in the calves of the present study. The majority of calves received colostrum. Nevertheless, about 33% of calves became sick and 17% of calves died. However, those values are not absolute as a number of calves were sold during the experiment and their health status could not be followed up. Although the majority of calves that became sick consumed some colostrum, calves that died had low plasma gamma-glutamyltransferase (GGT) concentrations indicative of failure of colostrum intake. However, plasma GGT concentrations do not seem to be a good indicator of immune status of newborn calves and thus no judgement can be made as to whether calves that remained healthy had a better immune status than calves that became sick or those that died. There were no significant differences between assisted and unassisted calves in any of the parameters measured, suggesting that the early assistance provided at difficult births had a beneficial effect on the health of assisted calves at least during the first few days after birth. A comparison between calves that were separated from their mothers at an age younger and older than 24 hours revealed that calves separated from their mothers early had a higher incidence of sickness and mortality. Also, the results of the present study suggest that calves staying with their mother for a longer period may be more likely to take in colostrum. Overall, the physiological status was adequate for the majority of calves in the present study up to four days after birth. However, calves that died had low plasma GGT concentrations which seem to indicate a failure of or insufficient consumption of colostrum before pick-up. The present work thus seems to support previous research in that ensuring the intake of colostrum adequate to acquire passive immunity is of major importance for the welfare of the newborn calf. In addition, other factors may predispose animals to become sick, such as hygiene and housing conditions as well as management, even though passive immunity has been acquired.

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3.1 Introduction

The welfare of calves sent to slaughter at 4 days of age remains acceptable when the *Code of Recommendations and Minimum Standards for the Welfare of Bobby Calves* is followed (Todd *et al.*, 2000a, b; Stafford *et al.*, 2001). Criteria have been established for assessing the status of calves on arrival at a slaughter plant, which improves the detection of calves that are not treated according to the code (Stafford *et al.*, 2001). However, there is little information available on possible welfare compromise in calves from birth to 4 days of age.

In the previous chapter it was established that the physiological status of calves in a dairy herd was fairly uniform at birth and that those calves had a good start in life. A significant proportion of disease and ill-thrift in calves might therefore be due to factors that occur after the immediate postnatal period. In lambs, a major postnatal factor contributing to debility and death was starvation (Barlow *et al.*, 1987; Mellor, 1988). Colostrum intake in the hours after birth fuels key metabolic processes such as heat production and provides the newborn lamb and calf with protective antibodies and factors that aid in gut development and growth. Starvation thus will impede heat production, increase the animals' susceptibility to disease and reduce gut development. This may increase the risk of debility and death in the short- or long-term (Mellor, 1988).

3.1.1 Starvation in the adult animal

In adult animals starvation has marked effects on substrate utilisation. The brain depends on a supply of glucose for its energy needs and other tissues stop using glucose as their major energy source and begin using fatty acids or ketone bodies (White &

Baxter, 1994) and finally protein. This shift of substrate use is mainly due to decreased glucose oxidation in tissues other than the nervous system.

White & Baxter (1994) reported that a fall in glucose concentrations is observed during the first few days of starvation, which is accompanied by a fall in plasma insulin concentrations and an increase in plasma glucagon concentrations. This reduces the uptake of glucose in tissues other than the nervous system. Glucose supplied to and utilised by the brain is provided by hepatic glycogenolysis, which is then followed by hepatic and renal gluconeogenesis, substrates for which include lactate from skeletal muscle, alanine from muscle protein and glycerol from adipose tissue (White & Baxter, 1994).

Lower plasma insulin concentrations reduce the inhibition of proteolysis, and muscle protein mobilisation can proceed leading to a release of alanine from muscle into the blood (White & Baxter, 1994). The increased plasma glucagon levels on the other hand, stimulate hepatic extraction of alanine from the blood, which provides one of the major substrates for gluconeogenesis (White & Baxter, 1994). However, muscle glycogen is not mobilised due to a protective action of increased glucagon levels (White & Baxter, 1994).

The reduction in insulin concentrations also increases the rate of lipolysis in adipose tissue and non-esterified fatty acids (NEFA) are released into the blood stream. NEFA are then used by some tissues (e.g. skeletal muscle) as a major energy source. Also, NEFA are used for ketogenesis by the liver (White & Baxter, 1994). These ketones (acetoacetate and 3- hydroxybutyrate) are then released into the plasma and interstitial fluid from where most tissues have access to them (White & Baxter, 1994). The metabolism of ketone bodies and fatty acids also seems to inhibit glucose oxidation in extrahepatic tissues, which contributes to the glucose sparing effect.

As starvation proceeds, there is an increase in gluconeogenesis in the kidney contributing about 50% of the total glucose synthesis. Also, after 4 to 5 weeks of starvation in adult animals, gluconeogenesis is reduced by 70% compared with the early stages of starvation and the brain also utilises ketone bodies as energy substrate (White & Baxter, 1994). When carbohydrate and lipid energy stores are exhausted, structural

protein becomes the primary energy source for the starved animal leading to muscle tissue degradation (White & Blaxter, 1994).

In newborn lambs starvation may be a rather rapid event with death occurring between 16 hours and 5 days (Alexander, 1962c), possibly due to the small energy reserves of lambs (Mellor & Cockburn, 1986; Okamoto *et al.*, 1986) and the relative immaturity of body systems compared to the adult animal. The same may be the case for newborn calves.

3.1.2 Starvation and its effects in the newborn animal

Colostrum is the first secretion of the mammary gland during lactation and is important for nutrient supply, immunoglobulin transfer, maturation of organs and the initiation of metabolic and endocrine changes (Blum & Hammon, 1999). Colostrum is markedly different from milk in that it has different amounts of substances such as immunoglobulins, growth factors, hormones, cytokines, polyamines and enzymes, and differing amounts of nutrients such as carbohydrates, lipids, proteins, minerals and vitamins (Hadorn *et al.*, 1997).

While these biologically active substances aid in gut differentiation and supply the newborn with immunoglobulins, the nutrient components are important for fuelling metabolic processes such as heat production (Mellor & Cockburn, 1986; Hadorn *et al.*, 1997; Rauprich *et al.*, 2000).

Insufficient intake of colostrum/milk can occur in lambs and calves when the newborn animal is too weak to suck, when colostrum supply is reduced, when mothering is inadequate or separation from the dam occurs. Also, inappetance and starvation may be observed in response to diseases, which may become established in newborn animals unprotected by failure of immunoglobulin transfer (Sawyer *et al.*, 1977).

Heat production

To survive the newborn must maintain normal body temperature by increasing its heat production immediately after birth if environmental temperatures are below its body temperature (Alexander, 1979). Even if ambient temperatures are only slightly below the animal's body temperature, heat production has to be increased in order to balance

heat loss from the evaporation of amniotic fluid as the animal's coat dries (Mellor & Cockburn, 1986). Energy-rich substrates are required to fuel heat production and they are derived from body energy reserves deposited before birth and from colostrum and milk taken in after birth (Mellor, 1992; Mellor, 1993). The major sources of energy for thermogenesis in newborn ruminants include glycogen and lipid, as protein catabolism is only small during the early postnatal period (Mellor & Cockburn, 1986).

Liver glycogen deposition increases as term approaches and a rapid increase occurs a few days before birth in lambs (Mellor, 1993). Liver glycogen is the newborn's only major source of circulating glucose before colostrum is ingested and is of major importance for the neonate's survival (Mellor & Cockburn, 1986). The concentration of muscle glycogen also increases before birth and is utilised during muscular activity for shivering heat production and locomotion (Mellor, 1993). Amino acid catabolism is minimal at least during the first day after birth, so that body glycogen and lipid stores as well as colostral lactose and lipids are the animals' major sources of fuel for heat production (Mellor & Cockburn, 1986). Heat production was found to be simultaneously fuelled by carbohydrate as well as lipid, but at high rates of heat production, carbohydrate use was found to be dominant, while at low rates of heat production, lipid is the predominant fuel (Mellor & Cockburn, 1986). In human infants, piglets and lambs it has been shown that carbohydrate is the rate limiting substrate for heat production and that exhaustion of carbohydrate energy stores will lead to hypoglycaemia and hypothermia or cerebral compromise (depending on ambient temperatures), even if lipid stores are still available (Mellor & Cockburn, 1986).

Some lambs that become hypothermic between 12 and 36 hours after birth were found to have experienced starvation, depleted energy reserves and a low rate of heat production (Eales *et al.*, 1982a). Starved newborn lambs were found to survive longer in warmer ambient temperatures than lower ambient temperatures (Alexander, 1962c). This could be explained by the lambs with higher rates of heat production using carbohydrate as the predominant fuel. Even if lipid stores were not depleted in these animals, the depletion of carbohydrate stores would inhibit heat production and cause hypothermia and eventually death. Alexander (1962c) observed that at the onset of coma, liver and muscle glycogen reserves were virtually exhausted. Although lipid was still present in the starved lambs, he found that lipid deposits were virtually exhausted

and concluded that the lipids which were still present were structural lipids. Protein was utilised as a source of energy after 3 days of starvation and was not sufficient to maintain life in the lambs (Alexander, 1962c). Alexander (1962c) concluded that before 3 days of age protein utilisation for energy was not fully developed. Also he found that at cold environmental temperatures hypoglycaemia was due to exhausted carbohydrate reserves and that the rate of gluconeogenesis from protein was too low to sustain life.

When starved and fed lambs were compared the basal metabolic rate was 37% higher and the summit metabolic rate 18% higher in the fed lambs (Eales & Small, 1980a). During basal metabolism, Eales & Small (1980a) also found that mean plasma levels of glucose and free fatty acids were significantly higher in the fed lambs, while during summit metabolism only the difference between glucose levels in the two groups of lambs remained significant. Eales & Small (1980a) showed that during summit metabolism carbohydrate was the major source of energy (respiratory quotient RQ=0.94) and suggested that availability of glucose may limit heat production in lambs. This is supported by Eales & Small (1981) who found that during the period from basal to summit metabolic rate in lambs aged only a few hours, there was a significant increase in respiratory quotient but no significant changes in plasma concentrations of urea or beta-hydroxybutyrate. They suggested that carbohydrate was the major fuel for heat production during summit metabolism and that fat and protein catabolism were of little significance (Eales & Small, 1981).

Starving calves survived for up to 10 days at temperatures below 11°C (Goodwin, 1957). The relatively longer period of survival than lambs may be due to their smaller surface area to body weight ratio (Alexander, 1962c). However, Okamoto *et al.* (1986) did not find any differences in resting or summit metabolic rate between calves that were fed and those that were starved, unlike the previously observations in lambs (Eales & Small, 1981). This though might have been due to the differing experimental set-ups used in the two studies. Lambs in the study by Eales & Small (1981) were 4.5 hours old when the metabolic rate readings started, while the calves of Okamoto *et al.* (1986) were between 2.5 and 15 hours of age. In addition the two studies had different feeding regimes, differing test procedures as well as different timing between feeding of colostrum and measuring summit metabolic rate.

In a study by Kinsbergen *et al.* (1994) it was observed that fasted calves that only received saline infusions, showed increased free fatty acid and urea concentrations and decreased plasma glucose concentrations. The authors assumed that, although lipid reserves in 1-week-old calves are relatively small [supported by Shannon & Lascelles (1966)], that the calves were obviously able to rapidly mobilise lipid for energy. Food withdrawal in their study was accompanied by those endocrine changes that would be expected for energy deficient animals. They found a marked decrease in insulin concentrations, while other hormones such as growth hormone and cortisol did not show any significant changes as these were shown to take several days to increase in fasted adult cattle (Kinsbergen *et al.*, 1994).

Passive Immunity

No passive transfer of immunoglobulins from the placenta to the bovine fetus takes place (Besser & Gay, 1994). Therefore, the ingestion of colostrum after birth is the only means for newborn calves to receive immunoglobulins for protection against infectious agents (Barrington *et al.*, 2001).

The four major classes of immunoglobulins in colostrum include IgG_1, IgG_2, IgM and IgA. IgM has a local effect in the gastrointestinal tract (GI) and acts against septicaemia, while IgA occurs in most external secretions and protects the respiratory and intestinal tracts (Wilkie, 1974). IgG_1 and IgG_2 are active in virus neutralisation, toxin neutralisation, bacterial agglutination and opsonisation (Wilkie, 1974). Although IgG_1 is the predominant immunoglobulin, the other classes are also of major importance for the well-being of the newborn calf (Besser & Gay, 1994).

Failure of immunoglobulin transfer

The transfer of colostral immunoglobulins to the systemic circulation of the newborn animal occurs via a non-selective macromolecular transport mechanism across the epithelium of the small intestine (Besser & Gay, 1994). Failure of this transfer may lead to some of the most common diseases in newborn calves, including septicaemia, diarrhoea and respiratory disease (Besser & Gay, 1994). Boyd *et al.* (1974) observed that calves that developed non-fatal diarrhoea and those that died had lower serum immunoglobulin levels than calves that were healthy, and calves that survived diarrhoea had higher immunoglobulin levels than those that did not. In a study by Campbell

(1974) lambs which were colostrum deprived were more likely to die and were less able to withstand exposure to *Escherichia coli* than lambs which were fed colostrum before or after *E.coli* exposure. Wittum & Perino (1995) reported that failure of passive immunisation was directly linked to a greater risk of health problems before and after weaning and was indirectly associated with poorer preweaning and feedlot growth performance through its effect on morbidity. They assumed that passive immunity allowed the controlled development of an active immune system and that this development in calves with low serum immunoglobulin levels may be overwhelmed by a large infectious challenge thus leading to future problems.

The failure of immunoglobulin transfer to the newborn calf may lead to an increased incidence of infectious diseases and mortality, and negatively influence growth, production and future performance of surviving animals. In a study by Robinson & Young (1988) it was shown that serum immunoglobulin concentrations at 24 and 48 hours after birth were significantly correlated with growth rate from birth to 180 days of age and hypogammaglobulinaemic calves were found to suffer increasing loss with each growth period through the first 180 days after birth. Proper growth and development during this period was shown to be critical for subsequent growth, development, reproduction and production (Robinson & Young, 1988). DeNise *et al.* (1989) found that cows that had the lowest Ig concentrations shortly after birth were culled extensively for low production reasons later on in life.

Factors affecting passive transfer

The most important factors influencing the acquisition of immunoglobulins are the timing of colostrum ingestion and the mass of colostrum ingested (Mohammed *et al.*, 1991), but dystocia, udder conformation and other factors are also important.

a) Timing of colostrum intake

The ability of the newborn to absorb immunoglobulins from the gastrointestinal tract rapidly declines after birth (Besser & Gay, 1994). By nine hours of age the absorptive capacity for immunoglobulins was about half that of eight hours earlier (Besser & Gay, 1994). Calves that had sucked within 6 hours postpartum achieved higher levels of all immunoglobulins in their serum than calves that did not suck during this period (Edwards *et al.*, 1982). The concentrations of all immunoglobulin classes were

negatively correlated with the time delay between birth and first sucking (Edwards *et al.*, 1982). A delay of colostrum intake to day 2 after birth markedly reduced globulin concentrations in comparison with calves that had colostrum intake on day 1 after birth (Hadorn *et al.*, 1997). In a New Zealand study by Wesselink *et al.* (1999), 48% of calves failed to suck within 6 hours after birth and this percentage was similar to the proportion of calves in which passive immunoglobulin transfer apparently failed. Mohammed *et al.* (1991) report that a large proportion of calves do not suck at all during the 12 to 24 hours before they are separated from their dams.

Gut closure, the cessation of the ability to absorb macromolecules via the epithelial cells of the small intestine (Besser & Gay, 1994), occurs at around 24 hours after birth, but there seems to be variation between studies, with some calves showing earlier closure (Patt, 1977), while it seems generally accepted that calves can absorb immunoglobulins for up to 36 hours after birth (Kruse, 1970a; Weaver *et al.*, 2000). Although Besser & Gay (1994) reported that gut closure was not affected by feeding or starvation, food intake does seem to play some role, as shown by Stott *et al.* (1979a), who found that closure occurred 2 to 3 hours earlier if colostrum feeding had taken place. Michanek *et al.* (1989) showed that calves given their first colostrum at 24 hours after birth could achieve acceptable levels of passive immunisation if not fed previously.

A delay before colostrum intake occurs will negatively influence the immunoglobulin status of the calf, and prolong the time for which the animal is unprotected while exposed to harmful micro-organisms.

b) Mass of Immunglobulins absorbed

The mass of immunoglobulins that is ingested by the calf is another factor of major importance for passive immunisation. If the absorption is not sufficient, then immunity will be poor even if the calf has sucked within a few hours after birth. Wittum & Perino (1995) report that calves with marginal levels of immunoglobulins obtained sufficient amounts to provide protection early during the neonatal period, but that levels may have declined steadily to values insufficient for later protection. Michanek *et al.* (1989) reported that calves need not necessarily have impaired transmission when they first suck and that low immunoglobulin levels after closure may be due to inferior transmission at later colostrum intake.

The mass of immunoglobulins ingested is determined by the volume of and concentration of immunoglobulins in ingested colostrum. In turn, colostral immunglobulin concentration itself is apparently a major factor in the rate of immunoglobulin absorption and the amount of absorption when fed to newborn calves (Stott & Fellah, 1983). There are differences in immunoglobulin concentration between breeds and in successive lactations (Mohammed *et al.*, 1991), with heifers having lower immunoglobulin concentrations than older cows (Kim *et al.*, 1983), and beef cows have higher colostral immunoglobulin concentrations than dairy cows (Besser & Gay, 1994).

Colostral immunoglobulin concentrations decrease with time after birth (Kruse, 1970b). In some cows, leakage of colostrum from the udder a few days before parturition may cause reduced immunoglobulin concentrations (and intakes) due to dilution of colostrum with milk (Petrie, 1984).

Protein and energy deprivation of the cow during late pregnancy may also have an effect on the immunoglobulin status of the calf, as this affects the volume of colostrum produced (Besser & Gay, 1994).

c) Dystocia and acidosis

Calves born after dystocia are often weak, may lack a sucking reflex and swallowing reflexes may be altered (Kasari, 1989). A reduced ability to absorb immunoglobulins and reduced calf vigour and sucking intensity may also occur in dystocic calves (Odde, 1988). Dystocic calves are at a higher risk of starvation and are more likely to receive inadequate colostral immunoglobulins (Kasari, 1989; Besser & Gay, 1994). Besser *et al.* (1990) found that respiratory acidosis may adversely affect immunoglobulin absorption even if adequate amounts of colostrum have been given early. However, calves were not sampled for IgG concentrations beyond 12 hours postpartum. In a study by Adams *et al.* (1995), serum immunoglobulin levels were not lower in calves with respiratory acidosis. They suggest that immunoglobulin absorption by individual calves was unpredictable. In addition, Weaver *et al.* (2000) report of a study where respiratory acidosis had no effect on serum IgG concentrations in calves at 13, 25 and 37 hours. They suggest that it may be possible that calves with metabolic or respiratory acidosis have the potential to absorb an adequate amount of immunoglobulin and that the high

rate of failure of passive transfer seen in these animals may be because they are less likely to get up and nurse in timely fashion.

d) Cow-calf interrelations

There seems to be evidence that calves left with their dams absorb immunoglobulins more efficiently than calves separated from their dams shortly after birth (Selman, 1973). Selman (1973) assumed that this might be a combined effect of earlier intake by calves staying with their mothers and possibly a greater colostrum intake. Stott *et al.* (1979b) also observed that early absorption and maximum absorption were greater in suckled calves rather than bottle-fed calves. They proposed that this might be due to a positive effect of colostrum intake on the pinocytotic activity in absorptive cells or an increased rate of transport of internalised immunoglobulins into the circulation. They also suggested that it may be possible that the stress of handling and separation experienced by calves removed from their mothers was not present, and that when such stress was present, this may have a negative effect on the absorption of immunoglobulins into the systemic circulation. However, Weaver *et al.* (2000) suggest that calves removed from the dam would be able to attain adequate amount of immunoglobulin if sufficient amounts of colostrum were fed. They considered that failure of passive transfer in naturally suckled calves would be greater because of the intake of inadequate volume and immunoglobulin mass.

Differences in cow behaviour between breeds may also affect the intake of colostrum. Beef cows seem to perform licking behaviour more vigorously and longer than dairy cows (Selman *et al.*, 1970a, b), which may lead to beef calves standing earlier and possibly sucking earlier compared to dairy calves which, in their turn, are often licked less vigorously or not at all (Selman *et al.*, 1970a, b).

e) Udder conformation

Although calves of older cows were observed to stand faster than those from heifers, sucking by these calves was delayed (Edwards, 1982). The dams' udder conformation and size may have contributed to this. Older cows were observed to be more likely to have pendulous udders and larger teats (Edwards & Broom, 1982). Derenbach *et al.* (1983) report that a high udder would lead to an earlier success of the calf finding the udder than a low udder in calves of multiparous cows. Calves of older cows were

observed to drink from only one quarter, which might have been due to the greater volume in cows' than heifers' udders or due to the fact that once sucking had occurred the teat became less swollen and easier to grip (Edwards & Broom, 1982). This could reduce the total intake of immunoglobulins due to the dilution of colostrum by milk in the one quarter that is emptied repeatedly. Kim *et al.* (1983) report that the front quarter of the udder has been shown to have less IgA and IgM levels than the hind quarters and that calves from younger dams were often found to suck predominantly from the front quarters. All of these factors may reduce the total intake and thus absorption of protective antibodies by the calves and increase their susceptibility to disease.

In contrast, Ventorp & Michanek (1992) found that, when adjustments were made for height above the ground, there was no significant effect of teat size on the time it took the calves to get the teat into their mouths. They also found that the shape of teats the animals managed to get into their mouths did not significantly differ from those they were unable to take into their mouths. However, the height of the udder from the ground did significantly affect the length of time a calf needed to search before it was able to suck for the first time (Ventorp & Michanek, 1992). Although the time to the first suck increased with lower udders, the delay might not be large enough to have any implications for the concentration of antibodies in colostrum or their intestinal absorption (Ventorp & Michanek, 1992). Nevertheless, if the delay is combined with other factors such as lack of maternal behaviour or low vitality of the calf, colostrum intake can be delayed considerably or might even be prevented (Ventorp & Michanek, 1992).

f) Environmental factors

Environmental factors also seem to have some effect on the passive immune status of the newborn calf. This is supported by studies by Stott *et al.* (1976) and Olson *et al.* (1980). Stott *et al.* (1976) showed that calves exposed to a hot and less desirable climate had lower serum immunoglobulin concentrations at days 2 and 10 after birth. Olson *et al.* (1980) reported that cold stressed newborn calves had a delay in onset of and a significant decrease in the rate of absorption of colostral immunoglobulins up to 15 hours after their first feed. They observed muscular weakness and reluctance to stand

and nurse in response to cold stress, which may cause a delay and decrease the total amount of colostrum ingested and absorbed.

A change of management system reduced calf mortality significantly even though plasma immunoglobulin concentration did not show an improvement (Boyd *et al.*, 1974). This suggests that not only the immunoglobulin status, but also the actual exposure to pathogens and possibly other environmental factors influence the welfare and survival of the newborn calf. It would also relate to an improved nutrient intake to support heat production and other metabolic processes.

Gut development

Vitamins

Vitamin A and beta-carotene were observed to enhance the immune system and provide protection against various diseases (Kume & Toharmat, 2001) and to have antioxidative properties (Blum *et al.*, 1997). The placental transfer of some vitamins is very limited and as colostrum is the primary source of these, their absorption from colostrum into the systemic circulation soon after birth is important (Kume & Toharmat, 2001). Kume & Toharmat (2001) found that calves suffering from diarrhoea showed lower plasma levels of vitamin A and beta-carotene. Although vitamin A was found to be transferred via the placenta during late pregnancy and was supplied in colostrum, beta-carotene was found to be low in calves at birth, so that the primary source of this vitamin was colostrum (Kume & Toharmat, 2001). Blum *et al.* (1997) report that calves had low levels of the fat-soluble vitamins beta-carotene, retinol and alpha-tocopherol in their tissues and blood due to limited transfer via the placenta, and that concentrations of these factors declined in colostrum from the first to the fourth milked colostrum sample. Thus, early intake of colostrum is important for passive immunity, and for the provision of adequate amounts of these vitamins. Calves fed colostrum 5 to 7 hours after birth had higher levels of fat-soluble vitamins up to seven days than calves that received colostrum no sooner than 24 hours after birth when colostrum of the same age and presumably immunoglobulin concentrations was fed in both groups (Blum *et al.*, 1997).

Colostral growth factors and hormones

Growth factors and hormones present in colostrum include epidermal growth factor (EGF), prolactin, insulin and insulin-like growth factors 1 and 2 (IGF-1 and IGF2)

(Mellor, 1992; Hammon & Blum, 1997; Blum & Hammon, 1999). Although these factors are barely absorbed in newborn calves, they seem to exert their effects primarily within the gastrointestinal tract, modifying gastrointestinal growth, differentiation and function (Blaettler *et al.*, 2001).

The developmental changes which are affected seem to include the development of the gastrointestinal tract and its functions, as well as digestive enzymes, gastrointestinal hormones and absorptive capacity (Blum & Hammon, 1999). Hammon & Blum (1997) observed that xylose absorptive capacity (used to evaluate absorptive capacity) was increased in calves after prolonged colostrum intake compared with calves fed only milk replacer. Blaettler *et al.* (2001) demonstrated that in calves fed colostrum, proximal duodenal villus size was increased during the first eight days after birth. They also assumed that their results indicated that maximising colostrum intake might reduce apoptosis of mucosal epithelial cells. Overall they concluded that colostrum intake differentially affected intestinal epithelial surface and proliferation as well as enzyme activities.

That the development of the gastrointestinal tract is affected by colostrum intake is also supported by studies in a range of species including pigs, sheep, dogs, rabbits, guinea pigs and human beings (Mellor & Stafford, 2003).

3.1.3 Infectious diseases

Diseases commonly encountered by calves

Septicaemia, diarrhoea and respiratory diseases are common in newborn calves (Besser & Gay, 1994). Rotavirus, coronavirus, enteropathogenic *E.coli* and cryptosporidia all play significant roles in the aetiology of calf diarrhoea (Snodgrass *et al.*, 1982), while *E.coli* and less frequently *Salmonella*, *Pasteurella* and *Leptospira* species are commonly associated with septicaemia (Aldridge *et al.*, 1993).

Septicaemia

Septicaemia is a disease state compounded of toxaemia, hyperthermia and the presence of large numbers of infectious microorganisms in the bloodstream (Blood and Radostits,

1989). Calves have been reported to be especially susceptible to septicaemia via the immature gut wall and the umbilicus (Webster, 1984). The umbilicus contains residual blood after birth, which is an ideal medium for bacterial growth (Schrag *et al.*, 1982). Also, sucking of the navel may enable invasion of bacteria, as it cannot dry out. As the navel remains connected to the blood circulation, bacteria may escape into the general circulation and cause generalised infections (Schrag *et al.*, 1982). Bacteraemia and localisation of infection may occur at joints, meninges, eyes, endocardium and end arteries of the feet, tail and ears (Blood and Radostits, 1989).

Once bacterial toxins affect the general circulation, damage can occur almost anywhere (Webster, 1984). In calves, septicaemia was found to lead to lethargy, poor sucking reflex, weakness and diarrhoea, nasal discharge and lameness (Aldridge *et al.*, 1993). Other common findings observed by Aldridge *et al.* (1993) included dehydration, cold extremities, inability or reluctance to rise as well as neurological, musculoskeletal and respiratory signs.

Diarrhoea

Enteritis, the inflammation of the intestinal tract, as well as enterotoxaemia, the infection of the wall of the intestinal tract by organisms that release toxins into the general circulation, may both lead to diarrhoea (Webster, 1984). *E. coli* species seem to be isolated from cases of enteritis most commonly, but seldom seem to be the primary cause of the disease (Webster, 1984), which is more likely to be viruses such as rota- and corona virus. *E.coli* species are thought of as opportunist pathogens colonising the intestinal wall after damage has occurred (Webster, 1984).

Rotavirus is usually confined to the gastrointestinal tract and attacks epithelial cells of the villi, especially in the duodenum. The cells subsequently die or slough off or become cuboidal or squamous so that the villi are stunted (Roy, 1990). Corona virus affects both the small and the large intestine and the infection in the small intestine is more in the ileum than the duodenum (Roy, 1990). All diarrhoeal viruses were reported to replicate in the epithelial cells of the small intestine, which results in a high degree of villous atrophy (Roy, 1990).

