Improving Triplet Lamb Survival in New Zealand

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Joanne Isabel Kerslake

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ABSTRACT

This thesis sets out to identify physical and physiological differences between lambs of different birth ranks at birth, and to use this information to identify practical on-farm management strategies which could improve triplet-born lamb survival.

Triplet-born lambs, especially the lightest-triplet-born lambs, not only had a greater capacity to lose heat but also had a reduced capacity to produce heat when compared to twin-born lambs. Due to their lighter birth weights, triplet-born lambs had lower plasma thyroxine (T_4) concentrations within the first 24 hours of life than twin-born lambs, and within twin- and triplet-born litters, the lightest- and medium-triplet-born lambs had greater plasma lactate concentrations than all twin-born lambs and the heaviest-triplet-born lambs. Independent of lamb birth weight, triplet-born lambs had lower plasma tri-iodothyronine (T_3) concentrations within five minutes of birth, and within twin- and triplet-born litters, the lightest- and medium-triplet-born lambs had lower plasma T_4 and T_3 concentrations within five minutes of birth than all twin-born lambs and the heaviest-triplet-born lambs. It was hypothesised that because triplet-born lambs had a lighter birth weight and lower plasma thyroid hormone concentrations, they would have inadequate thermoregulatory capabilities when compared to twin-born lambs. The lower rectal temperatures of triplet-born lambs within the first hour of life and the lower heat production on a per lamb basis at 24 to 36 hours of age, and the lack of difference in maximum heat production on a per kg of birth weight basis at 24 to 36 hours of age support this hypothesis.

Two practical on-farm management strategies trialled in this thesis to improve triplet-born lamb thermoregulation were offering concentrate supplement during late pregnancy to improve lamb birth weights, and maternal iodine supplementation to improve lamb plasma thyroid hormone concentrations. While offering concentrate showed positive effects such as increasing lamb birth weights, colostrum uptake and triplet-born lamb heat production on a per kg of birth weight basis, the results were either inconsistent across experiments or between birth ranks suggesting additional work is required to determine the repeatability and cost effectiveness of these findings. Maternal iodine supplementation offered no benefits in terms of lamb birth weights, plasma thyroid hormone concentrations or lamb heat production. Further investigations identified that lamb birth weights, thyroid hormone concentrations, glucose and NEFA concentrations are positively associated with maximum heat production at 24 to 36 hours of age. Practical on-farm management strategies which could target these physical and physiological factors may improve triplet-born lamb heat production, and therefore the survival rates of triplet-born lambs.

This thesis is dedicated to Geoff and Jill Kerslake

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INTRODUCTION

In New Zealand there has been a focus on increasing lambing percentage (Anon 2008) in order to increase farm production and profit. Research has shown however, that as lambing percentage increases there is an associated decline in the number of single-bearing ewes and an increase in the number of twin- and triplet-bearing ewes (Davis *et al* 1983). While an increase in the number of ewes bearing larger litters should offer greater financial gains, an increase in litter size is also associated with low lamb survival rates (Scales *et al* 1986; Kenyon *et al* 2002; Everett-Hincks *et al* 2004; Morris and Kenyon 2004; Thomson *et al* 2004; Kerslake *et al* 2005; Kleemann and Walker 2005; Gootwine *et al* 2007; Everett-Hincks and Dodds 2008). In an outdoor lambing system triplet-born lambs have the lowest survival rates at, or within three days of birth, followed by twin and then single-born lambs (Dalton *et al* 1980; Nicoll *et al* 1999; Kerslake *et al* 2005). This makes the survival rates of triplet-born lambs an economic (Amer *et al* 1999) and potential welfare concern for the New Zealand sheep industry (Mellor and Stafford 2004; Fisher 2007; Dwyer 2008b; Stafford and Gregory 2008).

The main factors which predispose newborn lambs to death have been investigated in pathological and physiological studies (McFarlane 1965; Barlow *et al* 1987; Kerslake *et al* 2005; Everett-Hincks and Duncan 2008), but there has been little research examining why lambs from a larger litter have greater mortality rates than those from a smaller litter. Understanding the physical and physiological differences between single-, twin- and triplet-born lambs at birth may help explain the negative association found between litter size and lamb survival. This information may then be used to help identify practical on-farm management strategies which could be used to improve triplet-born lamb survival. The objectives of this research was therefore to identify physical and physiological differences between single-, twin- and triplet-born lambs at birth, and to use this information to identify practical on-farm management strategies to improve triplet-born lamb survival. The list below briefly describes the objective of each chapter in this thesis;

Chapter one: The objective of the first chapter was to review the literature and to identify any known differences between single-, twin- and triplet-born lambs. Potential practical on-farm management strategies to improve the physical and physiological impairments identified in the triplet-born lamb were also reviewed.

Chapter 2: This chapter describes and compares the physical and physiological differences between single-, twin- and triplet-born lambs at birth. The objective here was to identify physical and physiological factors which may help explain the variation observed in single-, twin- and triplet-born lamb survival rates.

Chapter 3 and 4: It was hypothesised that improving triplet-bearing ewe nutrition during late pregnancy may be one practical management strategy which could improve triplet-born lamb birth weight and heat production. The main objectives of these chapters were therefore to offer concentrate during late pregnancy to twin- and triplet-bearing ewes and to monitor the effects on lamb birth weight, thermoregulation, colostrum intake and growth.

Chapter 5: Because supplementation with iodine has been shown to have a positive effect on the thyroid hormone concentrations of newborn lambs (Andrewartha *et al* 1980; Rose *et al* 2007, the objective of this chapter was to examine the effects of supplementing triplet-bearing ewes with iodine on lamb birth weight, plasma thyroid hormone concentrations and lamb heat production.

Chapters 6 and 7: These chapters attempted to identify why the lightest lambs within a twin- or triplet-born litter have the lowest survival rates.

Chapter 8: The main objective of this chapter was to identify which physical and physiological factors within the first 24 hours of life were negatively and positively associated with lamb heat production. This would provide valuable information on which physical and physiological factors need to be targeted by on-farm management strategies.

The majority of chapters in this thesis have been either published in or submitted to refereed journals. Because of this there is some repetition in the introduction, material and methods and discussion in some chapters. Publications and submissions are stated on the title pages of each chapter.

CHAPTER 1

Literature Review

Introduction

Triplet-born lamb survival rates are on average 10-20% less than single- and twin-born lambs (Scales *et al* 1986; Kenyon *et al* 2002; Everett-Hincks *et al* 2004; Morris and Kenyon 2004; Thomson *et al* 2004; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008). While it is well known that as litter size increases, the average birth weight declines, and lamb survival decreases (Christley *et al* 2003; Dwyer and Morgan 2006; Gardner *et al* 2007; Gootwine *et al* 2007; Everett-Hincks and Dodds 2008; Hatcher *et al* 2009), the exact mechanisms which cause reduced fetal growth rates and lower survival rates remains unclear (Wu *et al* 2006).

A reduction in fetal growth can result from maternal undernutrition during pregnancy and/or failure of the placenta to deliver an adequate amount of nutrition (Mellor 1983; Robinson and McDonald 1989; Redmer *et al* 2004; Gootwine *et al* 2007). An inadequate supply of nutrients to the fetus can result in lambs being born with light birth weights (Mellor and Murray 1981; Robinson *et al* 1999), inadequate thermoregulatory capabilities (Eales and Small 1980b; Eales and Small 1985) and inadequate post-natal behaviours, such as standing and sucking (Dwyer *et al* 2003; Everett-Hincks *et al* 2005a). All of these factors have a negative impact on the survival capacity of the newborn lamb after birth.

Understanding the physical and physiological differences between lambs born as single-, twin- or triplet may help explain the negative association found between litter size and lamb survival rates. Identification of physical and physiological impairments in the triplet-born lamb may help identify practical on-farm management strategies which could offer improvement. This literature review is therefore divided into two parts. The first part identifies the known literature on physical and physiological differences between lambs of different birth ranks and the second part discusses potential on-farm management strategies which may improve physical and physiological impairments identified in triplet-born lambs, which in turn, may offer some improvement in triplet-born lamb survival rates.

Physical and physiological differences between lamb birth ranks

The time and cause of lamb death has been investigated using both field observations and pathological autopsies (McFarlane 1965; Haughey 1993; Kerslake *et al* 2005; Everett-

Hincks and Duncan 2008). Most lamb deaths occur within three days of life (Hight and Jury 1970; Dalton *et al* 1980; Nicoll *et al* 1999; Everett-Hincks *et al* 2005b), with single-, twin- and triplet-born lamb deaths being mainly attributed to dystocia and/or starvation/exposure (Dalton *et al* 1980; Knight *et al* 1988; Tarbotton and Webby 1999; Kerslake *et al* 2005). The physical and physiological factors which predispose newborn lambs to these types of mortality include;

- light lamb birth weights, which can result in excessive heat loss during adverse weather conditions (Sykes *et al* 1976; Slee 1977; Alexander 1979; McCutcheon *et al* 1983b);

- placental insufficiency, intra-partum hypoxia and inadequate thermogenic mechanisms, which result in inadequate heat production (Comline and Silver 1972; Mellor and Pearson 1977; Eales and Small 1980b; Cabello 1983; Barlow *et al* 1987; Dwyer and Morgan 2006; Stafford *et al* 2007);

- inadequate udder-seeking behaviour, colostrum intake and/or colostrum availability, which result in an inability to replace utilised energy reserves and to sustain heat production (Mellor and Cockburn 1986; Mellor and Murray 1986; Dwyer *et al* 2005; Everett-Hincks *et al* 2005a; Nowak and Poindron 2006).

Light lamb birth weight

Lambs with a lighter birth weight not only have a greater surface-area-to-birth-weight ratio than lambs of a heavier birth weight (Alexander 1962c; Slee 1977; Alexander 1979; McCutcheon *et al* 1983b), they also have less energy reserves (Alexander 1962a) and are slower at standing and sucking after birth (Dwyer 2003). By having a greater surface-area-to-birth-weight ratio, the lighter lamb will lose more heat to the environment than the heavier lamb, making it more susceptible to excessive heat loss. Excessive heat loss occurs when exposure to cold weather conditions causes the lamb to lose more heat than it is able to produce (Sykes *et al* 1976; Alexander 1979; McCutcheon *et al* 1981). Such an event generally occurs directly after birth, where wind, rain and birth fluid on the coat can cause rapid heat loss to the environment (Alexander 1962b; Gregory *et al* 1999). If the lighter lamb is able to produce enough heat to counter the heat being lost, it is still at a disadvantage. This is because compared to the heavier lamb; the lighter lamb has a greater lower critical temperature below which it must generate heat to maintain a homoeothermic status (Sykes *et al* 1976; Slee 1977). This need to generate heat earlier than a heavier lamb places a greater demand on the energy reserves of a lighter lamb (Eales *et al* 1980). A lighter lamb will therefore exhaust its energy reserves faster than a heavier lamb during a cold stress event. Failure to produce enough heat to counteract heat loss will result in hypothermia.

Because of their lighter birth weights, triplet-born lambs are likely to lose a greater amount of heat to the environment than single- and twin-born lambs. They are also more likely to have less energy reserves and to be slower at standing and sucking after birth than single- and twin-born lambs (Dwyer *et al* 2003; Everett-Hincks *et al* 2005a). The lighter birth weight of triplet- and twin-born lambs compared to single-born lambs offers some explanation as to why the starvation/exposure syndrome is more commonly reported in litter of two or more (McCutcheon *et al* 1981). Further research into ways of increasing the birth weight of triplet-born lambs is likely to be beneficial for their survival.

Placental insufficiency

Placental insufficiency generally results from a small placenta which fails to deliver an adequate supply of nutrients and oxygen to the fetus while in the uterus (Mellor and Murray 1981; Mellor 1983; Mellor 1988; Reynolds and Redmer 1995). Placental insufficient lambs are often identified by their light birth weight, greater plasma lactate concentration and packed cell volume, which all indicate signs of prolonged hypoxemia (Barlow *et al* 1987; Mellor 1988). Placental insufficient lambs have a reduced ability to produce heat, where plasma lactate concentrations have been found to be negatively correlated with heat production in the first 6-8 hours of life (Eales and Small 1980b; Eales and Small 1985).

Lambs from twin- or triplet-born litters have fewer placentomes (Greenwood *et al* 2000; Dwyer *et al* 2005; Kenyon *et al* 2007) and a reduced utero-placental blood flow (Rhind *et al* 1980; Ferrell and Reynolds 1992; Vonnahme *et al* 2008) when compared to single-born lambs. Because the nutrient and oxygen transfer capacity of the placenta is reliant on the surface-area of the placenta, which in turn, is affected by the number of placentomes and utero-placental blood flow (Fowden *et al* 2006), triplet-born lambs may be at a greater risk of placental insufficiency when compared to single- or twin-born lambs. Both Stafford *et al* (2007) and Dwyer *et al* (2005), have suggested that compared to single- and twin-born lambs, triplet-born lambs may be subject to placental insufficiency. Dwyer *et al* (2005) showed that triplet-born lambs had fewer cotyledons per fetus, that they were slower at standing and sucking, and had lower rectal temperatures; Stafford *et al* (2007) showed that triplet-born lambs had lighter birth weights, greater plasma lactate concentrations, and lower rectal temperatures at birth. Barlow *et al* (1987) however, who identified placental insufficiency by lighter birth weights, greater plasma lactate concentrations, higher packed cell volume and lower rectal temperatures, found that the number of placental insufficient lambs were distributed evenly amongst single-, twin- and triplet-born lambs.

Intra-partum hypoxia

Difficult births, which in this review, have been defined as prolonged births, mal-presentation and births requiring assistance, can result in cord compression or prolonged labour, which in turn can result in acute hypoxia and greater plasma lactate concentrations (Comline and Silver 1972; Jones 1977; Mellor and Stafford 2004). In addition to this, lambs which endure difficult births have trouble maintaining their body temperature, and have inhibited behaviours such as teat searching and sucking (Dwyer 2003). All of these factors limit the ability of the lamb to produce an adequate amount of heat during a cold stress event, thus increasing the risk of hypothermia.

Mal-presented births have been shown to be more frequent in single- and triplet-born lambs than twin-born lambs (Dwyer 2003). While lamb birth weight is well recognised risk factor for explaining the difficulty of a singleton birth (Dalton *et al* 1980), the reason for difficulties in triplet-born lambs is less clear. It was suggested by Dwyer (2003) however, that in both single- and triplet-bearing ewes there is less space in the uterine horn and therefore less room for the fetus to move freely. This may result in the fetus being expelled in an incorrect presentation. More recent studies have shown that triplet-bon lambs which died at birth or within the first three days of life, 47% had localised moderate to severe oedema on the body at post-mortem (Kerslake *et al* 2005). The results of Kerslake *et al* (2005) suggest that triplet-born lambs are more prone to a difficult birth than twin-born lambs. While research has found associations between prolonged duration of birth, localised oedema and lower survival rates (Everett-Hincks *et al* 2007), there have been no published results on the effect of prolonged labour on the physiological status of the lamb after birth. An unpublished study conducted by Everett-Hincks (2005) however, showed that lambs which died with localised moderate to severe oedema before three weeks of age had greater plasma lactate concentrations, PCV and plasma creatine kinase concentrations post birth. These factors are used as indicators for prolonged hypoxemia and increased muscle damage. Further research is required to understand the factors that contribute to difficult birth for triplet-born litters and the impact that this may have on the physiological status and survival capabilities of the triplet-born lamb.

Impaired thermogenic mechanisms

After birth, when the core body temperature of the lamb falls below the thermo-neutral zone, the lamb must activate thermogenic mechanisms in order to produce extra heat and maintain its body temperature. Impaired thermogenic mechanisms resulting from immature births, and/or reduced cortisol, thyroid and noradrenaline concentrations after birth have been shown to result in inadequate heat production (Cabello 1983; Barlow *et al* 1987; Polk *et al* 1987; Symonds *et al* 1995; Mellor and Stafford 2004). Failure of thermogenic mechanisms would increase the susceptibility of the newborn lamb to hypothermia.

Recent research has suggested that compared to single-born lambs, twin-born lambs have a blunted hypothalamic-pituitary axis (HPA), where the onset of the cortisol surge before birth has been shown to be delayed (Edwards 2002; Gardner *et al* 2004). Because the cortisol surge before birth is important for maturation and biological activity of various tissues (Fowden 1995), it is possible that twin-born lambs, and therefore potentially triplet-born lambs, are in a relative state of physiological immaturity compared to single-born lambs. In addition to this, triplet-born lambs have been shown to have shorter gestation lengths making them more susceptible to premature births (Cabello and Levieux 1981; Dwyer and Morgan 2006). Research has shown that the cortisol surge before birth appears to occur asynchronously in twin pairs (Mellor *et al* 1977; Schwartz and Rose 1998). This may suggest that fetuses within a litter experience different durations of a cortisol surge before birth and therefore may be at different states of relative maturity when born. Mellor *et al* (1977) showed that the onset of the rise in cortisol is the same in single- and first-born twin-born lambs, but delayed for second-born lambs. It has been suggested that researchers investigating the interactions between fetal development and post-natal physiology need to be aware of the potential confounding effects of different *in-utero* growth trajectories in multiples which may influence post-natal physiology (Bloomfield *et al* 2007).

Compared to single- and twin-born lambs, triplet-born lambs had lower plasma T_4 concentrations after birth (Barlow *et al* 1987; Stafford *et al* 2007), where other research has shown that litter size has no effect on plasma thyroid hormones above and beyond that of lamb birth weight (Dwyer and Morgan 2006). Strong associations between lambs born light, placental insufficiency and low plasma thyroid hormones have been reported (Cabello and Levieux 1981; Symonds 1995). Because triplet-born lambs are lighter at birth and have lower plasma thyroid hormones, they may have a limited ability to thermoregulate effectively.

Inadequate colostrum intake

Failure of the newborn lamb to ingest an adequate amount of colostrum will result in depletion of energy reserves, and the onset of hypoglycaemia (Eales *et al* 1982; Mellor and Cockburn 1986). Once the lamb is hypoglycaemic, cerebral compromise will occur, and the associated inhibition of heat production will cause a decline in rectal temperature (Hamadeh *et al* 2000). Colostrum is essential as an additional source of energy substrates for cerebral function and heat production (Eales and Small 1981; Clarke *et al* 1997), especially when body reserves approach depletion (Mellor and Cockburn 1986). An insufficient intake of colostrum can also result in inadequate immunoglobulin transfer from mother to young (Campbell *et al* 1977) increasing the risk of infection after birth (Campbell 1974). Because energy reserves available for heat production are finite, and because passive immuno-globulin G (IgG) absorption in the newborn intestine ceases at 24 to 36 hours of life, an early intake of colostrum is vital for provision of extra energy reserves for thermoregulation and immunity to fight infections (Nowak 1996). Failure to consume colostrum will increase the risk of the newborn succumbing to hypothermia or infection after birth.

Colostrum intake is dependent on how much colostrum is available from the ewe, and the adequate udder-seeking behaviours of the lamb.

Colostrum availability

Colostrum production has been shown to vary widely amongst ewes (Pattinson *et al* 1995; Odoherty and Crosby 1997; Annett *et al* 2005), where ewes bearing larger litters have been shown to produce either similar or less amounts of colostrum (Shubber *et al* 1979; Hall *et al* 1990) than ewes bearing smaller litters. In addition to this, ewes bearing larger litters have been shown to have a delay in the onset of lactogenesis (Hall *et al* 1990; Holst *et al* 1996a; McNeill *et al* 1998). All of these factors combined may suggest that lambs born in large litters have less colostrum available on a per lamb basis. Triplet-born lambs may therefore have less colostrum available for consumption after birth, which is crucial for sustainable heat production (Mellor and Cockburn 1986). There is currently no data available in New Zealand on the onset of lactogenesis in triplet-bearing ewes and the amount of colostrum which is available to triplet-born lambs within the first 24 hours of birth. Further research on the concentration of lactational hormones, such as progesterone, prolactin and cortisol, in triplet-bearing ewes prior to birth, and the colostrum secretion rate and composition within the first 24 hours of birth is warranted.

Post-natal lamb behaviour

The intake of colostrum after birth is dependent on the development of such behaviours as standing quickly, actively seeking the udder and sucking (Nowak and Poindron 2006; Dwyer 2008a). These behaviours not only result in the successful intake of colostrum, but also help establish a strong mother-young bond, which limits the risk of separation, mis-mothering and starvation (Nowak *et al* 1997). Lambs which are slower at standing, actively seeking the udder and sucking are at the greater risk of never sucking (Alexander and Williams 1966). Lamb behaviour at birth is influenced by litter size, prenatal nutrition and the birth process, where behaviour tends to be negatively affected by larger litters, difficulty during birth, and maternal undernutrition during pregnancy (Dwyer 2003; Dwyer *et al* 2005; Dwyer and Lawrence 2005; Everett-Hincks *et al* 2005a).