The period from first appearance of pathogens in the intestine and visible signs of the disease is between 12 and 14 hours (Schrag *et al.*, 1982). After the partial loss of intestinal mucosa, utilisation of food is reduced or no longer possible. The undigested food with its high milk protein content is an ideal growth medium for bacteria (usually *E.coli*) and the bacteria and their toxins can penetrate the mucosa damaged by viruses and pass into the blood stream (Schrag *et al.*, 1982). Webster (1984) states that *E.coli* seems to do by far the most harm, especially if it is a strain that causes severe enterotoxaemia. Enterotoxigenic *E.coli* cause diarrhoea during the first few days after birth, and strains that cause the disease have special attributes of virulence that allow them to colonise the small intestine, proliferate rapidly and produce an enterotoxin which causes hypersecretion of fluid into the intestinal tract (Roy, 1990).

Although calves may remain bright and continue to eat in uncomplicated cases of enteritis, in more severe cases diarrhoea may be accompanied by abdominal pain, while in cases of enterotoxaemia the calf may be ill to varying degrees (Webster, 1984). Dehydration commonly accompanies diarrhoea due to the increased water loss in the faeces. Loss of sodium and potassium ions, which contribute to blood volume maintenance and normal cellular function, is also a common feature in diarrhoeic calves (Webster, 1984).

Respiratory infections

Respiratory infections may vary from subclinical pneumonia to an acute fatal disease and may result from one or more micro-organisms interacting with several predisposing factors such as stress of being moved, environment or nutrition (Roy, 1990). Sudden changes in climate may affect the colonisation of the respiratory tract by micro-organisms partly due to increased survival rates of pathogens and partly due to reduced host resistance (Roy, 1990). The importance of factors such as air temperature, air movement, relative humidity, ammonia concentration and cubic capacity per calf may have to be elucidated (Roy, 1990). Symptoms may be variable, but there may be nasal discharge, discharge from the eyes, dry cough and respiratory distress (Roy, 1990). Enzootic pneumonia is an infectious disease primarily in housed calves and is characterised by varying degrees of severity of viral pneumonia with or without secondary bacterial bronchopneumonia and occurs most commonly in calves aged 2 to 5 months and calves during the first week after birth (Blood and Radostits, 1989).

The primary cause for pulmonary disease is an infection with one or more of a number of viruses and other organisms such as *Mycoplasma*. Primary damage to the lungs is often followed by secondary bacterial invasion including bacteria such as *Pasteurella haemolytica* (Webster, 1984; Roy, 1990). In the review by Roy (1990) he reported that in Germany, *Pasteurella haemolytica* infection was an independent disease involving severe dyspnoea and pyrexia (40.5-41°C) and that the peracute form was characterised by a septicaemia, the acute form by fibrinous pleuropneumonia and the subacute form by a purulent necrotic pneumonia.

Effects of infectious diseases on calf physiological status

Infectious diseases in calves may lead to inappetance, catabolic metabolism and enhanced nutrient and energy needs (Kinsbergen *et al.*, 1994). In addition, a number of other sickness behaviour patterns occur, including fever, increased time sleeping, social isolation, mental confusion and impaired memory and learning capacity (Gregory, 1998).

Pneumonia, as mentioned above, may lead to pyrexia (Roy, 1990) and anorexia may be observed (Blood and Radostits, 1989), which when prolonged may lead to starvation involving lipid and eventually protein catabolism should the animal survive. However, fever may be beneficial in the short term as it has several effects such as enhancing leucocyte proliferation, enhancing antibody production and increasing B lymphocyte responsiveness, which might aid in curtailing bacterial growth (Gregory, 1998). Enterotoxic colibacillosis leads to dehydration, electrolyte imbalances, acidosis, hyperkalemia when acidosis is severe, circulatory failure and eventually death (Blood and Radostits, 1989). Hypoglycaemia may occur in diarrhoeic calves, which may be brought about by *E.coli* endotoxin inhibiting glucogenesis in the liver or from depletion of glycogen from the liver (Roy, 1990). Anorexia may also be observed (Blood and Radostits, 1989). Again, this may lead to lipid and protein catabolism if the calf survives the disease long enough. Diarrhoea has been shown to lead to dehydration, hyponatraemia, uraemia, metabolic acidosis and hypocalcaemia (Groutides & Michell, 1990).

In lambs, the pathogenesis of the disease watery mouth is implicated with endotoxic shock including lactic acidaemia and hypoglycaemia (Hodgson *et al.*, 1989). It may be possible that septicaemic calves close to endotoxic shock thus display similar pathological signs.

Overall, infectious diseases can have a variety of physiological effect, many of which can be beneficial in aiding the animal to recover from the disease. Others effects, on the other hand, such as dehydration may lead to a rapid death, while anorexia will compromise the chance of survival of the infected animal over the longer term due to increased lipid and protein catabolism.

3.1.4 Aims of the present experiment

The aims of the present experiment were as follows:

- To determine the physiological status of calves from 1 to 4 days of age.
- To determine factors that may predict the incidence of disease and death in newborn calves of this age.

3.2 Materials and Methods

3.2.1 Animals

Cows and blood sampling

A blood sample was taken by tail vein venepuncture into sodium-heparin vacutainers (10ml, Becton Dickinson, Franklin Lakes, NJ, USA; Ref no. 366480, Lot 0263678, Expiry 2002-09) from 99 Friesian heifers and 397 Friesian and Jersey mixed-aged cows at Massey University Dairy Farm No.4 during July 2001, two weeks before the peak of the calving season. The heifers had been mated by Angus bulls and their calves were Angus crosses (hereafter referred to as Angus calves), while mixed aged Friesian cows were mated by Friesian bulls thus giving birth to Friesian calves. Eight Jersey cows were mated by Jersey bulls. As only one Jersey calf was studied, it was included in the group of calves of mixed-aged cows. Of the 496 cows and heifers eight could not be identified and 15 missed a blood sample. The blood samples were put on ice immediately after being taken and were centrifuged at 3000rpm for 15 minutes using a Heraeus Sepatech centrifuge (Labofuge 400e, Biolab Scientific, NZ). The plasma of the centrifuged samples was pipetted, using disposable plastic pipettes (Samco ® transfer pipettes; cat No. 225, purchase ID. *+H56822533*; Samco Scientific Corp., San Fernando, Ca., USA) into two 1.5ml Eppendorff tubes (RayLab NZ Ltd., Auckland, NZ; code P4010-03, item code: 10301013, Batch 0 28 88). About 0.5ml of plasma was put into each tube. The remainder of the plasma was placed into a 5 ml screw top vial (Sarstedt, SA, Australia; No. 60.9921.532, CH-B/lot No.00976, Exp 11-2003, sterile). The samples were stored on ice in an upright position and were then frozen deep at -50°C until required for analysis.

Calves, blood sampling and management

Calves born to cows during the period of the 6th August to 27th August 2001 were picked up daily between 10am and 1pm according to farm practice, and cows that had calved were shifted from the calving paddock to the "colostrum mob". The calves were taken to a calf shed, where calves from all Massey University Dairy farms were kept. The shed consisted of a series of pens with a roof and walls on the back and the sides. The front of the pens was open as were the partitions between pens. Calves were bedded

on wood shavings that were replaced with fresh shavings at the beginning of the calving season and regularly during the trial. Calves had free access to water.

In the afternoon after pick-up, calves to be studied until day 4 after birth were identified. This was determined by referring to calf PCV, weight, rectal temperature, time to stand and general appearance as well as maternal body condition score (see below for details). Some calves were chosen when values seemed normal, while others were chosen to be followed up as they looked weak or strong or showed values that seemed very high or very low in the above parameters. Of the 35 assisted calves (Chapter 2), 21 calves were also chosen for follow-up. Also, 9 calves were chosen that became sick or died during the 4 days of follow-up. In all, 126 calves were chosen to be studied until four days of age.

These calves had a jugular blood sample taken using sodium-heparin vacutainers (see cows above for details) and rectal temperature was measured using a digital thermometer. The first blood samples and rectal temperatures were taken in the afternoon before feeding but after the calves were picked up from the field. Following this, samples were taken every morning and afternoon before feeding usually until four days of age. The morning samples were usually taken between 6 and 8am while the afternoon samples were taken between 1.30 and 3pm. Blood samples were spun in a refrigerated centrifuge (Heraeus Minifuge T, Biolab Scientific, NZ) at 3000rpm for 15 minutes and plasma was pipetted in the same fashion as for cows (see above). Samples were frozen in a deep freeze at –50°C until required for analysis.

Each calf was offered 2 litres of colostrum, or if this was not available, whole milk, twice daily. Sick calves were separated from healthy ones, and calves that did not drink satisfactorily were separated from calves drinking well. The incidence of disease and death was recorded for calves during the 4 days of follow-up and veterinary records on the incidence of disease and death were obtained from the farm veterinarian for a period of 1.5 months after the calves were studied. However, the records did not include animals that were sold, or animals that got sick but were not reported to the veterinarian. Information on which calves were sold and the date of sale was obtained from the farm supervisor.

Calves that did not become sick or die while they stayed on the farm have been referred to as 'healthy calves'. Calves that got sick and subsequently recovered between birth and 1.5 months of age have been referred to here as 'sick calves', while calves that died during this period have been referred to as 'dying calves'.

3.2.2 Plasma analyses

Cows

Blood samples from cows were only analysed if their calves had their blood samples analysed as well (see below). Altogether, 106 cow blood samples were analysed for plasma glucose, urea and beta-hydroxybutyrate concentrations on a Cobas Fara 2 analyser. Glucose was determined by the use of the Roche unimate7 kit no. 0736740 according to Trinder (1969). Urea was measured using the Roche unimate7 kit no. 0736856 (Talke & Schubert, 1965). Beta-hydroxybutyrate was determined using a sigma diagnostics kit, procedure No.310-UV (Williamson *et al.*, 1962).

Calves

Healthy calves were selected for study in the following manner. They included calves, which had the 20 highest and 20 lowest values in the following birth parameters: PCV, vigour, weight, rectal temperature, plasma lactate concentration, plasma glucose concentration and maternal body condition score (see Chapter 2). Only those calves that were healthy during the 4 days of follow-up, that were not assisted at birth and which did not have any missing values in any of the variables were included. Thus blood samples were analysed from 77 healthy animals. Of these animals, samples taken closest to 24, 48, 72 and 96 hours of age were analysed.

There were 21 assisted calves, 8 sick calves and one calf that died (between birth to 4 days) and samples taken closest to 24, 48, 72 and 96 hours of age were analysed for these animals.

Overall, the 24-hour samples were taken at mean time after birth of 25.1 ± 0.5 (SEM) hours, the 48-hour samples at 48 ± 0.4 hours, the 72-hour samples at 72.5 ± 0.4 hours and the 96-hour samples at 91.4 ± 0.6 hours after birth.

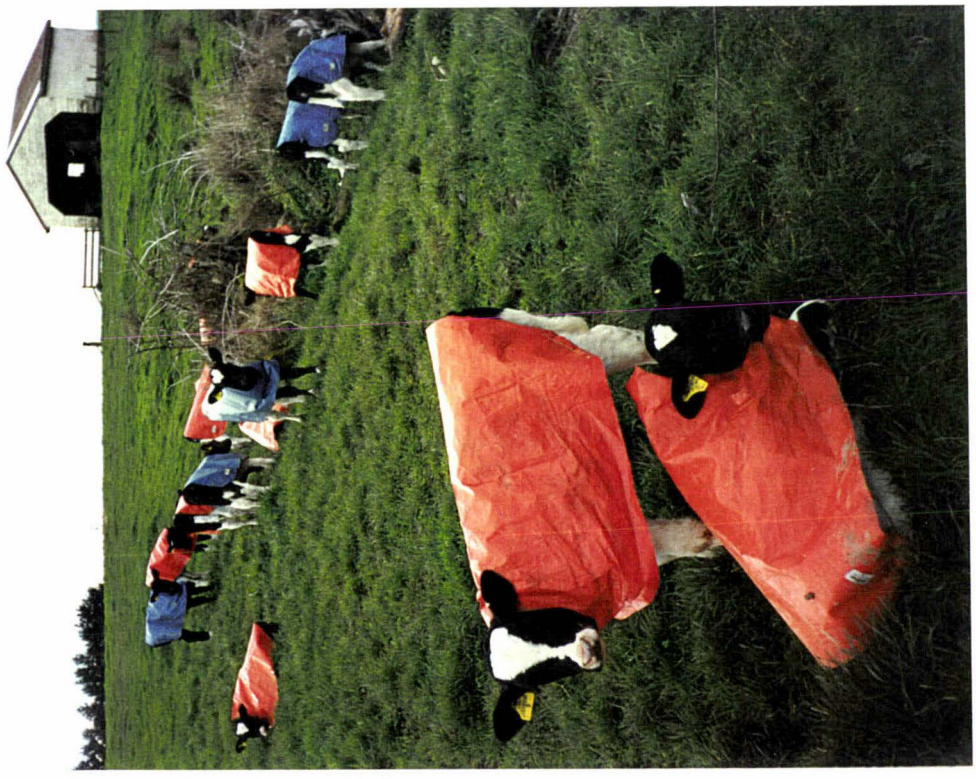
All blood samples were analysed for plasma glucose, urea, beta-hydroxybutyrate and gamma-glutamyl transferase (GGT) concentrations on a Cobas Fara 2 analyser. Glucose was determined by the use of the Roche unimate7 kit no. 0736740 according to Trinder (1969). Urea was measured using the Roche unimate7 kit no. 0736856 (Talke & Schubert, 1965). Beta-hydroxybutyrate was determined using a sigma diagnostics kit, procedure No.310-UV (Williamson *et al.*, 1962). Lastly, gamma-glutamyl transferase (GGT) activity was measured by using a Roche diagnostics kit cat. No. 2016788 (Shaw *et al.*, 1983).

Gamma-glutamyl transferase

Gamma-glutamyl transferase (GGT) is a membrane-associated protein involved in amino acid transport and GGT activity in milk and colostrum is high (Thompson & Pauli, 1981). Low total plasma protein and gamma globulin levels are apparently concurrent with low serum GGT, which suggested a relationship between colostrum ingestion and serum GGT (Thompson & Pauli, 1981). Braun *et al.* (1982) reported that plasma GGT activity was an easy and inexpensive way of testing colostrum intake. Perino *et al.* (1993) showed that GGT activity was high in calves that had sucked colostrum. They also found that, although GGT did not reflect the amount of colostrum and immunoglobulins consumed, a degree of activity greater than 200 IU/L suggested that the calf had consumed colostrum. Thus, calves, which have been referred to as having had low GGT levels in the following sections, are those in which GGT activity was below 200 IU/L, while those referred to as having high GGT levels were the calves with GGT activity above 200 IU/L.

Photograph e: Calf shed

Photograph f and g: Calves in shed and older calves in the field wearing calf coats.



Photograph h and i: Taking the rectal temperature and a blood sample from calves in the shed.

3.2.3 Statistical analyses

A repeated measures ANOVA was undertaken for assisted versus unassisted calves, healthy, sick and dying calves, calves that had high (>200IU/L) and low (<200IU/L) plasma GGT concentrations 24 hours after birth (hereafter referred to as calves with high and low plasma GGT concentrations) and calves that had their first blood sample taken in the shed when younger than 24 hours versus those which had their first blood sample taken in the shed when aged 24 hours old or older. These were not necessarily the 24-hour samples that were analysed, because some 24-hour samples were the second sample that was taken in the shed, as this was closer to 24 hours.

As no data were available on the health status of calves after they were sold, the healthy calves included only those calves that were not sold and remained healthy between birth and 1.5 months of age (n=23) (see Table A1.20 in Appendix 1 for further details).

The comparison between calves sampled at ages younger and older than 24 hours was undertaken to determine if there were significant differences in the parameters measured between calves depending on how much time they spent in the vicinity of their mothers. Staying with the mother for a longer period may have an important impact on colostrum intake or may be beneficial due to other factors. Subsequently, calves that had their first sample taken in the shed at an age younger than 24 hours will be referred to as 'calves younger than 24 hours at pick-up' and those that had their first sample taken at an older age than 24 hour will be referred to as 'calves older than 24 hours at pick-up'. However, the exact age at pick-up is not known. It has to be mentioned that although those calves might have been brought into the shed earlier, the blood sample that was analysed might not have been the first one taken before the first feed in the shed. It might have been the one taken before the feed the following morning, depending on which was closer to 24 hours. Thus, some of the calves that were brought into the shed at a younger age may not have sucked their mothers, but might have had a suck in the shed when offered food. Thus, it may be possible that an even greater percentage of calves that were brought into the shed earlier might not have had a feed when with their mothers.

Breed was also included in the repeated measures ANOVA model. The variables included plasma glucose, beta-hydroxybutyrate, urea and GGT concentrations as well as rectal temperature. The plasma concentrations of urea, beta-hydroxybutyrate and GGT were log-transformed to normalise the data, whereas plasma glucose and rectal temperature did not need to be transformed.

As unadjusted p-values were used to determine significant differences between groups as well as differences within the groups, significant levels were changed such that for example, in a repeated measures ANOVA where 16 comparisons were made, the cut-off point for significance was 0.003 (0.05/16). Thus any p-value greater than 0.003 did not indicate a significant difference. The unadjusted p-values are presented in Appendix 1.

Included in the glucose and rectal temperature repeated measures ANOVAs were the birth samples reported in Chapter 2.

Comparisons between groups were restricted to the same blood samples at one time only, for example the group one 24-hour sample versus the group two 24-hour sample; the group one 72-hour sample versus the group two 72-hour sample. Mean values for the five parameters in the different groups were graphed and standard errors calculated by the repeated measures ANOVA (standard errors depicted in the graphs are only useful for comparisons between groups not within groups). The means and standard errors for log urea, log beta-hydroxybutyrate and log GGT levels were converted to arithmetic values before graphing.

An ANCOVA was undertaken to determine if rectal temperatures measured in the morning and afternoon during the four days of follow-up were related to air temperature and weather condition. Air temperatures at the times of sampling were noted retrospectively from temperatures recorded at AgResearch, Palmerston North. The same was true for rainfall and solar radiation.

Three calves had a calf coat fitted for a few days in the shed. A one-way ANOVA was undertaken to determine if there were significant differences in rectal temperatures

between calves with coats (3) and those without (100). This may determine if coats are useful in resuscitating moderately hypothermic calves (none of the calves followed up were shown to have extremely low temperatures).

A one-way ANOVA was undertaken to determine if there were significant differences in body condition score and plasma glucose, urea and beta-hydroxybutyrate concentrations of the cows that gave birth to different classes of calves: between assisted and unassisted calves of the different breeds, and between healthy, sick or dying calves.

A Canonical Discriminant Analysis (CDA) was undertaken to determine if any of the parameters measured would be able to predict which calves may become sick or die. The variables glucose 96-hour, urea 96-hour, beta-hydroxybutyrate 96-hour and temperature at 91 hours were not included due to the high number of missing values at these sampling times. Also, only the 24-hour GGT sample was used for this analysis as means and standard deviations showed that GGT concentrations decreased over the 4 days and therefore, low concentrations on day one would have led to low concentrations over the following days in the same animal. Thus, GGT in the first sample would give an indication of the intake of colostrum by the calves in the present study.

3.2.4 Presentation of results

The results of the present study are presented first according to the parameters measured, however the summary of the results, presented last, is arranged by timing of events. The summary of results is itself divided into two parts, namely the results for the first four days after birth followed by the results from calves after that period to 1.5 months of age. The results of day 1 to day 4 after birth include all calves, while those of day 4 up to 1.5 months of age only include the calves that remained on the farm of origin as well as those that became sick before they were sold. Figures and tables in the results section include all calves that did not have missing values in any of the parameters, however the ones for healthy, sick and dying calves only included those calves that remained on the farm after the four days of examination or were sold after becoming sick.

3.3 Results

Of the 106 calves that were followed up between day 1 and day 4 after birth, 28 calves were Angus and 78 Friesian. Overall, 15 calves got sick and 8 calves died between birth and 1.5 months of age. Of the assisted calves, 21 were followed up and three of those subsequently became sick (2) or died (1). Overall, pneumonia was diagnosed in one calf, rotavirus in four calves, navel and joint ill in three calves, rotavirus as well as navel ill in one calf and enteritis in one calf. There were eight calves with diarrhoea of unknown cause, one with respiratory problems and four that appeared ill. Three calves had calf coats during the four days of follow up due to low rectal temperature upon arrival at to the calf shed.

Table 3.3.1 shows the percentages of healthy, sick and dying calves; calves assisted at birth; calves which had low or high GGT levels in the first jugular blood sample taken in the shed and the percentage of the unassisted and assisted calves that had low or high GGT levels. Of the calves that remained at the farm of origin or were sold after being sick, 50% of calves remained healthy, 17% died and 33% became sick. The majority of sick and dying calves (87 and 88%, respectively) were unassisted. Of the calves with low GGT concentrations 54% died. Of the calves with high GGT concentration, about 39% became sick while about 3% died.

The majority of calves were sampled for the first time at an age younger than 24 hours and thus were younger than 24 hours at pick-up (Table 3.3.2). Of the calves that died subsequently, 88% were younger than 24 hours at pick-up, compared with 73% of sick calves. 87% of calves over 24 hours of age at pick-up had high GGT concentrations compared with 57% of calves younger than 24 hours at pick-up. Also, there was a higher percentage of assisted calves among those older than 24 hours at pick-up (30%) compared to those younger than 24 hours at pick-up (13%).

Table 3.3.1: Percentages of healthy, sick and dying calves (not sold and remaining healthy for the first 1.5 months of age) in the various groups.

		Healthy Calves			Sick Calves			Dying Calves		
Group		*n*	*Group*	*Healthy (23)*	*n*	*Group*	*Sick (15)*	*n*	*Group*	*Dead (8)*
	Total	23	50.0%	100%	15	32.6%	100%	8	17.4%	100%
	Angus	3	6.5%	13.0%	3	6.5%	20.0%	1	2.2%	12.5%
	Friesian	20	43.5%	87.0%	12	26.1%	80.0%	7	15.2%	87.5%
Assisted	Total	3	50.0%	13.0%	2	33.3%	13.3%	1	16.7%	12.5%
	Angus	2	33.3%	8.7%	2	33.3%	13.3%	0	0	0
	Friesian	1	16.7%	4.3%	0	0	0	1	16.7%	12.5%
Unassisted	Total	20	50.0%	87.0%	13	32.5%	86.7%	7	17.5%	87.5%
	Angus	1	2.5%	4.3%	1	2.5%	6.7%	1	2.5%	12.5%
	Friesian	19	47.5%	82.6%	12	30.0%	80.0%	6	15%	75%
GGT >200IU/L	Total	19	57.6%	82.6%	13	39.4%	86.7%	1	3.0%	12.5%
	Angus	3	9.1%	13.0%	3	9.1%	20%	0	0	0
	Friesian	16	48.5%	69.6%	10	30.3%	66.7%	1	3.0%	12.5%
GGT <200IU/L	Total	4	30.8%	17.4%	2	15.4%	13.3%	7	53.8%	87.5%
	Angus	0	0	0	0	0	0	1	7.7%	12.5%
	Friesian	4	30.8%	17.4%	2	15.4%	13.3%	6	46.2%	75.0%
Assisted+GGT high	Total	3	60.0%	13.0%	2	40.0%	13.3%	0	0	0
	Angus	2	40.0%	8.7%	2	40.0%	13.3%	0	0	0
	Friesian	1	20.0%	4.3%	0	0	0	0	0	0
Assisted+GGT low	Total	0	0	0	0	0	0	1	100%	12.5%
	Angus	0	0	0	0	0	0	0	0	0
	Friesian	0	0	0	0	0	0	1	100%	12.5%
Unassisted+GGT high	Total	16	57.1%	69.6%	11	39.3%	73.3%	1	3.6%	12.5%
	Angus	1	3.6%	4.3%	1	3.6%	6.7%	0	0	0
	Friesian	15	53.6%	65.2%	10	35.7%	66.7%	1	3.6%	12.5%
Unassisted+GGT low	Total	4	33.3%	17.4%	2	16.7%	13.3%	6	50.0%	75.0%
	Angus	0	0	0	0	0	0	1	8.3%	12.5%
	Friesian	4	33.3%	17.4%	2	16.7%	13.3%	5	41.7%	62.5%

Table 3.3.2: Percentage of calves, younger than 24 hours or older than 24 hours at pick-up in the various groups. (Healthy calves included those remaining healthy for the first four days and being sold shortly thereafter and those remaining healthy between 12 hours and 1.5 months of age on the farm of origin).

	Healthy and sold calves (including 18 assisted calves)	*Sick calves (including 2 assisted calves)*	*Dying calves (including 1 assisted calf)*	*Assisted calves*	*Calves with high GGT levels*	*Calves with low GGT*
82 calves aged younger than 24 hours at pick-up	63/82	12/82	7/82	11/82	58/82	24/82
% of calves sampled at a younger age	77%	15%	8%	13%	71%	29%
24 calves older than 24 hours at pick-up	20/24	3/24	1/24	7/24	20/24	4/24
% of calves sampled at an older age	83%	13%	4%	30%	83%	17%
Total	**83**	**15**	**8**	**18**	**78**	**28**

	83 Healthy and sold calves (including 11 assisted calves)	*15 Sick calves (including 2 assisted calves)*	*8 Dying calves (including 1 assisted calf)*	*18 Assisted calves*	*78 Calves with high GGT levels*	*28 Calves with low GGT*
Calves younger than 24 hours at pick-up	63/83	12/15	7/8	11/18	58/78	24/28
% of calves within each groups	76%	80%	88%	61%	74%	86%
Calves older than 24 hours at pick-up	20/83	3/15	1/8	7/18	20/78	4/28
% of calves within each groups	24%	20%	12%	39%	26%	14%

3.3.1 Calf variables

3.3.1.1 Glucose

Means and standard errors for plasma glucose concentrations for all groups of calves are shown on Table 3.3.4 (page 130).

Assisted versus unassisted calves

The p-values for the repeated measures ANOVA for glucose comparing assisted and unassisted calves are shown on Table 3.3.3. No significant between-group differences existed overall or over time (Figure 3.3.1). Breed differences were also not apparent. There was a large time effect.

Table 3.3.3: Results of repeated measures ANOVA (p-values) for glucose.

Effect	Assisted versus unassisted calves
Breed	0.531
Time	<0.0001
*Time*breed*	0.103
Assisted	0.132
*Breed*assisted*	0.951
*Time*assisted*	0.258

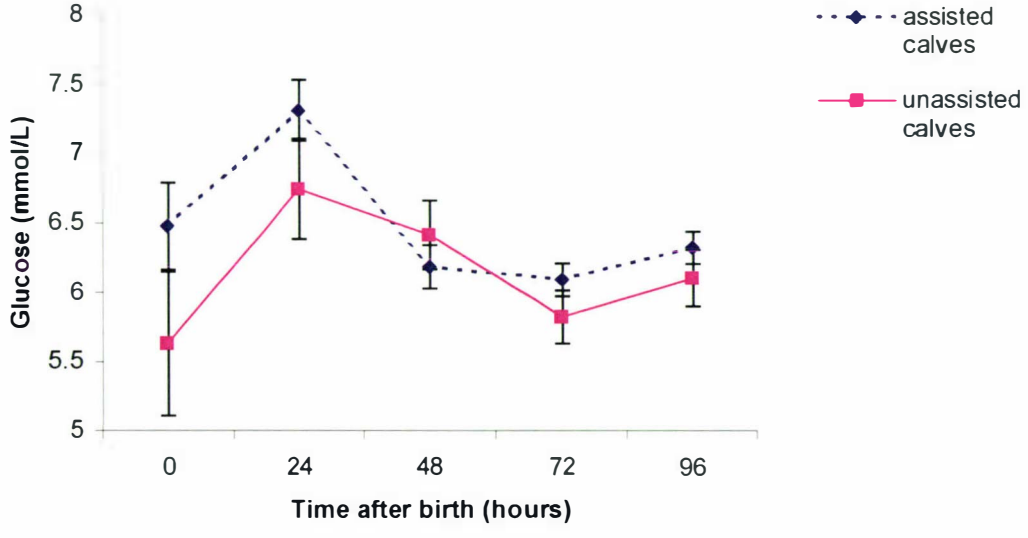


Figure 3.3.1: Means and standard errors for the plasma glucose concentrations in assisted (n= 21) and unassisted (n=85) calves calculated by repeated measures ANOVA.

Table 3.3.4: Means and standard errors of the mean (SEM) for plasma glucose concentrations (mmol/L) of the various groups (n=number of calves).