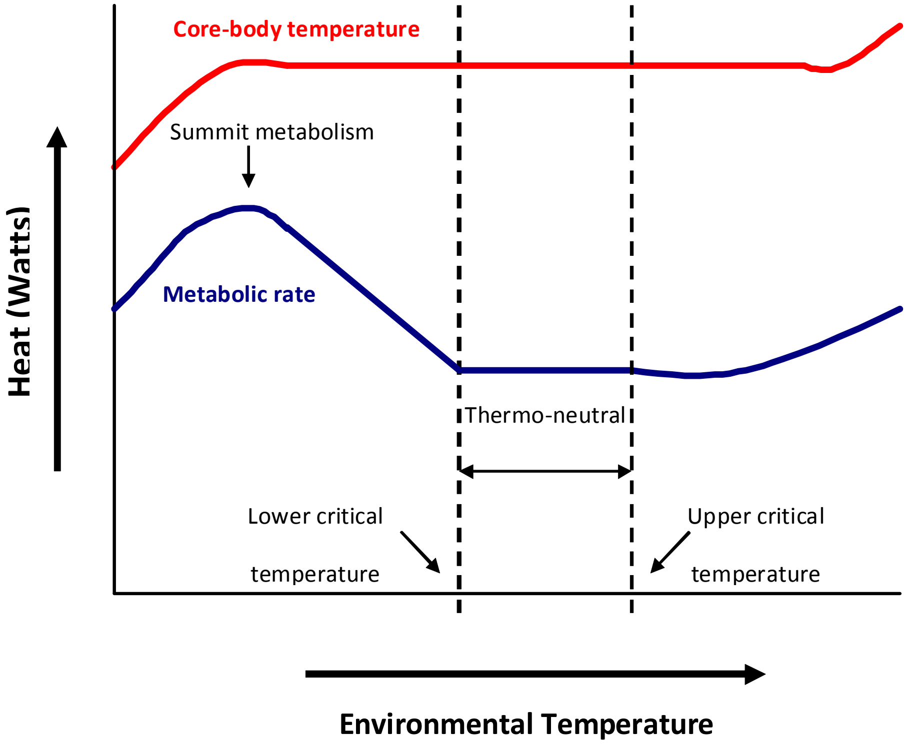
Compared to twin-born lambs, triplet-born lambs have been reported to be slower at standing and sucking (Dwyer *et al* 2005; Everett-Hincks *et al* 2005a). The ability of the lamb to consume colostrum may be affected by competition within litters, where it has been noted that when two lambs have initiated sucking, sucking is often terminated when the third lambs begins to suck (Hinch 1989). Lambs in a litter of three or more, are therefore forced to compete for colostrum supply, whose volume may only be suitable for rearing two lambs. Triplet-born lambs are therefore at the greatest risk of receiving an insufficient amount of colostrum after birth. While large variations in colostrum intake have been reported within triplet-born lamb litters (Halliday 1974; Hunter *et al* 1977; Kenyon *et al* 2005b), there is no research on the competitive behaviours of triplet-born lambs and what impact this has on an individual's intake of colostrum. Further research on the sucking behaviour of individual lambs within a litter of twin and triplets and the impact this has on their survival is needed.

Physical and physiological impairments of triplet-born lambs

Overall it appears that the lower survival rates observed in triplet-born lambs, when compared to single- and twin-born lambs may be a result of: a) lighter birth weight, b) greater susceptibility to placental insufficiency, intra-partum hypoxia, inadequate thermogenic mechanisms, and/or c) an inadequate intake and/or availability of colostrum. This suggests that they have a greater susceptibility to both excessive heat loss and/or inadequate heat production. Improvements in the ability of the triplet-born lamb to thermoregulate should therefore offer some improvement in their survival rates.

Thermoregulation is a dynamic system that balances heat loss and heat gain and keeps the lamb at an optimal core body temperature for survival (thermo-neutral zone). If lamb core body temperature increases above the upper critical limit or decreases below the lower critical limit, then the lamb must thermoregulate to re-establish its core body temperature (Figure 1).

Figure 1 *The effect of environmental temperature on the metabolic rate and core body temperature of the lamb (adapted from Pough et al (1990)).*



In a cold environment thermoregulation is achieved through an increase in metabolic rate which produces extra heat to warm the body. If the heat produced is insufficient to offset the loss of heat to the environment, the core body temperature of the lamb will not be maintained. In a cooling environment the metabolic rate will increase until it reaches a maximum (summit metabolism). If the environmental temperature continues to cool after the lamb has reached maximum heat production, the core body temperature and the metabolic rate of the lamb will decrease and the lamb will die of hypothermia (Cannon and Nedergaard 2004).

The ability of the triplet-born lamb to thermoregulate effectively after birth is dependent on its ability to minimise heat loss and maximise heat production. As already mentioned, the light birth weight of the triplet-born lambs, and thus their greater surface-area-to-birth-weight ratio, means they will lose a greater amount of heat than single- and twin-born lambs (Sykes *et al* 1976; Alexander 1979; McCutcheon *et al* 1981). They will also use a greater amount of energy reserves to produce heat to counter this heat loss. Improvements in lamb birth weight may therefore help reduce the amount of heat that a triplet-born lamb loses to the environment. The ability of triplet-born lamb to thermoregulate effectively is also dependent on their ability to maximise and sustain heat production during a cold stress event. Improvements in birth weight, energy reserve accumulation, efficient utilisation of energy reserves, and the intake of colostrum for sustainable heat production, may help improve the ability of the triplet-born lamb to increase and/or sustain heat production during a cold stress event.

Practical management solutions to improve triplet-born lamb survival

Improving triplet-bearing ewe nutrition during late pregnancy may be one practical management strategy which could have an over all impact on fetal growth and development (Stevens *et al* 1990; Budge *et al* 2000), energy reserve accumulation and utilisation (Stott and Slee 1987; Budge *et al* 2000; Symonds *et al* 2003), and the intake of colostrum via improvements in lamb post-natal behaviour and/or colostrum production (Murphy *et al* 1996; Banchero *et al* 2004a). In addition, supplementation with minerals, such as iodine, may have a positive effect on thyroid hormone concentrations of newborn lambs (Andrewartha *et al* 1980; Rose *et al* 2007), and therefore their production of heat after birth. Another practical management solution could be to reduce heat loss by using lamb covers and/or provision of shelter (Gregory 1995; Pollard 2006; Fisher 2007).

Improving triplet-bearing ewe nutrition during late pregnancy

In New Zealand triplet-bearing ewes are generally expected to meet the high energy demands of late pregnancy by grazing pasture only. While pasture grazing guidelines for late pregnancy have been developed for single- and twin-bearing ewes (Kenyon and Webby 2007), there has been limited research on the effect of pasture grazing on the energy intakes of triplet-bearing ewes. One study in New Zealand has shown that the dry matter intake of a triplet-bearing ewe is maximum on a ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* sward of 4 cm or higher (Morris and Kenyon 2004). From this study, the calculated energy intakes of triplet-bearing ewes grazing a sward height of 4 cm or higher suggest that they were unable to consume enough herbage to meet their theoretical energy requirements of their fetuses. In the same study, triplet-bearing ewes mobilised a greater amount of body reserves than twin-bearing ewes (Morris and Kenyon 2004) providing further evidence that triplet-bearing ewes were not consuming enough pasture to meet the energy requirements of their fetuses. Another New Zealand study has shown that insufficient glucose is available from *ad-libitum* ryegrass pasture diets to maintain triplet-fetal growth, and that amino acid requirements of triplet fetuses are likely to exceed net absorption of fresh forage diets, causing maternal tissues to be mobilised in order to meet the high energy demands of late pregnancy (Barry and Manley 1985). The results of these two studies suggest that triplet-bearing ewes cannot consume enough herbage in ryegrass and white clover swards to meet their nutrient requirements in late pregnancy. Failure of the ewe to provide adequate nutrients to the fetus, can result in newborn lambs with light birth weights (Robinson and McDonald 1989), low energy reserves (Budge *et al* 2000) and slow progressive behaviors, such as standing and suckling (Dwyer *et al* 2003). It can also result in ewes mobilizing body reserves, resulting in poor colostrum production and insufficient body reserves for lactation. (Mellor and Murray 1985b; Mellor 1987). Overall these results highlight the vulnerability of triplet-bearing ewes, and thus triplet-fetuses, to nutritional stress when grazing a ryegrass and white clover pasture. There is still a need to find an optimal feeding strategy during late pregnancy which will ensure the optimal growth and development of all three fetuses, and in addition, ensure the early onset of lactogenesis and optimal production of colostrum after birth.

Current feeding recommendations for feeding triplet-bearing ewes in New Zealand

The current recommendations for triplet-bearing ewes grazing pasture only diets, is to graze them on a sward height of 4 cm or higher. Morris and Kenyon (2004) found that twin and triplet-bearing ewe intake was maximum on a 4 cm sward height of ryegrass and white clover, and that offering a sward height higher than 4 cm had no effect on lamb production. While offering a 4 cm sward height or higher had no effect on lamb birth weight, higher sward heights did have a beneficial effect on triplet-born lamb vigour (Everett-Hincks *et al* 2005b). Further research has also shown that if herbage growth is inadequate to provide pasture sward heights of 4 cm or greater from pregnancy day 70 until parturition, restricting intake in mid-pregnancy by offering a 2 cm sward height, and providing a 4 cm sward height in late pregnancy is adequate in terms of twin- and triplet-born lamb birth weight (Corner *et al* 2008). While feeding regimens must provide enough nutrients to support adequate fetal growth and mammary gland development, it is important to remember that matching nutrient supply with individual demand is not necessarily practical or profitable. Extra nutrient requirements during pregnancy can be met by the mobilisation of maternal body reserves. For example, while twin-bearing ewes require 4 cm sward surface height to meet their nutritional requirements, Morris *et al* (1993) also showed that grazing twin-bearing ewes, which are in good condition, on a 2 cm sward height is adequate in terms of lamb production.

Offering concentrate supplement

Because herbage intake can be limited by physical constraint (bulk and high water content) and rate of forage removal from rumen (fibre), it can be difficult for high producing animals to consume enough herbage to meet their nutrient requirements (Forbes 1996; Weston 1996; Fisher 2002). This is especially true for triplet-bearing ewes whose ability to consume pasture may be restricted due to limited space in the abdomen or limited capacity to ingest dry matter at the end of pregnancy (Everts 1990). Offering energy and protein concentrates to grazing ewes has the potential to alter the metabolisable energy (ME) supply, the utilisation of ME, the amount of microbial protein synthesised, and the amount of dietary protein that escapes the rumen (Dove 2002). Adding concentrates to hay (Everts 1990), or grass silage diets (Orr and Treacher 1990) has been shown to increase total ME intakes of single, twin, and triplet-bearing ewes during late pregnancy. Offering concentrate to pasture fed ewes may therefore be a management option to improve total energy intake of the ewe and to improve the growth and development of all three fetuses.

Improving the intake of nutrients to meet nutritional requirements of pasture fed animals can be highly complex (Forbes 1996; Weston 1996; Fisher 2002). It is dependent on a number of factors, including the energy demand of the animal, the amount and quality of pasture on offer, ewe behaviour, the physical and physiological state of the animal, its capacity to use energy (ability to ingest and digest) and climate. When concentrate is offered to animals grazing pasture, animals can respond by consuming the supplement and increasing or maintaining herbage intake or reducing pasture intake (Holst *et al* 1996b). For animals grazing pasture, substitution of pasture for concentrate is likely to be greater when pasture availability is greater, when high quality supplements are fed, when a greater amount of supplements are fed, and when energy demand is less (Dove 2002). Because of the expense and the difficulty of measuring supplement intake in grazing animals, there have been limited studies looking at the effects of offering concentrate supplement on the substitution rates of pasture. The substitution rate has however, been looked at in metabolism or indoor pen studies, where offering concentrate has been shown to cause small reductions in the intakes of silage (Orr and Treacher 1989; Orr and Treacher 1990) and greater reductions in the intakes of hay (Orr and Treacher 1984). In pasture grazing studies, research has shown considerable variation in supplement intake between individual ewes (Holst *et al* 1994). In addition, triplet- and twin-bearing ewes have been shown to eat more supplement than twin- and single-bearing ewes, respectively (Hall *et al* 1992; Holst *et al* 1996b; Banchero *et al* 2007). All of these factors need to be taken into consideration when designing an optimal feeding strategy, as if substitution rates are too great, or intake behaviour is highly variable the production response may be small or non-existent.

While the effect of offering concentrate to ewes fed grass-silage diets in an indoor environment on ewe and lamb performance have been thoroughly investigated (Table 1), there has been limited research on the effect of offering concentrates to ewes grazing pasture-based diets in an outdoor environment on ewe and lamb performance (Table 2). The effects of offering concentrate to ewes in late pregnancy with restricted and/or unrestricted diets in either and indoor and/or outdoor environment has shown mixed results in terms of ewe and lamb production (Table 1 and 2). Differences are likely to be due to the high variability in the type of concentrate offered, the time the concentrate was offered and with different control diets. Overall, there is a lack of information in the literature on the effects of offering concentrate supplement to triplet-bearing ewe grazing unrestricted outdoor pastures in late pregnancy and the effects this may have on ewe and lamb performance.

Table 1 *The effect of offering concentrate with a restricted or un-restricted pasture-based diets in an indoor environment on ewe liveweight (EL), ewe body condition (EC), lamb birth weight (BW), colostrum production (CP), lamb growth (LG) and lamb survival (LS).*

Author	Litter size	Supplement (amount offered and GE or ME content of the diet)	Time offered	Control diet (amount offered and GE or ME content of the diet)	EL	EC	BW	CP	LG	LS
Restricted diet	1									
Stephenson & Bird (1992)		Cotton seed meal (150g/ewe/d; ME, na), molasses (50g/ewe/d, ME, na)	P113-Birth	Rhode grass (0.8kg/ewe/day, ME, na (low quality)	+		+	+		
Dawson *etal* (2005)	2	Concentrate (500g/ewe/d; GE, 17.8 MJ/kg DM)	P105-Birth	Pasture (1.3, 1.75 kg DM/ewe/d, ME, 11.1 MJ kg/DM)	+	+	+		ne	ne
Unrestricted diet										
Orr & Treacher (1989)	1, 2 & 3	Barley, soya bean & fish meal mix (450g/ewe/d; ME, na,)	P105-Birth	Grass silage (Ad-lib; ME, 10.4MJ/kg DM)	+	+	+		ne	
Orr & Treacher (1990)	1, 2 & 3	Barley, soya bean & fish meal mix (600g/ewe/d; ME, na)	P105-Birth	Grass & white clover silage (Ad-lib; ME, 10.9MJ/kg DM)	+	+	+ (3)		+ (2)	
Banchero *et al* (2004a)	1 & 2	Cracked maize (200-700g/ewe/d; ME, 13.6 MJ/kg DM)	P134-Birth	Lucerne hay (1-1.4kg/ewe/d; ME, 8.6 MJ/kg DM)	ne	ne	ne	+		
Banchero *et al* (2004b)	2	Cracked maize (800g/ewe/d; ME, 13.8 MJ/kg DM)	P138-Birth	Lucerne & Oat chaff (75g/kg $LW^{-0.75}$; ME, 9.3 MJ/kg DM)	ne	ne	+	+		
	2	Cracked Lupin (800g/ewe/d; ME, 13.8 MJ/ kg DM)	P138-Birth	Lucerne & Oat chaff (75g/kg $LW^{-0.75}$; ME, 9.3 MJ/kg DM)	ne	ne	+	ne		
Annett *et al* (2005)	3	Barley, soya-bean meal, urea (0.56 Kg DM/ewe/d; ME, 11.8 MJ/kg DM)	P105-Birth	Ryegrass (0.6 kg DM/ewe/d; ME, 11.6 MJ/kg DM)	ne	+	ne	+	+	+
Dawson *et al* (2005)	2	Concentrate (500g/ewe/d; GE, 17.8 MJ/kg DM)	P105-Birth	Pasture (2.2, 2.6 kg DM/ewe/d, ME, 11.1 MJ kg/DM)	+	+	+		ne	ne
Banchero *et al* (2007)	1, 2	Cracked maize or Lupin (200-500 g/ewe/d; ME, 13.4 MJ/kg DM),	P134-141	Lucerne hay (1-1.4 kg DM/ewe/d; ME, 9.0 MJ/kg DM)			ne	+		

+, positive effect; -, negative effect; ne, no effect; + (2) or + (3), positive effect in twin- or triplet-born lambs respectively; na, not available

Table 2 *The effect of offering concentrate with a restricted or un-restricted pasture-based diets in an outdoor pasture grazing environment on ewe liveweight (EL), ewe body condition (EC), lamb birth weight (BW), colostrum production (CP), lamb growth (LG) and lamb survival (LS).*

Author	Litter size	Supplement (amount offered GE or ME content of the diet)	Time offered	Control diet (amount offered and GE or ME content of the diet)	E L	E C	B W	C P	**L G**	**L S**
Restricted diet										
Hall *et al* (1992)	1, 2, 3, 4	Lupin grain (400g/ewe/d; ME, na)	P134-143	Legume grass (Restricted availability; ME, na)			+		ne	+
Hall & Holst (1996)	1, 2	Lupin, oat grain (183g/head/day; ME, na)	P100-Birth	Barley grass (1500-1700 kg green DM/Ha and 1500 kg Dead DM/Ha; ME, na)			ne		ne	ne
	1, 2	Lupin, oat grain (365g/head/day; ME, na)	P100-Birth	Barley grass (1500-1700 kg green DM/Ha and 1500 kg Dead DM/Ha; ME, na)			+		ne	ne
	1, 2	Lupin, oat grain (550g/head/day; ME, na)	P100-Birth	Barley grass (1500-1700 kg green DM/Ha and 1500 kg Dead DM/Ha; ME, na)			+		ne	ne
Unrestricted diet										
Hinch *et al* (1996)	1, 2, 3, 4	Cotton seed meal, bran pellets (80g/ewe/2nd d; ME, 9.7 MJ/kg DM)	P100-130	Green pasture (excess of 3000 kg DM/Ha; ME, na)	ne		+ 1,3,4		ne	+
Murphy *et al* (1996)	1	Lupin (1000g/ewe/d; ME, na)	P140-Birth	Pasture (Lush; ME, na)			ne	+		
Kerslake *et al* (2008)	2	Pellets (400g/ewe/d; ME, 12.3 MJ/kg DM)	P100-145	Ryegrass and white clover (6cm sward height; ME, na)	+		+		+	

+, positive effect; -, negative effect; ne, no effect; + (1, 3, 4) positive effect in single, triplet and quad-born lambs only; na, not available

Improving the thermogenic mechanisms of the newborn lamb

After birth, when the core body temperature of the lamb falls below the thermo-neutral zone, the lamb must activate thermogenic mechanisms in order to produce extra heat and maintain its body temperature. The success of this process relies on certain physiological mechanisms which occur directly before and after birth. Immediately before birth the fetal HPA axis is activated causing a surge of cortisol, which in turn causes a surge in tri-iodotyronine (T_3; Fraser and Liggins 1989; Forhead *et al* 2006). This surge of cortisol and T_3 is vital for the physical and physiological maturity of the fetus at birth (Liggins 1994; Fowden *et al* 1998). Both cortisol and T_3 ensure that tissues, such as brown adipose tissue, and organs, such as the liver and lung, are physiologically primed for the onset of thermoregulation after birth by having a positive affect on the expression of gluconeogenic enzymes, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (Fowden *et al* 2001; Forhead *et al* 2003) and proteins, such as uncoupling protein one (UCP-1; Mostyn *et al* 2003). After birth, the onsets of shivering and/or non-shivering thermogenesis are the two main mechanisms utilised for the production of heat (Ribeiro *et al* 2001; Silva 2001). The onset of shivering and non-shivering thermogenesis occurs when the cold stimulates the hypothalamus to involuntary increase the shivering of skeletal muscle, to stimulate the sympathetic nervous system to release noradrenaline from the adrenal cortex, to stimulate the pituitary-adrenal axis to release cortisol from the adrenal medulla, and to stimulate the pituitary-thyroid-axis to produce thyroid hormones from the thyroid glands. The release of noradrenaline, cortisol and thyroid hormones act independently and/or in synergism to mobilise and/or produce energy substrates, such as carbohydrates and fats (Silva 1995) , and to transcribe and activate important heat production genes, such as UCP-1(Mory *et al* 1984; Silva 1995; Mostyn *et al* 2003). Heat is successfully generated by shivering thermogenesis when the repeated tremor of the skeletal muscle converts the chemical energy created from carbohydrates or fats to mechanical work. Non shivering thermogenesis is able to generate a large amount of heat in brown adipose tissue, by mobilising energy substrates, such as free fatty acids, and uncoupling ATP synthesis from the electron transport chain via a specialised mitochondrial protein UCP 1 (Nedergaard *et al* 2001). Impaired thermogenic mechanisms resulting from immature births, and/or reduced cortisol, thyroid and noradrenaline concentrations after birth have been shown to result in inadequate heat production (Cabello 1983; Barlow *et al* 1987; Polk *et al* 1987; Mellor and Stafford 2004). Within five minutes of birth, triplet-born lambs, or lambs of a lighter birth weight, have been identified as having lower plasma thyroxine (T_4) concentrations than twin-born lambs or lambs of a heavier birth weight (Barlow *et al* 1987; Dwyer and Morgan 2006; Stafford *et al* 2007). This may indicate that triplet-born lambs have an impaired ability to generate heat directly after birth, which may make them more susceptible to a hypothermic death.

Maternal iodine supplementation

Thyroid hormone production is reliant on the thyroid gland absorbing iodine from the blood (Underwood and Suttle 1999). Iodine is actively transported across the placenta to the developing fetus, where from day 50 of pregnancy the fetus synthesises its own thyroid hormones from maternal iodine (Potter *et al* 1986). Lambs born from ewes supplemented with iodine have greater thyroxine concentrations at birth (Andrewartha *et al* 1980) and at 24 hours of age (Rose *et al* 2007) when compared to lambs born to non-supplemented ewes. They have also been reported as having greater rectal temperatures after birth (Donald *et al* 1994). Newborn lambs with greater plasma concentrations of thyroid hormones appear to be better at producing heat (Alexander 1970) and maintaining core body temperature when subjected to cold stress (Caple and Nugent 1983; Polk *et al* 1987). Increasing maternal plasma iodine concentration during pregnancy may therefore have a positive effect on the thyroid hormone concentrations of the triplet-born lamb, which in turn, may have a positive effect on triplet-born lamb heat production.

Provision of shelter

While improving lamb heat production is likely to be beneficial for triplet-born lamb thermoregulation, it may be more beneficial to reduce the triplet-born lambs' need to produce heat. This could be done by reducing the amount of heat it loses to the environment through the use of lamb covers or the provision of shelter. The potential use of shelter or lamb covers has been reviewed in depth by Gregory (1995), Pollard (2006) and Fisher (2007). The use of shelter is not covered in this review.

Purpose and Scope of this thesis

Triplet-born lambs appear to have a greater susceptibility to both excessive heat loss and/or inadequate heat production when compared to single- and twin-born lambs. This can result from a lighter birth weight, greater susceptibility to placental insufficiency, intra-partum hypoxia, inadequate thermogenic mechanisms, and/or an inadequate intake/availability of colostrum. Potential practical management strategies which could have a positive effect on lamb birth weights and/or thyroid hormone concentrations include the improvement of triplet-bearing ewe nutrition during late pregnancy, and the supplementation of the triplet-bearing ewe with minerals, such as iodine. Research into the effects that these strategies may have on triplet-born lamb thermoregulation is required.

The aims of this thesis were;

- To identify the physical and physiological differences between birth ranks, from birth to 24 hours of age, under outdoor pastoral conditions (chapter 2)
- To investigate the effect of offering a concentrate supplement, from pregnancy day 102 until pregnancy day 145, to twin- and triplet-bearing ewes grazing a 6cm sward height, on lamb birth weight, colostrum intake and growth (chapter 3)
- To investigate the effect of offering concentrate supplements to twin- and triplet-bearing ewes grazing a 6 cm sward height from pregnancy day 100 until parturition on lamb birth weight and heat production at 24 to 36 hours of age (chapter 4).
- To investigate the effect of maternal iodine supplementation on twin- and triplet-born lamb plasma thyroid hormone concentration, rectal temperature and their ability to produce heat at 24 to 36 hours after birth (chapter 5).
- To identify physiological differences from birth until 24 hours of age within twin- and triplet-born litters (chapter 6).
- To determine if lambs within a twin- or triplet-born lamb litter produce similar amounts of heat during cold exposure at 24 to 36 hours of age? (chapter 7).

- To identify physical and physiological factors associated with maximum heat production on a per lamb basis and a per kg of birth weight basis, and the likelihood of a lamb remaining homoeothermic during a cold stress event (chapter 8).

CHAPTER 2

Are triplet-born lambs different from single- and twin-born lambs from birth to 24 hour of age?

Related Publication;

Based on a submission to Animal

Kerslake JI, Kenyon PR, Morris ST, Stafford KJ, Morel PCH (2010) Are triplet-born lambs different from single- and twin-born lambs from birth to 24 hour of age?

Abstract

In an outdoor lambing system most lamb deaths occur at, or within three days of birth, with triplet-born lambs having the lowest survival rates when compared to twin- or single-born lambs. The exact reasons for lower survival rates in lambs from a large litter when compared to lambs from a small litter remain unclear. Further investigation of the physical and physiological differences between lamb birth ranks within the first 24 hours of life may help explain the differences observed in the survival rates of single-, twin- and triplet-born lambs. The aim of this experiment was to identify the physical and physiological differences between different birth ranks, from birth to 24 hours of age, under outdoor pastoral conditions. Sixty triplet-, 40 twin- and 20 single-bearing ewes, chosen at pregnancy day 50, were offered *ad-libitum* pasture during pregnancy until parturition. Lamb blood samples were taken at zero, three, 12 and 24 hours post-birth and rectal temperature was taken at zero, one, three, six and 12 hours post-birth. Lamb birth weight, crown-rump length and thoracic-girth circumference were measured at three hours of age. Triplet-born lambs had shorter ($P < 0.001$) gestation lengths than single- or twin-born lambs. Single- and twin-born lambs were heavier ($P < 0.001$), had longer crown-rump length ($P < 0.001$) and greater ($P < 0.001$) thoracic-girth circumference than triplet-born lambs. Within five minutes of birth, single- and twin-born lambs had greater ($P < 0.05$) plasma tri-iodothyronine concentrations than triplet-born lambs. Single-born lambs had greater ($P < 0.01$) plasma thyroxine concentrations than both twin- and triplet-born lambs. In addition, all birth ranks had different ($P < 0.01$) log transformed plasma fructose concentrations. Lactate, packed cell volume and cortisol plasma concentrations did not differ between birth ranks. Rectal body temperature from birth to 24 hours of age was lower ($P < 0.05$) in triplet-born lambs than single- or twin-born lambs. Log transformed plasma glucose concentrations from birth until 24 hours of age were different ($P < 0.05$) for all birth ranks. Birth rank therefore has a significant effect on the physiological status of the newborn lamb. Overall these results suggest that triplet-born lambs are likely to have less energy and stimulatory factors, such as glucose and thyroid hormones, for adequate thermoregulation than single- and twin-born lambs. This may offer some explanation as to why triplet-born lambs have a lower survival rate.

Introduction

In a New Zealand outdoor lambing system most lamb deaths occur at, or within three days of birth (Dalton *et al* 1980; Nicoll *et al* 1999), with triplet-born lambs having the lowest survival rates when compared to twin- or single-born lambs (Nicoll *et al* 1999; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008). Triplet-born lamb survival is therefore an economic (Amer *et al* 1999) and potential welfare concern (Fisher 2007; Dwyer 2008b) for the New Zealand sheep industry.

While lamb birth weight is a well known risk factor for lamb mortality (Yapi *et al* 1990; Christley *et al* 2003; Gootwine 2005; Gardner *et al* 2007), triplet-born lambs still have the lowest survival rate when compared to single- and twin-born lambs of the same birth weight (Everett-Hincks and Dodds 2008; Hatcher *et al* 2009). This suggests that other factors, other than lamb birth weight, play an important role in triplet-born lamb survival. A few studies have already examined the relationship between lamb birth rank and the physiological characteristics of newborn lambs which may influence neonatal survival (Barlow *et al* 1987; Dwyer and Morgan 2006; Stafford *et al* 2007). The exact reasons for lower survival rates in lambs from a greater litter size however, still remains unclear.

Further investigation into the physical and physiological differences between lamb birth ranks within the first 24 hours of life may help explain why triplet-born lambs have lower survival rates than single- or twin-born lambs. The aim of this experiment was therefore to identify physical and physiological differences between lambs of different birth ranks, from birth to 24 hours of age, under outdoor pastoral conditions.

Materials and Methods

This experiment was conducted at Massey University on Keeble farm, Palmerston North, New Zealand (40º2 S latitude; 175º3 E Longitude, elevation 44 m) from 1st April 2005 until 10th September 2005.

Oestrus in 620 mixed-age ewes (½ Romney, ¼ Finn and ¼ Texel) was synchronised using a progesterone releasing device (Eazi-breed CIDR ®, Pfizer, Auckland, New Zealand). The device was removed 13 days after insertion and ewes were weighed unfastened, condition scored (Jefferies 1961) and injected with 400 I.U PSMG (Folligon ®, Intervet, Boxmeer, Holland). Romney rams (n = 48) fitted with crayon mating harnesses were joined with the ewes for a five day breeding period and the crayon marks on the rump of the ewe were recorded daily. Ewes with a crayon-marked rump were pregnancy diagnosed using ultrasound scanning at 49 days (P49) after the mid-point of the five day breeding period. Ewes were diagnosed as being non-pregnant, single-, twin- or triplet-bearing.

Sixty triplet-, 40 twin- and 20 single-bearing ewes were randomly chosen at P50. From P50 until parturition ewes were grazed on a minimum 4 cm herbage sward height of ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* sward which allowed for unrestricted herbage intake (Morris and Kenyon 2004). From P138 humans walked around and between the mob of ewes twice daily in order to habituate ewes to the presence of humans. A 10 m1 blood sample was collected from each ewe at P141 using jugular venepuncture (Lithium heparin vacutainer, Becton Dickson Vacutainer Systems, USA).

At P145 ewes were moved into a 2.5 ha paddock and were supervised continually for the next ten days. Thirteen single-, 22 twin- and 18 triplet-bearing ewes which showed signs of parturition were moved quietly to a temporary pen (5 x 3 m) located in the front of their main grazing paddock. Ewes had access to *ad-libitum* herbage and water within the pens, and were released from the pen 24 hours after their last lamb was born. The remaining ewes gave birth in the main grazing paddock with no observations recorded or measurements taken.

Animal measurements

Ewes were allowed to give birth to their lambs with minimal human disturbance, but within five minutes of each lamb being born, the lamb was tagged and its birth-rank recorded. Two 5 ml blood samples were taken by jugular venepuncture (lithium heparin, and potassium oxalate vacutainer, Becton Dickson Vacutainer Systems, USA) from each lamb. The rectal temperature of each lamb was also recorded at this time, and at one, three, six and 12 hours after birth. At three hours after birth each lamb was weighed, the sex of the lamb determined and crown-rump length and thoracic-girth circumference was measured. Another 5 ml blood sample was collected by jugular venepuncture (Lithium heparin vacutainer, Becton Dickson Vacutainer Systems, USA) from each lamb at three, 12 and 24 hours after birth. At three and six hours after the birth of the last lamb within a litter, a 10 ml blood sample was taken from the ewe by jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA). To minimize disruption to ewe/lamb bonding, all blood samples were collected and rectal temperatures taken within the pen. This allowed continuous contact between the ewe and lambs during sampling.

The intensive lambing observations and measurements were undertaken from 4th September until 10th September 2005. During this time weather conditions consisted of an average wind speed of 1.74 m s^{-1}, an average temp of 11.7°C (max = 18.0°C, min = 5.4°C), an average wind chill of 919.0 kJ $m^{2\ -1}h^{-1}$, and no rainfall. Massey University Animal Ethics Committee approved this trial.

Assays

All blood samples were stored on ice immediately after being taken. Lamb blood samples taken immediately after birth were measured for packed cell volume (PCV; %). Samples remained on ice until centrifugation (15 min, 1000 *g*) when the plasma was separated and stored at -20°C.

Plasma glucose (Hexokinase method, Roche Diagnostics Ltd, Switzerland), non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BOH; Sigma, Illinois, USA) concentrations were analysed using kits. Glucose concentrations were determined using a hexokinase assay (Roche Diagnostics Ltd, Switzerland), fructose using an enzymatic assay (R Biopharm, Darmstadt, Germany) and lactate using a lactate oxidase/peroxidase assay (Roche Diagnostics Ltd, Switzerland). Lamb plasma thyroxine (T_4), tri-iodothyronine (T_3) and cortisol concentrations were analysed by a radioimmunoassay diagnostic kit (Coat-A-Count, Diagnostic Products Corporation, CA, USA), plasma Gamma-glutamyl-transferase (GGT) concentrations were analysed using a Roche kit (Roche Diagnostics Ltd, Mannheim, Germany) and immuno-globulin G (IgG) were analysed using a standard direct ELISA assay).

Statistical analysis

The datasets for metabolite and hormone concentrations and rectal temperatures contained some missing values because of the following reasons: a) if the lamb was dead at birth blood samples and rectal temperatures were not measured, b) blood samples that were not obtained after two attempts were not collected, c) blood samples and rectal temperatures that were not taken within 10 minutes of the required time were not included in the analyses, and d) if the rectal temperature of a lamb fell below 36°C during the observation period, no further measurements were taken and the lamb was removed from the remainder of the study.

Ewe live weight, body condition score, plasma metabolite concentrations (glucose, NEFA, BOH), progesterone at P141, and progesterone at three and six hours after parturition were analysed using a general linear model (PROC GLM, SAS, 2003). The model contained the fixed effect of litter size (Single, Twin, Triplet).

Lamb physical measurements and plasma metabolite concentrations measured directly after birth (glucose, fructose, lactate, T_4, T_3 and cortisol) were analysed using a general linear model (PROC GLM, SAS, 2003). This model contained the fixed effects of birth rank (Single, Twin, Triplet) and lamb sex (Ram, Ewe) and their interactions. Lamb birth weight was fitted as a covariate, which identified if the dependent variables were influenced more by lamb birth rank, or lamb birth weight. Lamb birth weight was only retained in the model if significant ($P < 0.1$). Plasma glucose, fructose and cortisol concentrations immediately after birth were not normally distributed. To achieve normal distribution plasma glucose, fructose and cortisol concentrations were transformed using the $\log^{10}$ function.

Lamb plasma glucose concentrations within five minutes of birth, and at three, 12 and 24 hours of age, and lamb rectal temperatures within five minutes of birth, and at one, three, six and 12 hours of age were analysed using a repeated measure analysis (PROC MIXED, SAS, 2003). This model contained the fixed effects of birth rank (Single, Twin, Triplet), sex (Ram, Ewe), time and the interactions of the main effects. Lamb birth weight was fitted as a covariate and was retained in the model if significant ($P <$ 0.1).

Plasma GGT concentrations were not normally distributed. Transformations were unable to normalise the dependent variable. Plasma GGT concentrations are presented as median and interquartile ranges. The non-parametric test of Kuskal-Wallis was used to determine the significance of the birth rank effect. As a result, significant differences between birth ranks were unable to be determined.

Results

Ewe live weight and body condition score

At P141, single-bearing ewes (74.8 ± 1.86 kg) were lighter ($P < 0.01$) than triplet-bearing ewes (82.6 ± 1.58 kg), whereas the live weight of twin-bearing ewes (78.8 ± 1.40 kg) did not differ ($P > 0.1$) from either single- or triplet-bearing ewes. Litter size had no effect ($P > 0.1$) on ewe body condition score (single-bearing, 2.9 ± 0.15; twin-bearing, 2.7 ± 0.11; triplet-bearing, 2.2 ± 0.12).

Ewe plasma metabolite and hormone concentrations

Twin- and triplet-bearing ewes had greater ($P < 0.001$) plasma BOH and NEFA concentrations than single-bearing ewes at P141. In contrast, plasma glucose concentration did not differ ($P > 0.1$) between single-, twin- and triplet-bearing ewes (Table 3).

Table 3 *The effect of litter size (Single, Twin, Triplet) on ewe plasma glucose (mmol l^{-1}), non-esterified-fatty acids (NEFA; mmol l^{-1}) and beta-hydroxybutyrate (BOH; mmol l^{-1}) concentrations at pregnancy day 141 (P141)*

	n	Glucose	NEFA	BOH
Litter size				
Single	13	4.1 ± 0.17	0.6 ± 0.97^{a}	0.4 ± 0.06^{a}
Twin	22	3.8 ± 0.13	0.9 ± 0.07^{b}	0.7 ± 0.04^{b}
Triplet	18	3.8 ± 0.14	1.1 ± 0.08^{b}	0.8 ± 0.05^{b}
P Value		ns	0.001	0.001

Means ± standard errors within litter size with differing superscripts are significantly different ($P < 0.05$).

Twin- and triplet-bearing ewes had greater ($P < 0.05$) plasma progesterone concentrations at P141, and at six hours after birth than single-bearing ewes (Table 4). At three hours after birth twin-bearing ewes has the greatest plasma progesterone concentrations, followed by triplet-bearing and then single-bearing ewes ($P < 0.05$).

Table 4 *The effect of litter size (Single, Twin. Triplet) on ewe plasma progesterone concentrations (ng ml^{-1}) at pregnancy day 141 (P141) and at three and six hours after the birth*

		Progesterone		
	n	P141	Three hours post-birth[1]	Six hours post-birth[1]
Litter size				
Single	13	14.1 ± 2.48^{a}	-0.30 ± 0.14 (0.74)^{a}	-0.26 ± 0.17 (0.77)^{a}
Twin	22	30.5 ± 1.88^{b}	0.40 ± 0.10 (1.49)^{c}	0.16 ± 0.12 (1.17)^{b}
Triplet	18	24.9 ± 2.30^{b}	0.07 ± 0.11 (1.07)^{b}	0.16 ± 0.13 (1.17)^{b}
P Value		0.05	0.05	0.05

Means ± standard errors within litter size with differing superscripts are significantly different ($P < 0.05$).

[1] Data were log-transformed to normalise distribution; values in parenthesis are the non-transformed means.

Lamb gestation length and size

Triplet-born lambs had shorter ($P < 0.001$) gestation lengths, were lighter in weight ($P < 0.001$), had shorter ($P < 0.01$) crown-rump lengths and smaller ($P < 0.001$) thoracic-girth circumferences than twin- and single-born lambs (Table 5). Twin-born lambs were also lighter ($P < 0.01$) than single-born lambs. The birth rank effect on lamb body dimensions was not significant ($P < 0.1$) after adjustment for birth weight.

Table 5 *The effect of lamb birth rank (Single-, Twin-, Triplet) on gestation length (day), birth weight (kg), crown-rump length (cm) and girth circumference (cm).*

	n	Gestation length	Birth weight	Crown-rump length	Thoracic-girth circumference
Birth rank					
Single	13	146.3 ± 0.43^b	5.7 ± 0.20^c	54.9 ± 1.51^b	41.5 ± 0.70^b
Twin	44	146.0 ± 0.22^b	5.0 ±0.10^b	51.8 ± 0.77^b	40.5 ± 0.36^b
Triplet	51	145.0 ± 0.20^a	4.0 ± 0.09^a	48.7 ± 0.73^a	37.0 ± 0.33^a
P Value		0.001	0.001	0.001	0.001

Means ± standard errors within birth rank with differing superscripts are significantly different ($P < 0.05$)

Lamb plasma metabolite concentrations within five minutes of birth

Single- and twin-born lambs had greater ($P < 0.01$) plasma T_3 concentrations and plasma fructose concentrations within five minutes of birth than triplet-born lambs (Table 6). Single-born lambs had greater ($P < 0.05$) plasma glucose and T_4 concentrations within five minutes of birth than twin- and triplet-born lambs. Birth rank had no effect on PCV (single-born, 47.0 ± 1.49 %; twin-born, 43.8 ± 0.81 %; triplet-born, 44.6 ± 0.78 %) and plasma T_3: T_4, lactate and cortisol concentrations. Lamb birth weight did not affect plasma glucose, fructose, T_4, T_3, T_3: T_4 and cortisol concentrations, but did have a negative association ($P < 0.05$) with plasma lactate concentration. For every one kg increase in lamb birth weight, plasma lactate concentrations decrease 1.1 ± 0.48 mmol l^{-1}.

Lamb plasma glucose concentrations from birth until 24 hours of age

The plasma glucose concentration of single-born lambs was greater ($P < 0.05$) than twin- and triplet-born lambs from birth until 24 hours of age (Figure 2). The plasma glucose concentration of twin-born lambs was also greater ($P < 0.05$) than triplet-born lambs at three and 12 hours of age. Lamb birth weight had no effect on plasma glucose concentrations ($P < 0.1$).

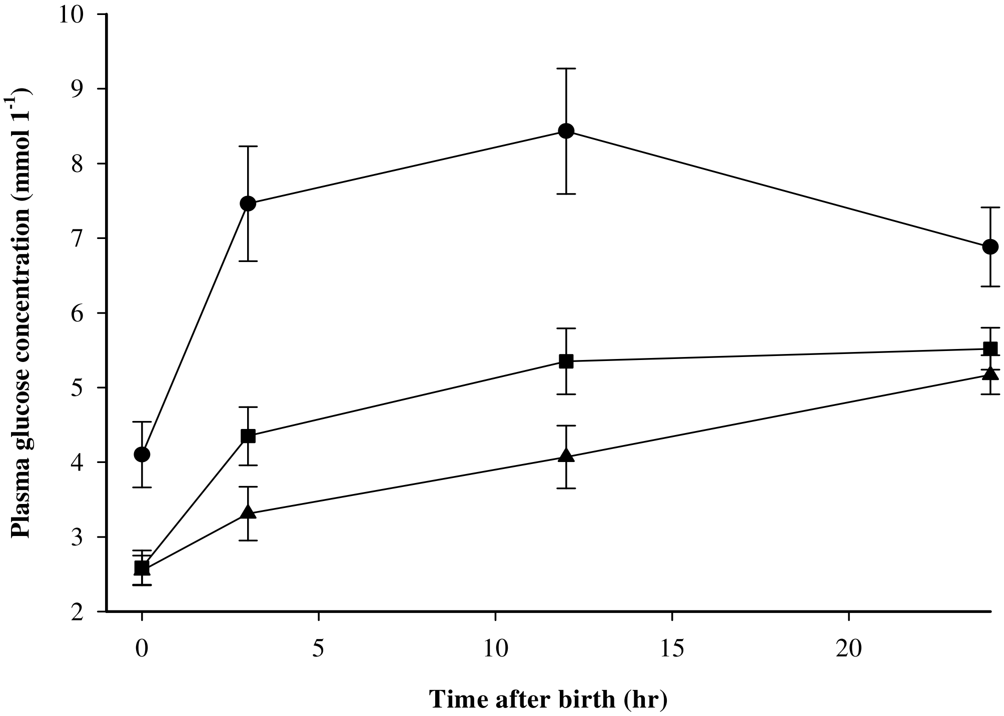
Table 6 *The effect of birth rank (Single, Twin, Triplet) on plasma glucose (mmol l^{-1}), fructose (mmol l^{-1}), lactate (mmol l^{-1}), thyroxine (T4; nmol l^{-1}), tri-iodothyronine (T3; nmol l^{-1}), T3:T4 ratio and cortisol concentrations (mmol l^{-1}) within five minutes of birth.*

	n	Glucose[1]	Fructose[1]	Lactate	T4	T3	T3:T4[1]	Cortisol[1]
Birth rank								
Single	12	1.2 ± 0.17^b (3.3)	1.1 ± 0.12^b (3.0)	7.9 ± 1.15	172.8 ± 11.95^b	3.5 ± 0.30^b	-3.9 ± 0.08 (0.02)	6.3 ± 0.05 (544.6)
Twin	43	0.9 ± 0.08^a (2.5)	0.9 ± 0.05^b (2.5)	7.7 ± 0.54	143.5 ± 6.27^a	2.9 ± 0.16^b	-3.9 ± 0.04 (0.02)	6.2 ± 0.03 (492.7)
Triplet	53	0.8 ± 0.08^a (2.2)	0.6 ± 0.06^a (1.8)	7.6 ± 0.57	129.6 ± 5.72^a	2.3 ± 0.14^a	-4.0 ± 0.04 (0.02)	6.2 ± 0.02 (492.7)
P Value		0.05	0.01		0.05	0.01		

Means ± standard errors within birth rank with differing superscripts are significantly different ($P < 0.05$).

[1] Data were log-transformed to normalise distribution; data presented is log-transformed means ± standard errors within non-transformed means in parenthesis.