	Birth Mean (n)	SEM	24hours Mean (n)	SEM	48hours Mean (n)	SEM	72hours Mean (n)	SEM	96hours Mean (n)	SEM
Assisted calves	6.48 (21)	0.32	7.31 (21)	0.22	6.18 (21)	0.15	6.09 (21)	0.12	6.32 (16)	0.11
Unassisted calves	5.63 (85)	0.52	6.74 (84)	0.36	6.40 (83)	0.25	5.82 (84)	0.19	6.10 (79)	0.21
Healthy calves	5.24 (83)	0.45	7.29 (85)	0.38	6.64 (82)	0.24	5.99 (83)	0.24	6.47 (78)	0.31
Sick calves	5.69 (15)	0.50	6.81 (15)	0.42	6.06 (15)	0.26	5.90 (15)	0.25	5.83 (11)	0.29
Dying calves	6.37 (8)	0.69	6.40 (8)	0.60	5.55 (7)	0.40	5.44 (7)	0.39	5.92 (6)	0.43
Calves younger than 24hours at pick-up	6.30 (82)	0.30	6.92 (82)	0.20	6.25 (81)	0.14	6.10 (82)	0.11	6.28 (74)	0.11
Calves older than 24 hours at pick-up	6.10 (24)	0.49	7.84 (24)	0.33	6.29 (23)	0.24	5.79 (23)	0.18	6.26 (21)	0.18
High GGT	6.04 (78)	0.28	7.54 (78)	0.18	6.45 (78)	0.14	6.26 (78)	0.10	6.47 (72)	0.10
Low GGT	6.81 (28)	0.45	6.05 (28)	0.29	5.67 (26)	0.22	5.32 (27)	0.17	5.65 (23)	0.17

Although there were no significant between-group differences over time, some significant differences were observed within one group at different times after birth (Table A1.1 in Appendix 1). The plasma glucose concentration at the 24 hours in unassisted calves was significantly higher those at 48, 72 and 96 hours after birth.

Calves with high and low plasma GGT concentrations

The p-values for the repeated measures ANOVA for glucose comparing calves with high and low plasma GGT concentrations are shown on Table 3.3.5. There were significant differences between the two groups overall and over time. Time also had a significant effect, however, breed did not have an effect. Calves with high plasma GGT concentrations had significantly higher glucose levels at 24, 48, 72 and 96 hours than calves with low GGT concentrations (Figure 3.3.2; Table A1.2 in Appendix 1).

Table 3.3.5: Results of repeated measures ANOVA (p-values) for glucose.

Effect	Calves with high versus calves with low plasma GGT concentrations
Breed	0.919
Time	<0.0001
*Time*breed*	0.171
GGT	0.0003
*Breed*GGT*	0.809
*Time*GGT*	0.0006

Calves with high plasma GGT levels had significantly lower plasma glucose concentrations at birth than at 24 hours and significantly higher plasma glucose levels at 24 hours than at 48, 72 and 96 hours after birth (see Table A1.2 in Appendix 1). Calves with low plasma GGT concentrations had significantly lower plasma glucose concentrations at 96 hours after birth than at birth (Table A1.2 in Appendix 1).

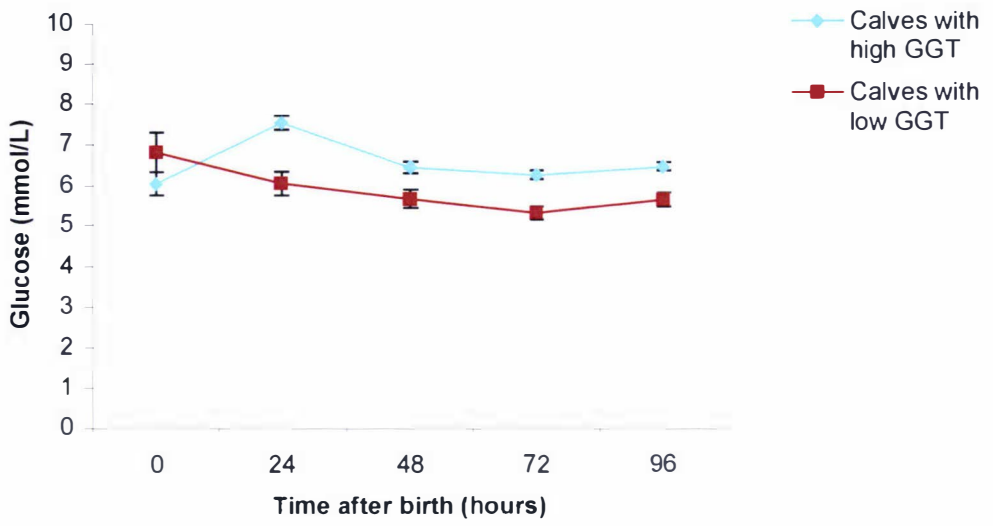


Figure 3.3.2: Means and standard errors for the plasma glucose concentrations in calves with high (n=78) and low (n=28) plasma GGT concentrations calculated by repeated measures ANOVA.

Healthy, sick and dying calves

There were no significant differences in plasma glucose concentrations overall and over time between healthy, sick and dying calves (Table 3.3.6). Breed also did not have an effect on plasma glucose concentrations. Dying calves though seemed to have had the lowest glucose levels of the three groups after 24 hours, although the difference was not significant (Figure 3.3.3). There was a time effect.

Table 3.3.6: Results for repeated measures ANOVA (p-values) for glucose.

Effect	Healthy, sick and dying calves
Breed	0.435
Sick	0.532
Time	0.024
*Sick*breed*	0.679
*Time*breed*	0.483
*Sick*time*	0.121

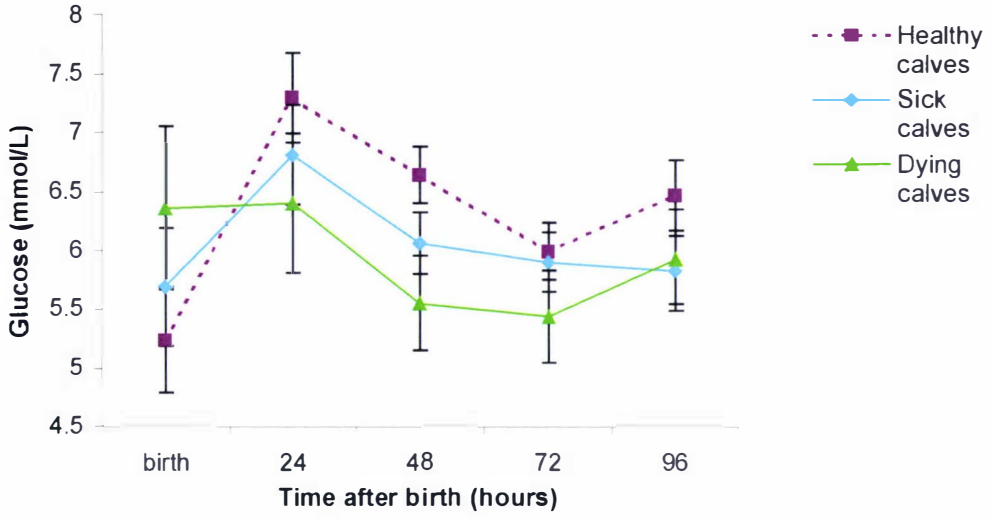


Figure 3.3.3: Means and standard errors plasma glucose concentrations in healthy (n=23), sick (n=15) and dying (n=8) calves calculated by repeated measures ANOVA.

Although there were no significant between-group differences over time, some significant differences were observed within the group of healthy calves between different times of sampling (Table A1.3 in Appendix 1). The plasma glucose concentration was significantly lower at birth than at 24 and 48 hours (marginally), and the concentration at 24 hours was significantly higher than at 72 hours after birth.

Calves younger and older than 24 hours at pick-up

Although there were no significant between-group differences in plasma glucose concentrations overall, there was a significant difference between the two groups over time (Table 3.3.7). Breed did not have an effect on plasma glucose concentrations. Figure 3.3.4 shows that plasma glucose concentrations between the two groups were different at 24 hours, however this was not significant (p-value=0.0165; Table A1.4 in Appendix 1).

Table 3.3.7: Results of repeated measures ANOVA (p-values) for glucose.

Effect	Calves younger and older than 24 of age at pick-up
Breed	0.784
Age	0.670
Time	<0.0001
*Age*breed*	0.489
*Breed*time*	0.131
*Age*time*	0.031

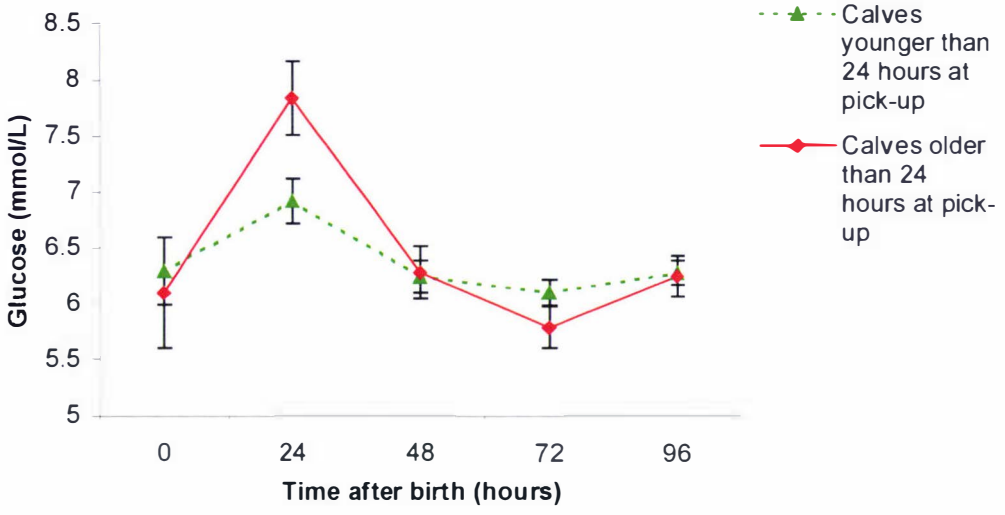


Figure 3.3.4: Means and standard errors for plasma glucose concentrations in calves younger (n=82) and older (n=24) than 24 hours at pick-up calculated by repeated measures ANOVA.

There were also some significant differences within the two groups between different times of sampling (Table A1.4 in Appendix 1). In the calves younger than 24 hours at pick-up the plasma glucose concentrations were higher at 24 than at 48 and 72 hours. Calves older than 24 hours at pick-up had significantly lower plasma glucose levels at birth than at 24 hours and the 24-hour concentration was also significantly higher than the values at 48, 72 and 96 hours.

3.3.1.2 Beta-hydroxybutyrate

Means and standard errors for plasma beta-hydroxybutyrate concentrations for all groups of calves are shown on Table 3.3.9 (page 136).

Assisted versus unassisted calves

There were no significant differences in plasma beta-hydroxybutyrate concentrations over time, but overall, assisted calves had significantly higher (p-value= <0.0001) plasma beta-hydroxybutyrate levels than unassisted calves (Figure 3.3.5). Breed did not have a significant effect on plasma beta-hydroxybutyrate levels in the two groups (Table 3.3.8).

Table 3.3.8: Results of repeated measure ANOVA (p-values) for beta-hydroxybutyrate.

Effect	Assisted versus unassisted calves
Breed	0.328
Time	0.082
*Breed*time*	0.668
Assisted	<0.0001
*Breed*assisted*	0.181
*Assisted*time*	0.363

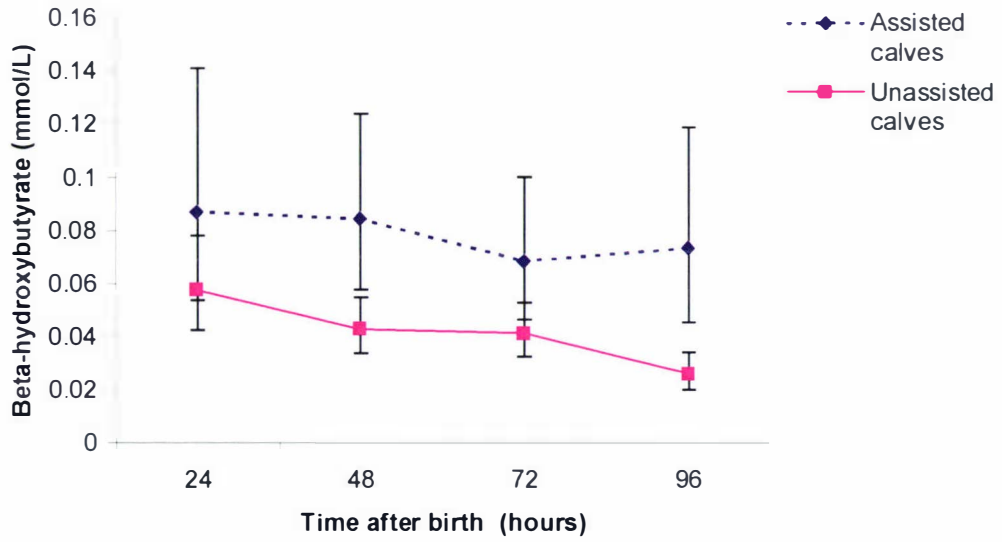


Figure 3.3.5: Means and standard errors for plasma beta-hydroxybutyrate concentration in assisted (n=21) and unassisted (n=85) calves calculated by repeated measures ANOVA.

Significant between-group differences in plasma beta-hydroxybutyrate concentration were present at 48 hours (marginal; p-value 0.0047) and 96 hours (p-value = 0.0004) (Table A1.5 in Appendix 1). In unassisted calves, the plasma concentration was significantly higher at 24 hours than at 96 hours.

Table 3.3.9: Means and standard errors of the mean (SEM) for plasma beta-hydroxybutyrate concentrations (mmol/L) of the various groups (n=number of calves). [As these values were transformed back form a log transformation, two SEM are given (+ and -)].

	24hours Mean (n)	-SEM	+SEM	48hours Mean (n)	-SEM	+SEM	72hours Mean (n)	-SEM	+SEM	96hours Mean (n)	-SEM	+SEM
Assisted calves	0.09 (21)	0.03	0.05	0.08 (21)	0.03	0.04	0.07 (21)	0.02	0.03	0.07 (16)	0.03	0.04
Unassisted calves	0.06 (85)	0.01	0.02	0.04 (83)	0.009	0.01	0.04 (84)	0.009	0.01	0.03 (79)	0.006	0.008
Healthy calves	0.12 (83)	0.04	0.06	0.07 (82)	0.02	0.04	0.05 (83)	0.02	0.03	0.06 (78)	0.03	0.04
Sick calves	0.09 (15)	0.03	0.05	0.06 (15)	0.03	0.04	0.08 (15)	0.03	0.04	0.07 (11)	0.0016	0.02
Dying calves	0.10 (8)	0.05	0.10	0.06 (7)	0.03	0.06	0.06 (7)	0.03	0.06	0.05 (6)	0.02	0.05
Calves younger than 24hours at pick-up	0.11 (82)	0.02	0.02	0.05 (81)	0.01	0.01	0.05 (82)	0.009	0.01	0.07 (74)	0.008	0.0009
Calves older than 24 hours at pick-up	0.06 (24)	0.02	0.02	0.07 (23)	0.02	0.03	0.06 (23)	0.02	0.02	0.07 (21)	0.01	0.02
High GGT	0.09 (78)	0.01	0.02	0.06 (78)	0.01	0.01	0.05 (78)	0.01	0.01	0.07 (72)	0.01	0.009
Low GGT	0.12 (28)	0.01	0.04	0.05 (26)	0.01	0.02	0.06 (27)	0.01	0.02	0.06 (23)	0.008	0.01

Calves with high and low plasma GGT concentrations

Although there were no overall differences in plasma beta-hydroxybutyrate concentrations between the two groups, there were significant differences in beta-hydroxybutyrate levels over time (Table 3.3.10; Figure 3.3.6; Table A1.6 in Appendix 1). There was a highly significant time effect, but breed did not have an effect.

Table 3.3.10: Results of repeated measure ANOVA (p-values) for beta-hydroxybutyrate.

Effect	Calves with high GGT versus low plasma GGT concentrations
Breed	0.921
Time	<0.0001
*Breed*time*	0.785
GGT	0.9913
*Breed*GGT*	0.461
*GGT*time*	0.023

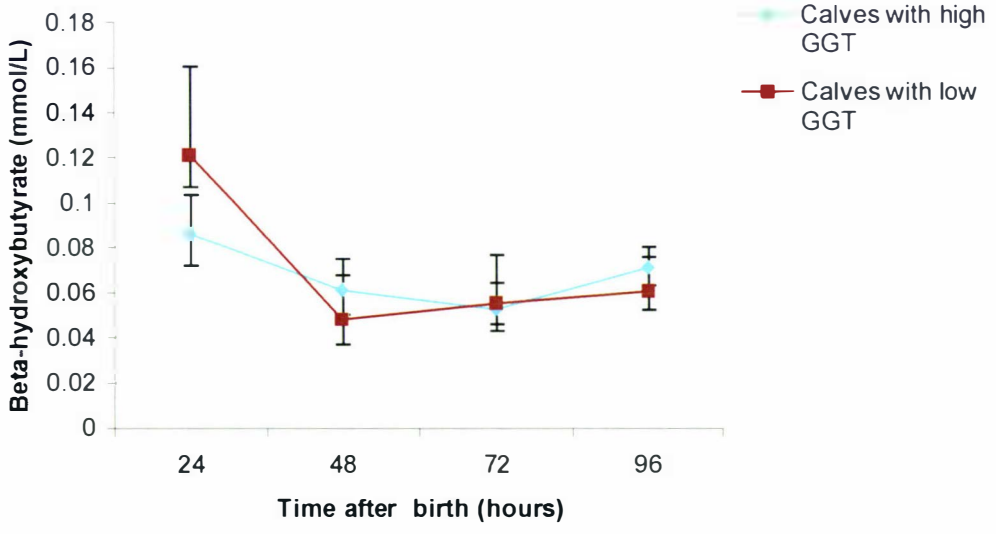


Figure 3.3.6: Means and standard errors for plasma beta-hydroxybutyrate concentrations in calves with high (n=78) and low (n=28) GGT concentrations calculated by repeated measures ANOVA.

Calves with high plasma GGT concentrations had significantly higher plasma beta-hydroxybutyrate concentrations at 24 than at 72 hours after birth. Calves that had low plasma GGT concentrations had significantly higher beta-hydroxybutyrate levels at 24 hours than at 48, 72 and 96 hours after birth.

Healthy, sick and dying calves

There were no significant differences in plasma beta-hydroxybutyrate concentrations between healthy, sick and dying calves overall and over time (Table 3.3.11; Figure 3.3.7). Also, breed did not have a significant effect. There were no significant differences within the three groups over time (Table A1.7 in Appendix 1). Time on its own had a significant effect.

Table 3.3.11: **Results of repeated measure ANOVA (p-values) for beta-hydroxybutyrate.**

Effect	Healthy, sick and dying calves
Breed	0.496
Time	0.013
*Breed*time*	0.563
Sick	0.808
*Breed*sick*	0.875
*Sick*time*	0.296

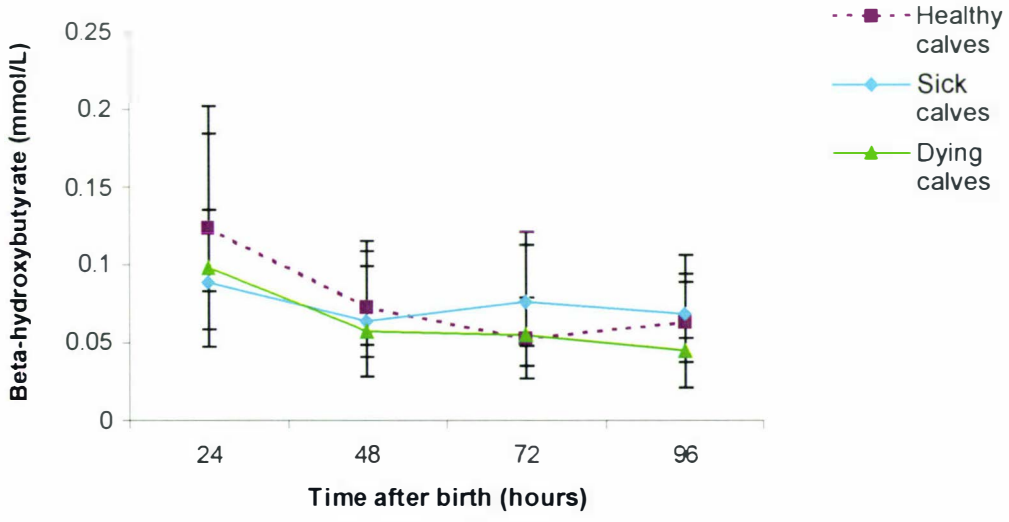


Figure 3.3.7: Means and standard errors for plasma beta-hydroxybutyrate concentrations in healthy (n=23), sick (n=15) and dying (n=8) calves calculated by repeated measures ANOVA.

Calves younger and older than 24 hours at pick-up

There was a significant difference in beta-hydroxybutyrate over time between the two groups, but not overall (Table 3.3.12). Breed did not have any effect on the plasma beta-hydroxybutyrate concentrations in the two groups. Time had a significant effect.

Table 3.3.12: Results of repeated measure ANOVA (p-values) for beta-hydroxybutyrate.

Effect	Calves younger and older than 24 at pick-up
Breed	0.428
Time	0.007
*Breed*time*	0.718
Age	0.820
*Breed*age*	0.505
*Age*time*	0.005

As shown by Figure 3.3.8 and Appendix 1 (Table A1.8), the difference between the two groups over time was primarily due to the difference at 24 hours and was only marginal (p-value=0.0078, where the cut-off value for significance is 0.003). Within the calves that were younger than 24 hours at pick-up, plasma beta-hydroxybutyrate concentration was significantly higher at 24 hours than at 48, 72 and 96 hours (Table A1.8 in Appendix 1).

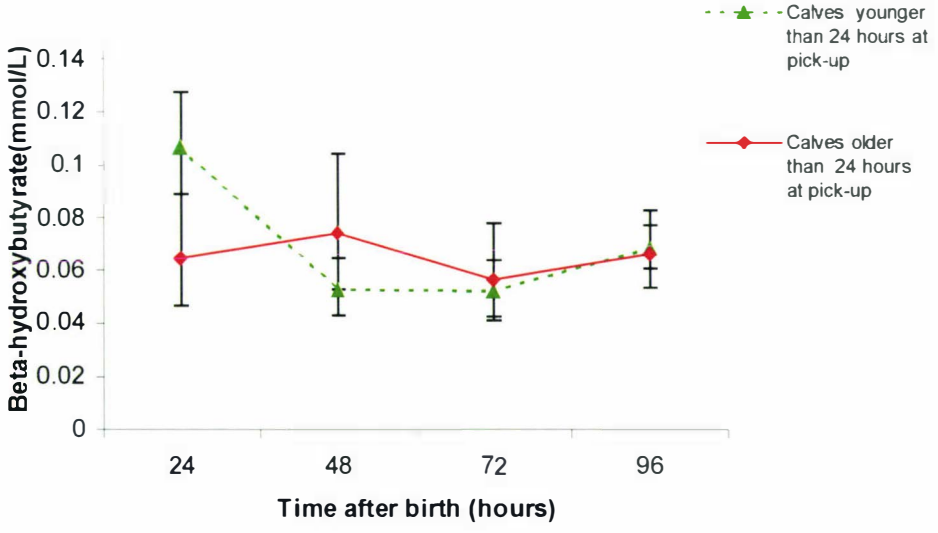


Figure 3.3.8: Means and standard errors for plasma beta-hydroxybutyrate concentrations in calves younger (n=24) and older (n=82) than 24 hours at pick-up calculated by repeated measures ANOVA.

3.3.1.3 Urea

Means and standard errors for plasma urea concentrations for all groups of calves are shown on Table 3.3.14 (page 142).

Assisted versus unassisted calves

There were no significant differences in plasma urea concentrations between assisted and unassisted calves overall or over time (Table 3.3.13). There was a large time effect (see Figure 3.3.9). Breed did not have an effect on plasma urea concentrations in the two groups (Table 3.3.13).

Table 3.3.13: Results of repeated measure ANOVA (p-values) for urea.

Effect	Assisted versus unassisted calves
Breed	0.311
Time	<0.0001
*Breed*time*	0.165
Assisted	0.695
*Breed*assisted*	0.898
*Assisted*time*	0.108

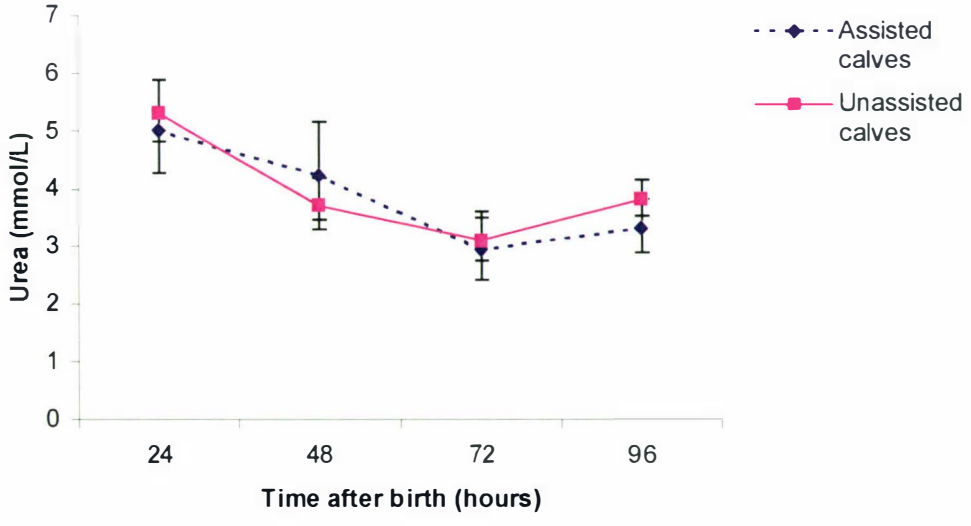


Figure 3.3.9: Means and standard errors for plasma urea concentrations in assisted and unassisted calves calculated by repeated measures ANOVA.

Although there were no significant between-group differences in plasma urea concentrations, differences could be found within the groups at different sampling times

(Table A1.9 in Appendix 1). In the assisted calves the plasma urea concentration at 24 hours was significantly higher than at 72 and 96 hours, and the 48-hour concentration was higher than the value at 72 hours. In unassisted calves the plasma urea concentrations was significantly higher at 24 hours that at 48, 72 and 96 hours. Also, the 48-hour concentration of unassisted calves was significantly higher than the value at 72 hours, and the 72-hour concentration was significantly lower than the value at 96 hours.

Table 3.3.14: Means and standard errors of the mean (SEM) for plasma urea concentrations (mmol/L) of the various groups (n=number of calves). |As these values were transformed back form a log transformation, two SEM are given (+ and -)|.

	24hours Mean (n)	-SEM	+SEM	48hours Mean (n)	-SEM	+SEM	72hours Mean (n)	-SEM	+SEM	96hours Mean (n)	-SEM	+SEM
Assisted calves	5.00 (21)	0.74	0.87	4.22 (21)	0.77	0.93	2.94 (21)	0.53	0.65	3.32 (16)	0.43	0.50
Unassisted calves	5.31 (85)	0.51	0.56	3.71 (83)	0.42	0.47	3.10 (84)	0.35	0.39	3.82 (79)	0.29	0.32
Healthy calves	4.95 (83)	0.90	1.10	3.71 (82)	0.96	1.30	2.60 (83)	0.68	0.91	3.39 (78)	0.67	0.83
Sick calves	5.26 (15)	1.04	1.29	3.94 (15)	1.08	1.48	3.16 (15)	0.86	1.19	4.06 (11)	0.74	0.90
Dying calves	4.76 (8)	1.03	1.79	3.10 (7)	1.14	1.81	2.48 (7)	0.92	1.45	3.06 (6)	0.84	1.56
Calves younger than 24 hours at pick-up	5.47 (82)	0.42	0.46	3.74 (81)	0.42	0.48	3.03 (82)	0.29	0.32	3.78 (74)	0.29	0.31
Calves older than 24 hours at pick-up	4.71 (24)	0.62	0.71	4.35 (23)	0.79	0.96	3.29 (23)	0.54	0.65	3.56 (21)	0.40	0.45
High GGT	5.26 (78)	0.40	0.44	4.01 (78)	0.46	0.52	3.13 (78)	0.30	0.33	3.74 (72)	0.29	0.31
Low GGT	5.05 (28)	0.66	0.76	3.32 (26)	0.60	0.74	2.89 (27)	0.48	0.57	3.53 (23)	0.40	0.45

Calves with high and low plasma GGT concentrations

There were no significant differences in plasma urea concentrations between the two groups of calves overall or over time (Table 3.3.15; Table A1.10 in Appendix 1). There was a large time effect (see Figure 3.3.10). Breed did not have an effect on plasma urea concentrations in the two groups (Table 3.3.15).

Table 3.3.15: Results of repeated measure ANOVA (p-values) for urea.

Effect	Calves with high GGT versus low plasma GGT concentrations
Breed	0.256
Time	<0.0001
*Breed*time*	0.381
GGT	0.181
*Breed*GGT*	0.694
*GGT*time*	0.284

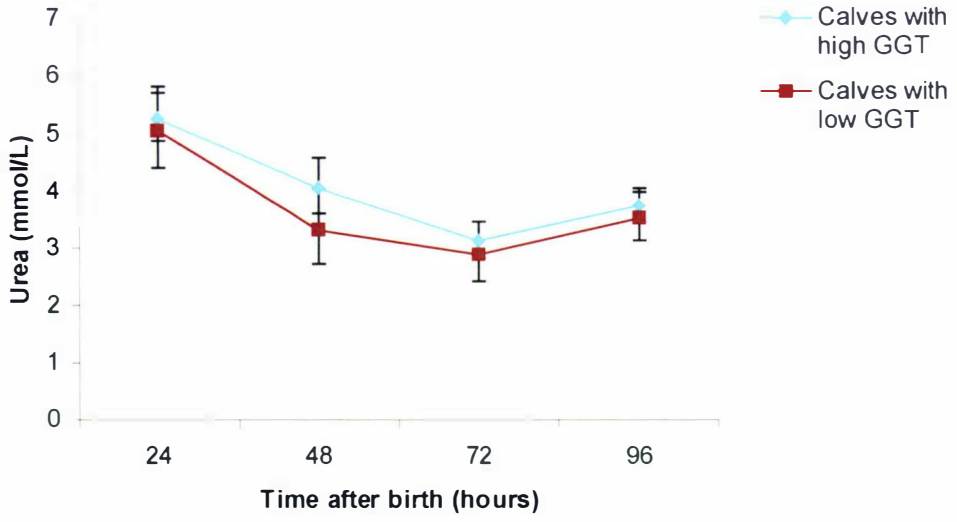


Figure 3.3.10: Means and standard errors for plasma urea concentrations in calves with high (n=78) and low (n=28) plasma GGT concentrations calculated by repeated measures ANOVA.

There were significant differences in plasma urea concentrations within the two groups (Table A1.10 in Appendix 1). Both, calves with high and low plasma GGT concentrations had significantly higher plasma urea levels at 24 hours than at 48, 72 and 96 hours. Calves with high GGT levels also had significantly higher levels at 48 hours than at 72 hours and significantly lower urea levels at 72 than at 96 hours.

Healthy, sick and dying calves

There were no significant differences in plasma urea concentrations between healthy, sick and dying calves overall and over time (Table 3.3.16). This can also be seen in Figure 3.3.11. Breed did not have an effect on plasma urea concentrations in the two groups. Time itself had a large significant effect. The plasma urea concentration in healthy calves was significantly higher at 24 hours than at 72 and 96 hours (Table A1.11 in Appendix 1). Also the urea level in sick calves was significantly higher at 24 hours than at 72 hours.

Table 3.3.16: Results of repeated measure ANOVA (p-values) for urea.

Effect	Healthy, sick and dying calves
Breed	0.725
Time	<0.0001
*Breed*time*	0.924
Sick	0.466
*Breed*sick*	0.655
*Sick*time*	0.929

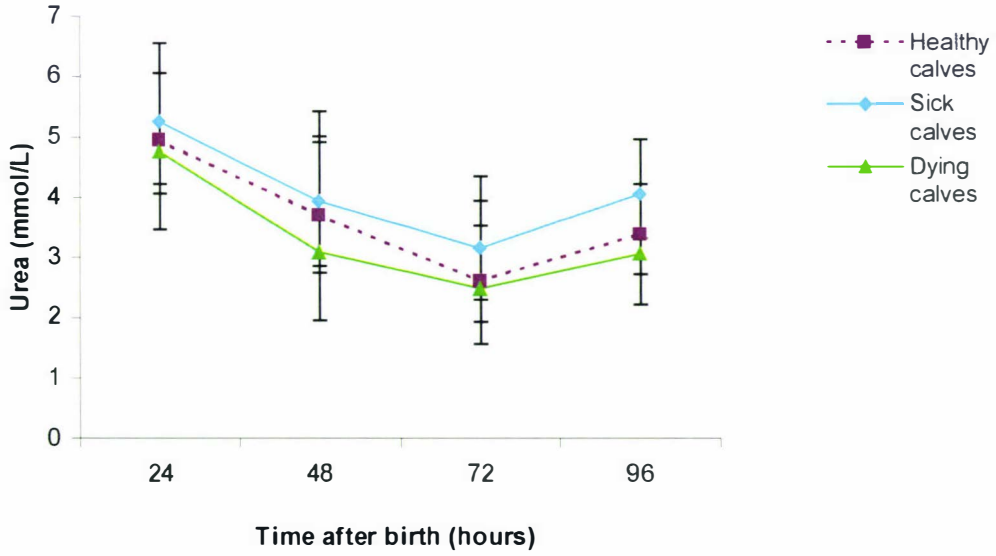


Figure 3.3.11: Means and standard errors for plasma urea concentrations in healthy (n=23), sick (n=15) and dying (n=8) calves calculated by repeated measures ANOVA.

Calves younger and older than 24 hours at pick-up

There was a significant difference between the two groups over time, but not overall. Breed had a significant effect overall as well as in combination with age at the first sample in the shed (Table 3.3.17). There were no significant differences between the groups in plasma urea concentrations (Figure 3.3.12 and Table A1.12 in Appendix 1). Time had a large significant effect.

Angus calves had significantly lower plasma urea concentrations than Friesian calves of this group (p-value=0.019). The significant p-value for the breed*age distinction was brought about by a significant difference in plasma urea concentration between Friesians and Angus calves which had their first blood sample taken at an age older than 24 hours (p-value=0.0043).

Table 3.3.17: Results of repeated measure ANOVA (p-values) for urea.

Effect	Calves younger and older than 24 of age at pick-up
Breed	0.019
Time	<0.0001
*Breed*time*	0.162
Age	0.934
*Breed*age*	0.015
*Age*time*	0.010

Although there were no significant differences between the two groups in plasma urea levels, there were significant differences within groups over time (Table A1.12 in Appendix 1). In the calves aged younger than 24 hours at pick-up, plasma urea concentration was significantly higher at 24 hours than at 48, 72 and 96 hours. The concentration at 48 hours was significantly higher than at 72 hours and the 72-hour concentration was significantly lower than that at 96 hours. In the group of calves older than 24 hours at pick-up, the urea concentration was significantly higher at 24 hours than at 72 and 96 hours, and the 48-hour value was significantly higher than that at 72 hours.

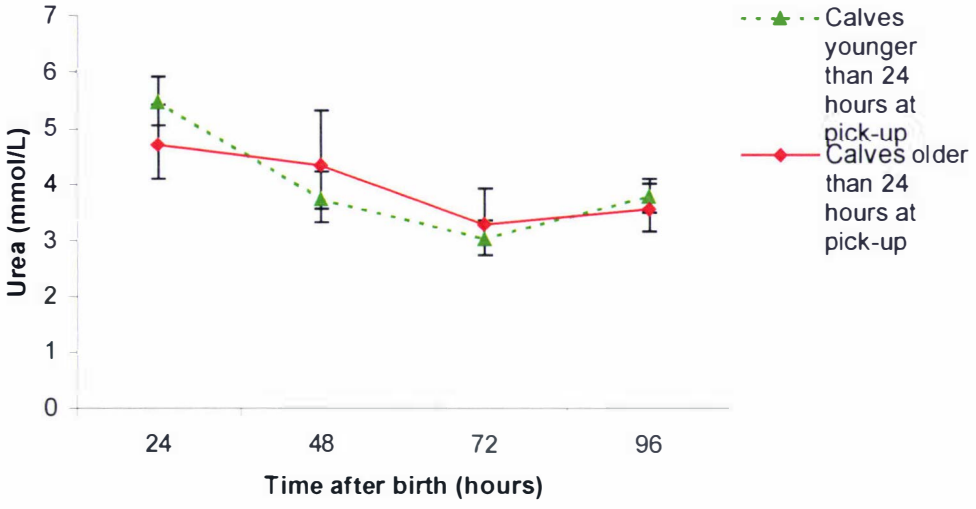


Figure 3.3.12: Means and standard errors for plasma urea concentrations in calves younger and older than 24 hours at pick-up calculated by repeated measures ANOVA.

3.3.1.4 Gamma glutamyl transferase

Means and standard errors for plasma GGT concentrations for all groups of calves are shown on Table 3.3.19 (page 148).

Assisted versus unassisted calves

There were no significant differences in plasma GGT concentrations between assisted and unassisted calves overall and over time (Table 3.3.18). Breed did not have a significant effect. In general, assisted calves had lower plasma GGT concentrations than unassisted calves, but the difference was not significant (Figure 3.3.13).

Table 3.3.18: Results of repeated measure ANOVA (p-values) for GGT.

Effect	Assisted versus unassisted calves
Breed	0.351
Time	<0.0001
*Breed*time*	0.929
Assisted	0.427
*Breed*assisted*	0.857
*Assisted*time*	0.318

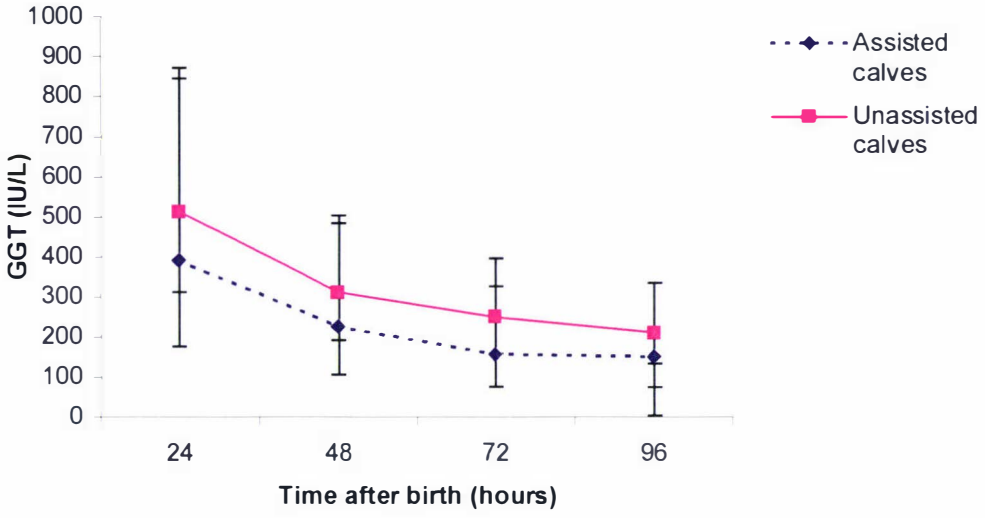


Figure 3.3.13: Means and standard errors for plasma GGT concentrations in assisted (n=21) and unassisted (n=85) calves calculated by repeated measures ANOVA.

Although there were no significant differences in plasma GGT concentrations between assisted and unassisted calves over time, there were significant differences within the groups over time (Table A1.13 in Appendix 1). In both assisted and unassisted calves, the GGT concentrations were significantly higher at 24 hours than at 48, 72 and 96 hours, and the 48-hour value was significantly greater than those at 72 and 96 hours. Also, in unassisted calves the value at 72 hours was significantly higher than that at 96 hours.

Table 3.3.19: Means and standard errors of the mean (SEM) for plasma GGT concentrations (IU/L) of the various groups (n=number of calves). [As these values were transformed back form a log transformation, two SEM are given (+ and -)].

	24hours Mean (n)	-SEM	+SEM	48hours Mean (n)	-SEM	+SEM	72hours Mean (n)	-SEM	+SEM	96hours Mean (n)	-SEM	+SEM
Assisted calves	392 (21)	216	480	226 (21)	120	257	156 (21)	81.6	171.0	150 (16)	77	158
Unassisted calves	513 (85)	202	333	311 (83)	119	192	250 (84)	92.0	146	211 (79)	78	123
Healthy calves	889 (83)	542	1387	498 (82)	295	726	351 (83)	211	529	351 (78)	208	512
Sick calves	982 (15)	614	1635	659 (15)	401	1027	384 (15)	237	619	395 (11)	558	231
Dying calves	30 (8)	24	114	29 (7)	22	104	22 (7)	17	79	16 (6)	13	54
Calves younger than 24 hours at pick-up	428 (82)	158	250	257 (81)	92	142	196 (82)	67	103	178 (74)	61	93
Calves older than 24 hours at pick-up	742 (24)	388	814	446 (23)	225	452	344 (23)	170	335	268 (21)	132	261

Healthy, sick and dying calves

There were significant differences in plasma GGT concentrations between the three groups overall (Figure 3.3.14), but not over time (Table 3.3.20). There was no effect of breed, but there was a significant overall time effect.

Table 3.3.20: Results of repeated measure ANOVA (p-values) for GGT.

Effect	Healthy, sick and dying calves
Breed	0.501
Time	<0.0001
*Breed*time*	0.122
Sick	0.002
*Breed*sick*	0.840
*Sick*time*	0.105

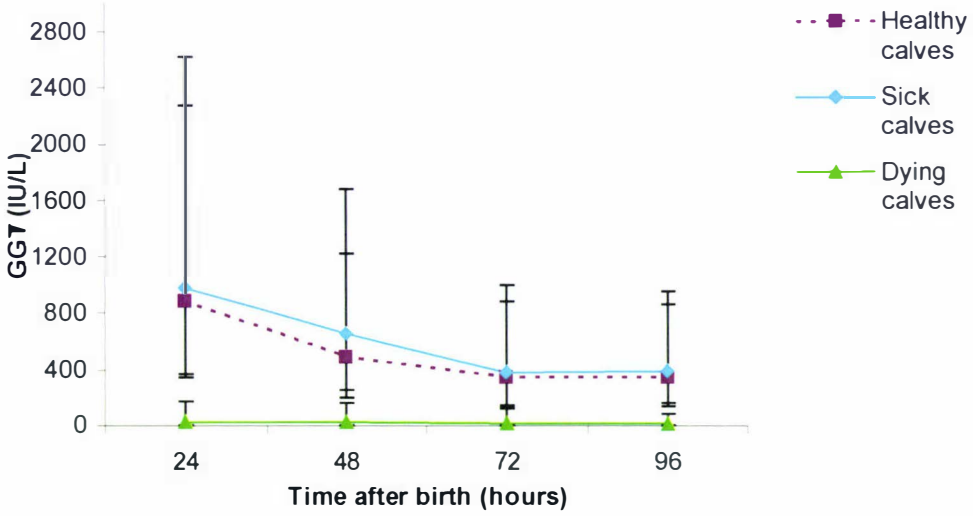


Figure 3.3.14: Means and standard errors for plasma GGT concentrations in healthy (n=23), sick (n=15) and dead (n=8) calves calculated by repeated measures ANOVA.

Appendix 1 (Table A1.14) shows the p-values for the significant differences between GGT levels between healthy, sick and dying calves. The values for GGT concentrations were significantly different between sick and dying calves and healthy and dying calves at all ages. The differences between sick and dying calves at 72 hours and those between healthy and dying calves at 48 and 72 hours were only marginally significant. However, there were no significant differences between healthy and sick calves. Also, Appendix 1 (Table A1.14) shows that there were significant differences within the

groups of healthy and sick calves. In healthy calves plasma GGT concentrations were significantly higher at 24 hours than at 48, 72 and 96 hours. In sick calves the GGT level at 24 hours was also significantly higher than that at 72 and 96 hours. Interestingly, the sick calves exhibited wide variation in plasma GGT concentrations (see standard error bars Figure 3.3.14).

Calves younger and older than 24 hours at pick-up

There were no significant differences in plasma GGT concentrations between the two groups overall and over time (Table 3.3.21, Figure 3.3.15). Also, breed did not have an effect on plasma GGT concentrations in the two groups. Time on its own had a significant effect.

Table 3.3.21: Results of repeated measure ANOVA (p-values) for GGT.

Effect	Calves younger and older than 24 hours at pick-up
Breed	0.996
Time	<0.0001
*Breed*time*	0.696
Age	0.196
*Breed*age*	0.349
*Age*time*	0.545

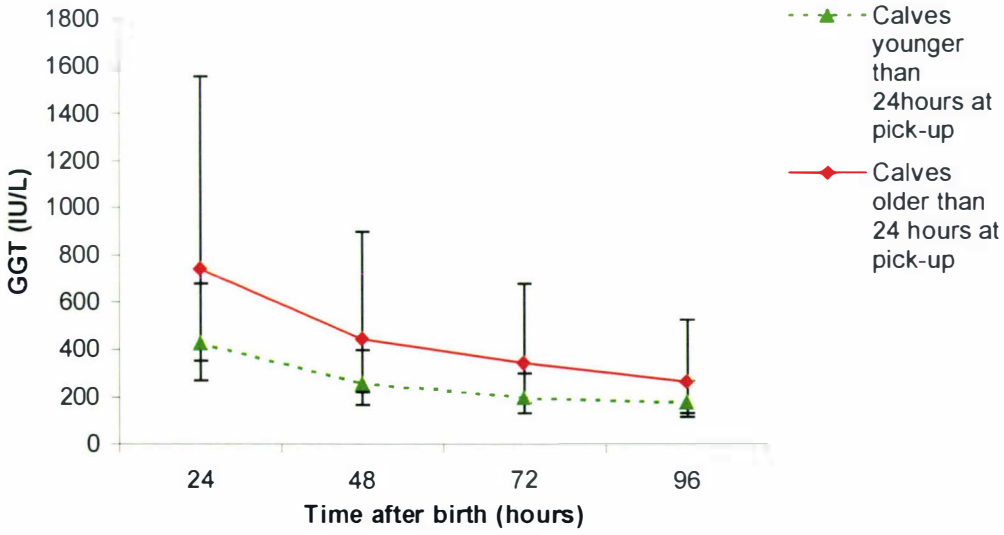


Figure 3.3.15: Means and standard errors for plasma GGT concentrations in calves younger (n=24) and older (n=82) than 24 hours at pick-up calculated by repeated measures ANOVA.

Although there were no significant differences between the two groups over time, there were some differences within the two groups over time (Table A1.15 in Appendix 1). In calves younger than 24 hours at pick-up there was a significant decrease in plasma GGT concentration between 24 and 48 hours, 48 and 72 hours, and 72 and 96 hours. In the calves older than 24 hours at pick-up the plasma GGT concentration at 24 hours was greater than those at 48, 72 and 96 hours, and the value at 48 hours was also significantly greater than the 96-hour value.

3.3.1.5 Rectal Temperature

Means and standard errors for rectal temperature for all groups of calves are shown in Table 3.3.23 (page 153).

All rectal temperature samples were included in the statistical analysis and were taken at the following times: rectal temperature sample 1 was taken at birth, sample 2 was taken at a mean time after birth of 19.4 ± 0.7 (SEM) hours, sample 3 at 35.6 ± 0.7 hours, sample 4 at 43.3 ± 0.7 hours, sample 5 at 59.6 ± 0.7 hours, sample 6 at 67.3 ± 0.7 hours, sample 7 at 83.2 ± 0.7 hours and sample 8 at 91.4 ± 0.8 hours after birth.

Therefore, the rectal temperature results will be referred to as the following: birth sample rectal temperature sample at 0 hours, sample 2 at 19 hours, sample 3 at 36 hours, sample 4 at 43 hours, sample 5 at 60 hours, sample 6 at 67 hours, sample 7 at 83 hours and sample 8 at 91 hours after birth.

Assisted versus unassisted calves

There was no significant difference between unassisted and assisted calves in rectal temperature overall, but there was a significant difference in rectal temperature between the two groups over time (Table 3.3.22). Also, breed had a small significant effect (p-value=0.04). It is apparent from Figure 3.3.16 that the significant difference between groups probably arose from between-group differences in rectal temperature at 19, 43 and 91 hours.

In unassisted calves, rectal temperature was significantly lower at 19 hours than at 60, 67, 83 and 91 hours (Table A1.16 in Appendix 1). The rectal temperature at 36 hours was also significantly lower than at 60, 67, 83 and 91 hours and the temperature at 43 hours was significantly lower than at 91 hours.

Table 3.3.22: Results of repeated measure ANOVA (p-values) for rectal temperature.

Effect	Assisted versus unassisted calves
Breed	0.244
Time	<0.0001
*Breed*time*	0.509
Assisted	0.533
*Breed*assisted*	0.040
*Assisted*time*	0.025

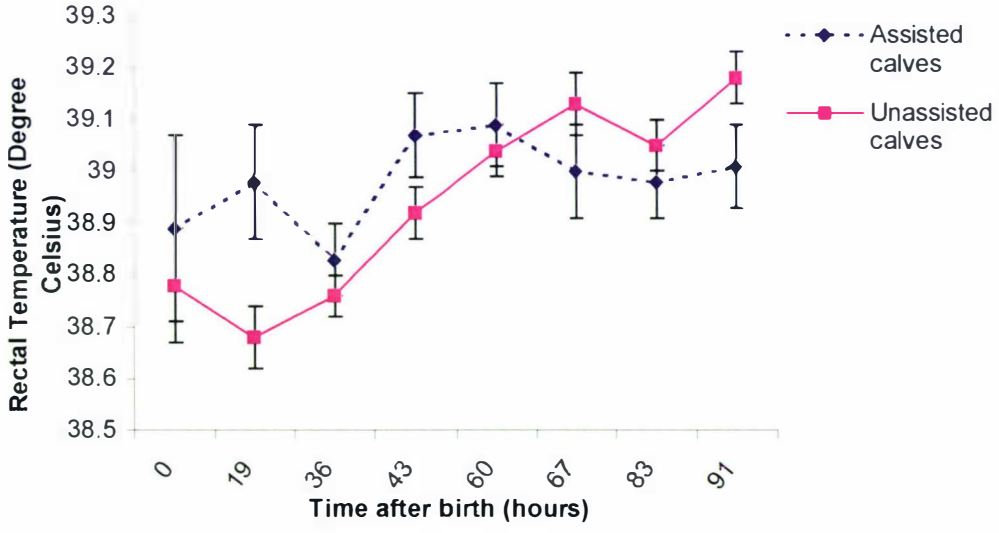


Figure 3.3.16: Means and standard errors for rectal temperature in assisted (n=21) and unassisted (n=85) calves calculated by repeated measures ANOVA.

Table 3.3.23: Means and standard errors of the mean (SEM) for rectal temperature (°C) of the various groups (n=number of calves).

	Rectal temp. birth		Rectal temp. 19 hours		Rectal temp. 36 hours		Rectal temp. 43 hours		Rectal temp. 60 hours		Rectal temp. 67 hours		Rectal temp. 83 hours		Rectal temp. 91 hours	
	Mean (n)	**SEM**	**Mean (n)**	**SEM**	**Mean (n)**	**SEM**	**Mean (n)**	**SEM**	**Mean (n)**	**SEM**	**Mean (n)**	**SEM**	**Mean (n)**	**SEM**	**Mean (n)**	**SEM**
Assisted calves	38.9 (21)	0.18	39.0 (20)	0.11	38.8 (21)	0.07	39.1 (21)	0.08	39.1 (21)	0.08	39.0 (21)	0.09	39.0 (21)	0.07	39.0 (21)	0.08
Unassisted calves	38.8 (85)	0.11	38.7 (83)	0.06	38.8 (84)	0.04	38.9 (84)	0.05	39.0 (84)	0.05	39.1 (84)	0.06	39.1 (84)	0.05	39.2 (82)	0.05
Healthy calves	39.3 (83)	0.21	38.7 (83)	0.17	38.8 (83)	0.08	39.0 (83)	0.10	39.1 (83)	0.10	38.9 (83)	0.13	39.1 (83)	0.10	39.1 (83)	0.10
Sick calves	39.2 (15)	0.23	38.8 (13)	0.19	39.0 (15)	0.09	39.2 (15)	0.11	39.2 (15)	0.11	39.2 (15)	0.14	39.1 (15)	0.11	39.2 (14)	0.11
Dying calves	38.7 (8)	0.31	38.5 (7)	0.26	38.8 (7)	0.14	39.1 (7)	0.16	39.0 (7)	0.17	38.8 (7)	0.21	39.1 (7)	0.16	39.3 (6)	0.16
Calves younger than 24hours at pick-up	38.8 (82)	0.10	38.7 (79)	0.06	38.8 (82)	0.04	38.9 (82)	0.05	39.1 (82)	0.05	39.1 (82)	0.05	39.1 (82)	0.04	39.2 (80)	0.05
Calves older than 24 hours at pick-up	38.9 (24)	0.17	39.0 (24)	0.10	38.9 (23)	0.06	39.2 (23)	0.08	39.1 (23)	0.08	39.1 (23)	0.09	39.0 (23)	0.07	39.1 (23)	0.08
High GGT	38.9 (78)	0.10	38.9 (76)	0.06	38.8 (77)	0.04	39.0 (77)	0.05	39.1 (77)	0.05	39.1 (77)	0.05	39.0 (77)	0.04	39.1 (75)	0.05
Low GGT	38.6 (28)	0.16	38.5 (28)	0.09	38.8 (28)	0.07	38.9 (28)	0.08	39.0 (28)	0.07	39.1 (28)	0.09	39.1 (28)	0.07	39.2 (28)	0.08

Calves with high and low plasma GGT concentrations

There was no significant difference between calves with high and low plasma GGT concentrations in rectal temperature overall, but there were significant differences over time (Table 3.3.24). Breed did not have an effect, however time had a great overall effect. It is apparent from Figure 3.3.17 that the significant difference between groups probably arose from between-group differences in rectal temperature at 19 hours after birth (Table A1.17 in Appendix 1, p-value=0.0002).

Table 3.3.24: Results of repeated measure ANOVA (p-values) for rectal temperature.

Effect	Calves with high GGT versus low plasma GGT concentrations
Breed	0.234
Time	<0.0001
*Breed*time*	0.068
GGT	0.095
*Breed*GGT*	0.188
*GGT*time*	0.007

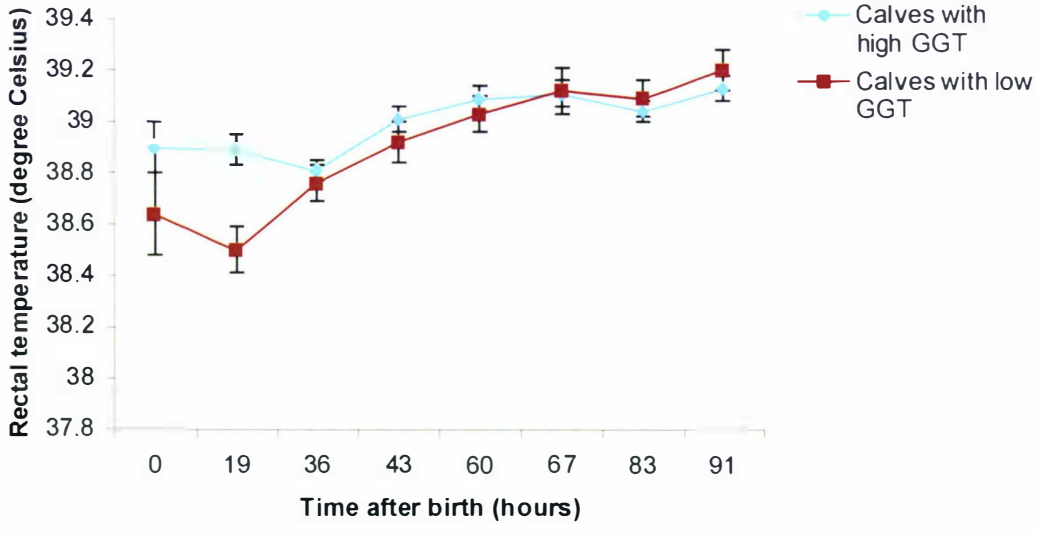


Figure 3.3.17: Means and standard errors for rectal temperature in calves with high (n=78) and low (n=28) plasma GGT concentrations calculated by repeated measures ANOVA.