Figure 2 *The effect of lamb birth rank (single (●), twin (■) and triplet (▲)) on plasma glucose concentration (mmol l^{-1}) directly after birth until 24 hours of age.*



Data were presented as means ± standard errors

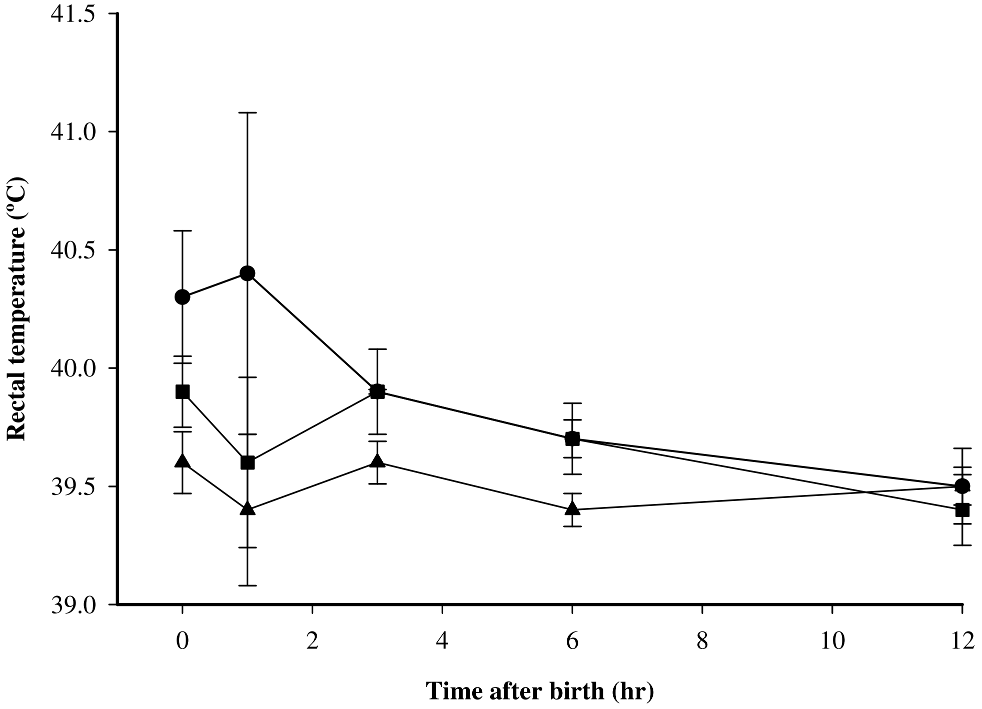
Lamb plasma GGT concentrations within five minutes of birth to 24 hours of age, and plasma IgG concentrations at 24 hours of age.

At three hours after birth, lamb plasma GGT concentration deceased with increasing litter size. However, within five minutes of birth, at 12 and 24 hours of age there were no significant differences between birth ranks (Table 7). Similarly, birth rank had no effect on plasma IgG concentrations at 24 hours of age (single-born, 52.0 ± 11.00 mg ml^{-1}; twin-born, 61.2 ± 5.33 mg ml^{-1}; triplet-born, 70.0 ± 6.03 mg ml^{-1}).

Lamb rectal temperature within five minutes of birth to 12 hours of age

The rectal temperature of triplet-born lambs was lower ($P < 0.05$) than single-born lambs within five minutes of birth, and lower ($P < 0.05$) than single- and twin-born lambs at three and six hours of age (Figure 3). There were no differences in birth ranks at one and 12 hours of age. Lamb birth weight had no effect on lamb rectal temperature ($P < 0.1$).

Figure 3 *The effect of lamb birth rank (single (●), twin (■) and triplet (▲)) on rectal temperature (ºC) within five minutes of birth to 24 hours of age*



Data were presented as means ± standard errors

Table 7 *The effect of lamb birth rank (Single, Twin, Triplet) on plasma gamma-glutamyl-transferase (GGT) concentrations (I U l^{-1}) directly after birth to 24 hours of age (median (interquartile range)).*

	Plasma GGT Concentrations							
	n	0hr	n	3hr	n	12hr	n	24hr
Birth rank								
Single	12	62.0 (53.3-63.4)	10	672.5 (202.0-1445.2)	12	3599.0 (1239.0-4726.0)	13	2126.1 (924.2-3260.0)
Twin	43	55.2 (45.1–64.1)	40	245.7 (85.2-823.7)	43	3244.0 (2024.0-5788.0)	44	1901.0 (818.4-3225.0)
Triplet	53	53.4 (45.1-63.7)	47	105.0 (53.8-428.3)	50	4218.0 (549.2-7960.0)	53	1852.0 (700.4-4006.0)
P value [1]		ns		0.01		ns		ns

[1] Significance was determined by Kruskal Wallis test

Discussion

The aim of this experiment was to identify physical and physiological differences between lambs of different birth ranks from birth until 24 hours of age which may explain the differences in single-, twin- and triplet-born lamb survival rates.

In this experiment, as in other experiments (Barlow *et al* 1987; Dwyer and Morgan 2006; Stafford *et al* 2007), triplet-born lambs had lower rectal temperatures within the first 6 hours of life when compared to single- and twin-born lambs. This suggests that triplet-born lambs may not be as capable at maintaining their body temperature as single- or twin-born lambs. While triplet-born lamb rectal temperatures were lower, in this experiment, the rectal temperatures of all newborn lambs were within a healthy physiological range (Barlow *et al* 1987). This would be expected however; as all newborn lambs were exposed to relatively mild environmental conditions where heat loss to the environment would have been minimal. In the Barlow *et al* (1987) experiment, where environmental conditions were a lot colder, triplet-born lambs were also reported as having lower rectal temperatures than single- and twin-born lambs. Overall, these results combined suggest that in both mild and cold environments, triplet-born lambs may have inadequate thermoregulatory capabilities when compared to single- and twin-born lambs. The 97 % survival rate of triplet-born lambs from birth until 24 hours of age in this experiment and 74 % survival rate of triplet-born lambs in the Barlow et al (1987) experiment suggest that inabilities of the triplet-born lamb to maintain their body temperature may be detrimental in cold environmental conditions only.

Inadequate thermoregulatory capabilities after birth can result from placental insufficiency, intra partum hypoxia or inadequate thermoregulatory mechanisms (Comline and Silver 1972; Mellor and Pearson 1977; Eales and Small 1980b; Cabello 1983; Barlow *et al* 1987; Mellor 1988; Dwyer and Morgan 2006; Stafford *et al* 2007). In this experiment, single-, twin- and triplet-born lambs had the same plasma lactate concentrations and PCV percentages suggesting that chronic or acute hypoxemia, resulting from placental insufficiency or intra partum hypoxia (Barlow *et al* 1987; Mellor 1988) were unlikely to be the main reason for thermoregulatory differences between birth ranks. This finding is in contrast to a similar experiment conducted by Stafford *et al* (2007), which showed that triplet-born lambs had greater plasma lactate concentrations and greater packed cell volumes then single- and twin-born lambs. These results combined suggested that compared to single- and twin-born lambs, triplet-born lambs showed signs of chronic hypoxemia, which was likely to have resulted from placental insufficiency. In this experiment, the lower rectal temperature of triplet-born lambs when compared to single- and twin-born lambs, and the lack of difference between plasma lactate concentrations and PCV percentages between lambs of different birth ranks suggest that triplet-born lambs may have inadequate thermoregulatory mechanisms (Barlow *et al* 1987; Mellor 1988). Inadequate thermoregulatory mechanisms can result from excessive heat loss or inadequate heat production. The lighter birth weights of triplet-born lambs, and consequently their greater surface-area-to-birth weight ratio (Alexander and McCance 1958; Alexander 1979; McCutcheon *et al* 1981), means that they would lose the greatest amount of heat to the environment than single- or twin-born lambs during a cold stress event. In addition to their lighter birth weights, triplet-born lambs also had lower concentrations of glucose when compared to single- and twin-born lambs. This may indicate that triplet-born lambs have less energy available for heat production or that utilisation of energy reserves is poor (Mellor and Cockburn 1986). To utilise energy reserves and generate glucose for heat production, a mature and functional hypothalamic pituitary axis (HPA) axis is needed to release thyroid hormones to stimulate lipid and carbohydrate metabolism through gluconeogenesis and glycogenlysis (Dauncey 1990). Compared to single- and twin-born lambs, triplet-born lambs had shorter gestation lengths and lower plasma T_3 concentrations. In addition, larger litters have been reported to have a depressed HPA axis at the adrenal level *in-utero* (Edwards and McMillen 2002; Gardner *et al* 2004). These factors combined raise the possibility that thermoregulation mechanism in triplet-born lambs may be immature compared to single- and twin-born lambs, and that they may have a reduced thyroidal stimulation for *post-natal* use of energy reserves (Mellor and Stafford 2004). These factors may have a negative impact on the ability of the triplet-born lamb to deal with cold stress (Mellor 1983; Symonds 1995).

To maintain thermoregulation the newborn lamb must stand, move to the udder, consume colostrum in order to replenish energy reserves and conserve pre-natal storage of glycogen and lipid (Mellor and Cockburn 1986; Nowak and Poindron 2006). The lower glucose concentrations from three hours after birth, and lower rectal temperatures at three and six hours after birth may be associated with poorer immediate post-natal behaviour of triplet-born lambs and reduced colostrum intake. At three hours of age, triplet-born lambs had plasma glucose concentrations below fed levels of 4-8 mmol l^{-1} (Mellor and Pearson 1977; Eales *et al* 1980; Eales *et al* 1982). Triplet-born lambs have also been found to be slower at standing and sucking after birth when compared to single and twin-born lambs (Dwyer 2003; Dwyer and Morgan 2006). While these results may suggest that triplet-born lambs are slower at replenishing energy reserves, plasma GGT and IgG concentrations at 24 to 36 hours of age, which have previously been used as an indicator of colostrum intake (Maden *et al* 2003; Kenyon *et al* 2005b), suggest that colostrum intake did not differ between births ranks. While appropriate feeding behaviours is important for the lambs to be able to consume adequate amounts of colostrum for maintenance of heat production, it is also important that the ewe has sufficient amounts of colostrum available for consumption (Nowak and Poindron 2006). It has been suggested that for colostrum to be produced in a sufficient amount, progesterone concentrations must fall below 1 ng ml^{-1} on the day before birth (Hartmann *et al* 1973). Twin- and triplet-bearing ewe plasma progesterone concentrations at three and six hours after birth were above this threshold while singles were not. This may indicate that twin- and triplet-bearing ewes have a delay in lacto-genesis, which may have a negative effect on twin- and triplet-born lamb colostrum intake. This needs to be further investigated.

Conclusions

Triplet-born lambs had lower birth weights than their single- and twin-born counterparts. They also have lower rectal temperatures and lower plasma glucose and thyroid hormone concentrations within five minutes of birth. These results may indicate that triplet-born lamb had less energy and stimulatory factors for adequate thermogenesis. These physiological weaknesses may be detrimental in a cold post-natal environment and could explain why triplet-born lambs have a lower survival rate when compared to single- or twin-born lambs. Further investigations into practical on-farm management strategies which offer improvements in lamb birth weights, plasma glucose or thyroid hormone concentrations may offer improvements in triplet-born lamb survival.

CHAPTER 3

The effect of offering concentrate supplement from pregnancy day 100 until pregnancy day 145 on twin- and triplet-bearing ewe and lamb performance

Related Publication;

Based on a submission to New Zealand Journal of Agricultural Research

Kerslake JI, Kenyon PR, Morris ST, Stafford KJ, Morel PCH (2010) The effect of offering concentrate supplement from pregnancy day 100 until pregnancy day 145 on twin- and triplet-bearing ewe and lamb performance.

Abstract

This experiment investigated the effect of offering concentrate supplement, from day 102 to day 145 of pregnancy, to twin- and triplet-bearing ewes grazing a 6 cm sward height, on ewe and lamb performance. Twin- ($n = 40$) and triplet-bearing ($n = 21$) ewes were grazed on a 6 cm sward height from day 70 of pregnancy until parturition. From pregnancy day 102, half of the ewes from each litter size were offered 400 g $\text{ewe}^{-1}\text{day}^{-1}$ of concentrate sheep pellets. From day 102 until day 145 of pregnancy, ewes offered concentrate gained 60 g day^{-1} more live weight than ewes offered pasture only ($P < 0.01$). Ewes offered concentrate were also under less metabolic stress in late pregnancy, as indicated by lower ($P < 0.05$) plasma beta-hydroxybutyrate and non-esterified fatty acid concentrations. Offering concentrate increased lamb birth weight from 3.9 to 4.2 kg ($P < 0.05$) and tended to increase lamb plasma gamma-glutamyl-transferase concentrations at 24 to 36 hours of age ($P = 0.08$). It had no effect however, on lamb plasma glucose or immuno-globulin G concentrations within 24 to 36 hours of age or lamb growth or survival from birth until day 52 of lactation. Compared to ewes offered pasture, ewes offered concentrate reared 3.3 kg more ($P < 0.05$) of lamb from birth until day 52 of lactation.

Introduction

The herbage intake of twin- and triplet-bearing ewes is unrestricted when grazing ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* swards that are 4 cm or greater in height (Morris and Kenyon 2004). At these sward heights however, triplet-bearing ewes still mobilise energy from their body reserves in late pregnancy (Morris and Kenyon 2004). Other research has also shown that the glucose supplied by an *ad-libitum* ryegrass diet is insufficient to maintain triplet fetal growth, and that calculated amino acid requirements of triplet fetuses are likely to exceed net absorption from fresh forage diets, causing maternal tissue to mobilise (Barry and Manley 1985). These results combined suggest that ewes with large litters may be unable to meet their nutritional requirements when grazing a ryegrass and/or white clover sward. The higher energy demand during pregnancy of triplet-bearing ewes compared to twin-bearing ewes, and the potential inability of the triplet-bearing ewe to consume enough nutrients in late pregnancy (Everts 1990), may explain why triplet-born lamb birth weights (Kenyon *et al* 2005b; Dwyer and Morgan 2006), survival rates (Thomson *et al* 2004; Kerslake *et al*

2005) and liveweight gain to weaning (Morris and Kenyon 2004) are often disappointing.

Triplet-bearing ewes may find it difficult to consume enough pasture during late pregnancy because of limited space in the rumen (Everts 1990), and/or the bulk, high water content, and greater fibre content of the pasture. By offering concentrate supplement, which compared to pasture is less bulky, has lower water content and less fibre, the nutrient intake of the ewe could be increased (Dove 2002). This is supported by the finding of Orr and Treacher (1990), which showed that the total nutrient intake of twin- and triplet-bearing ewes is increased by offering concentrate supplement with a grass silage diet.

While a number of studies have shown that offering concentrates, such as cottonseed meal or lupins, to ewes grazing low quality or low herbage allowances in late pregnancy can have a positive effect on ewe and/or lamb performance (Hall *et al* 1992; Stephenson and Bird 1992; Banchero *et al* 2004a), only a few studies have looked at offering concentrate on a high herbage allowance to improve ewe and lamb performance (Hinch *et al* 1996; Kerslake *et al* 2008). The aim of this study was therefore to investigate the effects of offering concentrate supplement, from pregnancy day 102 until pregnancy day 145, to twin- and triplet-bearing ewes grazing a 6 cm sward height, on ewe and lamb performance.

Materials and methods

Experimental design

The experiment was carried out at Kebble Farm, Massey University, Palmerston North, New Zealand (longitude 40.2°S; latitude 175.3°E , elevation 64 m). The experiment period was from the 8th March - 10th October 2005. The experimental design was a replicated 2 x 2 factorial design which included two litter sizes (twin- and triplet-bearing ewes) and two nutritional treatments (6 cm herbage sward height with concentrate supplement (Concentrate) or 6 cm herbage sward height only (Non-concentrate)).

The oestrus of 509 (307 two-tooth and 282 mixed-aged) Romney ewes was synchronised using a progesterone-releasing device (Eazi-breed CIDR ®, Pfizer, Auckland, New Zealand). The CIDR was inserted for 13 days, and on removal, ewes were injected with 400 I.U. of PSMG (Folligon ®, Intervet, Boxmeer, Holland). Also at this time, ewe live weight and body condition score (scale 1-5 units, where one describes a ewe that is extremely emaciated and five describes a ewe that is very fat (Jefferies 1961) was recorded, and Romney ram hoggets, fitted with crayon mating harnesses, were introduced for a five-day breeding period. At 49 days (P49) after the mid-point of the first oestrous cycle, crayon marked ewes were weighed, body condition scored and pregnancy diagnosed using ultrasound technology. Ewes were marked as being either non-pregnant, single-, twin- or triplet-bearing for future identification. All ewes were grazed as one group under commercial grazing conditions from the commencement of the breeding period until P70.

On P70, 20 twin and 10 triplet-bearing ewes were allocated to a 6 cm herbage sward height (Non-concentrate) and a total of 20 twin- and 11 triplet-bearing ewes were allocated to a 6 cm herbage sward height with concentrate supplement (Concentrate). A 6 cm sward height was chosen as previous research has shown that twin- and triplet-bearing ewe intake is unrestricted when grazing a sward height greater than 4 cm (Morris and Kenyon 2004). Each ewe nutritional treatment had two replicates and was balanced for ewe live weight and body condition (pooled means of ewe liveweight and body condition for Concentrate and Non-concentrate treatment groups were 63.8 and 62.8 kg, and 2.9 and 2.9 units, respectively). Throughout the remainder of the study, ewes were rotationally grazed around four 2.0 ha paddocks at a stocking rate of 7 or 8 ewes ha^{-1}. Rotational grazing limited any potential paddock effects, such as the potential sparing of pasture from pregnancy into lactation during supplementation.

From P102 until P145, concentrate fed ewes were offered 400 g day^{-1} ewe^{-1} of sheep pellets (903 g kg^{-1} dry matter (DM), 141 g kg^{-1} DM crude protein (CP), 126 g kg^{-1} DM of lipid, calculated metabolisable energy content (ME) of 12.3 MJ kg^{-1} DM; Universal stock feed, Harvey Farms Ltd, Wanganui, New Zealand) in two 2.5 × 0.4 m troughs. An amount of 400 g day^{-1} was chosen because previous research has shown that offering 400 g day^{-1} to twin-bearing ewes grazing a restricted herbage sward height increases lamb birth weight (Kerslake *et al* 2008), whereas offering 200 g day^{-1} did not (Kenyon *et al* 2005a). During the concentrate feeding period, troughs were checked daily for residuals. There were none however, during the entire feeding period. Four weeks after the mid-point of lambing, all ewes were grazed as one group under commercial grazing conditions. Three triplet-bearing ewes and one twin-bearing ewe died during pregnancy.

The lambing period was from the 12th August – 22nd August 2005. During the lambing period the average wind speed was 2.0 m s^{-1}, total rainfall of 0.4 mm, average temperature was 9.1ºC (min=4.8ºC and max=15.3ºC) and an average cold stress index was 966.49 $kJm^{2-1}h^{-1}$ (Donnelly 1984).

Pasture management

Throughout the study ewes and lambs were grazed on a ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* sward that was at least four years old. To ensure that ewes were grazed within a controlled range of sward heights, sward surface heights were measured at weekly intervals using a sward stick (Jenquip, New Zealand, 100 readings per paddock). If sward height was found to be below 5 cm, ewes were placed in an alternative paddock which had been prepared at the appropriate sward height. If the sward height was above 7 cm, additional ewes were introduced to control the sward height. The aim was therefore to maintain the defined sward conditions irrespective of size of area grazed. Herbage mass was measured at two-weekly intervals using a rising plate meter (Ashgrove Pastoral Products, New Zealand, 50 readings per paddock) and was calculated using the following formula:

$$Herbage\ mass\left(kg\ DM\ /\ ha\right) = (158 \times MR) + 200$$

where MR is the meter reading (Hodgson *et al* 1999).

Animal measurements

Unfasted live weight and body condition scores of ewes were measured on P70, P87, P102, P116, and P140. On P144, ewe back-fat depth on the left-hand side above the loin area of the last rib (Ultrasound, Auckland (Purchas and Beach 1981) and ewe udder size was measured. Three measurements of udder size were taken from the posterior margin to the anterior margin of the udder. These included one along the midline of the udder and two parallel to the midline immediately medial to each teat (Mellor and Murray 1985b). A 10 ml blood sample was collected by jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA) on P102, P116, P130 and P140.

Within 12 hours of birth, all lambs were identified to their dam, tagged and their sex and birth rank determined. In addition, lamb birth weight, crown-rump length and thoracic-girth circumference were measured. Surface area (SA) of the lamb was calculated using the mathematical formula for the total SA of a cylinder,

$$SA\left(cm^2\right) = 2\pi r h + 2\pi r^2$$

where r is the thoracic-girth circumference of the lamb divided by 2π , h is the crown-rump length of the lamb.

Between 24 and 36 hours old a 5 ml blood sample (Lithium heparin, Becton Dickson Vacutainer Systems, USA) was collected by jugular venepuncture from all lambs. All ewes and lambs remained in their treatment groups and were rotationally grazed on a 6 cm sward height until weaning. All ewes and lambs were weighed 29 days (L29) and L52 days after the midpoint of lambing (15 August 2005).

Blood Analysis

All blood samples were placed on ice until they were centrifuged at 1000 *g* for 15 minutes. The plasma was removed and frozen at -20ºC. Ewe plasma samples were analysed using diagnostic kits for glucose (Hexokinase method, Roche Diagnostics Ltd, Switzerland), beta-hydroxybutyrate (BOH) and non esterified fatty acid (NEFA) (Sigma, Illinois, USA) concentrations. Lamb plasma samples were analysed for immuno-globulin G (IgG) (Standard direct ELISA assay), gamma-glutamyl transferase (GGT) (Roche, diagnostics Ltd, Mannheim, Germany), glucose (hexokinase method, Roche Diagnostics Ltd, Switzerland), thyroxine (T_4) and triiodothyronine (T_3) (Radioimmunoassay kit, Coat-a-count Diagnostic Products Corporation, CA, USA) concentrations.

Statistical analysis

Herbage sward surface heights and mass were analysed using a general linear model (PROC GLM, SAS, SAS Institute, Cary, NC) which contained the fixed effect of replicate (one, two) and concentrate supplement (Non-concentrate, Concentrate). Ewe liveweight gain, back-fat depth, ewe udder size, live weight at L52 and total live weight of lamb produced at L52 were analysed using a general linear model (PROC GLM). Fixed effects included replicate (One, Two), concentrate supplement (Non-concentrate, Concentrate), litter size (Twin, Triplet) and the interactions between the main effects. Interactions were retained in the model if significant ($P < 0.05$). If non-significant, interactions were removed and model re-fitted.