There were also significant differences in rectal temperature within the two groups over time (Table A1.17 in Appendix 1). Calves that had high plasma GGT concentrations had significantly lower rectal temperature at 19 hours than at 91 hours after birth, and

significantly lower rectal temperature at 36 hours than at 43, 60, 67, 83 and 91 hours. Calves with low plasma GGT concentrations had significantly lower rectal temperature at birth than at 91 hours, significantly lower rectal temperature at 19 hours than 43, 60, 67, 83 and 96 hours and significantly lower rectal temperature at 36 than at 67, 83 and 96 hours after birth.

Healthy, sick and dying calves

There were no significant differences between healthy, sick and dying calves in rectal temperature overall and over time (Table 3.3.25). Breed had an effect on rectal temperature in the three groups over time. Although no significant differences were found in rectal temperature between the three groups, it is apparent from Figure 3.3.18 that dying calves had a generally lower rectal temperature than healthy and sick calves. Time had a significant effect. There were no significant differences within the groups over time (Table A1.18 in Appendix 1).

Table 3.3.25: Results of repeated measure ANOVA (p-values) for rectal temperature.

Effect	Healthy, sick and dying calves
Breed	0.954
Time	0.004
*Breed*time*	0.027
Sick	0.219
*Breed*Sick*	0.134
*Sick*time*	0.828

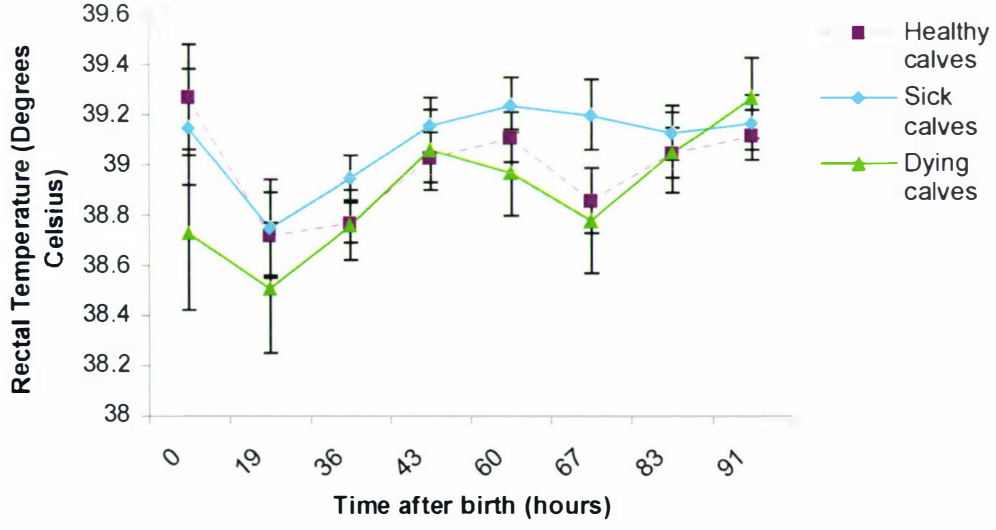


Figure 3.3.18: **Means and standard errors for rectal temperature in healthy (n=23), sick (n=15) and dying (n=8) calves calculated by repeated measures ANOVA.**

Calves younger and older than 24 hours at pick-up

Although there was no significant difference in rectal temperature between the two groups overall, there was a significant difference in rectal temperature between the two groups over time (Table 3.3.26). It is apparent from Figure 3.3.19 that this difference only exists over the first two days after birth, where calves younger than 24 hours at pick-up had a lower rectal temperature compared to calves from the other group.

There were significant differences in rectal temperature within the group of calves younger than 24 hours at pick-up (Table A1.19 in Appendix 1). The rectal temperature at birth was significantly lower than that at 91 hours, that at 19 hours was significantly lower than at 60, 67, 83 and 91 hours, the rectal temperature at 36 hours was significantly lower than that at 60, 67, 83 and 91 hours and temperature at 43 hours was also significantly lower than at 91 hours after birth.

Table 3.3.26: Results of repeated measure ANOVA (p-values) for rectal temperature.

Effect	Calves younger and older than 24 at pick-up
Breed	0.841
Time	<0.0001
*Breed*time*	0.178
Age	0.103
*Breed*age*	0.141
*Age*time*	0.005

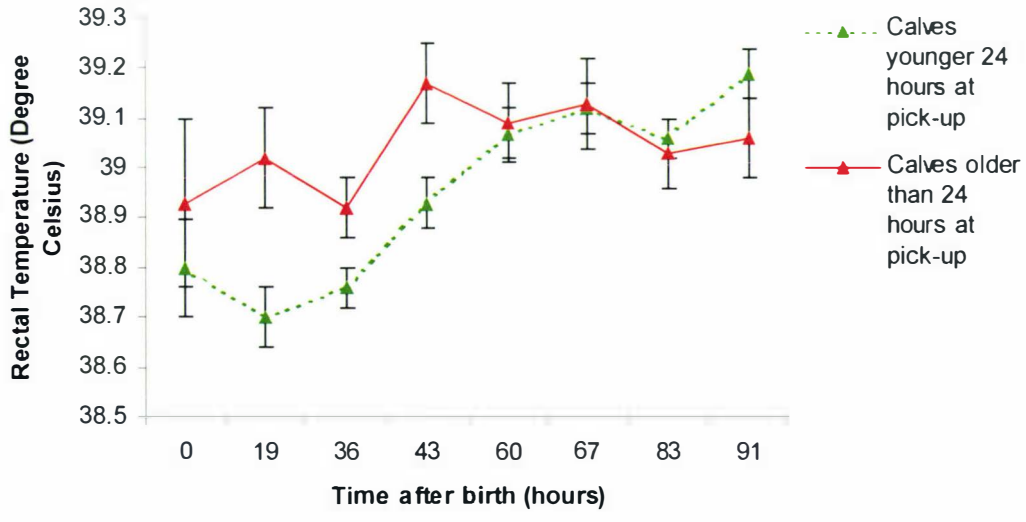


Figure 3.3.19: Means and standard errors for rectal temperature in calves younger (n=24) and older (n=82) than 24 hours at pick-up calculated by repeated measures ANOVA.

Other statistical tests for rectal temperature

Table 3.3.27 shows the results for the analysis of co-variance for rectal temperature. At 19 hours after birth, air temperature together with weather had a significant effect on rectal temperature (p-value <0.0001 air temperature and weather combined). Also, although air temperature seemed to have a significant effect on rectal temperature at 43 hours, weather seemed to be a better predictor of the relationship judged by univariate tests not presented here. Thus, there were no significant effects of weather and air temperature on rectal temperature at that time. Rectal temperature at 91 hours seemed to have been highly affected by air temperature and weather (p-value 0.01), but air temperature and weather individually had no significant effect. In comparison, the univariate analysis showed that both had a highly significant effect on rectal temperature when only one of the two factors was fitted to the model at a time. Thus, it may be possible that there may be some values where air temperature and weather are highly correlated as adding either one does not change the effect on rectal temperature. Table 3.3.28 shows the results for the non-parametric ANOVA undertaken to determine differences in rectal temperatures between calves that wore coats and those that did not. It can be seen that rectal temperatures were marginally lower in calves with coats at the first sample taken in the shed, which is the reason why a coat was put on, and that subsequently there were no significant differences between the two groups in rectal temperature.

Table 3.3.27: Results of the ANCOVA for rectal temperature (relationship between rectal temperature with both air temperature and weather together).

Source	Temperature at 19 hours (n=103)	Temperature at 36 hours (n=105)	Temperature at 43 hours (n=105)	Temperature at 60 hours (n=105)	Temperature at 67 hours (n=105)	Temperature at 83 hours (n=105)	Temperature at 91 hours (n=103)
Rectal temperature (°C)	<0.0001	0.48	0.07	0.51	0.30	0.133	0.01
Air temperature (°C)	0.05	0.32	0.03	0.30	0.26	0.92	0.11
Weather	0.02	0.77	0.10	0.78	0.53	0.06	0.78

Table 3.3.28: Results of non-parametric one-way ANOVA (p-values) (coats n=3, no coats n=103).

Group	Temperature at 19 hours	Temperature at 36 hours	Temperature at 43 hours	Temperature at 60 hours	Temperature at 67 hours	Temperature at 83 hours	Temperature at 91 hours
Calves with coats versus calves without coats	0.046	0.63	0.59	0.67	0.46	0.87	0.66

3.3.1.6 Multivariate statistics

The canonical discriminant analysis (CDA) showed a high degree of separation between sick and dying calves (12.99) and between healthy and dying calves (14.25), but only a small degree of separation between healthy and sick calves (4.17). The first canonical discriminant function contributed 67.53% of the overall variation, while the second function only contributed 32.47% to the overall variation in the data. The canonical coefficients (Table 3.3.29) for the first canonical function showed that it was dominated by the 48-hour glucose, the 48- and 72-hour urea, 24-hour beta-hydroxybutyrate and the 24-hour GGT concentrations. The second canonical function was dominated by the 24-hour glucose, 48-hour urea, 72-hour beta-hydroxybutyrate and the 72-hour beta-hydroxybutyrate concentrations and the 36- and 83-hour temperature.

Table 3.3.29: Pooled Within-Class Standardized Canonical Coefficients for CDA of follow-up data.

Label	Can1	Can2
Glucose 24-hour	0.04	**-0.68**
Glucose 48-hour	**0.68**	-0.04
Glucose 72-hour	-0.18	-0.14
Urea 24-hour	-0.20	0.47
Urea 48-hour	**-0.89**	**-0.57**
Urea 72-hour	**0.94**	0.37
Beta- hydroxybutyrate 24-hour	**0.60**	-0.29
Beta- hydroxybutyrate 48-hour	-0.25	0.40
Beta- hydroxybutyrate 72-hour	0.10	**0.54**
GGT 24-hour	**1.41**	0.15
Temperature 19 hours	-0.29	0.08
Temperature 36 hours	-0.08	**0.75**
Temperature 43 hours	-0.30	-0.30
Temperature 60 hours	-0.11	-0.01
Temperature 67 hours	-0.06	**0.59**
Temperature 83 hours	0.08	-0.07

Figure 3.3.20 shows the canonical scores of the calf follow-up data. Calves scoring high on the first canonical discriminant function had high plasma glucose concentrations at 48 hours, low plasma urea levels at 48 hours, high urea concentrations at 72 hours, high plasma beta-hydroxybutyrate concentrations and high plasma GGT concentrations at 24 hours. The calves scoring low on this function showed the opposite trends. Calves scoring high on the second canonical discriminant function have low plasma glucose concentration at 24 hours, low plasma urea concentrations at 48 hours, high plasma beta-hydroxybutyrate concentrations at 72 hours and high rectal temperature at 36 and 83 hours. The calves scoring low on this function showed the opposite trends.

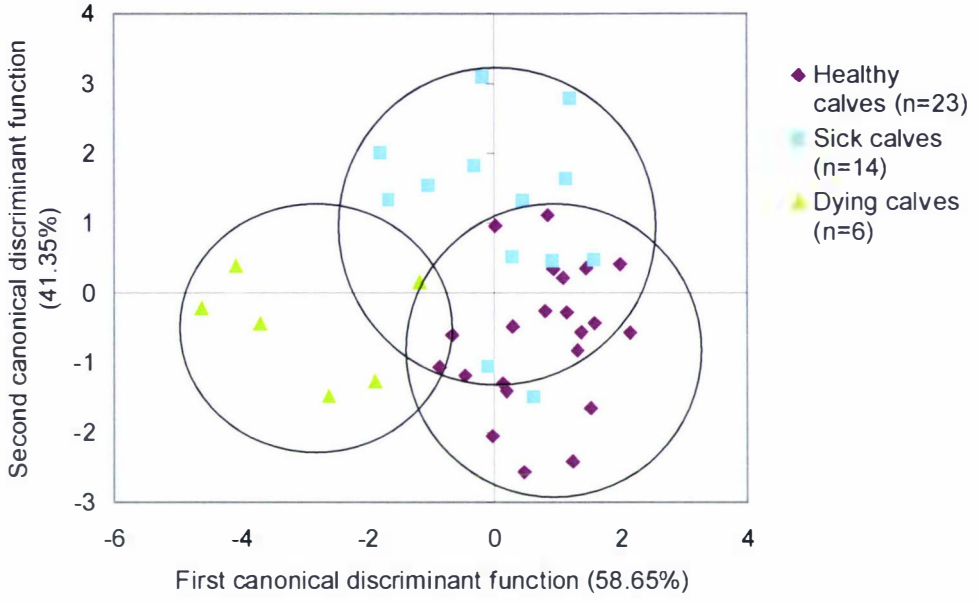


Figure 3.3.20: Canonical scores of calf follow-up data.

The first canonical discriminant function seemed to separate dying calves from healthy and sick calves. Thus, dying calves seemed to have had lower plasma GGT levels at 24 hours than calves that remained healthy or became sick. Dying calves also had lower plasma glucose levels at 48 hours, higher plasma urea levels at 48 hours, lower plasma urea concentrations at 72 hours and higher plasma beta-hydroxybutyrate concentrations at 24 hours compared to calves that remained healthy and those that became sick. Calves that became sick seemed to be separated from healthy calves along the second canonical discriminant function. Healthy calves seemed to have higher plasma glucose levels at 24 hours and higher plasma urea concentrations at 48 hours than sick calves. Also, they had higher plasma beta-hydroxybutyrate concentrations than sick calves at 24 hours, lower plasma urea concentrations at 72 hours. Rectal temperatures at 36 and 83 hours were higher for sick calves than for healthy calves. Overall, the plasma GGT concentrations at 24 hours was the major factor separating dying calves from healthy and sick calves, while all of the factors of the second canonical discriminant function seemed to influence the separation between healthy and sick calves equally.

3.3.2 Cow variables

3.3.2.1 Assisted versus unassisted calves

Table 3.3.30 shows that there were significant differences between assisted and unassisted calves in the corresponding maternal body condition score (BCS), and in maternal plasma glucose, urea and beta-hydroxybutyrate concentrations. The mean separation test (Tukey) showed that assisted (n=13) and unassisted (n=15) Angus calves were born to mothers with significantly higher body conditions scores than assisted (n=8) and unassisted (n=70) Friesian calves (p-value=<0.0001). Moreover, the mothers of assisted Angus calves had significantly lower plasma glucose concentrations (p-value=0.021) and significantly higher plasma urea concentrations (p-value=0.031) than mothers of unassisted Friesian calves. Also, mothers of assisted and unassisted Angus calves had higher plasma beta-hydroxybutyrate concentrations than mothers of assisted and unassisted Friesian calves (p-value=<0.0001). Weight did not have an effect. Means and standard errors are shown in Table 3.3.32.

Table 3.3.30: One-way ANOVA for maternal variables and birth weight (breed and assistance separation). BCS, body condition score

Parameters	p-values
BCS	<0.0001
Birth weight	0.116
Cow-glucose	0.021
Cow-urea	0.031
Cow-beta-hydroxybutyrate	<0.0001

3.3.2.2 Healthy, sick and dying calves

There were no significant differences in any of the parameters between mothers of healthy, sick or dying calves (Table 3.3.31). Means and standard errors are shown in Table 3.3.33.

Table 3.3.31: One-way ANOVA for maternal variables and birth weight for healthy, sick and dying calves (no breed separation). BSC, body condition score

Parameters	p-values
BCS	0.3337
Birth weight	0.0738
Cow-glucose	0.8165
Cow-urea	0.2993
Cow-beta-hydroxybutyrate	0.8417

Table 3.3.32: **Means and standard errors of the mean (SEM) for the maternal variables of mothers of assisted and unassisted calves (n=number of calves).**

	Cow-glucose (mmol/L)		Cow-beta hydroxy-butyrate (mmol/L)		Cow-urea (mmol/L)		Cow BCS		Calf birth weight (kg)	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Unassisted Angus calves (n=15)	4.09	0.05	0.54	0.03	8.03	0.27	5.0	0.09	35.2	1.05
Unassisted Friesian calves (n=70)	4.06	0.03	0.38	0.01	7.80	0.13	4.5	0.05	37.7	0.50
Assisted Angus calves (n=13)	3.87	0.08	0.58	0.03	8.81	0.39	5.1	0.09	37.8	1.02
Assisted Friesian calves (n=8)	3.95	0.07	0.35	0.06	8.19	0.32	4.3	0.2	39.3	1.6

BCS, body condition score

Table 3.3.33: Means and standard errors of the mean (SEM) for the maternal variables of mothers of healthy, sick and dying calves.

	Cow-glucose (mmol/L)		Cow-beta hydroxy-butyrate (mmol/L)		Cow-urea (mmol/L)		Cow BCS		Calf birth weight (kg)	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Healthy calves (n=83)	4.01	0.05	0.42	0.03	8.27	0.30	4.5	0.08	36.1	0.74
Sick calves (n=15)	4.07	0.07	0.40	0.03	7.91	0.25	4.7	0.15	38.1	1.02
Dying calves (n=8)	4.04	0.07	0.43	0.05	7.49	0.28	4.4	0.10	39.6	1.75

BCS, body condition score

3.3.3 Summary of results

3.3.3.1 Birth to 4 days of age

- The maternal physiological status was significantly different for cows where labour had to be assisted compared with those where labour was not assisted.
- Overall, assisted calves were not markedly different from unassisted calves, however assisted calves had significantly higher plasma beta-hydroxybutyrate concentrations at 48 and 96 hours, and higher rectal temperature at 19 hours after birth compared to unassisted calves although this was not significant.
- Calves that were younger than 24 hours of age at pick-up had lower plasma glucose concentrations at 24 hours after birth than calves that were older than 24 hours at pick-up. In addition, calves picked up at a younger age seemed to have had lower plasma GGT concentrations, and lower rectal temperatures between 19 and 43 hours after birth. However, none of these differences were significant. 87% of calves that were older than 24 hours at pick-up had high plasma GGT concentrations, while only 71% of calves younger than 24 hours at pick-up had high plasma GGT concentrations. In addition, a higher percentage of calves became sick or died when picked up early compared to calves picked up later (80% of calves that became sick and 88% of dying calves were picked up younger than 24 hours of age).
- Calves that had high plasma GGT concentrations at 24 hours after birth had significantly higher plasma glucose concentrations over the four days of examination compared to calves with low plasma GGT concentrations. In addition, calves with high plasma GGT levels had lower beta-hydroxybutyrate concentrations at 24 hours (not significant) and higher rectal temperature at 19 hours after birth (significant) compared with calves that had low plasma GGT levels.
- Overall, calf plasma glucose concentrations were fairly high, beta-hydroxybutyrate concentrations were fairly low and rectal temperatures were fairly high (Table 3.3.34). This suggests that calves were well fed and did not become hypothermic.
- Weather as well as air temperature influenced rectal temperature on the first day after pick-up, but not subsequently.

- The use of calf coats might be beneficial in restoring normal rectal temperatures in calves with mild to moderate hypothermia.

Table 3.3.34: Means and standard errors of the mean for all calves followed up during the first four days after birth for all parameters measured.

Parameter		Mean	Standard Error (SEM)	Number of animals (n)
Glucose (mmol/L)	*Birth*	6.04	0.22	106
	24 hours	7.23	0.15	106
	48 hours	6.34	0.11	104
	72 hours	6.04	0.09	105
	96 hours	6.29	0.09	95
Beta-hydroxybutyrate (mmol/L)	*24 hours*	0.10	0.01	106
	48 hours	0.07	0.01	104
	72 hours	0.06	0.00	105
	96 hours	0.04	0.00	95
Urea (mmol/L)	*24 hours*	5.49	0.17	106
	48 hours	4.34	0.18	104
	72 hours	3.46	0.14	105
	96 hours	3.97	0.12	95
GGT (mmol/L)	*24 hours*	1123	107	106
	48 hours	653	65	101
	72 hours	480	46	105
	96 hours	399	41	95
Rectal temperature (°C)	*Birth*	38.81	0.08	106
	19 hours	38.74	0.05	103
	36 hours	38.76	0.03	105
	43 hours	38.97	0.04	105
	60 hours	39.08	0.04	105
	67 hours	39.14	0.04	105
	83 hours	39.07	0.03	105
	91 hours	39.14	0.03	103

3.3.3.2 Four days to 1.5 months of age

- Only three assisted calves have been reported to have become sick or die between 1 day and 1.5 months of age. However, the majority of assisted calves that were examined over the first four days after birth (16/21) were sold and no record of disease incidence or death was available.
- Of the calves that remained on the farm for the first 1.5 months after birth, 50 % remained healthy while about 33% became sick and subsequently recovered and 17% became sick and subsequently died.
- There were no significant differences in calf plasma glucose, beta-hydroxybutyrate and urea concentrations or in rectal temperature between healthy, sick and dying calves.
- Of the calves that remained on the farm up to 1.5 months of age, none seemed to have experienced starvation during the four days of examination as judged by relatively high plasma glucose, relatively low plasm beta-hydroxybutyrate and fairly low plasma urea concentrations.
- Healthy calves were well fed for the first four days after birth, the majority (83%) had intake of colostrum and none of the healthy calves were hypothermic.
- Calves that became sick between 1 day and 1.5 months of age did not differ greatly from healthy calves during the four days of examination. The majority (87%) had colostrum intake, were well fed and did not experience hypothermia.
- The majority (87.5%) of calves that died between day 1 and 1.5 months of age had insufficient colostrum intake. Although not significantly different, their plasma glucose concentrations were lower than those in healthy calves and calves that became sick. Also, plasma glucose concentrations in calves dying between 1 day and 1.5 months of age did not show a peak at 24 hours of age suggesting that significant colostrum intake did not take place until after 24 hours. Dying calves did not become hypothermic.
- Maternal physiological status did not seem to have an effect on the incidence of disease and mortality in the calves of this study.

3.4 Discussion

The major findings and conclusions from this research were as follows:

- Overall, the physiological and physical status of calves between day 1 and day 4 after birth was good and the majority of calves (91.5%) remained healthy during this period.
- Starvation and hypothermia were not major issues in the calves of the present study during the first four days after birth.
- In addition to plasma GGT concentrations, measuring plasma glucose and beta-hydroxybutyrate concentrations as well as rectal temperature at 24 hours after birth gave some indication whether calves received colostrum or not.
- Early assistance of dystocic cows may have had beneficial effects on the physiological and physical status of assisted calves over the first four days after birth.
- Calves that were separated from their mothers earlier seemed to be less likely to take in colostrum than calves that were separated from their mothers later after birth. In addition, a greater number of calves in the early-separation group became sick or died.
- Overall, calves that remained healthy, became sick or died between day 1 and 1.5 months of age did not differ significantly in their physiological and physical status between day 1 and day 4 after birth. However, even though healthy calves and calves that became sick did not differ significantly in plasma GGT concentrations, dying calves had significantly lower plasma GGT concentrations than healthy and sick calves.
- There is some doubt as to whether plasma GGT concentrations are an adequate measure for immune status of newborn calves.
- Maternal physiological status did not seem to have an effect on the incidence of disease and mortality in the calves of this study.

The findings of this study are discussed in detail in the following sections.

3.4.1 Calf energy and immune status between day 1 and 4 after birth

Calf energy status

In the adult animal, starvation or other situations where energy needs exceed energy intake, such as disease, may eventually lead to lipid and protein catabolism and an increase of plasma free fatty acid, ketone bodies and urea concentrations in the circulation. Fat is stored as triacylglycerol in white adipose tissue, from which free fatty acids are mobilised in times of energy need (White and Baxter, 1994). When lipolysis is increased and the rate of beta-oxidation of free fatty acids exceeds the rate at which acetyl-CoA enters the citric acid cycle, the liver will synthesis ketone bodies, such as beta-hydroxybutyrate (Girard *et al.*, 1992; White and Baxter, 1994). Ketone bodies can then be rapidly used in extrahepatic tissues as energy source.

In lambs it has been found that carbohydrate and lipid are important sources for energy production in the neonate animal and that both are used simultaneously if available (Mellor & Cockburn, 1986). Before colostrum is taken in by the neonate, liver and muscle glycogen and depot lipid will provide energy for thermogenesis (Carstens, 1994). Mellor & Cockburn (1986) report that in the unfed newborn the respiratory quotient (RQ) is usually higher immediately after birth and that this indicates a greater dependence on carbohydrate. The major carbohydrate sources in the unfed newborn are liver and muscle glycogen stores, while in the fed newborn the major carbohydrate source is colostral lactose (Mellor & Cockburn, 1986). Mellor & Cockburn (1986) suggest that at the usual intake of colostrum the amounts of lactose could provide sufficient carbohydrate for only about half a day and that lambs that are fully fed are also obliged to draw on their body glycogen reserves in order to make up the difference. Beyond the first day after birth milk must rapidly become the only source of carbohydrate, lipid and protein (Mellor & Cockburn, 1986).

It seems that next to liver and muscle glycogen the only other major sources of energy in the unfed newborn animal are non-structural body lipids (Mellor & Cockburn, 1986). Protein catabolism in un-fed newborns was found to be minimal, only representing 4 to 8% of the rate of heat production (Mellor & Cockburn, 1986).

Calves are expected to exhibit similar features to lambs with respect to the energy sources and rates of use during the immediate postnatal period (Mellor & Stafford, 2003).

Calves with an adequate energy status would thus be expected to have relatively high plasma glucose, low plasma beta-hydroxybutyrate and low urea concentrations. Plasma glucose is the rate-limiting factor for heat production in the newborn animal (Mellor & Cockburn, 1986) and thus unfed calves with very low plasma glucose concentrations would be expected to have lower rectal temperatures or be more prone to hypothermia than fed calves with higher plasma glucose concentrations. This is supported by studies by Eales & Small (1980a and 1981) who showed that starved lambs had a lower summit metabolic rate than fed lambs and that, judged by the respiratory quotient (RQ=0.94), starved lambs derived heat produced during summit metabolism mainly from carbohydrate. They suggest that during summit metabolism, when carbohydrate was apparently the major source of energy, availability of glucose may limit heat production in lambs.

The following observations support the notion that the physiological and physical status of calves for the four days of examination was good and that starvation and hypothermia were not significant problems.

1. Of the 106 calves examined over the four-day period, all had plasma glucose concentrations above 4 mmol/L between 24 and 96 hours after birth, except for one calf that had concentrations between 3 and 4 mmol/L at 72 hours, and two calves that had concentrations between 3 and 4 mmol/L at 96 hours. Wesselink *et al.* (1999) observed that in more severely hypoglycaemic calves, which were hypothermic, plasma glucose concentrations were around 2 mmol/L. Accordingly, none of the calves in the present study were severely hypoglycaemic, a proposition supported by the fact that hypothermia did not occur (see below). In a study by Todd *et al.* (2000a), fed calves had mean plasma glucose concentrations between 5 and 6 mmol/L, while calves that were fasted developed hypoglycaemia progressively 12 hours after food withdrawal

and plasma glucose concentrations were reported to decline to values of about 3.4 mmol/L. As only two calves had plasma glucose concentrations below 4 mmol/L, starvation, as indicated by extremely low plasma glucose concentrations (probably below 2 mmol/L), was not observed in the calves of the present study. This is consistent with the findings that the present calves did not show elevated plasma beta-hydroxybutyrate or urea concentrations (see below). However, 4, 11 and 11 calves had plasma glucose concentrations between 4 and 5 mmol/L at 24, 48 and 72 hours, respectively. It may be possible that these experienced slightly lower plasma glucose concentrations as blood samples were taken before the morning or afternoon feed, some hours after the previous feed. In the present calves there seemed to be a trend for plasma glucose levels to increase between birth and 24 hours after birth, followed by a subsequent fall of plasma glucose concentrations (Table 3.3.34). This was also observed in a study by Shannon & Lascelles (1966) who showed that plasma glucose concentrations were relatively low at birth and increased sharply to reach a peak at 2 days of age; subsequently the concentrations decreased until about 2 months of age. Daniels *et al.* (1979) also observed that glucose concentrations increased after colostrum intake to reach a peak around 12 to 14 hours after birth. This observation and the high plasma glucose concentrations in the present calves suggest that they received significant quantities of colostrum during the first 24 hours after birth. The different results in the present study and the study by Shannon & Lascelles (1966) compared to that by Daniels *et al.* (1979) regarding the timing of the plasma glucose peak might have been due to the timing of blood samples. Daniels *et al.* (1979) sampled their calves more regularly (every 30 min for the first 6 hours and then hourly until calves were 24 hours old). Thus, the peak observed in plasma glucose concentrations at about 24 hours in the present study and in the study by Shannon & Lascelles (1966) may actually be part of the declining slope of overall plasma glucose concentrations and the overall peak might have occurred as observed by Daniels *et al.* (1979) around 12 to 14 hours.