Lamb birth weight, SA, crown-rump length, thoracic-girth circumference and lamb plasma metabolites (T_3, T_4, Glucose, GGT and IgG) at 24 to 36 hours of age were analysed using a general linear model (PROC GLM). Fixed effects included replicate (one, two) concentrate supplement (Non-concentrate, Concentrate), birth rank (Twin, Triplet), lamb sex (Ewe, Ram) and interactions between fixed effects. Lamb birth weight was added as a covariate for all dependent variables apart from lamb birth weight and surface-area-to-birth-weight ratio. Live weight at L29 and L52 and liveweight gain from birth until L29, L52 or from L29-L52 were analysed using the same general linear model (PROC GLM). The fixed effect of birth rank (twin, triplet) however, was removed and replaced with rearing rank (Twin reared as a single or twin, Triplet reared as a twin or triplet). No triplet-born lambs were reared as singles. Lamb birth weight was also not adjusted for in these models.

The T_3, T_4, GGT and IgG plasma concentrations were not normally distributed. To achieve a normal distribution, T_3, T_4, GGT and IgG were subjected to a $\log^{10}$ transformation before being analysed using a general linear model. Back-transformed means are presented in the tables.

All ewe measurements that were taken over time (live weight, body condition score, and BOH and NEFA concentrations), from day P102 to P140, were analysed using a repeated measure (PROC MIXED) analysis. This model contained the fixed effects of replicate (one, two), concentrate supplement (Non-concentrate, Concentrate), litter size (Twin, Triplet), lamb sex (Ewe, Ram), time and the interactions of the fixed effects. If significant ($P < 0.05$), interactions were retained in the model. If non-significant, interactions were removed and the model refitted.

The proportion of lambs surviving from birth to weaning were analysed as a binomial trait using the SAS procedure for categorical modelling (PROC GENMOD). Fixed effects included replicate (One, Two), concentrate supplement (Non-concentrate, Concentrate), litter size (Twin, Triplet), lamb sex (Ewe, Ram) and their interactions. If not significant ($P > 0.05$), interactions were removed from the model.

There were no significant differences found between replicates throughout this experiment. For the remainder of this paper only the differences between ewe nutritional treatments and birth ranks will be presented.

Results

Herbage sward surface height and mass

For both ewe nutritional treatments, herbage measurements from P70 until P145 did not differ ($P > 0.1$) in sward height (Non-concentrate 6.6 ± 0.21 cm, Concentrate 6.1 ± 0.22 cm) or mass (Non-concentrate 1671 ± 108 kg DM / ha, Concentrate 1539 ± 108 kg DM ha^{-1}).

Ewe live weight and body condition score

On P116 and P140 ewes that were offered concentrate were heavier ($P < 0.05$) than ewes offered pasture only (Table 8). Litter size had no effect ($P > 0.1$) on ewe live weight at P102, P116 and P140. From P102 until P145 concentrate fed ewes gained 60 g more per day than ewes offered pasture only ($P < 0.01$). Litter size and nutritional treatment had no effect ($P > 0.1$) on ewe live weight on L29 or L52 (pooled means and standard deviations of ewe liveweight at L29 and L52 were 67.7 ± 7.05 kg and 61.9 kg ± 7.31 kg, respectively).

Ewe body condition score at P102, P116, P140 and ewe back fat depth measured on P144 were not influenced ($P > 0.1$) by litter size or nutritional treatment (pooled means and standard deviations of ewe body condition score at P102, P116 and P140, and ewe back fat depth measurements on P144 were 2.9 ± 0.38, 2.9 ±0.43 and 2.8 ± 0.42 units, and 5.2 ± 1.82 cm, respectively).

Ewe metabolic status

Ewes offered concentrate had greater ($P < 0.05$) plasma NEFA concentrations on P102 and P116, but lower ($P < 0.05$) plasma NEFA concentrations on P130 and P140 (Table 9). Compared to ewes offered pasture only, offering concentrate had no effect ($P > 0.1$) on ewe plasma BOH concentrations at P102, increased plasma BOH concentrations on P116 and decreased plasma BOH concentrations on P130 and P140 ($P < 0.01$; Table 10).

Ewe nutritional treatment had no effect ($P > 0.1$) on plasma glucose concentrations at P102, P116, P130 or P140 (pooled means and standard deviations of plasma glucose concentrations at P102, 116, 130 and 140 were 3.7 ± 0.46, 3.9 ± 0.64, 3.8 ± 0.44 and 3.9 ± 0.47 mmol l^{-1}, respectively.). All ewe plasma metabolites measured were unaffected by litter size on P102 and 116, 130 and 140 ($P > 0.1$).

Table 8 *The effect of offering concentrate (Non-concentrate, Concentrate) and litter size (Twin, Triplet) on ewe live weight (kg) at pregnancy day 102 (P102), 116 (P116) and 140 (P140) and on liveweight gain (kg day^{-1}) between pregnancy day 102 and 140 (P102-P140)*

	Live weight					Liveweight gain
	n	P102	P116	n	P140	P102-P140
Nutritional treatment						
Non-concentrate	30	68.6 ± 0.44	73.2 ± 0.55	29	78.8 ± 0.70	0.27 ± 0.01
Concentrate	31	68.2 ± 0.43	74.8 ± 0.54	28	81.0 ± 0.71	0.33 ± 0.01
P Value		ns	0.05		0.05	0.01
Litter size						
Twin	40	67.9 ± 0.37	73.5 ± 0.47	39	79.4 ± 0.58	0.30 ± 0.01
Triplet	21	68.9 ± 0.51	74.4 ± 0.63	18	80.4 ± 0.85	0.30 ± 0.02
P value		ns	ns		ns	ns

Data is presented as means ±.standard errors; ns, non significant

Table 9 *The effect of offering concentrate (Non-concentrate, Concentrate) and litter size (Twin, Triplet) on ewe plasma non-esterified fatty acid (NEFA) (mmol/l) concentrations at pregnancy day 102 (P102), 116 (P116), 130 (P130) and 140 (P140)*

	NEFA[1]				
	n	P102	P116	P130	P140
Nutritional treatment					
Non-concentrate	30	-0.76 ± 0.07 (0.47)	-1.18 ± 0.12 (0.31)	-0.55 ± 0.12 (0.55)	-0.71 ± 0.13 (0.49)
Concentrate	31	-0.53 ± 0.07 (0.59)	-0.84 ± 0.12 (0.43)	-1.06 ± 0.12 (0.34)	-1.23 ± 0.13 (0.29)
P Value		0.05	0.05	0.05	0.05
Litter size					
Twin	40	-0.70 ± 0.06 (0.50)	-1.08 ± 0.11 (0.34)	-0.92 ± 0.10 (0.44)	-1.02 ± 0.11 (0.37)
Triplet	21	-0.59 ± 0.08 (0.55)	-0.95 ± 0.15 (0.39)	-0.68 ± 0.14 (0.43)	-0.91 ± 0.16 (0.38)
P Value		ns	ns	ns	ns

Data is presented as means ±.standard errors; ns, non significant

[1] Data were log-transformed, transformed data with SE are presented with back-transformed means in parentheses

***Table 10** The effect of offering concentrate (Non-concentrate, Concentrate) and litter size (Twin, Triplet) on ewe plasma beta-hydroxybutyrate (BOH; mmol l^{-1}) concentrations at pregnancy day 102 (P102), 116 (P116), 130 (P130) and 140 (P140)*

	BOH[1]				
	n	P102	P116	P130	P140
Nutritional treatment					
Non-concentrate	30	-1.01 ± 0.05 (0.36)	-1.07 ± 0.04 (0.34)	-0.96 ± 0.05 (0.38)	-0.56 ± 0.05 (0.57)
Concentrate	31	-0.90 ± 0.05 (0.41)	-0.90 ± 0.04 (0.40)	-1.15 ± 0.05 (0.33)	-0.94 ± 0.05 (0.39)
P Value		ns	ns	0.01	0.01
Litter size					
Twin	40	-0.97 ± 0.04 (0.38)	-0.97 ± 0.04 (0.38)	-1.03 ± 0.04 (0.36)	-0.75 ± 0.04 (0.47)
Triplet	21	-0.94 ± 0.06 (0.39)	-1.00 ± 0.05 (0.37)	-1.07 ± 0.06 (0.34)	-0.74 ± 0.06 (0.47)
P Value		ns	ns	ns	ns

Data is presented as means ±.standard errors; ns, non significant

[1] Data were log-transformed, transformed data with SE are presented with back-transformed means in parentheses

Ewe udder size

At P144, ewe nutritional treatment tended (*P* = 0.08) to have an effect on ewe udder size (Concentrate ewes 56.8 ± 1.49 cm, Non concentrate ewes 53.2 ± 1.47 cm, respectively). Triplet-bearing ewes had (*P* < 0.05) larger udder sizes than twin-bearing ewes (57.2 ± 2.05 cm vs. 51.2 ± 1.03 cm, respectively).

Lamb live weight and dimensions

Lambs born to ewes offered concentrate had heavier (*P* < 0.05) birth weights than lambs born to ewes offered pasture only. In addition, twin-born lambs were heavier (P < 0.001) than triplet-born lambs. Twin-born lambs had longer crown-rump lengths (P < 0.001) and greater thoracic-girth circumference (*P* < 0.001) than triplet-born lambs. The birth rank effect on lamb body dimensions, however, was not apparent after adjustment for lamb birth weight (Table 11).

Table 11 *The effect of offering concentrate (Non-concentrate, Concentrate) and lamb birth rank (Twin, Triplet) on lamb birth weight (kg), surface-area-to-birth-weight ratio(SA:BW; (cm^2 kg^{-1}), crown-rump length (cm) and thoracic-girth circumference (cm)*

	n	Birth weight	SA:Bw	Crown-rump length	Thoracic-girth circumference
Nutritional treatment					
Non-concentrate	66	3.9 ± 0.09	179.8 ± 7.08	51.6 ± 0.47	36.3 ± 0.37
Concentrate	63	4.2 ± 0.10	174.1 ± 6.82	52.6 ± 0.48	37.0 ± 0.38
P Value		0.05	ns	ns	ns
Lamb birth rank					
Twin	78	4.5 ± 0.09	165.8 ± 7.04	53.4 ± 0.43	38.0 ± 0.34
Triplet	51	3.6 ± 0.11	188.1 ± 6.88	50.8 ± 0.53	35.3 ± 0.41
P Value		0.001	0.001	0.001	0.001

Values presented as means ± standard errors; ns, non significant

Ewe nutritional treatment had no effect (*P* > 0.1) on lamb live weight at L29, L52 or liveweight gain from birth until L29 or L52 (Table 12). Offering concentrate did however, have positive effects on liveweight gain from L29 to L52 ($P < 0.05$). On L29, triplet-born lambs reared as triplets were lighter ($P < 0.05$) than all other rearing ranks. On L52, triplet-born lambs reared as triplets were lighter ($P < 0.05$) than all twin-born lambs. Twin-born lambs reared as singles were heavier ($P < 0.05$) at L52 than all other rearing ranks. From birth until L29, twin-born lambs reared as singles gained a greater ($P < 0.05$) amount of live weight than all other rearing ranks. Twin-and triplet-born lambs reared as twins gained a greater ($P < 0.05$) amount of weight than triplet-born lambs reared as triplets. From birth to L52 and from L29 to L52, triplet-born lambs reared as triplets gained less ($P < 0.05$) live weight than twin-born lambs reared as either singles or twins.

Lamb plasma thyroid hormone concentrations

There was a significant interaction between ewe nutritional treatment and birth rank for both plasma T_4 and T_3 concentrations ($P < 0.01$; $P < 0.05$, respectively; Table 13). Triplet lambs born to pasture-fed ewes had lower ($P < 0.05$) plasma T_4 and T_3 concentrations compared to twin lambs born to ewes offered pasture only. There were no differences however, between twin and triplet lambs born to ewes offered concentrate ($P > 0.1$). Twin lambs born to concentrate-fed-ewes had lower ($P < 0.05$) plasma T_4 and T_3 concentrations than twin lambs born to ewes offered pasture only. There were no differences in plasma T_4 and T_3 concentrations between triplet lambs born to ewes offered concentrate or ewes offered pasture only ($P > 0.1$). Lamb birth weight had a positive effect ($P < 0.001$) on plasma T_4 concentrations, where a one kg increase in lamb birth weight increased plasma T_4 concentrations by 0.09 (± 0.03 (se)) nmol l^{-1}.

Table 12 *The effect of offering concentrate (Non-concentrate, Concentrate) and lamb rearing rank (Twin-born reared as single or twin vs. triplet-born reared as a twin or triplet) on lamb live weight (kg) at lactation day 29 (L29) and 52 (L52), and on liveweight gain (kg day^{-1}) from birth until L29, birth until L52 and L29 to L52.*

	Live weight				Liveweight gain		
	n	L29	n	L52	Birth – L29	Birth – L52	L29 – L52
Nutritional treatment							
Non-concentrate	50	12.1 ± 0.35	48	15.8 ± 0.41	0.27 ± 0.013	0.22 ± 0.008	0.16 ± 0.017
Concentrate	51	11.5 ± 0.32	50	16.2 ± 0.40	0.25 ± 0.013	0.22 ± 0.008	0.21 ± 0.018
P Value		ns		ns	ns	ns	0.05
Lamb rearing rank [1]							
Twin							
Single	5	12.4 ± 0.74^{a}	5	18.3 ± 0.99^{a}	0.30 ± 0.032^{c}	0.29 ± 0.021^{b}	0.28 ± 0.043^{c}
Twin	62	12.2 ± 0.27^{a}	61	16.0 ± 0.30^{bc}	0.27 ± 0.009^{b}	0.23 ± 0.006^{b}	0.17 ± 0.012^{b}
Triplet							
Twin	22	12.0 ± 0.55^{a}	20	15.1 ± 0.56^{cd}	0.24 ± 0.016^{b}	0.21 ± 0.011^{ab}	0.17 ± 0.022^{ab}
Triplet	12	10.6 ± 0.53^{b}	12	14.5 ± 0.71^{d}	0.22 ± 0.021^{a}	0.17 ± 0.014^{a}	0.11 ± 0.028^{a}
P Value		0.05		0.05	0.05	0.05	0.05

Data is presented as means ±.standard errors; ns, non significant

[1] Means ± standard errors within lamb rearing rank with differing superscripts are significantly ($P < 0.05$) different

Table 13 *The effect of offering concentrate (Non concentrate, Concentrate) and lamb birth rank (Twin, Triplet) on lamb plasma thyroid hormones (T_4 (nmol l^{-1}) and T_3 (nmol l^{-1}) at 24-36 hours after birth*

		Thyroid Hormones[1]	
	n	T_4	T_3
Nutritional treatment			
Non-concentrate	55	4.7 ± 0.03 (111.1)	1.6 ± 0.04 (4.8)
Concentrate	53	4.7 ± 0.03 (109.9)	1.5 ± 0.04 (4.6)
P Value		ns	ns
Lamb birth rank			
Twin	68	4.7 ± 0.03 (116.4)	1.7 ± 0.04 (5.3)
Triplet	40	4.7 ± 0.03 (109.9)	1.4 ± 0.05 (4.2)
P Value		0.05	0.001
Interactions			
Twin			
Non-concentrate	38	4.8 ± 0.04 (122.7)^{b}	1.8 ± 0.05 (5.7)^{c}
Concentrate	30	4.7 ± 0.04 (109.9)^{a}	1.6 ± 0.05 (4.9)^{b}
Triplet			
Non-concentrate	17	4.6 ± 0.06 (100.5)^{a}	1.4 ± 0.07 (4.0)^{a}
Concentrate	23	4.7 ± 0.04 (108.9)^{a}	1.5 ± 0.06 (4.3)^{ab}
P Value		0.01	0.05

Data were presented as means ± standard errors; means ± standard errors within interaction with differing superscripts are significantly ($P < 0.05$) different; ns, non significant

[1] Data were log-transformed, transformed data with SE are presented with back-transformed means in parenthesis.

Lamb plasma glucose GGT and IgG concentrations

Ewe nutritional treatment had no effect *($P > 0.1$)* on plasma glucose and IgG concentrations of the lamb at 24 to 36 hours of age. There was a tendency ($P = 0.08$)

for plasma GGT concentrations to be greater in lambs born to concentrate-fed ewes than lambs born to ewes fed pasture only (Concentrate 7.4 ± 0.10 IU l^{-1} (1685.8), Non-concentrate 7.4 ± 0.13 IU l^{-1} (1603.6) (transformed data with SE are presented with back-transformed means in parentheses)). Plasma glucose concentrations were greater ($P < 0.01$) in twin-born lambs than triplet-born lambs at 24 to 36 hours of age (Twin-born 6.7 ± 0.18 mmol l^{-1}, Triplet-born 5.6 ± 0.23 mmol l^{-1}). Birth rank had no effect on plasma GGT and IgG concentrations at 24 to 36 hours of age ($P > 0.1$). Lamb plasma GGT concentration was positively correlated ($R = 0.29$; $P < 0.001$) with plasma IgG concentration at 24 to 36 hours of age. No correlations were found between plasma GGT and glucose or IgG and glucose ($P > 0.1$).

Lamb survival was not affected ($P > 0.1$) by ewe nutritional treatment (Table 14). Triplet-born lambs had lower ($P < 0.01$) survival rates than twin-born lambs. Ewes offered concentrate reared a heavier ($P < 0.05$) total weight of lamb to L52 than ewes offered pasture only.

Table 14 *The effect of offering concentrate (Non concentrate, Concentrate) and lamb birth rank (Twin, Triplet) on lamb survival from birth until lactation day 52 (L52; %) and total weight of lamb produced per ewe (kg ewe^{-1}) at L52.*

	Lamb Survival		Total weight of lamb	
	n	Birth to L52	n	L52
Nutritional treatment				
Non-concentrate	48	0.9 ± 0.29[#] (70.1[$])	29	28.7 ± 1.32
Concentrate	50	1.4 ± 0.33 (80.0)	28	32.0 ± 1.35
P Value		ns		0.05
Lamb birth rank				
Twin	66	1.8 ± 0.32 (85.2)	39	30.0 ± 0.92
Triplet	32	0.5 ± 0.29 (62.0)	18	30.7 ± 1.87
P Value		0.01		ns

Total weight of lamb is presented as means ± standard errors; ns, non-significant

[#]Log10 transformed; [$] Back-transformed (%)

Discussion

The aim of this study was to investigate the effect of offering a concentrate supplement, from day 102 of pregnancy until day 145 of pregnancy, to twin- and triplet-bearing ewes grazing a 6 cm sward height, on ewe and lamb performance. Supplementing a ryegrass and white clover sward with concentrate led to a moderate improvement in ewe liveweight, a reduction in ewe plasma NEFA and BOH concentrations in late pregnancy, indicating ewe were mobilising less energy from body reserves, and a 300 g increase in lamb birth weight. When offered concentrate supplement with pasture, ewes will either respond by eating the concentrate and increasing or maintaining herbage intake (supplementation), or eating the concentrate and substituting herbage intake (Holst *et al* 1996a). If the substitution of pasture for concentrate is too great, the production benefits of offering concentrate will be either small or non-existent. Although ewe herbage and concentrate intakes were not measured in this trial, improvements in ewe liveweight gain, a reduction of ewe plasma NEFA and BOH concentrations and an increase in lamb birth weight suggests that offering concentrate increased the total nutrient intake of twin- and triplet-bearing ewes, and that substitution of herbage was minimal. Improvements in ewe live weight, body condition and lamb birth weights of concentrate-fed ewes, when compared to ewes fed silage only, have been previously attributed to the greater nutrient intake of concentrate-fed ewes (Orr and Treacher 1990).

During late pregnancy, nutrients are not only partitioned towards fetal growth but also towards mammary development and colostrum synthesis (Robinson 1983). While offering concentrate had no effect on plasma IgG concentrations, it tended to increase ewe udder size and lamb plasma GGT concentrations. Because ewe udder size and plasma GGT concentrations have been previously used as an indirect measure of colostrum production (Mellor and Murray 1985b) and colostrum intake (Parker and Nicol 1990; Kenyon *et al* 2005b), respectively, these results tentatively suggest that offering concentrate to ewes grazing a 6 cm sward height may have a positive effect on colostrum let down or production, and consequently the colostrum intake of the lamb. Offering concentrate supplement during late pregnancy to ewes grazing a low (Stephenson and Bird 1992) or high (Murphy *et al* 1996) herbage allowance has previously been shown to increase colostrum or milk production.

Ewe nutrition during pregnancy can influence the thyroid status of the fetus (Symonds 1995). Thyroid hormones T_3 and T_4 are important for the maturation and functional development of brown adipose tissue and heat production directly after birth (Polk 1988; Symonds 1995). In the present study, triplet lambs born to pasture-fed ewes had lower plasma thyroid hormone concentrations than twin lambs born to pasture-fed ewes. This may indicate that triplet-born lambs have a lower potential for heat production at 24 to 36 hours of age (Symonds 1995). Dwyer and Morgan (2006) however, have reported no differences between birth ranks in plasma thyroid hormone concentrations at 24 to 36 hours of age and over the first three days of life. Interestingly, offering concentrate to twin-bearing ewes decreased plasma T_3 concentrations in their offspring. This relationship was not observed in triplet-born lambs and the reason for this decrease in twin-born lambs is unknown.

Offering concentrate during late pregnancy to ewes grazing high herbage allowances has been shown to improve lamb survival to weaning (Hinch *et al* 1996). In the present study, offering concentrate had no effect on lamb survival to weaning. Caution should be taken here however, as the numbers of lambs in the present study were insufficient to accurately determine survival differences between nutritional treatments. Nevertheless, ewes offered concentrate reared a greater total live weight of lamb at L52 than ewes fed pasture only. Total live weight of lamb weaned takes into account both lamb growth and survival. To be economical, concentrate supplementation must result in a production difference that covers the expense of the concentrate and the associated labour and equipment required. While it is probable that the production gains observed in this trial, approximately 3.3 kg of lamb live weight weaned per ewe were not large enough to justify the costs associated with offering the concentrate, the degree, if any, of substitution is not known. Therefore the biological efficiency and economic viability of offering such a concentrate to twin- and triplet-bearing ewes grazing a 6 cm sward can only be hypothesised.