2. Beta-hydroxybutyrate concentration in plasma would be expected to increase during starvation. The mean beta-hydroxybutyrate concentrations that were reported for 5- to 10-day-old calves fasted for 30 hours were 0.12 to 0.14

mmol/L (Todd *et al.*, 2000a). In the present study, 34 calves had plasma beta-hydroxybutyrate concentrations of 0.1 mmol/L or higher at 24 hours, 18 at 48 hours, 18 at 72 hours and 14 at 96 hours. Of the calves with plasma beta-hydroxybutyrate concentrations 0.1 mmol/L or higher, only one had plasma glucose concentrations below 4 mmol/L at the same time (at 96 hours). According to the means recorded by Todd *et al.* (2000a) it may seem that a fairly high proportion of calves in the present study might have experienced some degree of fasting. However, this is not supported by plasma glucose concentrations and could be explained by the present observation that beta-hydroxybutyrate concentrations seemed to decline over the four days of study (Table 3.3.34). Therefore, plasma beta-hydroxybutyrate concentrations in fed younger calves may be similar to levels found in fasted 5- to 10-day-old calves by (Todd *et al.*, 2000a). Girard *et al.* (1992) noted that blood ketone bodies in fed newborn lambs remain very low and suggested that they were not likely to play an important role as substrates for energy production. If so, this might help to explain the fairly low beta-hydroxybutyrate concentrations in the majority of calves over the four days of the present study.

3. Mean plasma urea concentrations of the calves in the present study were relatively higher at all times (Table 3.3.34) than those reported for 1-week-old calves (2.1 ± 0.2 mmol/L) at the beginning of the study by Kinsbergen *et al.* (1994), and higher at 24 and 48 hours in the present calves than in the 5- to 10-day-old calves (3.13 ± 0.35 mmol/L in control calves) studied by Todd *et al.* (2000a). Age differences between the calves in the different studies may be a significant factor here, rather than differences in protein catabolism, as glucose concentrations of the calves of the present study were fairly high. Higher plasma urea concentrations in the present calves may be an artefact of changes from the intrauterine to the extrauterine environment, such as reduced renal clearance rate. In addition, as reported by Mellor and Cockburn (1986), protein catabolism is not likely to play a significant role in unfed newborns. The likelihood that calves would have mobilised protein for energy supply is therefore small. This is also supported by Todd *et al.* (2000a), who showed that the calves in their study did not show an increase in protein catabolism in

response to fasting for 30 hours although calves became significantly hypoglycaemic by 18 hours.

4. Rectal temperatures of the calves during this study indicated that hypothermia was not a problem between 24 and 96 hours after birth. As reported by Anderson & Bates (1984) mild hypothermia is indicated by a rectal temperature between about 36-37°C. There was only one calf that had a rectal temperature below 37°C (36.8°C) and seven calves that had rectal temperatures between 37°C and 38°C at 19 hours. Only one calf had a rectal temperature between 37°C and 38°C at about 36 hours and one at 43 hours after birth. Subsequently no calves had rectal temperatures below 38°C.

Calf immune status

The failure of immunoglobulin absorption increases the newborn's risk of contracting infectious diseases and dying (Campbell, 1974; Sawyer *et al.*, 1977; Besser & Gay, 1994; Wittum & Perino, 1995). Thus immunoglobulin intake is of major importance for the neonate's future welfare and survival. In lambs, colostrum intake sufficient to avoid starvation and hypothermia is normally sufficient to provide protection against infectious diseases (Mellor, 1990), and this would also be expected to be case in calves (Mellor & Stafford, 2003).

Plasma glucose and beta-hydroxybutyrate concentrations as well as rectal temperature could therefore not only be used as indicators of the well-being of an animal, but may also help to distinguish calves which have fed from those which have not. Unfed calves might have lower rectal temperatures as heat production may be impaired by reduced availability of energy substrates, as indicated by low plasma glucose and elevated plasma beta-hydroxybutyrate concentrations. In comparison, fed calves will be able to produce enough heat to sustain thermal balance unless challenged by disease or other adverse environmental factors.

Thus, in addition to plasma GGT concentrations, measuring plasma glucose and beta-hydroxybutyrate concentrations as well as rectal temperature at 24 hours may give some

indication on the intake of colostrum of calves before they were picked up. This idea is supported by the following observations from the present study.

1. Calves that apparently received colostrum (plasma GGT concentrations >200 IU/L) had significantly higher plasma glucose concentrations over the four days of examination compared to calves which apparently did not or which had insufficient colostrum intake (plasma GGT concentrations <200 IU/L) (Figure 3.3.2). In addition, calves with low plasma GGT concentrations at 24 hours also did not show a peak in plasma glucose concentration at 24 hours, which may be indicative of a lower colostrum intake (Daniels *et al.*, 1979). It is surprising though that calves with low GGT concentrations had lower glucose concentrations over the whole four days of follow-up rather than just at 24 hours. It may however be possible that calves that had low GGT concentrations at 24 hours after birth were calves that were too weak to suck from birth. In these calves a weak sucking drive may have persisted and they may have been bad competitors for colostrum, which might predispose them to take in less colostrum than other calves.
2. At 24 hours after birth, calves that had apparently received colostrum had lower beta-hydroxybutyrate concentrations than calves that apparently did not or received insufficient colostrum (Figure 3.3.6), but this difference was not significant. Nevertheless it may be indicative that colostrum intake may not have taken place and that the calves thus had to increase their rate of lipid catabolism in order to fuel basic metabolism and heat production.
3. At 19 hours after birth, calves that had apparently received colostrum had significantly higher rectal temperatures than those calves that apparently had not or that had received insufficient colostrum (Figure 3.3.17).

Therefore combining common measures of colostrum intake, such as plasma GGT concentrations, with measures of plasma glucose and beta-hydroxybutyrate concentrations and rectal temperature measurements may help to distinguish calves that have taken in colostrum sufficient to prevent starvation and hypothermia and possibly hypogammaglobulinaemia. In addition, it may also be possible to detect calves that had

received some, but insufficient, colostrum to maintain plasma GGT concentrations over the cut-off limit, and insufficient colostrum to raise plasma glucose and lower plasma beta-hydroxybutyrate concentrations.

Energy and immune status of assisted calves

Calves born after dystocia are often weak, may lack a sucking reflex and their swallowing reflexes may be altered (Kasari, 1989). In addition, a reduced ability to absorb immunoglobulins may also occur in dystocic calves (Odde, 1988). Dystocic calves are thus more likely to experience starvation and are more likely to receive inadequate amounts of colostral immunoglobulins (Kasari, 1989; Besser and Gay, 1994). However, assisted calves in the present study did not experience starvation and did not have significantly different plasma GGT concentrations than unassisted calves.

Early assistance of dystocic cows may thus have had beneficial effects on the physiological and physical status of assisted calves over the first four days after birth. This is supported by the following observations.

1. There were no significant differences in plasma glucose concentration between assisted and unassisted calves (Figure 3.3.1 and Table A2.1 in Appendix 1). Also, the idea that animals in both groups were well fed is supported by the fact that the majority of calves had plasma glucose concentrations above 4 mmol/L. In addition, there were no significant differences in plasma urea concentrations between the two groups. Nevertheless, beta-hydroxybutyrate concentrations were significantly higher in assisted calves at 48 and 96 hours after birth (Figure 3.3.5). This though, is unlikely to be due to starvation as plasma glucose concentrations were shown to be relatively high. There may be a breed effect or the differences might have been affected by parity of the dam, as assisted calves were mainly Angus crosses (primiparous cows) rather than Friesian crosses (multiparous cows). This might also have been a fortuitous occurrence with little biological significance.
2. In lambs, severe hypoxaemia has been observed to lead to depressed heat production capacity and thus hypothermia (Eales & Small, 1985; Mellor, 1988).

However, the assisted calves of the present study did not experience hypothermia as none of the calves had rectal temperatures below 37°C (i.e. mild hypothermia; Anderson & Bates (1984)). Assisted calves even had higher rectal temperatures at 19 hours than unassisted calves although this difference was not significant (Figure 3.3.13). It may be possible that early assistance reduced the extent of intrapartum hypoxaemia (and metabolic acidosis) and its negative effect on heat production. In addition, starvation was not observed in these animals and thus the absence of hypothermia may not be surprising.

3. There were no significant differences in plasma GGT concentrations between assisted and unassisted calves. This, in addition to the absence of significant differences in plasma glucose concentrations and rectal temperature, seems to suggest that assisted calves did receive colostrum. Although assisted calves were observed to take longer to stand (Chapter 2) than unassisted calves, this did not seem to have influenced colostrum intake. It may be possible that the delay in standing for the first time was not long enough to delay colostrum intake sufficiently to cause failure of immunoglobulin absorption. Also, calves of the present study might not have been too weak to suck, as was suggested by Kasari (1989), due to the early assistance given.

Thus early assistance may offset the negative effects of difficult labour on thermogenesis and colostrum intake.

Energy and immune status of calves younger and older than 24 hours at pick-up

As suggested by Mohammed *et al.* (1991), one of the most important factors influencing the acquisition of immunoglobulins is the timing of colostrum intake. They report that a large proportion of calves may not suck at all during the first 12 to 24 hours before they are separated from their dams. In addition, there seems to be some evidence that calves left with their mothers absorb immunoglobulins more efficiently than calves separated from their dams shortly after birth, which maybe due to the combined effect of earlier

and possibly greater intakes in calves staying with their mothers for a longer period (Selman, 1973).

The following observations tentatively support the proposition that calves that were separated from their mothers earlier seemed to be less likely to take in colostrum than calves separated later.

1. 29% of the calves younger than 24 hours at pick-up were found to have GGT values below 200 IU/L (Table 3.3.2), which was determined to be the cut-off point for successful intake of colostrum (Perino *et al.*, 1993). 86% of calves with low plasma GGT concentrations were calves that were picked up before they were 24 hours of age (Table 3.3.2). Thus, judging by this information, calves of this group seem to be less likely to take in colostrum before 24 hours. This seems to be supported by the observation that calves younger than 24 hours at pick-up showed a flatter peak of plasma glucose concentration at 24 hours than calves older than 24 hours at pick-up (Figure 3.3.4). This difference however was not significant.
2. The above finding is also supported by the observations that calves younger than 24 hours at pick-up had lower rectal temperatures at 19, 36 and 43 hours after birth than calves of an older age at pick-up, although these differences were not significant (Figure 3.3.19). Rectal temperature may give an indication of whether a calf received colostrum or not and thus the lower rectal temperature in calves picked up earlier may be due to those calves not receiving colostrum before being picked up.
3. Of the calves that became sick, 80% were picked up at an age younger than 24 hours and of the calves dying 88% were picked up at an age younger than 24 hours (Table 3.3.2), which seems to suggest that these calves might not have had sufficient intake of colostrum to acquire adequate levels of immunity.

Following these results it seems that there might be a beneficial effect of leaving the calf with its dam for a longer period than practiced on some New Zealand farms. Benefits may include increased numbers of calves that received colostrum before being brought to the shed, which was shown here to have effects GGT concentrations. Other

studies seem to support the beneficial effect of separating the calf later rather than earlier from its dam. Calves left with their dams seem to absorb immunoglobulins more effectively than calves separated from their dams shortly after birth (Selman, 1973). Selman (1973) assumed that this might be a combined effect of earlier intake by calves staying with their mothers and possibly a greater colostrum intake. Stott *et al.* (1979b) also observed that early absorption and maximum absorption were greater in suckled calves rather than bottle-fed calves. They proposed that this might be due to a positive effect of colostrum intake on the pinocytotic activity in absorptive cells or an increased rate of transport of internalised immunoglobulins into the circulation. They also suggested that when the stress of handling and separation experienced by calves removed from their mothers was absent, any associated negative effects on the absorption of immunoglobulins into the systemic circulation would also be avoided. Weary & Chua (2000) showed that calves which spent more time with their dams before separation tended to experience fewer bouts of diarrhoea for the first 3 weeks after birth. They assumed that this might have been due to the fact that older calves drank more colostrum.

Although it seems that increasing the period a calf spends with its mother may be beneficial for the calf's health, it is difficult to determine when the right time for cow-calf separation may be. If calves stay with their dams for a longer period of time, health status may be improved; but on the other hand, removal of calves after a longer period of time was shown to be more stressful for both animals (Weary & Chua, 2000). Weary & Chua (2000) thus suggest that ' judging the best date for the animals welfare will require a balancing of these factors'.

3.4.2 Energy and immune status of healthy, sick and dying calves between 4 days and 1.5 months of age

Calf energy status

Overall, calves that remained healthy, became sick or died between one day and 1.5 months of age did not differ significantly in their physiological and physical status between day 1 and day 4 after birth. This is supported by the fact that calves of the three

groups did not differ significantly in plasma glucose, urea and beta-hydroxybutyrate concentrations or in rectal temperature (Figures 3.3.3; 3.3.7; 3.3.11; 3.3.18).

The fact that there were no significant differences between healthy, sick and dying calves in plasma glucose, beta-hydroxybutyrate and urea concentrations and rectal temperature might indicate that diseases were not far enough advanced to have a negative effect on energy balance or, that disease occurred after the calves were studied and that plasma glucose, beta-hydroxybutyrate and urea concentrations and rectal temperature were not good indicators of whether calves remain healthy or may become sick or die. This is also emphasised by the canonical discriminant analysis (pages 159 and 160), where separation of healthy, sick and dying calves was determined mainly by GGT levels rather than any of the other variables examined.

Calf immune status

Calves that died had significantly lower plasma GGT concentrations than healthy and sick calves. However, there were calves that remained healthy or became sick which also had low plasma GGT concentrations, but which survived. It may be possible that calves that died experienced more serious disease than the calves that became sick and subsequently survived or that calves which died were in general weaker calves less able to cope physically with disease. It may also be possible that calves that died were fed with milk if colostrum was unavailable and that the local protection of IgA and IgM in colostrum was lost to them in addition to them being hypogammaglobulinaemic. On the other hand, it may also be possible that calves that did not survive were unable to build up an active immune response strong enough to fight the infection, while those calves that had low plasma GGT concentrations and survived infections were able to do so. Although plasma GGT concentrations were lower in dying calves than in healthy and sick calves, plasma glucose and beta-hydroxybutyrate concentrations and rectal temperatures did not significantly differ. This seems to be in contrast to our results from previous sections where it was found that low plasma GGT concentrations were associated with low plasma glucose, high plasma beta-hydroxybutyrate concentrations and high rectal temperatures. However, included in the groups of healthy and sick calves were calves that also had low plasma GGT concentrations and it may be possible that the presence of these animals in these two groups might have led to the similarities in all three groups.

As there were calves with low plasma GGT concentrations that remained healthy it seems that calves with low plasma GGT concentrations at 24 hours may nevertheless be able to thrive and survive subsequently. It has been mentioned by Robinson & Young (1988) that calves that fail to absorb sufficient amount of immunoglobulins must mount an active immune response for defence against pathogens. Ishikawa & Konishi (1982) for example found that two hypogammaglobulinaemic calves started synthesising IgM as early as one week of age. Moreover, intake of colostrum after the absorptive period may also provide the newborn with local protection from infectious enteric diseases (Logan *et al.*, 1974), which may be another explanation for the success of hypogammaglobulinaemic calves.

Healthy and sick calves did not differ significantly in plasma GGT concentrations. This fact is surprising as it has often been reported that many newborn calves become sick and die due to the failure of passive transfer of immunoglobulins (Selman, 1973; Besser & Gay, 1994; Weaver *et al.*, 2000). Thus, calves that became sick in the present study would have been expected to have low plasma GGT concentrations indicative of failure of passive immunisation. One possible explanation for the fact that the majority of calves that became sick had high plasma GGT concentrations may be that those calves might have taken in low quality or pooled colostrum with low concentrations of immunoglobulins. Even if calves had achieved satisfactory immunoglobulin concentrations, other factors may also play a role in the incidence of disease including pathogen load, hygiene, environmental influences, nutrition (Besser & Gay, 1994; Tyler *et al.*, 1999) and other managerial factors.

Also, as reported by Perino *et al.* (1993), serum GGT activity showed only a low association with actual serum immunoglobulin concentrations. Thus, calves of the present study might have had colostrum intake as shown by high plasma GGT as well as high glucose concentrations, but the quality of the colostrum might have been too low for significant passive immunity to be acquired. Although plasma GGT concentrations decline over time due to dilution, Perino *et al.* (1993) showed that calves which had levels below 100 IU/L at day 4 after birth also failed to receive colostrum. Thus, in some calves colostrum intake might have been sufficient to raise plasma GGT concentration at 24 hours, but GGT concentrations might have declined afterwards

indicative of immunisation failure. There were some calves (n=4) that went from high plasma GGT concentrations at 24 hours to low GGT concentrations up to day 4. However, none of them had low plasma glucose concentrations suggesting that they might have taken in colostrum of lower quality although their colostrum intake as such might have been sufficient for them to acquire passive immunity if the colostrum quality had been adequate. Apart from one of those calves, however, all remained healthy. That calves can remain healthy despite being hypogammaglobulinaemic is supported by Rea *et al.* (1996) who observed low mortality rates, although the calves in their study commonly exhibited failure of immunoglobulin transfer. This again seems to point to local protection of colostrum intake after gut closure, early attainment of an active immune system and possibly managerial factors.

Overall, it seems hard to determine why calves that became sick had higher plasma GGT concentrations than calves that remained healthy, even though this was not significant, and why healthy and sick calves did not differ in plasma GGT concentrations. It may be possible that GGT is not a very good measure of IgG and immune status in calves, although it is possible to determine that some colostrum has been consumed (Weaver *et al.*, 2000). Thus, the fact that calves that became sick had higher plasma GGT concentrations than those that remained healthy is not to say that sick calves had higher concentrations of plasma IgGs, but rather that sick as well as healthy calves received colostrum while calves that died did not. Therefore, no concrete conclusions can be drawn regarding the immune status of calves in the present study.

3.4.3 Maternal status

Maternal physiological status did not seem to have an effect on the incidence of disease and mortality in the calves of this study as shown by the one-way ANOVA (Table 3.3.31). However, there were significant differences in maternal variables between assisted and unassisted calves (Table 3.3.30).

Whitaker (2000) states that the optimum levels of plasma glucose concentrations in cows are over 3 mmol/L. The optimum beta-hydroxybutyrate level for dry cows at the end of pregnancy was reported to be below 0.6 mmol/L (Whitaker, 2000). Optimum plasma urea concentrations are between 3.6 and 10 mmol/L for dry cows.

In all of the cows of the present study, mean plasma glucose (4.04 ± 0.02 mmol/L) and urea (7.99 ± 0.11 mmol/L) concentrations were at acceptable levels. However, 16 cows had plasma beta-hydroxybutyrate concentrations of 0.6 mmol/L or higher. In cows within the last 1 to 2 weeks of gestation beta-hydroxybutyrate concentrations above the optimum were reported to be a sign of negative energy balance (Whitaker, 2000). Thus, the status of the majority of cows in the present study was fairly good 2 weeks before the start of the peak of the calving season although there were some with beta-hydroxybutyrate concentrations indicative of a negative energy balance; however body condition scores of those cows were all above 4.0.

Mean beta-hydroxybutyrate concentrations were close to the cut-off point of 0.6 mmol/L in mothers of Angus calves (Table 3.3.32) and thus may indicate a slight tendency towards a negative energy balance in cows in their first pregnancy. This may be supported by the fact that 11 of the 16 cows with high plasma beta-hydroxybutyrate concentrations were cows in their first pregnancy.

Mothers of assisted calves had significantly lower plasma glucose concentrations and significantly higher urea concentrations and maternal body condition scores than the mothers of unassisted calves. This might have been due to higher birth weights in assisted calves. Calves with higher weight at birth would place a higher physiological burden on their mothers, and this might have led to a negative energy balance in the mother, as indicated here by lower plasma glucose and increased plasma urea concentrations. However, birth weight of the calves in this study did not have a significant effect (Table 3.3.30), which suggests that differences between maternal parameters were not due to different sizes of calves.

As overall cow energy status was adequate it could be assumed that colostrum production was adequate as well, which may be one reason why cow physiological status and the incidence of disease and death in the calves were not linked in this study. Underfed ewes were found to produce reduced amounts of colostrum for the first 18 hours after birth (Mellor & Murray, 1985) which may thus have an effect on the energy and immune status of the newborn lamb due to insufficient colostrum intake. Also,

underfeeding during late pregnancy was shown to reduce fetal growth in lambs (Mellor & Murray, 1982a) leading to lambs born to underfed ewes being lighter at birth and having reduced energy stores. This may be of importance as lighter lambs will have a proportionally higher heat loss than heavier lambs due an increased surface area to weight ratio (Alexander, 1979) and reduced energy reserved will reduce the time for which such lambs can remain normothermic without food intake (Mellor & Cockburn, 1986).

In lambs hypothermia was associated with a reduction in sucking drive (Alexander & Williams, 1966). This may increase the susceptibility of newborn lambs to infections if lambs have not had intake of colostrum before they became hypothermic. Thus, the risks of contracting infectious diseases may increase due to maternal underfeeding during late pregnancy.

Even if these observations were to apply for calves as well, the majority of cows in the present study were not found to be underfed in late pregnancy, which might explain the absence of significant differences in maternal parameters between healthy, sick and dying calves.

3.5 General discussion

The majority of calves studied over the first four days after birth showed no signs of starvation and hypothermia, as is shown by relatively high plasma glucose concentrations, relatively low plasma urea and beta-hydroxybutyrate concentrations and relatively high rectal temperatures. In addition, the majority of healthy and sick calves had colostrum intake. However, calves that died had significantly lower plasma GGT concentrations than sick and healthy calves and were likely to have failed to acquire sufficient passive immunity.

The results of the present study also suggest that there might be a beneficial effect of separating calves later than usual from their mothers. Calves that stayed with their mothers for longer in the present study might have been more likely to ingest more colostrum than calves that were removed from their mother before 24 hours of age. This

is supported by the fact that plasma glucose concentrations of calves separated from their mother earlier did not show as high a peak as those separated later, they also had lower GGT concentrations than calves separated later and lower rectal temperatures during the first 60 to 70 hours after birth.

Assisted and unassisted calves did not differ significantly in any of the parameters measured, which, when compared to the negative impact of unassisted difficult birth, seems to suggest that early assistance is also beneficial for the longterm health of the newborn calf. However, knowledge about the health status of assisted calves, due to their sale, was limited and thus, although healthy during the study period, they might have became sick later on.

Thus, there seems to be a benefit in assisting calves early as already noted in Chapter 2 and in separating calves from their mothers later than is usual farm practice.

Overall, ensuring that the newborn calf receives adequate amounts of colostrum to acquire passive immunity is also of major importance. However, as seen in the present study, calves, which acquired passive immunity, may nevertheless become sick. Thus, other factors may also play a significant role in the incidence of disease and death and ensuring proper hygiene and housing also seem of major importance next to ensuring the animal has taken in sufficient colostrum to acquire passive immunity.

3.5.1 Problems with experimental set-up

- GGT activity might not have been the best indicator to test for passive immunity (Weaver *et al.*, 2000). GGT is also used as an indicator for liver disturbances, during which GGT concentrations rise (Braun *et al.*, 1982) and thus high values in the present study might have been due to liver disturbances in some animals rather than colostrum intake. In addition, GGT does not reflect the amount of colostrum consumed, but rather a degree of activity where values greater than 200 IU/L suggest that the calf has consumed some colostrum (Perino *et al.*, 1993).

- Calves were not followed up after they were sold and thus no information was available on the incidence of disease and death in the calves after they were sold, which potentially reduced the strength of the statistical analysis.

- Blood samples were not taken at the same age in every calf as they were born at different times of the day and pick up at different ages.

3.5.2 Future research

Future research regarding the welfare of newborn dairy calves over the first few days, weeks and possibly years after birth could include the following:

- Research to determine which age would be best for the separation of calves from their dams and from what age onwards no additional benefit may be achieved.

- The use of other parameters to determine immunoglobulin status of newborn calves, so calves that remain healthy, become sick or die can be better compared with regard to immune status.

- Research to determine the adequate amount of colostrum necessary for calves to acquire sufficient passive immunity.

- Research to determine the effect that maternal nutritional status has on the welfare of the newborn calf or its ability to cope with stress.

- Research to determine which factors may be involved in the longterm welfare and health of the dairy cow, such as feeding practices, competition during feeding and sucking ability of calves.

- Research to determine differences between herds, and possibly breeds, in all the parameters measured in the present study.

Chapter Four:

General Discussion

4

General Discussion

The welfare of newborn calves is important for the economical success of a farm, and also because of the intrinsic value of the calves. Neonatal calf welfare can be improved by stockhandlers knowing about the causes of welfare compromise and by applying remedies accordingly to restore the animals to good health and welfare. However, until now, no detailed accounts have been available on the physiological and physical status of dairy calves at and following birth and how this could affect the neonatal calf's welfare. In comparison, such an account has been available for lambs for some time (Chapter 2). Thus, studies in lambs were used as a guideline for the present study of newborn calves. Although we expected similar problems to occur in calves as were observed in lambs, including placental insufficiency, intrapartum hypoxaemia, inadequate heat production and starvation (Barlow *et al.*, 1987; Mellor, 1988), this was not the case in the calves of the present study. We found that the physiological and physical status of calves corresponded more closely to that observed in single lambs (Chapter 2), which seems to suggest that twins and triplets are more likely to encounter problems than singles. Therefore, litter size should be taken into consideration when comparing the physiological status of newborn animals of different species.

Overall, the major conclusions of this research are presented below.

4.1 Major conclusions

In the present study the physiological and physical status of calves at birth to 4 days of age was examined. Under good New Zealand farming conditions, including well-fed cows, early assistance of dystocic cows and mild weather conditions, the following can be concluded for the calves of the present study:

- Placental insufficiency was not a major problem in the calves of the present study and their physiological status was fairly uniform.

a) This is likely to have been the case as all calves examined were single calves and thus did not have to compete with another embryo for placental implantations sites (Mellor, 1983). In addition, the cows of the present study were all in good condition and generally well fed during pregnancy, as indicated by medium to high body condition scores and the levels of the measured blood parameters at the end of pregnancy. This suggests that cows did not experience undernutrition during early pregnancy and thus during the period of placental development and growth (Mellor, 1988). These facts are likely to have contributed to the low incidence of placental insufficiency in the calves of the present study.

b) Weather conditions and management of calves in the shed may also have contributed to this uniformity. Weather conditions were mild during most of the study and the majority of days were dry and warm. Thus, most calves were born into an environment that might not have been very challenging for them, enabling them to cope well with the change from intrauterine to extrauterine life. Calves were held in a shed, which was open and thus well ventilated. Also, calves were fed colostrum for as long as possible, which would have provided local protection even though gut closure had occurred. In addition, wood shavings on the ground in the calf shed were changed before and once during the trial to ensure adequate hygiene. This may have contributed to the low incidence of calves dying and the fairly uniform status of calves during the first 4 days of life.

c) In lambs the physiological status seems to be more variable (Barlow *et al.* 1987), but pregnant sheep are not usually as intensively managed as dairy cows and are thus more likely to show greater variation in maternal body condition score, which may indicate maternal undernutrition during the time of placental development and growth (Mellor, 1983). Sheep also have a higher incidence of twin and triplet pregnancies compared to dairy cows, which may also affect the physiological and physical status of the newborn animal (Barlow *et al.*, 1987).

- Calves have a good chance of survival if they do not experience placental insufficiency or intrapartum hypoxaemia. Early assistance of dystocic cows may reduce the adverse effects of intrapartum hypoxaemia on the status of the neonatal calf, as shown by the low incidences of hypothermia and of failed colostrum intake. The fact that calves were assisted early during difficult labour evidently prevented the more adverse effects of prolonged intrapartum hypoxaemia from developing. Cows in New Zealand dairy herds may not be assisted during difficult labour as early as was done in this study. Thus, it was not possible in the present study to determine the welfare compromise experienced by calves that are assisted later during difficult labour, as is more likely to be farm practice than early assistance given in the present trial.

- The majority of calves studied between day 1 and 4 after birth were healthy. Half of the calves that stayed on the farm of origin subsequently remained healthy. Healthy calves and calves that became sick received some colostrum, were well fed and were normothermic.

- Calves that died between birth and 1.5 months of age were found to have had insufficient intake of colostrum to attain passive immunity compared to calves that remained healthy and those that became sick.

- Calves that were left with their mothers for a longer period of time seemed to be more likely to suck colostrum from their mothers than calves that were separated at an earlier age.