Conclusion

Offering concentrates to twin- and triplet-bearing ewes grazing a 6 cm high pasture moderately improved both ewe and lamb performance. Offering concentrate supplement during late pregnancy resulted in an increase in ewe liveweight gain from P102 until P140, a decrease in metabolic stress, and an increase in the birth weight of their offspring. While these positive effects suggest that offering concentrate increased the total nutrient intake of twin- and triplet-bearing ewes and that substitution of the 6 cm high herbage was minimal, concentrate and herbage intakes were not measured in this trial and can therefore only be hypothesised. In addition to increasing lamb birth weight, offering concentrate tended to have a positive effect on the colostrum uptake by the lamb. Offering concentrate also had a positive effect on total lamb weight at L52. Further studies are required to look at the substitution rate and the economic viability of offering twin- and triplet-bearing ewes concentrate when grazing a 6 cm sward height.

CHAPTER 4

The effect of offering concentrate supplement to twin- and triplet-bearing ewes grazing a 6 cm sward height on lamb birth weight and heat production.

Related Publication;

Based on a publication in the Journal of Agricultural Science (Cambridge)

Kerslake JI, Kenyon PR, Morris ST, Stafford KJ, Morel PCH (2009) The effect of offering concentrate supplement to twin- and triplet-bearing ewes grazing a 60 mm herbage sward height on lamb birth weight, heat production and post-natal growth. *Journal of Agricultural Science (Cambridge)* **147**, 1-12.

Abstract

This experiment investigated the effect of offering concentrate supplement to ewes in late pregnancy on twin- and triplet-born lamb heat production at 24 to 36 hours of age and their performance from birth until lactation day 94 (L94). Twin- ($n = 40$) and triplet-bearing ($n = 28$) ewes were grazed on a 6 cm sward height from day 70 of pregnancy (P70) until L94. From P100, half of the ewes from each litter size were offered 400 g ewe^{-1} day^{-1} of concentrate sheep pellets. Ewe live weight and body condition were recorded on P50, 100, 130, 135 and 140. Ewe blood samples were also collected on P130, 135 and 140, and ewe herbage intake was estimated from P133 to 136 using the n-alkane method. Lamb measurements included live weight and body size at birth, production of heat using indirect open-circuit calorimetry at 24 to 36 hours of age and live weight at L94. Blood samples were also collected from lambs at 24 to 36 hours of age and directly before and after calorimetry measurements. While estimates of ewe herbage intake suggested that substitution of herbage for concentrate did not occur, offering concentrate supplement failed to improve ewe liveweight gain, or birth weight of lambs ($P > 0.1$). Offering concentrate supplement however, did have a positive effect ($P < 0.05$) on the maximum amount of heat a triplet-born lamb can produce on a per kg of birth weight basis (Concentrate 20.8 ± 1.31 W kg^{-1}, Non-concentrate 16.7 ± 0.62 W kg^{-1}). It also had a positive effect ($P < 0.05$) on lamb square-root-transformed plasma gamma-glutamyl-transferase concentrations, an indicator of colostrum uptake (Concentrate 46.2 ± 3.11 U l^{-1}, Non concentrate 38.1 ± 2.93 U l^{-1}). Irrespective of lamb birth rank, offering concentrate supplement had a positive effect ($P < 0.01$) on liveweight gain per day from birth until L94 (Concentrate 261 ± 5.7 g day^{-1}, Non concentrate 239 ± 5.8 g day^{-1}), although there was no effect on the total live weight of lamb reared per ewe ($P > 0.1$). Supplementation with concentrate resulted in triplet-born lambs that produced more heat which may have positive effects on the ability of the newborn lamb to deal with cold stress and potentially its survival.

Introduction

Triplet-born lambs are lighter at birth (Gootwine 2005; Gardner *et al* 2007; Gootwine *et al* 2007) and have lower rectal temperatures (Dwyer and Morgan 2006; Stafford *et al* 2007) than single- or twin-born lambs. In addition, lighter lambs have a greater surface-area-to-birth-weight ratio (Alexander 1962c) and less body energy reserves (Alexander

1978) than heavier lambs. Thus, in a cold environment, lighter lambs lose more heat to the environment and have less energy reserves to produce heat to maintain rectal body temperature after birth. A light birth weight and poor thermoregulation may therefore be important factors influencing the lower survival rates of triplet-born lambs (Thomson *et al* 2004; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008).

The herbage intake of twin- and triplet-bearing ewes is unrestricted when grazing ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* sward heights that are 4 cm or greater (Morris and Kenyon 2004). Triplet-bearing ewes however, still mobilize body reserves and lose body condition when grazing these pasture heights (Morris and Kenyon 2004), suggesting that pasture-only diets may be unable to meet the nutritional requirements of a triplet-bearing ewe in late pregnancy. Insufficient ewe nutrition during mid- to late-pregnancy can result in lighter lamb birth weights (Robinson and McDonald 1989), utilization of fetal endogenous energy reserves (Dalinghaus *et al* 1991; Milley 1993), which are used for post-natal heat production (Mellor and Cockburn 1986), and poor colostrum production after birth (Mellor and Murray 1985a). In contrast, improved ewe nutrition or the infusion of glucose during late pregnancy has been shown to increase lamb birth weight (Stevens *et al* 1990; Budge *et al* 2000), increase the amount or thermogenic capacity of endogenous energy reserves used for post-natal heat production (Stott and Slee 1987; Stevens *et al* 1990; Budge *et al* 2000) and increase colostrum production in ewes (Murphy *et al* 1996; Banchero *et al* 2004a). In this thesis, chapter three showed that offering concentrate supplement to twin- and triplet-bearing ewes grazing *ad-libitum* pasture moderately improved ewe and lamb performance by increasing lamb birth weight, potentially increasing colostrum uptake of the lamb, and total live weight of lamb at lactation day 52. Hinch *et al* (1996) has also shown that offering concentrate on green pasture excess of 3000 kg DM ha^{-1} has positive effects on lamb birth weight and lamb survival to weaning. Because improvements in lamb birth weight and colostrum uptake are positively associated with heat production (Alexander 1962c; Eales and Small 1980a; Eales and Small 1981), offering concentrate supplement may have a positive effect on lamb thermoregulation and therefore twin- and triplet-born lamb survival.

The aim of this experiment was to investigate the effect of offering concentrate supplement to twin- and triplet-bearing ewes grazing 6 cm sward height from pregnancy day 100 until parturition on lamb birth weight, heat production at 24 to 36 hours of age and post-natal growth.

Materials and Methods

The experiment was carried out at Keebles Farm, Massey University, Palmerston North, New Zealand (latitude: 40°S, longitude: 175°E, 44 m asl). The experimental period was from 24 January–10 September 2006. The experimental design was a 2 × 2 factorial design which included two litter sizes (Twin- and Triplet-bearing ewes) and two nutritional treatments (6 cm sward height (Non-concentrate), and 6 cm high sward with concentrate supplement (Concentrate)). A 6 cm sward height was chosen as previous research has shown that twin- and triplet-bearing ewe intake is unrestricted when grazing a sward height greater than 4 cm (Morris and Kenyon 2004).

Six hundred and seventy six ewes were bred with five Romney rams, fitted with crayon mating harnesses, for a breeding period of three oestrous cycles. At 54 days (P54) after the mid-point of the first oestrous cycle, crayon marked ewes from the first oestrous cycle were weighed, body condition scored (scale 1–5, where, one describes a ewe that is extremely emaciated and five describes a ewe that is very fat (Jefferies 1961)) and pregnancy diagnosed using ultrasound technology. All ewes were grazed as one group under commercial grazing conditions from the commencement of the breeding period until P70. On P70, 40 twin-bearing and 28 triplet-bearing ewes were balanced for live weight and body condition score and were allocated to either a 6 cm pasture sward height (Non-concentrate) or 6 cm pasture sward height with concentrate supplement (Concentrate). From P102 until parturition, concentrate supplemented ewes were offered 400g day^{-1} ewe^{-1} of sheep pellets (903 g kg^{-1} dry matter (DM), 141 g kg^{-1} DM crude protein (CP), 126 g kg^{-1} DM of lipid, calculated metabolisable energy content (ME) of 12.3 MJ / kg DM; Universal stock feed, Harvey Farms Ltd, Wanganui, New Zealand) in two 2.5 × 0.4 m troughs. An amount of 400 g day^{-1} was chosen because previous research has shown that offering this amount to twin-bearing ewes grazing a restricted herbage sward height increases lamb birth weight (Kerslake *et al* 2008), whereas offering 200 g day^{-1} did not (Kenyon *et al* 2005a). Throughout pregnancy, ewes were set stocked in two 2.0 ha paddocks at a rate of 17 ewes ha^{-1}. Ewes were rotated weekly to limit potential paddock effects. During the concentrate feeding period, troughs were checked daily for residuals; however, there were none during this period.

Twenty-four hours after parturition, ewes and lambs were moved from their paddock to an indoor pen. Lambs were removed from their dams for heat production measurements and were returned within 3 hours. Ewes and lambs remained in the indoor pen for 24 to 36 hours to ensure the mother–young bond was re-established and then were returned to the paddock. Between P100 and 140, two triplet-bearing ewes aborted and were removed from the study.

The lambing occurred from 28 August–10 September. During the lambing period the average wind speed was 3.0 m s^{-1}, total rainfall 0.7 mm, average temperature of 11.8°C (min=8.0°C and max=15.5°C) and the average cold stress index was 994.5 kJ m^{2-1} h^{-1}.

Herbage management

Throughout the study ewes and lambs were grazed on a ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* sward that was sown the previous year. Herbage sward heights were measured at weekly intervals (Sward stick, Jenquip, New Zealand, 100 readings per paddock) to ensure that ewes were grazed within a controlled range of sward heights. If the sward height was below 5 cm, ewes were placed in an alternative paddock which had been prepared at the appropriate sward height. If the sward height was above 7 cm, additional ewes were introduced to control sward height.

Herbage mass was measured at two weekly intervals using a rising plate meter (Ashgrove Pastoral Products, New Zealand, 50 readings per paddock) and was calculated using the following formula:

$$Herbage\ mass\left(kg\ DM\ /\ ha\right) = (158 \times MR) + 200$$

where MR is the meter reading (Hodgson *et al* 1999).

To estimate the amount of neutral detergent fibre (NDF) and crude protein (CP) in the sward, sward samples were plucked on P116, freeze died, ground to pass a 1 mm sieve, and analysed using near-infra-red-reflectance spectrometry (FeedTech, AgResearch, Grasslands, Palmerston North, New Zealand). The sward had 400 g kg^{-1} DM of NDF, 249 g kg^{-1} DM of CP, and a calculated metabolizable energy content of 12.8 MJ kg^{-1} DM.

Animal measurements

Unfasted live weight and body condition scores of ewes were measured on P52, 70, 100, 130, 135 and 140. A 10 ml blood sample was collected by jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA) on P100, 130, 135 and 140.

From P125 until P136, triplet-bearing ewe herbage intake was estimated using the n-alkane method (Dove and Mayes 2005). On P125, triplet-bearing ewes were dosed with an intra-rumenal alkane controlled release faecal marker capsule (Alkane CRC, Captec NZ Ltd ®, Auckland, New Zealand), which released the alkanes, dotriacontane (C_{32}) and hexatriacontane (C_{36}), at a rate of 50 mg day^{-1}. From P133 until P136, between the period 08.00 and 10.00 hours, a faecal sample was collected from all triplet-bearing ewes. These samples were then dried in an oven at 65°C for 48 h. Dried faecal samples were ground, and 0.5 g sub-samples from each sample taken over the 4-day period were bulked for each ewe. Herbage samples were plucked on P116 to a similar height grazed by the sheep. Samples were washed, oven dried at 65°C for 48 hours and ground. Faecal and herbage alkane concentrations were measured using gas chromatography (Dexcel, Hamilton, New Zealand) and intakes were calculated using procedures of Dove & Mayes (1996).

Within 12 hours of birth, all lambs were identified to their dam, tagged and their sex and birth rank determined. In addition, lamb birth weight, crown-rump length and thoracic-girth circumference was recorded. Surface area (SA) of the lamb was calculated using the mathematical formula for the total SA of a cylinder,

$$SA\left(cm^2\right) = 2\pi r h + 2\pi r^2$$

where r is the thoracic-girth circumference of the lamb divided by 2π , h is the crown-rump length of the lamb.

Between 24 and 36 hours of age a 5 ml blood sample was collected by jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA). At this time, 35 twin- and 11 triplet-born lamb sets were chosen randomly to determine maximum heat production (summit metabolism) for each lamb. An average cold stress index (CSI) within the first 24–36 hour of life was also calculated using the following equation (Donnelly 1984):

$$CSI(kJ/m^2/h) = (11.7 + 3.1W^{0.5})x(40 - T) + 481 + 418(1 - e^{-0.04R})$$

where W is the mean windspeed (m s^{-1}), T is the mean temperature (°C), R is the mean rainfall (mm).

A 10 ml blood sample was taken from the lamb via jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA) immediately prior to the lamb being placed in a crate within an indirect open circuit calorimeter (McCutcheon *et al* 1983a). The lamb's head was placed in a plastic hood, which was sealed around the neck with a collar. Air was drawn into and from the hood for measurement of oxygen consumption. A temperature probe was inserted in the rectum of the lamb for measurement of body temperature. The lamb was exposed to an environmental temperature that ranged from 6–8°C, and was allowed to acclimatize to these conditions for a total of 12 min. This period of time is referred to as the adjustment period. During this time rectal temperature and oxygen consumption measurements were taken over three successive 4 min periods to obtain a stabilized metabolic rate so a base heat production could be calculated. At the end of 12 min, lambs were exposed to wet and windy conditions to induce maximum heat production (summit metabolism). This period of time is referred to as the onset of severe cold stress. Artificial chilled rain (1°C) was applied through sprinklers at a standardized rate of (1.08 l min^{-1}) and cold air was passed over the lamb by a fan positioned behind the animal at a rate of 1.0 m s^{-1}. After 20 min cold air was passed over the lamb at a rate of 1.5 m s^{-1}, and after another 20 min at a rate of 2.0 m s^{-1}. The rate of cold air then stayed constant for the remaining period of time. Rectal temperature and oxygen consumption measurements were taken at 4 min intervals for 88 min or until the lamb reached maximum heat production, whichever occurred first. Maximum heat production was assumed to have been met when the rectal temperature of lamb declined at the rate of 1°C / 20 min and there was no further increase in the consumption rate of oxygen (Alexander 1962c). To facilitate heat loss and to encourage heat production to reach a maximum, all lambs with a birth weight above 4 kg had the wool removed from their back and sides leaving a wool depth of 3 mm. Maximum heat production in Watts (W) was calculated from oxygen consumption using the following formula (Revell *et al* 2002):

$$Maximal\ heat\ production\,(W) = oxygen\ consumption\,(l/h) \times 20.46/3.6$$

where it was assumed that 20.46 kJ of heat is produced per litre of oxygen consumed (McLean 1972). Division of oxygen consumption by 3.6 converts from kJ h^{-1} to W.

After the lamb reached maximum heat production or after 88 min in the calorimeter, the lamb was removed, dried and a 5 ml blood sample collected via jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA). Lambs were then placed in a heated room for 1 h before being returned to their dams. Ewes and lambs were weighed 94 days after the midpoint of lambing (lactation day 94 (L94)).

Laboratory analysis

All blood samples were placed on ice until they were centrifuged at 1000 *g* for 15 min. The plasma was removed and frozen at –20ºC. Ewe plasma samples were analysed using diagnostic kits for glucose (Hexokinase method, Roche Diagnostics Ltd, Switzerland), beta-hydroxybutyrate (BOH) and non esterified fatty acid (NEFA) (Sigma, Illinois, USA) concentrations. Lamb plasma samples were analysed for gamma-glutamyl-transferase (GGT) (Roche, diagnostics Ltd, Mannheim, Germany), glucose (hexokinase method, Roche Diagnostics Ltd, Switzerland) and NEFA (Sigma, Illinois, USA).

Statistical analysis

Pasture sward height and herbage mass, and triplet-bearing ewe herbage intake, were analysed using a general linear model (PROC GLM, SAS, 2003) which contained the fixed effect of nutritional treatment (Non-concentrate, Concentrate).

Ewe live weight (mating, P52, 70, 100 and 140, and L94) and liveweight gain (P100-140), body condition score (P52, 70, 100 and 140) and change in body condition score (P100-P140), BOH, NEFA and glucose concentrations at P135 and P140 and total weight of lamb produced at L94 were analysed using a general linear model (PROC GLM, SAS, 2003). Fixed effects included nutritional treatment (Non-concentrate, Concentrate), litter size (Twin, Triplet) and the interactions between the main effects. Interactions were retained in the model if significant ($P<0.05$). If not significant they were removed and the model re-fitted.

Lamb birth weight, crown–rump length, thoracic-girth circumference and lamb plasma metabolites (glucose, NEFA and GGT) were analysed using a general linear model (PROC GLM). Fixed effects included nutritional treatment (Non-concentrate, Concentrate), birth rank (Twin, Triplet), lamb sex (Ewe, Ram) and interactions between fixed effects. Live weight at L94 and liveweight gain from birth until L94 were analysed using the same general linear model (PROC GLM, SAS, 2003). The fixed effect of birth rank (Twin, Triplet) however, was removed and replaced with rearing rank (Twin reared as a single or twin, Triplet reared as a single, twin or triplet). Interactions were retained in the model if significant at $P<0.05$, If not significant they were removed and the model re-fitted

The base heat production and maximum heat production (W lamb^{-1}), the base heat production and maximum heat production on a per kg of birth weight basis (W kg^{-1}), rate to reach maximum heat production from the base heat production (W kg^{-1} min^{-1}), rectal temperature before the onset of severe cold stress, plasma glucose and NEFA concentrations immediately before or after calorimetry and the change from before to after calorimetry were analysed using a general linear model (PROC GLM, SAS, 2003). Fixed effects included nutritional treatment (Non-concentrate, Concentrate), birth rank (Twin, Triplet), lamb sex (Ewe, Ram) and the interactions between the main effects. Birth weight, plasma GGT concentration, average cold stress index within the first 24 h of life, and rectal temperature before the onset of severe cold stress were all fitted as covariates. Interactions and covariates were retained in the model if significant at $P < 0.05$. If not significant they were removed and the model re-fitted.

The plasma GGT concentrations were not normally distributed. To achieve a normal distribution, plasma GGT concentrations were subjected to square root transformation before being analysed.

The proportion of lambs reaching summit metabolism and surviving from birth to weaning were analysed as a binomial trait using the SAS procedure for categorical modelling (PROC GENMOD, SAS, 2003). Data were analysed as logit transformed values and presented in results as back-transformed percentages. Fixed effects included nutritional treatment (Non-concentrate, Concentrate), birth rank (Twin, Triplet), lamb sex (Ewe, Ram) and the interactions between the main effects.

Results

Herbage measurements

Pasture measurements from P70 until parturition for both ewe nutritional treatments did not differ (Sward height, Non-concentrate 7.6 ± 0.56 cm and Concentrate 6.3 ± 0.56 cm; Herbage mass, Non-concentrate 1207 ± 74.5 kg DM ha^{-1}, Concentrate 1007 ± 74.5 kg DM ha^{-1}; $P > 0.1$).

Ewe live weight, body condition and estimated herbage intake

Live weight at mating, and live weight and body condition score at P52 and P70 did not differ between ewe nutritional treatments and litter size groups ($P > 0.1$; pooled means of ewe live weight at mating and ewe live weight and body condition score at P52 and P70 were 59.2 kg, 62.4 kg and 3.10, and 64.3 kg and 3.21 respectively). Offering concentrate supplement and litter size had no effect ($P > 0.1$) on ewe live weight at P100 and P140 (Table 15). Twin-bearing ewes had a greater ($P < 0.05$) body condition score than triplet-bearing ewes at P100. This effect was not observed at P140 ($P > 0.1$). Offering concentrate supplement and litter size also had no effect on ewe liveweight gain and change in body condition score from P100 until P140 ($P > 0.1$; data not shown). Offering concentrate supplement had no effect on triplet-bearing ewe herbage intake during late pregnancy ($P > 0.1$; Non-concentrate 2.5 ± 0.27 kg DM ewe^{-1} day^{-1}, Concentrate 2.4 ± 0.23 kg DM ewe^{-1} day^{-1}).

At P135, offering concentrate supplement had no effect on ewe plasma BOH, NEFA and glucose concentrations ($P > 0.1$). Triplet-bearing ewes had greater ($P < 0.05$) plasma BOH concentrations than twin-bearing ewes at P135 (Triplet, 0.6 ± 0.03 mmol^{-1}; Twin, 0.5 ± 0.02 mmol l^{-1}) but had the same plasma NEFA and glucose concentrations as twin-bearing ewes (pooled means of plasma NEFA and glucose concentrations at P135 were 0.4 and 3.6 mmol l^{-1} respectively). At P140, offering concentrate supplement and litter size had no effect on plasma BOH, NEFA and glucose concentrations (pooled means of plasma BOH, NEFA and glucose concentrations at P140 were 0.5, 0.5 and 3.8 mmol l^{-1} respectively).

***Table 15** The effect of offering concentrate supplement (Non-concentrate, Concentrate) and litter size (Twin, Triplet) on ewe live weight (kg) and body condition score (1-5 scale) at pregnancy day 100(P100) and P140*

	Live weight				Body condition Score	
	n	P100	n	P140	P100	P140
Nutritional treatment						
Non-concentrate	34	73.5 ± 0.98	32	89.8 ± 1.25	3.0 ± 0.08	3.1 ± 0.06
Concentrate	34	72.1 ± 0.98	34	90.8 ± 1.26	3.1 ± 0.09	3.1 ± 0.06
P Value		ns		ns	ns	ns
Litter size						
Twin	40	72.7 ± 0.89	40	90.1 ± 1.13	3.2 ± 0.08	3.1 ± 0.05
Triplet	28	72.9 ± 1.07	26	90.4 ± 1.39	2.9 ± 0.09	3.0 ± 0.07
P Value		ns		ns	0.05	ns

Data were presented as means ± standard errors; ns, non-significant

Lamb size and plasma concentrations of glucose and GGT

Offering concentrate supplement had no effect on lamb birth weight, surface-area-to-birth-weight ratio, crown–rump length or thoracic-girth circumference ($P > 0.1$; Table 16). Triplet-born lambs however, had lighter birth weights, a greater surface-area-to-birth-weight ratio and a shorter crown–rump length and thoracic-girth circumference than twin-born lambs ($P < 0.001$). Birth rank still had a significant ($P < 0.05$) effect on body size measurements after correction for lamb birth weight.

Lambs born to concentrate-fed ewes had greater ($P < 0.001$) plasma GGT concentrations between 24 and 36 hours of age than lambs born to ewes offered pasture only (Table 17). Offering concentrate supplement had no effect on lamb plasma glucose concentration between 24 and 36 hours of age ($P > 0.1$). Triplet-born lambs had lower ($P < 0.001$) plasma glucose concentrations than twin-born lambs.

Table 16 *The effect of offering concentrate supplement (Non-concentrate, Concentrate) and birth rank (Twin, Triplet) on lamb birth weight (kg), surface-area-to-birth-weight ratio (cm^2 kg^{-1}), crown–rump length (cm) and thoracic-girth circumference (cm)*

	n	Birth weight	SA:BWt	Crown–rump length	Thoracic-girth circumference
Nutritional treatment					
Non-concentrate	79	4.6 ± 0.09	161.0 ± 1.72	53.5 ± 0.34	38.2 ± 0.30
Concentrate	75	4.7 ± 0.10	160.4 ± 1.77	53.9 ± 0.35	38.4 ± 0.31
P value		ns	ns	ns	ns
Birth Rank					
Twin	76	5.2 ± 0.09	155.0 ± 1.75	55.7 ± 0.35	40.4 ± 0.31
Triplet	78	4.1 ± 0.09	166.4 ± 1.73	51.6 ± 0.35	36.2 ± 0.31
P value		< 0.001	< 0.001	< 0.001	< 0.001

Data is presented as means ±.standard errors; ns, non significant

Table 17 *The effect of offering concentrate supplement (Non-concentrate, Concentrate) and birth rank (Twin, Triplet) on lamb plasma gamma-glutamyl-transferase (GGT; IU l^{-1}) and glucose (mmol l^{-1}) concentration at 24 to 36 hours of age*

		GGT*	Glucose
Nutritional treatment	n		
Non-concentrate	60	38.1 ± 2.93 (1451)	5.8 ± 0.19
Concentrate	52	46.2 ± 3.11 (2134)	6.2 ± 0.21
P Value		0.001	ns
Birth Rank			
Twin	58	41.2 ± 2.88 (1697)	6.5 ± 0.19
Triplet	54	43.1 ± 3.16 (1858)	5.4 ± 0.20
P Value		ns	0.001

Data were presented as means ± standard errors; ns, non-significant

*Transformed data with standard errors are presented with back-transformed least square means in parenthesis.

Rectal temperature before onset of severe cold stress

Offering concentrate supplement and birth rank had no effect on lamb rectal temperature before the onset of severe cold stress ($P > 0.1$). The average cold stress index in the first 24 hours of life however, had a negative effect ($P < 0.01$) on the rectal temperature of the lamb, with a 0.008 KJ m^{2-1} h^{-1} increase in the cold stress index being associated with a 1°C decrease in rectal temperature. Plasma glucose concentrations immediately before calorimetry measurements also had a positive effect ($P < 0.001$) on lamb rectal temperature before the onset of severe cold stress, with a 1 mmol l^{-1} increase in plasma glucose associated with a 0.1°C increase in lamb rectal temperature.

Calculated heat production during calorimetry

Offering concentrate supplement had no effect on the base heat production of the twin-born lamb ($P > 0.1$; Table 18). Triplet-lambs born to concentrate-fed ewes, however, had a greater ($P < 0.05$) base heat production than triplet-lambs born to ewes fed pasture only. Twin and triplet-lambs born to pasture-only-fed ewes had the same base heat production ($P > 0.1$). Within the concentrate treatment group, however, triplet-born lambs had a greater ($P < 0.05$) base heat production than twin-born lambs. There was a positive relationship between the base heat production and lamb body weight, with a one kg increase in birth weight being associated with an 8.0 W increase in the base heat production ($P < 0.05$).

Offering concentrate supplement had a negative effect ($P < 0.05$) on the base heat production on per kg of birth weight basis of a twin-born lamb but had a positive effect ($P < 0.01$) on the base heat production on a per kg of birth weight basis of a triplet-born lamb (Table 18). Twin-lambs born to concentrate-fed ewes had greater ($P < 0.05$) base heat production on a per kg of birth weight basis than triplet-lambs born to ewes fed pasture only, and triplet-lambs born to concentrate fed ewes had greater ($P < 0.05$) base heat production on a per kg of birth weight basis than twin-lambs born to ewes fed pasture only.

Offering concentrate supplement and birth rank had no effect on maximum heat production ($P > 0.1$; Table 18). A one kg increase in twin- or triplet-born lamb birth weight was associated ($P < 0.001$) with an increase in maximum heat production of 13.3 and 25.5 W respectively (Figure 4). In addition, a one degree increase ($P < 0.001$) in the rectal temperature of the lamb before the onset of severe cold stress was associated with an 11.2 W increase in maximum heat production. Triplet-lambs born to concentrate fed ewes had a greater ($P < 0.01$) maximum heat production on a per kg of birth weight basis than triplet-lambs born to pasture only fed ewes and twin-lambs born to ewes from both nutritional treatment groups (Table 18). A 1 kg increase in triplet-born lamb birth weight was associated with a 4.5 W increase in maximum heat production on a per kg of birth weight basis. Twin-born lamb birth weight had no effect on maximum heat production on a per kg of birth weight basis ($P > 0.1$; Figure 5). In addition, a 1°C increase in lamb rectal temperature before the onset of severe cold stress was associated with a 2.0 W kg^{-1} increase in maximum heat production.

Offering concentrate supplement and birth rank had no effect on the lambs' rate to reach maximum heat production on a W min^{-1} kg^{-1} of birth weight basis from the base heat production during the adjustment period. The rectal temperature of the lamb before the onset of severe cold exposure had a positive effect ($P < 0.01$) on the rate to reach maximum heat production on a watt per min per kg of birth weight basis, with a 1°C increase in rectal temperature being associated with a 0.01 W kg^{-1} min^{-1} increase in the rate to reach maximum heat production.

Offering concentrate supplement had no effect on the percentage of lambs reaching maximum heat production during severe cold stress (Non-concentrate, 67.9%, Concentrate, 65.2%). A greater ($P < 0.05$) proportion of twin-born lambs reached maximum heat production when compared to triplet-born lambs (Twin-born, 76.8%, Triplet-born, 54.6%).

Table 18 *The effect of offering concentrate supplement (Non-concentrate, Concentrate) and birth rank (Twin, Triplet) on the base heat production (W and W kg^{-1}), maximum heat production (W and W kg^{-1}) and the rate to reach maximum heat production (W kg^{-1}min^{-1})*

		Base heat production W	Base heat production W kg^{-1}		Maximum heat production W	Maximum heat production W kg^{-1}	Rate to reach maximum heat production
Nutritional treatment							
Non-concentrate	52	48.3 ± 1.82	10.0 ± 0.38	37	86.2 ± 2.67	17.2 ± 0.56	0.24 ± 0.020
Concentrate	50	52.9 ± 1.81	11.0 ± 0.38	34	89.8 ± 3.16	19.0 ± 0.73	0.25 ± 0.020
P value		ns	ns		ns	< 0.05	ns
Birth Rank							
Twin	69	49.7 ± 1.54	10.2 ± 0.31	53	85.4 ± 2.04	17.5 ± 0.40	0.24 ± 0.015
Triplet	33	51.5 ± 2.24	10.8 ± 0.44	18	90.6 ± 3.85	18.7 ± 0.78	0.25 ± 0.026
P value		ns	ns		ns	ns	ns
Interactions[1]							
Twin							
Non-concentrate	36	51.4 ± 2.08^{ab}	10.8 ± 0.42^{b}	29	-	17.7 ± 0.53^{a}	-
Concentrate	33	48.1 ± 2.26^{a}	9.6 ± 0.45^{a}	24	-	17.3 ± 0.98^{a}	-
Triplet							
Non concentrate	16	45.3 ± 2.99^{a}	9.2 ± 0.63^{a}	8	-	16.7 ± 0.62^{a}	-
Concentrate	17	57.7 ± 3.22^{b}	12.3 ± 0.63^{c}	10	-	20.8 ± 1.31^{b}	-
P value		< 0.05	< 0.05		-	< 0.05	-

Data is presented as means ±.standard errors; ns, non significant

[1] Means ± standard errors within interaction with differing superscripts are significantly ($P < 0.05$) different

Figure 4 *The association between twin- and triplet- lamb birth weight (kg) and maximum heat production (MHP; W) after adjustment for ewe nutritional treatment (Non-concentrate, Concentrate) and initial rectal temperature (°C) before the onset of severe cold stress.*

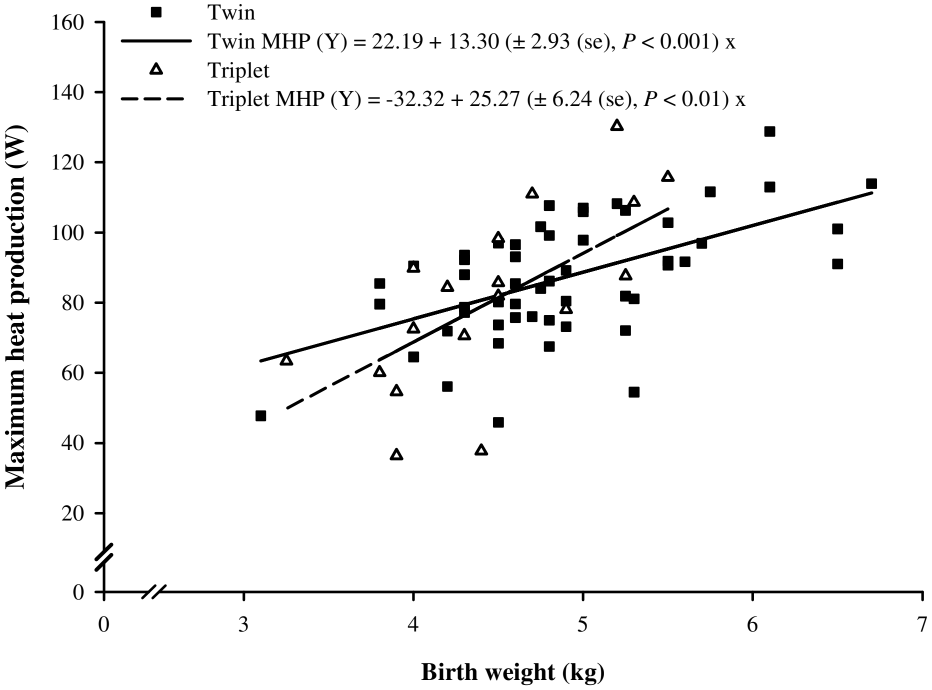
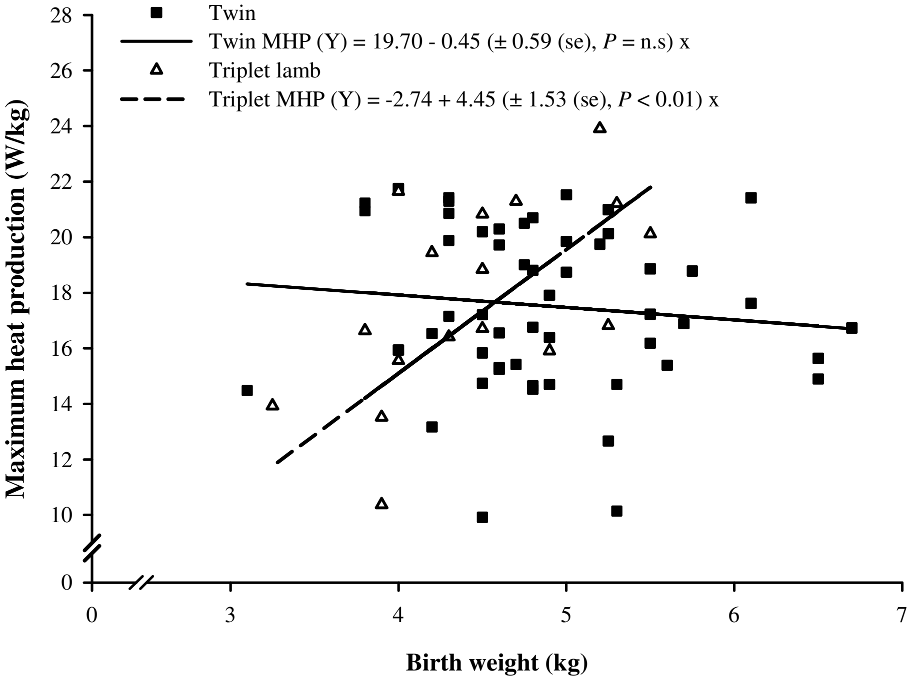


Figure 5 *The association between twin- and triplet- lamb birth weight (kg) and maximum heat production (MHP) on a per kg of birth weight basis (W kg $^{-1}$) after adjustment for ewe nutritional treatment (Non-Concentrate, Concentrate) and initial rectal temperature before the onset of severe cold stress (°C).*



ns = not significant.

Lamb plasma concentrations of glucose and NEFA before and after calorimetry

Of the lambs that reached maximum heat production, offering concentrate supplement had no effect on lamb plasma glucose concentrations immediately before or directly after the calorimetry procedure or on the change of plasma glucose concentrations from immediately before until after calorimetry procedure ($P > 0.1$; Table 19). Birth rank also had no effect on lamb plasma glucose concentrations immediately before calorimetry ($P > 0.1$). Twin-born lambs, however, had greater ($P < 0.05$) plasma glucose concentrations than triplet-born lambs immediately after calorimetry and a greater ($P < 0.05$) change in glucose concentration from immediately before calorimetry until directly after calorimetry. Of the lambs that reached maximum heat production, offering concentrate supplement and birth rank had no effect on lamb plasma NEFA concentrations immediately before or directly after calorimetry, or the change of plasma NEFA concentrations from immediately before until directly after calorimetry ($P > 0.1$; pooled means were 0.7, 1.0 and 0.3 mmol l^{-1} respectively).

Table 19 *The effect of offering concentrate supplement (Non concentrate, Concentrate) and birth rank (Twin, Triplet) on lamb plasma glucose concentrations immediately before calorimetry (mmol* l^{-1}*), immediately after calorimetry (mmol* l^{-1}*) and on the change in plasma glucose concentrations from before until after calorimetry (Δ before-after; mmol* l^{-1}*))*

		Lamb plasma glucose concentrations		
	n	Before calorimetry	After calorimetry	Δ before-after
Nutritional treatment				
Non-concentrate	52	6.1 ± 0.35	9.3 ± 0.46	3.3 ± 0.35
Concentrate	50	6.9 ± 0.34	9.9 ± 0.47	2.9 ± 0.31
P value		ns	ns	ns
Birth Rank				
Twin	69	6.6 ± 0.28	10.5 ± 0.35	3.8 ± 0.25
Triplet	33	6.3 ± 0.43	8.7 ± 0.61	2.4 ± 0.40
P value		ns	0.05	0.05

Data were presented as least square means ± standard error; ns, non-significant

Lamb growth and survival

Live weight gain per day from birth until L94 was greater ($P < 0.01$) for lambs born to ewes fed concentrate than lambs born to ewes fed pasture only (Concentrate 261 ± 5.7 g day^{-1}, Non-concentrate 239 ± 5.8 g day^{-1}). Triplet-born lambs reared as a twin (224 ± 9.1 g day^{-1}) or triplet (219 ± 7.6 g day^{-1}) gained less ($P < 0.05$) live weight per day than twin-born lambs (Twin-born lamb reared as single 260 ± 13.2 g day^{-1}, Twin-born lamb reared as twin 250 ± 4.8 g day^{-1}) and triplet-born lambs reared as a single (297 ± 13.3 g day^{-1}). Triplet-born lambs reared as a single gained more ($P < 0.05$) live weight per day than twin-born lambs and triplet-born lambs reared as a twin- or triplet-born lamb.

Offering concentrate supplement had no effect on lamb survival to L94 ($P > 0.1$; data not shown). Triplet-born lambs had a lower ($P < 0.05$) survival rate than twin-born lambs (Triplet-born 73.1 %, Twin-born 93.7 %). Offering concentrate supplement and litter size had no effect on total live weight of lamb reared/ewe from birth until L94 ($P > 0.1$; pooled mean was 50.9 kg).

Discussion

The aim of this experiment was to investigate the effect of offering concentrate supplement to twin- and triplet-bearing ewes grazing *ad-libitum* herbage from pregnancy day 100 until parturition on twin- and triplet-born lamb birth weight, heat production at 24–36 hours of age and post-natal growth.

Although individual concentrate intake was not measured, herbage intake measurements of triplet-bearing ewes indicated that substitution of herbage for concentrate supplement did not occur. While this may suggest that total dry matter intake was greater in ewes offered concentrate supplement, offering concentrate supplement had no effect on ewe liveweight gain or body condition during pregnancy or the birth weight of the offspring when compared to ewes offered a pasture only diet. This is inconsistent with chapter 3 which showed that offering concentrate supplement to twin- and triplet-bearing ewes grazing a 6 cm sward improved ewe liveweight gain by 60 g day^{-1} and twin- and triplet-born lamb birth weight by 300 g. While the reasons for the inconsistencies across these two studies are unclear, in the current study ewes were heavier throughout pregnancy and were grazed on 1-year-old pasture rather than 4–6-year-old pasture.

An improvement in lamb thermoregulation can occur through either a reduction in heat loss or an increase in heat production. While heat loss was not measured in the present experiment, the lack of difference in birth weight and surface-area-to-birth-weight ratio between lambs born to either concentrate- or non-concentrate-fed ewes suggests that lambs from both nutritional treatments would lose a similar amount of heat during cold stress. Compared to twin-born lambs, the lighter birth weights and greater surface-area-to-birth-weight ratio of triplet-born lambs would suggest that triplet-born lambs would lose more heat to the environment than twin-born lambs (Alexander 1962c; Mount and Stephens 1970). Within litter size, offering concentrate supplement had a positive effect on the triplet-born lambs' maximum heat production on a W kg^{-1} of birth weight basis. The same effect was not seen in twin-born lambs, which may suggest that endothermic mechanisms of the triplet-born lamb are more sensitive to ewe nutrition during pregnancy. Interestingly, the amount of prolactin and prolactin receptors, which play a role in thermogenesis (Stephenson *et al* 2001), have been shown to be influenced by fetal number and maternal nutrition (Mostyn *et al* 2004). An increase in fetal number, independent of maternal nutrition, has been shown to be positively associated with an increase in the amount of prolactin receptors in fetal brown adipose tissue (Budge *et al* 2003). In addition, plasma prolactin concentrations of the fetus have been shown to be positively influenced by an increase in nutrient supply (Harding and Johnston 1995). While offering concentrate may have had a positive effect on the thermogenic efficiency of the triplet-born lamb, the thermogenic efficiency of brown adipose tissue was not measured in the present experiment. It is also of interest to note that while triplet-lambs born to concentrate-fed ewes had a greater ability to produce a maximum amount of heat on a per kg of birth weight basis, their base heat production on a per kg of birth weight basis, which was required to maintain homoeothermic status at a constant environmental temperature, was elevated compared to lambs from all other treatments. This may indicate that the endothermic mechanisms of triplet-lambs born to concentrate fed ewes are not as energy efficient. Because triplet-born lambs have the potential to lose a greater amount of heat to the environment, and are likely to have less energy reserves than single- and twin-born lambs, the efficient use of energy reserves will be of particular importance to sustain high rates of heat production during cold stress. While offering concentrate may improve the maximum heat production of the triplet-born lambs, further research into the ability of the lamb to sustain a homoeothermic state during severe cold stress would be beneficial.

Both twin- and triplet-born lamb birth weights were positively associated with maximum heat production. This is in agreement with Alexander (1962c), who also showed that maximum heat production on a per kg of birth weight basis for single-, twin-, and triplet-born lambs at 24 to 36 hours old were independent of lamb birth weight. This is somewhat inconsistent with the current results, which showed that maximum heat production on a per kg of birth weight basis was positively associated with an increase in triplet-born lamb birth weight, but was constant over a range of body weights for twin-born lambs. This suggests that increasing the birth weight of triplet-born lambs should increase their ability to cope with a cold stress event.

The rectal temperature of the lamb during the adjustment period was also positively associated with maximum heat production on a per kg of birth weight basis. Furthermore, this rectal temperature was positively related to plasma glucose concentration prior to calorimeter procedure and negatively related to the exposure of colder outdoor conditions. These results indicate that ensuring lambs are adequately fed, to maintain plasma glucose concentrations, and keeping lambs sheltered from cold conditions would have a positive effect on the lambs' ability to maintain body temperature during adverse environmental conditions.

It is well known that an inadequate intake of colostrum will limit the ability of the lamb to maintain high rates of heat production during periods of cold stress (Eales and Small 1980a; Eales and Small 1981; Eales *et al* 1982; Mellor and Cockburn 1986). In the current experiment, and others (Stafford *et al* 2001; Kenyon *et al* 2005b), plasma GGT concentrations, which are positively correlated with plasma IgG concentrations (Tessman *et al* 1997; Maden *et al* 2003; Britti *et al* 2005), were used as an indicator of colostrum uptake. The plasma GGT concentrations of lambs may therefore indicate that offering concentrate to twin- and triplet-bearing ewes has a positive effect on the colostrum intake of the lamb. Caution should be taken here however, as passive transfer of IgG is not only influenced by colostrum intake, but also by the amount of IgG in the milk, and the efficiency of colostral absorption (Boland *et al* 2004; Boland *et al* 2005). While colostrum intake has been shown to increase summit metabolism of the lamb by 17–20 % (Eales and Small 1981), associations between plasma GGT concentrations and summit metabolism were not significant in this current experiment.

Offering concentrate supplement had a positive effect on twin- and triplet-born lamb growth rate from birth until L94. A positive relationship between ewe nutrition and milk production (Treacher 1970), and between milk production and lamb liveweight gain (Morgan *et al* 2007), have previously been reported. It may therefore be possible that ewes offered concentrate had greater milk production to improve lamb growth rates. To clarify this association however, further research on the effects of offering concentrate supplement to twin- and triplet-bearing ewes grazing 6 cm herbage sward height on milk production of the ewe and lamb growth is needed.