4.2 Experimental design and limitations

If there had been complete control of the experiment and sufficient funds the experimental design could have been adjusted in the following way to obtain high precision of experimental results in a scientific context. However, such an approach probably would not have been representative of the natural situation, as the more interference the less normal the situation would have become. Thus, the present experiment, although it might have been scientifically imprecise in some respects, will

have more value for on-farm purposes and may more easily lead to practical advice for farmers.

- Dystocias would have been assisted at different times (for example assistance at 1, 2, 3 or more hours after no progress in labour occurred) to determine the effect of duration of dystocia on the physiological and physical status of newborn calves and on their survival. This would have allowed us to determine more precisely what benefits accrue from assisting dystocic cows at different stages during difficult labour.

- Blood samples would have been taken twice after birth, 15 minutes apart, instead of once, to be able to better determine the effect of pre-, intra- and postpartum factors on blood parameters measured. In addition, rectal temperature would have been measured four times during the first hour after birth and again during the second hour after birth to ensure every case of hypothermia was detected. Overall, all samples and rectal temperatures would be taken at specific times in relation to birth, so that they related to that event and not clock time.

- The first intake of colostrum could be timed and behavioural recordings could be taken to determine the sucking ability of the calf. In addition, the calf could be weighed before and after the first sucking bout to determine the amount taken in at the first drinking bout. Also, colostrum intake could be controlled so that calves with no intake and those with adequate intakes could be compared to determine differences in physiological and physical status as well as health status between the two groups. Timing of colostrum intake could be varied (early, medium and late during the first day after birth) to determine the effects of timing of colostrum intake on immune status and susceptibility to disease in later life. For comparisons between those groups to be effective, actual immune status would need to be measured rather than plasma GGT concentrations (Weaver *et al.*, 2000).

- Pick-up time would be controlled and calves would be picked up at a young, medium and older age to determine the differences in physiological, physical and immune status as well as health status between these groups. Thus, the optimum age for pick up could be determined. In addition, the effect of separation at different ages on calf and cow distress (monitored behaviourally and physiologically) would be examined to determine the best timing of separation to minimise separation stress for the cow and calf, but also to yield the highest standard of immune, health and physiological status (Weary & Chua, 2000).

- Once calves are picked up, feeding would occur at set times after birth for each calf in order to minimise variation due to differences in age at feeding. The sucking ability of calves and their ability to compete at feeders would be noted. In addition, they would be weighed before and after each feed to determine intake volume if that could not be measured directly.

- Calves of the various colostrum groups and pick-up groups would be housed in a variety of sheds to determine possible effects shed design might have on physiological, physical and health status of the calves.

- All calves would be followed for several months to determine the longterm effects of several assistance regimes, colostrum feeding regimes, pick-up regimes and housing on health and production.

Although a number of limitations exist for the present study, these were accepted by the need to use a commercial farm so that results of the present study would be applicable to the farm context. In addition, pros and cons of parameters used are listed.

- The staff number employed was relatively large and thus individual differences in handling calves at birth and timing of treatments might have

led to variation in the data. In addition, there was the odd occasion where the two staff members on each shift were not enough to deal with all calves born at the same time leading to a time delay of blood sampling and rectal temperature recording and possibly missing recordings for the time it took the calf to stand.

- It was impossible to control for weather conditions, however these were monitored and taken into consideration. In addition, calf nutritional status was not controlled for and therefore might have been quite variable which could have introduced bias in to the metabolite data.

- Cows, which could not be assisted to give birth in the field were taken to a shed to be assisted; this interfered with data collection and resulted in some missing data points.

- The design of the calf shed might have had an influence on the outcome of the present study. However, this is not known, as we did not compare various designs of calf sheds.

- Glucose measurements are a good indicator of carbohydrate metabolism showing if the animal is in an absorptive, post-absorptive or fasting state. However, it is not possible to detect the size of liver and muscle glycogen stores. Also, plasma glucose concentrations are affected by other parameters such as stress which causes increased plasma glucose concentrations due to cortisol secretion (Todd *et al.*, 2000a). However, plasma glucose concentrations will only be useful as indicators of energy balance in monogastric animals. In the ruminant animal, glucose is not as sensitive to changes in energy balance as beta-hydroxybutyrate (Whitaker, 2000).

- Although measurements of beta-hydroxybutyrate are useful in detecting undernutrition, it has to be mentioned that they are less sensitive than free fatty acid concentrations for detecting moderate degrees of underfeeding, but

are apparently good indicators for more sever underfeeding (Russel *et al.*, 1967).

- Although plasma urea concentrations can give a good indication of protein catabolism, urea concentrations can increase independently of protein breakdown. Dehydration or impaired kidney function (Guyton and Hall, 1996) may lead to reduced urea clearance in the kidney and thus to build-up of urea in plasma independently of protein breakdown.

- Plasma GGT concentrations are valuable to determine whether an animal has taken in colostrum or not, however, immune status cannot be determined as such as plasma GGT concentrations do not give an indication on the amount of immunoglobulins consumed. Although the interpretation of plasma GGT concentration and colostrum intake is enhanced by plasma glucose concentrations, it may be helpful to know about the feeding history of the animal. If a calf has fed early, plasma glucose and GGT concentrations may have declined to levels indicative of failure of colostrum intake although it might have taken in enough colostrum to acquire passive immunity. Problems with plasma GGT concentrations as a measure of colostrum may arise in animals with liver disturbances as GGT concentrations will rise in response to such disturbances (Braun *et al.*, 1982).

- Although rectal temperature can give an indication of the integrated effect of heat production and heat loss, it is not able to measure heat production and heat loss as such. Thus, our knowledge about the thermogenic ability of the newborn calves in the present study is limited.

4.3 Practical considerations

From the results of the present study, it could be demonstrated that the present calves, born to cows in good conditions and into good environmental conditions, did not experience great welfare problems. As shown in the present study, calves had a good chance of survival shortly after birth if they were not compromised by placental insufficiency or intrapartum hypoxaemia. Thus, feeding cows adequately during all

stages of pregnancy as well as giving early assistance to cows with difficult labour could prevent major welfare compromise to the calf (see chapter 1). Also, if calves are generally more likely to suck when left with their mothers for longer than 24 hours, it may be beneficial to the calves' welfare to prolong the time spent with the mother to maximise their chances of maternal colostrum intake and thus survival. Calves that died in the present study failed to take in colostrum before 24 hours of age. Thus, ensuring colostrum intake may help to prevent calves from dying even though it may not prevent disease totally.

Overall, recommendations for farmers from the results of this study may thus include the following advice:

1. Feed pregnant cows adequate amounts during the whole of pregnancy in order to avoid placental insufficiency or maternal undernutrition during late pregnancy, which may both have negative effects on the welfare of the neonatal calf.

2. Assist cows with difficult labour early to avoid adverse effects of intrapartum hypoxaemia and death.

3. Keep calves in well-ventilated, clean sheds and do not mix calves with other animals from another farm or herd. Disinfect umbilical cords as soon as possible after birth and when calves are brought into the calf shed.

4. Separate calves from their mothers after a longer time period than usual to increase the likelihood that calves will drink colostrum from their mother.

5. Ensure that calves receive sufficient colostrum to attain passive immunity to reduce incidence of death in the group. This may be achieved by feeding at pickup by stomach tube.

However, as the results of the present study were only obtained from calves of one dairy herd, one cannot extrapolate to all herds in New Zealand and the above advice should only be given after further study has been undertaken on a larger scale. The present research nevertheless seems to provide guidelines for further study and could be seen as a pilot study for a wider national investigation.

4.4 Where do we go from here?

Although we now know more about the status of calves at birth and following birth in regards to their physiological status, many questions still remain unanswered. We do not know if the situation of calves in the present study is similar for other herds in New Zealand. Also, we do not know what the status of calves is under different farming conditions, in beef herds, on organic farms or in other countries, especially where calves are born indoors or outdoors and under adverse environmental conditions. We do not have in depth records on the effect of placental insufficiency and maternal underfeeding on the physiology and welfare of the neonatal calf. Although the literature on colostrum and its effect on disease and death in calves is considerable, we do not know much about intake capacity, competition for food supply in the shed and how this might influence the attainment of passive immunity, growth and welfare of the calf. In addition, we have to look into other areas to determine the possible welfare compromise in neonatal calves, such as disease. The knowledge about disease in newborn animals is vast, but only limited information about the welfare of the newborn animal in regards to suffering before death, during sickness or poor growth resulting from different causes, is available. In addition, the effect of early assistance and late separation from the dam and their effect on calf welfare have to be further clarified.

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Appendix 1: Chapter 3

For glucose tables sample 1 = birth sample; sample 2 = 24-hour sample; sample 3 = 48-hour sample; sample 4 = 72-hour sample; sample 5 = 96-hour sample. For all other tables (excluding rectal temperature tables) sample 1 = 24-hour sample; sample 2 = 48-hour sample; sample 3 = 72-hour sample and sample 4 = 96-hour sample.

Table A1.1: Results of repeated measures ANOVA for glucose (p-values) to determine significant differences between groups and samples (25 comparisons, 0.05/25=0.002, thus any p-value smaller than 0.002 is significant).

Group	p-values
Sample 1 assisted versus sample 1 unassisted	0.1779
Sample 2 assisted versus sample 2 unassisted	0.1947
Sample 3 assisted versus sample 3 unassisted	0.4399
Sample 4 assisted versus sample 4 unassisted	0.2483
Sample 5 assisted versus sample 5 unassisted	0.3572
Sample 1 assisted versus sample 2 assisted	0.0353
Sample 1 assisted versus sample 3 assisted	0.1557
Sample 1 assisted versus sample 4 assisted	0.7252
Sample 1 assisted versus sample 5 assisted	0.3960
Sample 2 assisted versus sample 3 assisted	0.3645
Sample 2 assisted versus sample 4 assisted	0.0190
Sample 2 assisted versus sample 5 assisted	0.1170
Sample 3 assisted versus sample 4 assisted	0.0231
Sample 3 assisted versus sample 5 assisted	0.3062
Sample 4 assisted versus sample 5 assisted	0.2356
Sample 1 unassisted versus sample 2 unassisted	0.0096
Sample 1 unassisted versus sample 3 unassisted	0.3753
Sample 1 unassisted versus sample 4 unassisted	0.2425
Sample 1 unassisted versus sample 5 unassisted	0.6373
Sample 2 unassisted versus sample 3 unassisted	<0.0001
Sample 2 unassisted versus sample 4 unassisted	<0.0001
Sample 2 unassisted versus sample 5 unassisted	<0.0001
Sample 3 unassisted versus sample 4 unassisted	0.5573
Sample 3 unassisted versus sample 5 unassisted	0.4324
Sample 4 unassisted versus sample 5 unassisted	0.0825

Table A 1.2: Results of repeated measures ANOVA for glucose (p-values) to determine significant differences between groups and samples (25 comparisons, 0.05/25=0.002, thus any p-value smaller than 0.002 is significant).

Group	p-value
Sample 1 high GGT versus sample 1 low GGT	0.1289
Sample 2 high GGT versus sample 2 low GGT	<0.0001
Sample 3 high GGT versus sample 3 low GGT	0.0023
Sample 4 high GGT versus sample 4 low GGT	<0.0001
Sample 5 high GGT versus sample 5 low GGT	<0.0001
Sample 1 high GGT versus sample 2 high GGT	<0.0001
Sample 1 high GGT versus sample 3 high GGT	0.1755
Sample 1 high GGT versus sample 4 high GGT	0.4656
Sample 1 high GGT versus sample 5 high GGT	0.1490
Sample 2 high GGT versus sample 3 high GGT	<0.0001
Sample 2 high GGT versus sample 4 high GGT	<0.0001
Sample 2 high GGT versus sample 5 high GGT	<0.0001
Sample 3 high GGT versus sample 4 high GGT	0.1769
Sample 3 high GGT versus sample 5 high GGT	0.9093
Sample 4 high GGT versus sample 5 high GGT	0.0753
Sample 1 low GGT versus sample 2 low GGT	0.1027
Sample 1 low GGT versus sample 3 low GGT	0.0189
Sample 1 low GGT versus sample 4 low GGT	0.0018
Sample 1 low GGT versus sample 5 low GGT	0.0156
Sample 2 low GGT versus sample 3 low GGT	0.2153
Sample 2 low GGT versus sample 4 low GGT	0.0213
Sample 2 low GGT versus sample 5 low GGT	0.2199
Sample 3 low GGT versus sample 4 low GGT	0.1405
Sample 3 low GGT versus sample 5 low GGT	0.9636
Sample 4 low GGT versus sample 5 low GGT	0.0891

Table A1.3: Results of repeated measures ANOVA for glucose (p-values) to determine significant differences between groups and samples (45 comparisons, 0.05/45=0.001, thus any p-value smaller than 0.001 is significant).

Group	p-values
Sample 1 healthy versus sample 1 dying	0.1397
Sample 1 sick versus sample 1 dying	0.3959
Sample 1 sick versus sample 1 healthy	0.4537
Sample 2 healthy versus sample 2 dying	0.1693
Sample 2 sick versus sample 2 dying	0.5429
Sample 2 sick versus sample 2 healthy	0.3436
Sample 3 healthy versus sample 3 dying	0.0194
Sample 3 sick versus sample 3 dying	0.2734
Sample 3 sick versus sample 3 healthy	0.0904
Sample 4 healthy versus sample 4 dying	0.4638
Sample 4 sick versus sample 4 dying	0.3121
Sample 4 sick versus sample 4 healthy	0.7993
Sample 5 healthy versus sample 5 dying	0.4540
Sample 5 sick versus sample 5 dying	0.8899
Sample 5 sick versus sample 5 healthy	0.2831
Sample 1 sick versus sample 2 sick	0.0502
Sample 1 sick versus sample 3 sick	0.4557
Sample 1 sick versus sample 4 sick	0.6656
Sample 1 sick versus sample 5 sick	0.7923
Sample 2 sick versus sample 3 sick	0.0839
Sample 2 sick versus sample 4 sick	0.0362
Sample 2 sick versus sample 5 sick	0.0312
Sample 3 sick versus sample 4 sick	0.5978
Sample 3 sick versus sample 5 sick	0.4812
Sample 4 sick versus sample 5 sick	0.8123
Sample 1 healthy versus sample 2 healthy	0.0001
Sample 1 healthy versus sample 3 healthy	0.0028
Sample 1 healthy versus sample 4 healthy	0.0982
Sample 1 healthy versus sample 5 healthy	0.0116
Sample 2 healthy versus sample 3 healthy	0.0951
Sample 2 healthy versus sample 4 healthy	0.0011
Sample 2 healthy versus sample 5 healthy	0.0525
Sample 3 healthy versus sample 4 healthy	0.0179
Sample 3 healthy versus sample 5 healthy	0.5982
Sample 4 healthy versus sample 5 healthy	0.1288
Sample 1 dying versus sample 2 dying	0.9726
Sample 1 dying versus sample 3 dying	0.2300
Sample 1 dying versus sample 4 dying	0.1737
Sample 1 dying versus sample 5 dying	0.5224
Sample 2 dying versus sample 3 dying	0.1519
Sample 2 dying versus sample 4 dying	0.1049
Sample 2 dying versus sample 5 dying	0.4379
Sample 3 dying versus sample 4 dying	0.8015
Sample 3 dying versus sample 5 dying	0.4232
Sample 4 dying versus sample 5 dying	0.2970

Table A1.4: Results of repeated measures ANOVA for glucose (p-values) to determine significant differences between groups and samples (25 comparisons, 0.05/25=0.002, thus any p-value smaller than 0.002 is significant).

Group	p-values
Sample 1 younger 24 hours versus sample 1 older 24 hours	0.7278
Sample 2 younger 24 hours versus sample 2 older 24 hours	0.0165
Sample 3 younger 24 hours versus sample 3 older 24 hours	0.8889
Sample 4 younger 24 hours versus sample 4 older 24 hours	0.1504
Sample 5 younger 24 hours versus sample 5 older 24 hours	0.9066
Sample 1 younger 24 hours versus sample 2 younger 24 hours	0.0374
Sample 1 younger 24 hours versus sample 3 younger 24 hours	0.8739
Sample 1 younger 24 hours versus sample 4 younger 24 hours	0.5330
Sample 1 younger 24 hours versus sample 5 younger 24 hours	0.9539
Sample 2 younger 24 hours versus sample 3 younger 24 hours	0.0011
Sample 2 younger 24 hours versus sample 4 younger 24 hours	0.0002
Sample 2 younger 24 hours versus sample 5 younger 24 hours	0.0044
Sample 3 younger 24 hours versus sample 4 younger 24 hours	0.3235
Sample 3 younger 24 hours versus sample 5 younger 24 hors	0.8488
Sample 4 younger 24 hours versus sample 5 younger 24 hours	0.1589
Sample 1 older 24 hours versus sample 2 older 24 hours	0.0005
Sample 1 older 24 hours versus sample 3 older 24 hours	0.7252
Sample 1 older 24 hours versus sample 4 older 24 hours	0.5479
Sample 1 older 24 hours versus sample 5 older 24 hours	0.7698
Sample 2 older 24 hours versus sample 3 older 24 hours	<0.0001
Sample 2 older 24 hours versus sample 4 older 24 hours	<0.0001
Sample 2 older 24 hours versus sample 5 older 24 hours	<0.0001
Sample 3 older 24 hours versus sample 4 older 24 hours	0.0460
Sample 3 older 24 hours versus sample 5 older 24 hours	0.9130
Sample 4 older 24 hours versus sample 5 older 24 hours	0.0305

Table A1.5: Results of repeated measures ANOVA for beta-hydroxybutyrate (p-values) to determine significant differences between groups and samples (16 comparisons, 0.05/16=0.003, thus any p-value smaller than 0.003 is significant).

Group	p-values
Sample 1 assisted versus sample 1 unassisted	0.1637
Sample 2 assisted versus sample 2 unassisted	0.0047
Sample 3 assisted versus sample 3 unassisted	0.0327
Sample 4 assisted versus sample 4 unassisted	0.0004
Sample 1 assisted versus sample 2 assisted	0.9094
Sample 1 assisted versus sample 3 assisted	0.4311
Sample 1 assisted versus sample 4 assisted	0.6106
Sample 2 assisted versus sample 3 assisted	0.4119
Sample 2 assisted versus sample 4 assisted	0.6453
Sample 3 assisted versus sample 4 assisted	0.8132
Sample 1 unassisted versus sample 2 unassisted	0.096
Sample 1 unassisted versus sample 3 unassisted	0.0719
Sample 1 unassisted versus sample 4 unassisted	<0.0001
Sample 2 unassisted versus sample 3 unassisted	0.7987
Sample 2 unassisted versus sample 4 unassisted	0.0047
Sample 3 unassisted versus sample 4 unassisted	0.0052

Table A1.6: Results of repeated measures ANOVA for beta-hydroxybutyrate (p-values) to determine significant differences between groups and samples (16 comparisons, 0.05/16=0.003, thus any p-value smaller than 0.003 is significant).

Group	p-values
Sample 1 high GGT versus sample 1 low GGT	0.0399
Sample 2 high GGT versus sample 2 low GGT	0.2054
Sample 3 high GGT versus sample 3 low GGT	0.7706
Sample 4 high GGT versus sample 4 low GGT	0.2160
Sample 1 high GGT versus sample 2 high GGT	0.005
Sample 1 high GGT versus sample 3 high GGT	<0.0001
Sample 1 high GGT versus sample 4 high GGT	0.0505
Sample 2 high GGT versus sample 3 high GGT	0.2410
Sample 2 high GGT versus sample 4 high GGT	0.1719
Sample 3 high GGT versus sample 4 high GGT	0.0055
Sample 1 low GGT versus sample 2 low GGT	<0.0001
Sample 1 low GGT versus sample 3 low GGT	<0.0001
Sample 1 low GGT versus sample 4 low GGT	<0.0001
Sample 2 low GGT versus sample 3 low GGT	0.4782
Sample 2 low GGT versus sample 4 low GGT	0.2014
Sample 3 low GGT versus sample 4 low GGT	0.6249

Table A1.7: Results of repeated measures ANOVA for beta-hydroxybutyrate (p-values) to determine significant differences between groups and samples (30 comparisons, 0.05/30=0.002, thus any p-value smaller than 0.002 is significant).

Group	p-values
Sample 1 sick versus sample 1 dying	0.8064
Sample 1 healthy versus sample 1 dying	0.5723
Sample 1 sick versus sample 1 healthy	0.2538
Sample 2 sick versus sample 2 dying	0.7924
Sample 2 healthy versus sample 2 dying	0.5489
Sample 2 sick versus sample 2 healthy	0.6548
Sample 3 sick versus sample 3 dying	0.4269
Sample 3 healthy versus sample 3 dying	0.9288
Sample 3 sick versus sample 3 healthy	0.2017
Sample 4 sick versus sample 4 dying	0.2828
Sample 4 healthy versus sample 4 dying	0.3664
Sample 4 sick versus sample 4 healthy	0.7833
Sample 1 sick versus sample 2 sick	0.1690
Sample 1 sick versus sample 3 sick	0.5460
Sample 1 sick versus sample 4 sick	0.2217
Sample 2 sick versus sample 3 sick	0.4631
Sample 2 sick versus sample 4 sick	0.7498
Sample 3 sick versus sample 4 sick	0.6219
Sample 1 healthy versus sample 2 healthy	0.0179
Sample 1 healthy versus sample 3 healthy	0.0003
Sample 1 healthy versus sample 4 healthy	0.0112
Sample 2 healthy versus sample 3 healthy	0.1531
Sample 2 healthy versus sample 4 healthy	0.5896
Sample 3 healthy versus sample 4 healthy	0.4984
Sample 1 dying versus sample 2 dying	0.1412
Sample 1 dying versus sample 3 dying	0.1394
Sample 1 dying versus sample 4 dying	0.0424
Sample 2 dying versus sample 3 dying	0.9122
Sample 2 dying versus sample 4 dying	0.5041
Sample 3 dying versus sample 4 dying	0.6021

Table A1.8: Results of repeated measures ANOVA for beta-hydroxybutyrate (p-values) to determine significant differences between groups and samples (16 comparisons, 0.05/16=0.003, thus any p-value smaller than 0.003 is significant).

Group	p-values
Sample 1 younger 24 hours versus sample 1 older 24 hours	0.0078
Sample 2 younger 24 hours versus sample 2 older 24 hours	0.0752
Sample 3 younger 24 hours versus sample 3 older 24 hours	0.6779
Sample 4 younger 24 hours versus sample 4 older 24 hours	0.8100
Sample 1 younger 24 hours versus sample 2 younger 24 hours	<0.0001
Sample 1 younger 24 hours versus sample 3 younger 24 hours	<0.0001
Sample 1 younger 24 hours versus sample 4 younger 24 hours	<0.0001
Sample 2 younger 24 hours versus sample 3 younger 24 hours	0.9512
Sample 2 younger 24 hours versus sample 4 younger 24 hours	0.0136
Sample 3 younger 24 hours versus sample 4 younger 24 hours	0.0101
Sample 1 older 24 hours versus sample 2 older 24 hours	0.4971
Sample 1 older 24 hours versus sample 3 older 24 hours	0.5242
Sample 1 older 24 hours versus sample 4 older 24 hours	0.8514
Sample 2 older 24 hours versus sample 3 older 24 hours	0.1916
Sample 2 older 24 hours versus sample 4 older 24 hours	0.5547
Sample 3 older 24 hours versus sample 4 older 24 hours	0.3623

Table A1.9: Results of repeated measures ANOVA for urea (p-values) to determine significant differences between groups and samples (16 comparisons, 0.05/16=0.003, thus any p-value smaller than 0.003 is significant).

Group	p-values
Sample 1 assisted versus sample 1 unassisted	0.5051
Sample 2 assisted versus sample 2 unassisted	0.2872
Sample 3 assisted versus sample 3 unassisted	0.6322
Sample 4 assisted versus sample 4 unassisted	0.0610
Sample 1 assisted versus sample 2 assisted	0.0451
Sample 1 assisted versus sample 3 assisted	<0.0001
Sample 1 assisted versus sample 4 assisted	<0.0001
Sample 2 assisted versus sample 3 assisted	<0.0001
Sample 2 assisted versus sample 4 assisted	0.0158
Sample 3 assisted versus sample 4 assisted	0.1450
Sample 1 unassisted versus sample 2 unassisted	<0.0001
Sample 1 unassisted versus sample 3 unassisted	<0.0001
Sample 1 unassisted versus sample 4 unassisted	<0.0001
Sample 2 unassisted versus sample 3 unassisted	0.0014
Sample 2 unassisted versus sample 4 unassisted	0.5803
Sample 3 unassisted versus sample 4 unassisted	<0.0001

Table A1.10: Results of repeated measures ANOVA for urea (p-values) to determine significant differences between groups and samples (16 comparisons, 0.05/16=0.003, thus any p-value smaller than 0.003 is significant).

Group	p-values
Sample 1 high GGT versus sample 1 low GGT	0.5934
Sample 2 high GGT versus sample 2 low GGT	0.0678
Sample 3 high GGT versus sample 3 low GGT	0.3989
Sample 4 high GGT versus sample 4 low GGT	0.3668
Sample 1 high GGT versus sample 2 high GGT	<0.0001
Sample 1 high GGT versus sample 3 high GGT	<0.0001
Sample 1 high GGT versus sample 4 high GGT	<0.0001
Sample 2 high GGT versus sample 3 high GGT	<0.0001
Sample 2 high GGT versus sample 4 high GGT	0.1736
Sample 3 high GGT versus sample 4 high GGT	<0.0001
Sample 1 low GGT versus sample 2 low GGT	<0.0001
Sample 1 low GGT versus sample 3 low GGT	<0.0001
Sample 1 low GGT versus sample 4 low GGT	<0.0001
Sample 2 low GGT versus sample 3 low GGT	0.0790
Sample 2 low GGT versus sample 4 low GGT	0.5073
Sample 3 low GGT versus sample 4 low GGT	0.0051

Table A1.11: Results of repeated measures ANOVA for urea (p-values) to determine significant differences between groups and samples (30 comparisons, 0.05/30=0.002, thus any p-value smaller than 0.002 is significant).

Group	p-values
Sample 1 sick versus sample 1 dying	0.5949
Sample 1 healthy versus sample 1 dying	0.7930
Sample 1 sick versus sample 1 healthy	0.7181
Sample 2 sick versus sample 2 dying	0.5949
Sample 2 healthy versus sample 2 dying	0.7930
Sample 2 sick versus sample 2 healthy	0.7181
Sample 3 sick versus sample 3 dying	0.3784
Sample 3 healthy versus sample 3 dying	0.5011
Sample 3 sick versus sample 3 healthy	0.7560
Sample 4 sick versus sample 4 dying	0.3700
Sample 4 healthy versus sample 4 dying	0.8281
Sample 4 sick versus sample 4 healthy	0.3497
Sample 1 sick versus sample 2 sick	0.0255
Sample 1 sick versus sample 3 sick	0.0011
Sample 1 sick versus sample 4 sick	0.0461
Sample 2 sick versus sample 3 sick	0.1146
Sample 2 sick versus sample 4 sick	0.8287
Sample 3 sick versus sample 4 sick	0.0523
Sample 1 healthy versus sample 2 healthy	0.0114
Sample 1 healthy versus sample 3 healthy	<0.0001
Sample 1 healthy versus sample 4 healthy	0.0015
Sample 2 healthy versus sample 3 healthy	0.0079
Sample 2 healthy versus sample 4 healthy	0.5336
Sample 3 healthy versus sample 4 healthy	0.0404
Sample 1 dying versus sample 2 dying	0.0202
Sample 1 dying versus sample 3 dying	0.0030
Sample 1 dying versus sample 4 dying	0.0122
Sample 2 dying versus sample 3 dying	0.2552
Sample 2 dying versus sample 4 dying	0.9457
Sample 3 dying versus sample 4 dying	0.2458

Table A1.12: Results of repeated measures ANOVA for urea (p-values) to determine significant differences between groups and samples (16 comparisons, 0.05/16=0.003, thus any p-value smaller than 0.003 is significant).

Group	p-values
Sample 1 younger 24 hours versus sample 1 older 24 hours	0.0684
Sample 2 younger 24 hours versus sample 2 older 24 hours	0.1885
Sample 3 younger 24 hours versus sample 3 older 24 hours	0.4350
Sample 4 younger 24 hours versus sample 4 older 24 hours	0.4066
Sample 1 younger 24 hours versus sample 2 younger 24 hours	<0.0001
Sample 1 younger 24 hours versus sample 3 younger 24 hours	<0.0001
Sample 1 younger 24 hours versus sample 4 younger 24 hours	<0.0001
Sample 2 younger 24 hours versus sample 3 younger 24 hours	<0.0001
Sample 2 younger 24 hours versus sample 4 younger 24 hours	0.9076
Sample 3 younger 24 hours versus sample 4 younger 24 hours	<0.0001
Sample 1 older 24 hours versus sample 2 older 24 hours	0.2566
Sample 1 older 24 hours versus sample 3 older 24 hours	<0.0001
Sample 1 older 24 hours versus sample 4 older 24 hours	0.0004
Sample 2 older 24 hours versus sample 3 older 24 hours	0.0010
Sample 2 older 24 hours versus sample 4 older 24 hours	0.0345
Sample 3 older 24 hours versus sample 4 older 24 hours	0.2970

Table A1.13: Results of repeated measures ANOVA for GGT (p-values) to determine significant differences between groups and samples (16 comparisons, 0.05/16=0.003, thus any p-value smaller than 0.003 is significant).

Group	p-values
Sample 1 assisted versus sample 1 unassisted	0.5702
Sample 2 assisted versus sample 2 unassisted	0.4821
Sample 3 assisted versus sample 3 unassisted	0.2827
Sample 4 assisted versus sample 4 unassisted	0.4334
Sample 1 assisted versus sample 2 assisted	<0.0001
Sample 1 assisted versus sample 3 assisted	<0.0001
Sample 1 assisted versus sample 4 assisted	<0.0001
Sample 2 assisted versus sample 3 assisted	<0.0001
Sample 2 assisted versus sample 4 assisted	0.0026
Sample 3 assisted versus sample 4 assisted	0.7338
Sample 1 unassisted versus sample 2 unassisted	<0.0001
Sample 1 unassisted versus sample 3 unassisted	<0.0001
Sample 1 unassisted versus sample 4 unassisted	<0.0001
Sample 2 unassisted versus sample 3 unassisted	0.0001
Sample 2 unassisted versus sample 4 unassisted	<0.0001
Sample 3 unassisted versus sample 4 unassisted	0.0019

Table A1.14: Results of repeated measures ANOVA for GGT (p-values) to determine significant differences between groups and samples (30 comparisons, 0.05/30=0.002, thus any p-value smaller than 0.002 is significant).

Group	p-values
Sample 1 sick versus sample 1 dying	0.0004
Sample 1 healthy versus sample 1 dying	0.0005
Sample 1 sick versus sample 1 healthy	0.8915
Sample 2 sick versus sample 2 dying	0.0010
Sample 2 healthy versus sample 2 dying	0.0023
Sample 2 sick versus sample 2 healthy	0.6748
Sample 3 sick versus sample 3 dying	0.0025
Sample 3 healthy versus sample 3 dying	0.0031
Sample 3 sick versus sample 3 healthy	0.8964
Sample 4 sick versus sample 4 dying	0.0006
Sample 4 healthy versus sample 4 dying	0.0010
Sample 4 sick versus sample 4 healthy	0.8496
Sample 1 sick versus sample 2 sick	0.0039
Sample 1 sick versus sample 3 sick	<0.0001
Sample 1 sick versus sample 4 sick	0.0001
Sample 2 sick versus sample 3 sick	<0.0001
Sample 2 sick versus sample 4 sick	0.0077
Sample 3 sick versus sample 4 sick	0.8208
Sample 1 healthy versus sample 2 healthy	<0.0001
Sample 1 healthy versus sample 3 healthy	<0.0001
Sample 1 healthy versus sample 4 healthy	<0.0001
Sample 2 healthy versus sample 3 healthy	0.0039
Sample 2 healthy versus sample 4 healthy	0.0483
Sample 3 healthy versus sample 4 healthy	0.9905
Sample 1 dying versus sample 2 dying	0.8171
Sample 1 dying versus sample 3 dying	0.2183
Sample 1 dying versus sample 4 dying	0.0668
Sample 2 dying versus sample 3 dying	0.1626
Sample 2 dying versus sample 4 dying	0.0453
Sample 3 dying versus sample 4 dying	0.1860

Table A1.15: Results of repeated measures ANOVA for GGT (p-values) to determine significant differences between groups and samples (16 comparisons, 0.05/16=0.003, thus any p-value smaller than 0.003 is significant).

Group	p-values
Sample 1 younger 24 hours versus sample 1 older 24 hours	0.2025
Sample 2 younger 24 hours versus sample 2 older 24 hours	0.1777
Sample 3 younger 24 hours versus sample 3 older 24 hours	0.1636
Sample 4 younger 24 hours versus sample 4 older 24 hours	0.2904
Sample 1 younger 24 hours versus sample 2 younger 24 hours	<0.0001
Sample 1 younger 24 hours versus sample 3 younger 24 hours	<0.0001
Sample 1 younger 24 hours versus sample 4 younger 24 hours	<0.0001
Sample 2 younger 24 hours versus sample 3 younger 24 hours	<0.0001
Sample 2 younger 24 hours versus sample 4 younger 24 hours	<0.0001
Sample 3 younger 24 hours versus sample 4 younger 24 hours	0.0517
Sample 1 older 24 hours versus sample 2 older 24 hours	<0.0001
Sample 1 older 24 hours versus sample 3 older 24 hours	<0.0001
Sample 1 older 24 hours versus sample 4 older 24 hours	<0.0001
Sample 2 older 24 hours versus sample 3 older 24 hours	0.0035
Sample 2 older 24 hours versus sample 4 older 24 hours	<0.0001
Sample 3 older 24 hours versus sample 4 older 24 hours	0.0057

For rectal temperature, sample 1 = birth sample; sample 2 = temperature at 19 hours; sample 3 = at 36 hours; sample 4 = at 43 hours ; sample 5 = at 60 hours ; sample 6 = 67 hours ; sample 7 = 83 hours and sample 8 = at 91 hours.

Table A1.16: Results of repeated measures ANOVA for rectal temperature (p-values) to determine significant differences between groups and samples. Values are significant if smaller than 0.0008 (0.05/64 comparisons).

Group	p-values
Sample 1 assisted versus sample 1 unassisted	0.5983
Sample 2 assisted versus sample 2 unassisted	0.0218
Sample 3 assisted versus sample 3 unassisted	0.3970
Sample 4 assisted versus sample 4 unassisted	0 1423
Sample 5 assisted versus sample 5 unassisted	0.6123
Sample 6 assisted versus sample 6 unassisted	0.2352
Sample 7 assisted versus sample 7 unassisted	0 4054
Sample 8 assisted versus sample 8 unassisted	0.0771
Sample 1 assisted versus sample 2 assisted	0.6468
Sample 1 assisted versus sample 3 assisted	0 7242
Sample 1 assisted versus sample 4 assisted	0.3388
Sample 1 assisted versus sample 5 assisted	0.2991
Sample 1 assisted versus sample 6 assisted	0.5850
Sample 1 assisted versus sample 7 assisted	0.6555
Sample 1 assisted versus sample 8 assisted	0.5295
Sample 2 assisted versus sample 3 assisted	0.2004
Sample 2 assisted versus sample 4 assisted	0 4817
Sample 2 assisted versus sample 5 assisted	0 4065
Sample 2 assisted versus sample 6 assisted	0.9144
Sample 2 assisted versus sample 7 assisted	0 9508
Sample 2 assisted versus sample 8 assisted	0.8282
Sample 3 assisted versus sample 4 assisted	0.0172
Sample 3 assisted versus sample 5 assisted	0.0090
Sample 3 assisted versus sample 6 assisted	0 1250
Sample 3 assisted versus sample 7 assisted	0.1219
Sample 3 assisted versus sample 8 assisted	0.0651
Sample 4 assisted versus sample 5 assisted	0.8969
Sample 4 assisted versus sample 6 assisted	0.5217
Sample 4 assisted versus sample 7 assisted	0.3506
Sample 4 assisted versus sample 8 assisted	0.5581
Sample 5 assisted versus sample 6 assisted	0.4360
Sample 5 assisted versus sample 7 assisted	0.2690
Sample 5 assisted versus sample 8 assisted	0.4596
Sample 6 assisted versus sample 7 assisted	0.8448
Sample 6 assisted versus sample 8 assisted	0.9116
Sample 7 assisted versus sample 8 assisted	0.7305
Sample 1 unassisted versus sample 2 unassisted	0.4093
Sample 1 unassisted versus sample 3 unassisted	0.8387
Sample 1 unassisted versus sample 4 unassisted	0.2080
Sample 1 unassisted versus sample 5 unassisted	0.0224
Sample 1 unassisted versus sample 6 unassisted	0.0024
Sample 1 unassisted versus sample 7 unassisted	0.0159
Sample 1 unassisted versus sample 8 unassisted	0.0005
Sample 2 unassisted versus sample 3 unassisted	0.2994
Sample 2 unassisted versus sample 4 unassisted	0.0018
Sample 2 unassisted versus sample 5 unassisted	<0.0001
Sample 2 unassisted versus sample 6 unassisted	<0.0001
Sample 2 unassisted versus sample 7 unassisted	<0.0001
Sample 2 unassisted versus sample 8 unassisted	<0.0001

Sample 3 unassisted versus sample 4 unassisted	0.0078
Sample 3 unassisted versus sample 5 unassisted	<0.0001
Sample 3 unassisted versus sample 6 unassisted	<0.0001
Sample 3 unassisted versus sample 7 unassisted	<0.0001
Sample 3 unassisted versus sample 8 unassisted	<0.0001
Sample 4 unassisted versus sample 5 unassisted	0.0737
Sample 4 unassisted versus sample 6 unassisted	0.0030
Sample 4 unassisted versus sample 7 unassisted	0.0411
Sample 4 unassisted versus sample 8 unassisted	<0.0001
Sample 5 unassisted versus sample 6 unassisted	0.1619
Sample 5 unassisted versus sample 7 unassisted	0.8277
Sample 5 unassisted versus sample 8 unassisted	0.0228
Sample 6 unassisted versus sample 7 unassisted	0.2165
Sample 6 unassisted versus sample 8 unassisted	0.5028
Sample 7 unassisted versus sample 8 unassisted	0.0333

Table A1.17: Results of repeated measures ANOVA for rectal temperature (p-values) to determine significant differences between groups and samples. Values are significant if smaller than 0.0008 (0.05/64 comparisons).

Group	p-values
Sample 1 high GGT versus sample 1 low GGT	0.1418
Sample 2 high GGT versus sample 2 low GGT	0.0002
Sample 3 high GGT versus sample 3 low GGT	0.4766
Sample 4 high GGT versus sample 4 low GGT	0.3177
Sample 5 high GGT versus sample 5 low GGT	0.4913
Sample 6 high GGT versus sample 6 low GGT	0.9509
Sample 7 high GGT versus sample 7 low GGT	0.5044
Sample 8 high GGT versus sample 8 low GGT	0.4523
Sample 1 high GGT versus sample 2 high GGT	0.9097
Sample 1 high GGT versus sample 3 high GGT	0.3796
Sample 1 high GGT versus sample 4 high GGT	0.3072
Sample 1 high GGT versus sample 5 high GGT	0.0748
Sample 1 high GGT versus sample 6 high GGT	0.0462
Sample 1 high GGT versus sample 7 high GGT	0.1905
Sample 1 high GGT versus sample 8 high GGT	0.0243
Sample 2 high GGT versus sample 3 high GGT	0.2336
Sample 2 high GGT versus sample 4 high GGT	0.0875
Sample 2 high GGT versus sample 5 high GGT	0.0039
Sample 2 high GGT versus sample 6 high GGT	0.0022
Sample 2 high GGT versus sample 7 high GGT	0.0279
Sample 2 high GGT versus sample 8 high GGT	0.0003
Sample 3 high GGT versus sample 4 high GGT	0.0007
Sample 3 high GGT versus sample 5 high GGT	<0.0001
Sample 3 high GGT versus sample 6 high GGT	<0.0001
Sample 3 high GGT versus sample 7 high GGT	<0.0001
Sample 3 high GGT versus sample 8 high GGT	<0.0001
Sample 4 high GGT versus sample 5 high GGT	0.1952
Sample 4 high GGT versus sample 6 high GGT	0.1092
Sample 4 high GGT versus sample 7 high GGT	0.6325
Sample 4 high GGT versus sample 8 high GGT	0.0350
Sample 5 high GGT versus sample 6 high GGT	0.6668
Sample 5 high GGT versus sample 7 high GGT	03792
Sample 5 high GGT versus sample 8 high GGT	0.3958
Sample 6 high GGT versus sample 7 high GGT	0.2176
Sample 6 high GGT versus sample 8 high GGT	0.7322
Sample 7 high GGT versus sample 8 high GGT	0.0811
Sample 1 low GGT versus sample 2 low GGT	0.3917
Sample 1 low GGT versus sample 3 low GGT	0.4517
Sample 1 low GGT versus sample 4 low GGT	0.0905
Sample 1 low GGT versus sample 5 low GGT	0.0183
Sample 1 low GGT versus sample 6 low GGT	0.0047
Sample 1 low GGT versus sample 7 low GGT	0.0062
Sample 1 low GGT versus sample 8 low GGT	0.0008
Sample 2 low GGT versus sample 3 low GGT	0.0099
Sample 2 low GGT versus sample 4 low GGT	0.0001
Sample 2 low GGT versus sample 5 low GGT	<0.0001
Sample 2 low GGT versus sample 6 low GGT	<0.0001
Sample 2 low GGT versus sample 7 low GGT	<0.0001
Sample 2 low GGT versus sample 8 low GGT	<0.0001

Sample 3 low GGT versus sample 4 low GGT	0.0831
Sample 3 low GGT versus sample 5 low GGT	0.0026
Sample 3 low GGT versus sample 6 low GGT	0.0003
Sample 3 low GGT versus sample 7 low GGT	0.0002
Sample 3 low GGT versus sample 8 low GGT	<0.0001
Sample 4 low GGT versus sample 5 low GGT	0.2597
Sample 4 low GGT versus sample 6 low GGT	0.0592
Sample 4 low GGT versus sample 7 low GGT	0.0731
Sample 4 low GGT versus sample 8 low GGT	0.0047
Sample 5 low GGT versus sample 6 low GGT	0.3763
Sample 5 low GGT versus sample 7 low GGT	0.5053
Sample 5 low GGT versus sample 8 low GGT	0.0730
Sample 6 low GGT versus sample 7 low GGT	0.7634
Sample 6 low GGT versus sample 8 low GGT	0.4427
Sample 7 low GGT versus sample 8 low GGT	0.2346

Table A1.18: Results of repeated measures ANOVA for rectal temperature (p-values) to determine significant differences between groups and samples. Values are significant if smaller than 0.0005 (0.005/103 comparisons).

Group	p-values
Sample 1 healthy versus sample 1 dying	0.1118
Sample 1 sick versus sample 1 dying	0.2422
Sample 1 sick versus sample 1 healthy	0.6551
Sample 2 healthy versus sample 2 dying	0.4625
Sample 2 sick versus sample 2 dying	0.4386
Sample 2 sick versus sample 2 healthy	0.9015
Sample 3 healthy versus sample 3 dying	0.9270
Sample 3 sick versus sample 3 dying	0.2298
Sample 3 sick versus sample 3 healthy	0.1235
Sample 4 healthy versus sample 4 dying	0.8573
Sample 4 sick versus sample 4 dying	0.5708
Sample 4 sick versus sample 4 healthy	0.3067
Sample 5 healthy versus sample 5 dying	0.4446
Sample 5 sick versus sample 5 dying	0.1506
Sample 5 sick versus sample 5 healthy	0.3224
Sample 6 healthy versus sample 6 dying	0.7293
Sample 6 sick versus sample 6 dying	0.0862
Sample 6 sick versus sample 6 healthy	0.0555
Sample 7 healthy versus sample 7 dying	0.9969
Sample 7 sick versus sample 7 dying	0.6592
Sample 7 sick versus sample 7 healthy	0.5377
Sample 8 healthy versus sample 8 dying	0.4067
Sample 8 sick versus sample 8 dying	0.6160
Sample 8 sick versus sample 8 healthy	0.6752
Sample 1 sick versus sample 2 sick	0.1196
Sample 1 sick versus sample 3 sick	0.3847
Sample 1 sick versus sample 4 sick	0.9676
Sample 1 sick versus sample 5 sick	0.7186
Sample 1 sick versus sample 6 sick	0.8744
Sample 1 sick versus sample 7 sick	0.9206
Sample 1 sick versus sample 8 sick	0.9334
Sample 2 sick versus sample 3 sick	0.3003
Sample2 sick versus sample 4 sick	0.0543
Sample 2 sick versus sample 5 sick	0.0268
Sample 2 sick versus sample 6 sick	0.0634
Sample 2 sick versus sample 7 sick	0.0857

Sample 2 sick versus sample 8 sick	0.0552
Sample 3 sick versus sample 4 sick	0.0635
Sample 3 sick versus sample 5 sick	0.0282
Sample 3 sick versus sample 6 sick	0 1320
Sample 3 sick versus sample 7 sick	0.1776
Sample 3 sick versus sample 8 sick	0.0941
Sample 4 sick versus sample 5 sick	0.5307
Sample 4 sick versus sample 6 sick	0.8489
Sample 4 sick versus sample 7 sick	0.8096
Sample 4 sick versus sample 8 sick	0.9412
Sample 5 sick versus sample 6 sick	0.7519
Sample 5 sick versus sample 7 sick	0.4248
Sample 5 sick versus sample 8 sick	0.6398
Sample 6 sick versus sample 7 sick	0.6559
Sample 6 sick versus sample 8 sick	0.8994
Sample 7 sick versus sample 8 sick	0.7154
Sample 1 healthy versus sample 2 healthy	0.0160
Sample 1 healthy versus sample 3 healthy	0.0216
Sample 1 healthy versus sample 4 healthy	0.2731
Sample 1 healthy versus sample 5 healthy	0.4628
Sample 1 healthy versus sample 6 healthy	0.0871
Sample 1 healthy versus sample 7 healthy	0.3155
Sample 1 healthy versus sample 8 healthy	0.4905
Sample 2 healthy versus sample 3 healthy	0.7577
Sample2 healthy versus sample 4 healthy	0.0947
Sample 2 healthy versus sample 5 healthy	0.0433
Sample 2 healthy versus sample 6 healthy	0.5081
Sample 2 healthy versus sample 7 healthy	0.0854
Sample 2 healthy versus sample 8 healthy	0.0364
Sample 3 healthy versus sample 4 healthy	0.0155
Sample 3 healthy versus sample 5 healthy	0.0063
Sample 3 healthy versus sample 6 healthy	0.5568
Sample 3 healthy versus sample 7 healthy	0.0244
Sample 3 healthy versus sample 8 healthy	0.0048
Sample 4 healthy versus sample 5 healthy	0.4930
Sample 4 healthy versus sample 6 healthy	0.2655
Sample 4 healthy versus sample 7 healthy	0.8827
Sample 4 healthy versus sample 8 healthy	0.4876
Sample 5 healthy versus sample 6 healthy	0.0733
Sample 5 healthy versus sample 7 healthy	0.6446
Sample 5 healthy versus sample 8 healthy	0.9307
Sample 6 healthy versus sample 7 healthy	0 1655
Sample 6 healthy versus sample 8 healthy	0.077
Sample 7 healthy versus sample 8 healthy	0.5219
Sample 1 dying versus sample 2 dying	0.5285
Sample 1 dying versus sample 3 dying	0.9392
Sample 1 dying versus sample 4 dying	0.3333
Sample 1 dying versus sample 5 dying	0.4226
Sample 1 dying versus sample 6 dying	0.8987
Sample 1 dying versus sample 7 dying	0.3539
Sample 1 dying versus sample 8 dying	0 1203
Sample 2 dying versus sample 3 dying	0.3402
Sample 2 dying versus sample 4 dying	0.0576
Sample 2 dying versus sample 5 dying	0.1258
Sample 2 dying versus sample 6 dying	0.4135
Sample 2 dying versus sample 7 dying	0.0706
Sample 2 dying versus sample 8 dying	0.0123
Sample 3 dying versus sample 4 dying	0.0669
Sample 3 dying versus sample 5 dying	0.2666
Sample 3 dying versus sample 6 dying	0.9244
Sample 3 dying versus sample 7 dying	0.1274
Sample 3 dying versus sample 8 dying	0.0101
Sample 4 dying versus sample 5 dying	0.6121
Sample 4 dying versus sample 6 dying	0.2425
Sample 4 dying versus sample 7 dying	0.9548
Sample 4 dying versus sample 8 dying	0.3235
Sample 5 dying versus sample 6 dying	0.3851

Sample 5 dying versus sample 7 dying	0.6938
Sample 5 dying versus sample 8 dying	0.1630
Sample 6 dying versus sample 7 dying	0.2094
Sample 6 dying versus sample 8 dying	0.0465
Sample 7 dying versus sample 8 dying	0.2279

Table A1.19: Results of repeated measures ANOVA for rectal temperature (p-values) to determine significant differences between groups and samples. Values are significant if smaller than 0.0008 (0.05/64 comparisons).

Group	p-values
Sample 1 younger 24 hours versus sample 1 older 24 hours	0.5058
Sample 2 younger 24 hours versus sample 2 older 24 hours	0.0052
Sample 3 younger 24 hours versus sample 3 older 24 hours	0.0397
Sample 4 younger 24 hours versus sample 4 older 24 hours	0.0072
Sample 5 younger 24 hours versus sample 5 older 24 hours	0.8391
Sample 6 younger 24 hours versus sample 6 older 24 hours	0.9011
Sample 7 younger 24 hours versus sample 7 older 24 hours	0.7420
Sample 8 younger 24 hours versus sample 8 older 24 hours	0.1432
Sample 1 younger 24 hours versus sample 2 younger 24 hours	0.3685
Sample 1 younger 24 hours versus sample 3 younger 24 hours	0.6931
Sample 1 younger 24 hours versus sample 4 younger 24 hours	0.2453
Sample 1 younger 24 hours versus sample 5 younger 24 hours	0.0120
Sample 1 younger 24 hours versus sample 6 younger 24 hours	0.0043
Sample 1 younger 24 hours versus sample 7 younger 24 hours	0.0152
Sample 1 younger 24 hours versus sample 8 younger 24 hours	0.0004
Sample 2 younger 24 hours versus sample 3 younger 24 hours	0.3812
Sample 2 younger 24 hours versus sample 4 younger 24 hours	0.0020
Sample 2 younger 24 hours versus sample 5 younger 24 hours	<0.0001
Sample 2 younger 24 hours versus sample 6 younger 24 hours	<0.0001
Sample 2 younger 24 hours versus sample 7 younger 24 hours	<0.0001
Sample 2 younger 24 hours versus sample 8 younger 24 hours	<0.0001
Sample 3 younger 24 hours versus sample 4 younger 24 hours	0.0039
Sample 3 younger 24 hours versus sample 5 younger 24 hours	<0.0001
Sample 3 younger 24 hours versus sample 6 younger 24 hours	<0.0001
Sample 3 younger 24 hours versus sample 7 younger 24 hours	<0.0001
Sample 3 younger 24 hours versus sample 8 younger 24 hours	<0.0001
Sample 4 younger 24 hours versus sample 5 younger 24 hours	0.0171
Sample 4 younger 24 hours versus sample 6 younger 24 hours	0.0047
Sample 4 younger 24 hours versus sample 7 younger 24 hours	0.0238
Sample 4 younger 24 hours versus sample 8 younger 24 hours	<0.0001
Sample 5 younger 24 hours versus sample 6 younger 24 hours	0.4894
Sample 5 younger 24 hours versus sample 7 younger 24 hours	0.8565
Sample 5 younger 24 hours versus sample 8 younger 24 hours	0.0474
Sample 6 younger 24 hours versus sample 7 younger 24 hours	0.3867
Sample 6 younger 24 hours versus sample 8 younger 24 hours	0.2687
Sample 7 younger 24 hours versus sample 8 younger 24 hours	0.0274
Sample 1 older 24 hours versus sample 2 older 24 hours	0.5997
Sample 1 older 24 hours versus sample 3 older 24 hours	0.9436
Sample 1 older 24 hours versus sample 4 older 24 hours	0.1641
Sample 1 older 24 hours versus sample 5 older 24 hours	0.3629
Sample 1 older 24 hours versus sample 6 older 24 hours	0.2669
Sample 1 older 24 hours versus sample 7 older 24 hours	0.5536
Sample 1 older 24 hours versus sample 8 older 24 hours	0.4551
Sample 2 older 24 hours versus sample 3 older 24 hours	0.3274
Sample 2 older 24 hours versus sample 4 older 24 hours	0.2020
Sample 2 older 24 hours versus sample 5 older 24 hours	0.5819
Sample 2 older 24 hours versus sample 6 older 24 hours	0.4014
Sample 2 older 24 hours versus sample 7 older 24 hours	0.9485
Sample 2 older 24 hours versus sample 8 older 24 hours	0.7620
Sample 3 older 24 hours versus sample 4 older 24 hours	0.0072
Sample 3 older 24 hours versus sample 5 older 24 hours	0.0658
Sample 3 older 24 hours versus sample 6 older 24 hours	0.0431

Sample 3 older 24 hours versus sample 7 older 24 hours	0.2032
Sample 3 older 24 hours versus sample 8 older 24 hours	0.1234
Sample 4 older 24 hours versus sample 5 older 24 hours	0.3971
Sample 4 older 24 hours versus sample 6 older 24 hours	0.6862
Sample 4 older 24 hours versus sample 7 older 24 hours	0.1530
Sample 4 older 24 hours versus sample 8 older 24 hours	0.2571
Sample 5 older 24 hours versus sample 6 older 24 hours	0.7113
Sample 5 older 24 hours versus sample 7 older 24 hours	0.5636
Sample 5 older 24 hours versus sample 8 older 24 hours	0.7722
Sample 6 older 24 hours versus sample 7 older 24 hours	0.3706
Sample 6 older 24 hours versus sample 8 older 24 hours	0.5271
Sample 7 older 24 hours versus sample 8 older 24 hours	0.7765

Table A1.20: Summary of calves healthy, sick and dying when on farm and which calves were sold and thus not included in the statistical analysis.

calf #	healthy (while on the farm)	sick	dying	age when sold (days)	calves included in statistical analysis
88	x			not sold	yes
113	x			not sold	yes
119	x			not sold	yes
130	x			not sold	yes
132	x			not sold	yes
133	x			not sold	yes
149	x			not sold	yes
150	x			not sold	yes
158	x			not sold	yes
180	x			not sold	yes
183	x			not sold	yes
185	x			not sold	yes
193	x			not sold	yes
200	x			not sold	yes
209	x			not sold	yes
259	x			not sold	yes
274	x			not sold	yes
275	x			not sold	yes
290	x			not sold	yes
311	x			not sold	yes
312	x			not sold	yes
321	x			not sold	yes
324	x			not sold	yes
298	x			32	no
277	x			30	no
283	x			24	no
258	x			23	no
262	x			23	no

calf #	healthy (while on the farm)	sick	dying	age when sold (days)	calves included in statistical analysis
314	x			22	no
322	x			18	no
330	x			17	no
142	x			10	no
166	x			10	no
112	x			9	no
131	x			9	no
254	x			9	no
123	x			8	no
261	x			8	no
139	x			8	no
140	x			7	no
169	x			7	no
201	x			7	no
216	x			7	no
271	x			7	no
276	x			7	no
117	x			7	no
103	x			6	no
154	x			6	no
157	x			6	no
181	x			6	no
191	x			6	no
192	x			6	no
220	x			6	no
250	x			6	no
252	x			6	no
282	x			6	no
285	x			6	no
136	x			6	no

calf #	healthy (while on the farm)	sick	dying	age when sold (days)	calves included in statistical analysis
161	x			6	no
255	x			6	no
167	x			5	no
202	x			5	no
206	x			5	no
208	x			5	no
226	x			5	no
231	x			5	no
300	x			5	no
304	x			5	no
92	x			5	no
96	x			5	no
162	x			5	no
228	x			5	no
229	x			5	no
240	x			5	no
84	x			4	no
217	x			4	no
219	x			4	no
221	x			4	no
223	x			4	no
315	x			4	no
306	x			4	no
307	x			4	no
318	x			4	no
114		x			yes
116		x		9	yes
122		x			yes
125		x		8	yes
141		x			yes

calf #	healthy (while on the farm)	sick	dying	age when sold (days)	calves included in statistical analysis
145		x		7	yes
151		x			yes
173		x			yes
227		x		16	yes
236		x		5	yes
249		x			yes
333		x			yes
338		x		12	yes
339		x			yes
342		x		12	yes
218			x		yes
247			x		yes
278			x		yes
284			x		yes
316			x		yes
329			x		yes
335			x		yes
337			x		yes