Conclusion

Offering concentrate supplement in late pregnancy to ewes grazing *ad libitum* pasture had a positive effect on triplet-born lamb heat production on a per kg of birth weight basis and twin- and triplet-born growth rate to weaning. Concentrate supplement may therefore be a way of increasing the capabilities of the triplet-born lambs to deal with cold stress when offered under similar conditions. Triplet-born lamb birth weight also had a positive effect on the maximum amount of heat produced per kg of body weight. These effects were not seen in twin-born lambs. This may indicate that offering concentrate and increasing birth weight is beneficial for triplet-born lamb heat production only.

CHAPTER 5

Can maternal iodine supplementation improve twin- and triplet-born lamb plasma thyroid hormone concentrations and thermoregulation capabilities in the first 24 to 36 hours of life?

Related Publication;

Based on a publication in the Journal of Agricultural Science (Cambridge)

Kerslake JI, Kenyon PR, Morris ST, Stafford KJ, Morel PCH (2010) Can maternal iodine supplementation improve twin- and triplet-born lamb plasma thyroid hormone concentrations and thermoregulation capabilities in the first 24 to 36 hours of life? *Journal of Agricultural Science (Cambridge. In press*

Abstract

This study investigated the effect of maternal plasma iodine concentration on twin- and triplet-born lamb plasma thyroid hormone concentrations, rectal temperature, and maximum heat production. At pregnancy day 68 (P68), 16 twin- and 14 triplet-bearing ewes were randomly chosen from ewes which were injected intramuscularly with 1.5 ml of iodised-peanut oil and ewes which were not. Selected ewes were grazed on *ad-libitum* pasture from P68 until parturition. After parturition lamb blood samples were collected within five minutes of birth, and at three, 12, and 24 to 36 hours post birth. Lamb rectal temperatures were measured within five minutes of birth, and at one, three and 12 hours after birth. Other lamb measurements included lamb birth weight, crown-rump length and thoracic-girth circumference at three hours of age and the production of maximum heat at 24 to 36 hours of age using indirect open-circuit calorimetry. Maternal iodine supplementation successfully increased plasma iodine concentrations of twin- and triplet-bearing ewes throughout pregnancy ($P < 0.05$), but had no effect ($P > 0.05$) on the rectal temperature, thyroid hormone concentration and maximum heat production of twin- or triplet-born lambs. Compared to twin-born lambs, triplet-born lambs had lighter birth weights ($P < 0.001$), and lower rectal temperatures and plasma thyroxine and tri-iodothyronine concentrations within five minutes of birth ($P < 0.05$). Under the conditions of the present study, maternal iodine supplementation offered no benefit in improving lamb heat production at 24 to 36 hours of age.

Introduction

In chapter two of this thesis, triplet-born lambs were found to be lighter at birth, and to have lower thyroid hormone concentrations and lower rectal temperatures within the first 24 hours of life than twin- or single-born lambs. This is in agreement with others (Dwyer and Morgan 2006; Darwish and El-Bahr 2007; Stafford *et al* 2007). Newborn lambs with lighter birth weights or lower rectal temperatures can display reduced vigour (Dwyer 2003; Dwyer and Morgan 2006) and less of a drive to suckle (Alexander and Williams 1966), making them more susceptible to hypothermia (Dalton *et al* 1980). This may offer some explanation as to why triplet-born lambs have a lower survival rate than twin- or single-born lambs (Morris and Kenyon 2004; Thomson *et al* 2004; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008).

Thyroid hormones have been shown to play a critical role in the initiation of independent thermoregulation and maintenance of body temperature after birth (Symonds *et al* 1995). Newborn lambs with greater plasma concentrations of thyroid hormones appear to be better at producing heat (Alexander 1970) and maintaining core body temperature when subjected to cold stress (Caple and Nugent 1983; Polk *et al* 1987). Thyroid hormone production is reliant on the thyroid gland absorbing iodine from the blood (Underwood and Suttle 1999). Iodine is actively transported across the placenta to the developing fetus, where from day 50 of pregnancy the fetus synthesises it's own thyroid hormones from maternal iodine (Potter *et al* 1986). Lambs born from ewes supplemented with iodine have greater thyroxine (T_4) concentrations at birth (Andrewartha *et al* 1980) and at 24 hours of age (Rose *et al* 2007) when compared to lambs born to non-supplemented ewes. They have also been reported as having greater rectal temperatures after birth (Donald *et al* 1994). Increasing maternal plasma iodine concentration during pregnancy could therefore increase the thyroid hormone concentration in the fetus and/or newborn lamb, and thus improve newborn lamb heat production.

This experiment investigates the effect of maternal iodine supplementation on twin- and triplet-born lamb plasma thyroid hormone concentration, rectal temperature and their ability to produce heat within 24 to 36 hours after birth.

Materials and Methods

This experiment was conducted at Massey University Keeble farm, Palmerston North, New Zealand (lat. 40º, long. 175º, elevation: 44 m) from 8 February 2006 until 17 September 2006. The experiment was a 2 x 2 factorial design, which included two litter sizes (Twin- and Triplet) and two maternal iodine supplementation treatments (Supplemented and Non-supplemented). A sheep fertility enhancing vaccine (Androvax ®, AgVax, Upper Hutt, New Zealand) was given to 1150 mixed aged Romney cross ewes 63 and 35 days prior to the start of mating (13 April 2006). Half of the ewes (n=575) were injected intramuscularly in the anterior half of the neck with 1.5 ml ewe^{-1} of iodised peanut oil (Flexidine® (26% w/w of iodine bound to ethyl esters of unsaturated fatty acids in oil), Bomac Laboratories Ltd, Auckland, New Zealand) 35 days prior to the start of mating. Romney rams (n = 10) fitted with crayon mating harnesses joined the ewes for a 10 day breeding period. Ewes with a crayon-marked rump were pregnancy diagnosed using ultrasound scanning at 64 days (P64) after the mid-point of the breeding period (18 April 2006). Non-pregnant, single-, twin- and triplet-bearing ewes were marked for future identification. All of the ewes remained in one mob on *ad-libitum* pasture from the beginning of mating until P68. At P68, 16 twin- and 14 triplet-bearing ewes were randomly selected from each supplemented and non-supplemented treatment group. These ewes were removed from the main flock and were grazed together on *ad-libitum* pasture until parturition.

At P145 twin- and triplet-bearing ewes were moved into a 2.5 ha paddock and supervised continually for the next 10 days. Twenty five twin-bearing ewes (Supplemented, n = 13; and Non-supplemented, n = 12) and 22 triplet-bearing ewes (Supplemented, n = 12; and Non-supplemented, n = 10) which showed signs of parturition were moved quietly to small individual pens (5 x 3 m) located in the paddock. The ewes remained in these pens during parturition. Measurements and samples were taken from lambs within five minutes of birth and up until 24 to 36 hours of age (described later). All ewes had access to herbage and water within the pens and were released from the pen 24 hours after the last lamb was born. The remaining ewes gave birth in the main grazing paddock with no observations recorded or measurements taken.

The intensive lambing observations and measurement were undertaken from 6 September 2006 until 16 September 2006. During this period the weather conditions consisted of an average wind speed of 3.87 m s^{-1}, total rainfall of 0.01 mm, average temp of 10.3ºC (max=12.3ºC, min= 7.25ºC), and an average wind chill of 997.1 kj m^{2-1} h^{-1}.

Ewe measurements

Unfasted ewe live weight and body condition score (scale 1 - 5, half units (Jefferies 1961)) were measured at mating, P68, P100, P120 and P141. A 10 ml blood sample was also collected by jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA) from a sub-sample of the ewes (Supplemented, n = 12; and Non-supplemented, n = 14) on P100, P120 and P141.

Lamb measurements

The lambs were tagged and their birth-rank was recorded within the first five minutes of birth. At the same time, two 5 ml blood samples were collected from each lamb by jugular venepuncture (lithium heparin, and potassium oxalate vacutainer, Becton Dickson Vacutainer Systems, USA). Rectal temperature was also measured within the first five minutes of birth and this measurement was repeated at one, three, six and 12 hours after birth. If the rectal temperature of a lamb fell below 36°C (Alexander 1962c), no further measurements were taken and the lamb was removed from the experiment. At three hours after birth each lamb was weighed, its sex determined and crown-rump length and thoracic-girth circumference measured. Additional 5 ml blood samples were collected from each lamb by jugular venepuncture (Lithium heparin vacutainer, Becton Dickson Vacutainer Systems, USA) at three, 12 and 24 hours after birth.

At 24 to 36 hours of age, 28 twin- (Supplemented, n = 14; Non supplemented, n = 14) and 30 triplet-born (Supplemented, n = 17; Non supplemented, n = 13) lambs were randomly selected to determine their maximum heat production (summit metabolism) by using indirect open-circuit calorimetry. An average cold stress index (CSI) within the first 24 to 36 hours of life was calculated using the following equation (Donnelly 1984),

$$CSI\,(kJ/m^2/h) = (11.7 + 3.1W^{0.5})\,x\,(40 - T) + 481 + 418(1 - e^{-0.04R})$$

where, W is the mean wind speed (m s^{-1}), T is the mean temperature (ºC), and R is the mean rainfall (mm).

A 10 ml blood sample was taken from the lamb’s via jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA) immediately prior to the lamb being placed in a crate within an indirect open circuit calorimeter (McCutcheon *et al* 1983a). The lambs head was placed in a plastic hood, which was sealed around the neck with a collar. Air was drawn into and from the hood for measurement of oxygen consumption. A temperature probe was inserted in the rectum of the lamb to record body temperature. The lamb was exposed to an environmental temperature that ranged from 6-8ºC, and was allowed to acclimatise to these conditions for a total of 12 minutes. This period of time is referred to as the adjustment period. During this time rectal temperature and oxygen consumption measurements were recorded over three successive four-minute periods to obtain a stabilised metabolic rate so a base heat production level could be calculated. At the end of 12 minutes, lambs were exposed to wet and windy conditions to induce maximum heat production (summit metabolism). This period of time is referred to as the onset of severe cold stress. Artificial chilled rain

(1 ℃) was applied from the 12 minute mark through sprinklers at a standardised rate of (1.08 l min^{-1}) and cold air was passed over the lamb by a fan positioned behind the animal at a rate of 1.0 m s^{-1}. After 20 minutes cold air was passed over the lamb at a rate of 1.5 m s^{-1}, and after another 20 minutes at a rate of 2.0 m s^{-1}. The rate of cold air then stayed constant for the remaining period of time. Rectal temperature and oxygen consumption measurements were taken at 4 minute intervals for 88 minutes or until the lamb reached maximum heat production, whichever occurred first. Maximum heat production was assumed to have been met when the rectal temperature of the lamb declined at the rate of one degree per 20 minutes and there was no further increase in the consumption rate of oxygen (Alexander 1962c). To facilitate heat loss to stimulate heat production to its maximum, all lambs with a birth weight above 4 kg had the wool clipped from their back and sides leaving a wool depth of 3 mm. Maximum heat production in Watts (W) and was calculated from oxygen consumption using the following formula (Revell *et al* 2002),

$$Maximal\ heat\ production(W) = oxygen\ consumption\,(L/h)\, x\, 20.46/3.6$$

where it was assumed that 20.46 kJ of heat is produced per litre of oxygen consumed (McLean 1972). Dividing oxygen consumption by a constant of 3.6 converts kj h^{-1} to W.

After the lamb reached its maximum heat production, or after 88 minutes had elapsed in the calorimeter, the lamb was removed from the calorimeter, thoroughly dried, and a 5 ml blood sample was taken via jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA). Thereafter, the lambs were placed in a heated room for one hour before being returned to their dams.

Plasma analysis

All blood samples were stored on ice until they were centrifuged at 1000 *g* for 15 minutes. The plasma was removed and frozen at -20 °C. A random sample of ewe (8 twin- and 7 triplet-bearing ewes from non-supplemented treatment, and 7 twin- and 7 triplet-bearing ewes from supplemented treatment) and lamb blood samples (14 twin- and 13 triplet-born lambs born to non-supplemented ewes, and 14 twin- and 17 triplet-born lambs born to supplemented ewes) were analysed. Ewe plasma samples were analysed using a diagnostic kit for iodine (Tetramethylammonium hydroxide micro

(TMAH) digestion, 90ºC for 1 hour, filtration (Fecher *et al* 1998; Hill laboratories, Hamilton, New Zealand)). Lamb plasma samples were analysed using diagnostic kits for glucose (hexokinase method, Roche Diagnostics Ltd, Switzerland), non-esterfied fatty acid (NEFA) (Sigma, Illinois, USA), total T_4 and total tri-iodothyronine (T_3) (radioimmunoassay diagnostic kit, Coat-A-Count, Diagnostic Products Corporation, CA, USA), Gamma-glutamyl-transferase (GGT) (Roche kit, Roche Diagnostics Ltd, Switzerland), and Immuno-globulin (IgG) (Standard direct ELISA assay).

Statistical analysis

The dataset for metabolite and hormone concentrations and rectal temperatures contained some missing values due to the following reasons, a) if the lamb was dead at birth blood samples and rectal temperatures were not measured, b) blood samples that were not obtained after two attempts were not collected, c) blood samples and rectal temperatures that were not taken within 10 minutes of the required time, were not included in the analyses and d) if the rectal temperature of a lamb fell below 36ºC during the observation periods, no further measurements were taken and the lamb was removed from the remainder of the study.

All ewe measurements that were taken over time (plasma iodine concentrations (P68, P120 and P141), ewe live weight (P50, P100, P120 and P141) and ewe body condition score (P100, P120 and P141)) were analysed using a repeated measure (PROC MIXED, SAS, 2003) analysis. These models contained the fixed effects of ewe iodine supplementation (Non-supplemented, Supplemented) and litter size (Twin, Triplet), plus their interactions. Non-significant ($P > 0.1$) interactions were removed from the general linear model. Lamb plasma T_4 and T_3 concentrations that were taken over time (within five minutes of birth, at three hours of age, at 24 to 36 hours of age) were also analysed using a repeated measure (PROC GLM, SAS, 2003) analysis. These models contained the fixed effect of ewe iodine supplementation (Non-supplemented, Supplemented) and birth rank (Twin, Triplet), plus their interactions. After running the model, the model was re-run with lamb birth weight as a covariate. The interactions or covariate only remained in the general linear model if significant ($P < 0.1$).

Lamb physical measurements (birth weight, crown-rump length and thoracic-girth circumference), lamb plasma glucose, GGT and IgG concentrations at 24 to 36 hours of age, and lamb plasma glucose and NEFA concentration before and after the onset of calorimetry were analysed using a general liner model (PROC GLM, SAS, 2003). These models contained the fixed effects of ewe iodine supplementation (Non-supplemented, Supplemented) and lamb birth rank (Twin, Triplet), plus their interactions. Lamb birth weight was fitted as a covariate, which identified if the dependent variables were influenced more by lamb birth rank, or lamb birth weight. The interactions or covariate only remained in the general linear model if significant ($P < 0.1$).

Plasma NEFA concentrations before and after the onset of calorimetry were not normally distributed. To achieve a normal distribution the concentrations were $\log^{10}$ transformed. Lamb rectal temperatures measured within five minutes of birth, and at three and 12 hours of age were not normally distributed. Log, squared, cubed and square-root transformations were unable to normalise the data. Lamb rectal temperatures from birth until 12 hours of age were therefore presented as median and inter-quartile ranges. The non-parametric test of Kuskal-Wallis was used to determine the significance of ewe iodine supplementation (Non-supplemented, Supplemented) and lamb birth rank (Twin, Triplet) on lamb rectal temperatures.

The base heat production and maximum heat production (W), the base heat production and maximum heat production on a per kg of birth weight basis (W kg^{-1}), and the rate to reach maximum heat production (W $kg^{-1}min^{-1}$) were analysed using a general linear model (PROC GLM, SAS, 2003). The model contained the fixed effects of calorimeter (One, Two), iodine supplementation (Non-supplemented, Supplemented) and birth rank (Twin, Triplet), plus their interactions. Plasma GGT concentrations at 24 to 36 hours of age, and average CSI within the first 24 hours of life were fitted as covariates. After these models were run, the models were re-run with lamb birth weight as a covariate. Interactions and covariates were retained in the model if significant at $P < 0.1$. If not significant they were removed and the model refitted.

The proportion of lambs reaching maximum heat production were analysed as a binomial trait using the SAS procedure for categorical modelling (PROC GENMOD, SAS, 2003). Data were analysed as logit transformed values and presented in results as back-transformed percentages. The model contained the fixed effects of ewe iodine supplementation (Non-supplemented, Supplemented), and birth rank (Twin, Triplet), plus their interactions.

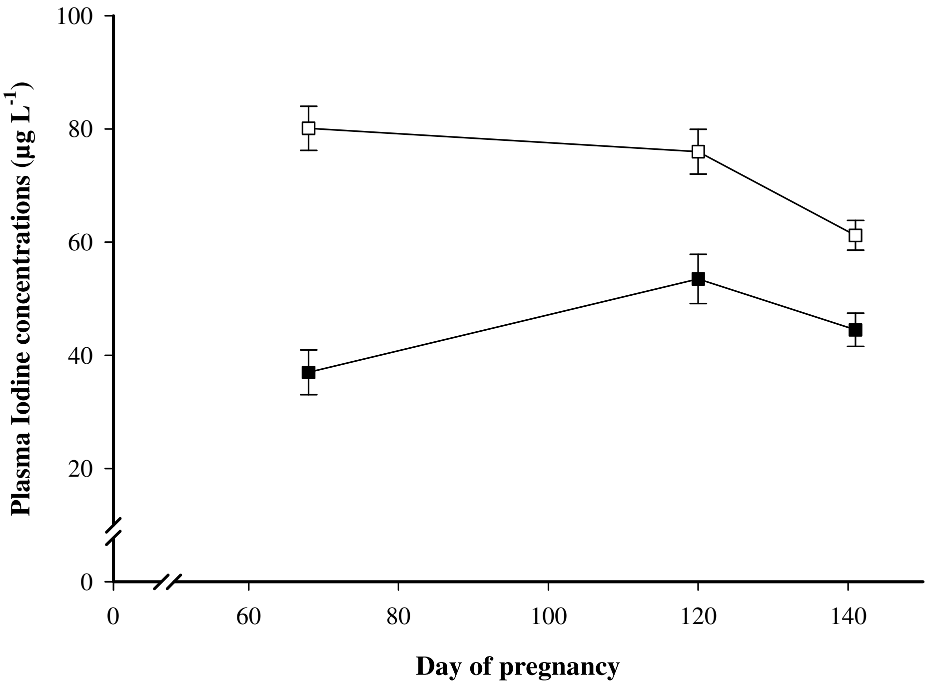
Results

Ewe plasma iodine concentrations

Ewes that were supplemented with iodine had greater (*P* < 0.001) plasma iodine concentrations at P68, P120 and P141 than ewes that were not supplemented (Figure 6). While plasma iodine concentrations of supplemented ewes did not differ (*P* > 0.1) between P68 and P120, they decreased (*P* < 0.01) from P120 until 141. In contrast, the plasma iodine concentrations of non-supplemented ewes increased (*P* < 0.01) from P68 to P120, but did not differ at P140 (*P* > 0.1) from 68 or P120.

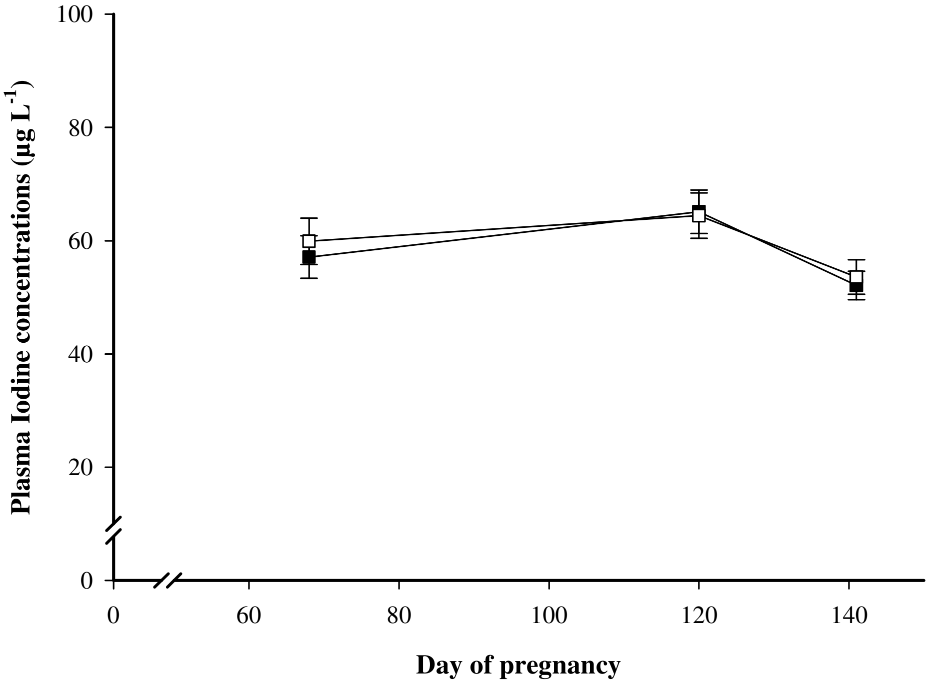
Litter size had no effect on ewe plasma iodine concentrations (*P* > 0.1; Figure 7). While plasma iodine concentrations of twin- and triplet-bearing ewes did not differ (P > 0.1) between P68 and P120, they did decrease (P < 0.01) from P120 and P141.

Figure 6 *The effect of maternal iodine supplementation (Non-supplemented (●, n = 15) and Supplemented (○, n = 15)) on ewe plasma iodine concentrations (μg L^{-1}) on pregnancy day 68 (P68), P120 and P141.*



Data were presented as means ± standard errors

Figure 7 *The effect of litter size (Twin-bearing (■, n = 16) and triplet-bearing (□, n = 14)) on ewe plasma iodine concentrations (µg L*$^{-1}$*) on pregnancy day 68 (P68), P120 and P141.*



Data were presented as means ± standard errors

Ewe live weight and body condition score during pregnancy

Ewe iodine supplementation and litter size had no effect on ewe live weight (kg) at P50, P100, P120 and P141 ($P > 0.1$; pooled means ± standard error, 60.5 ± 0.70, 72.5 ± 0.83, 76.1 ± 0.87 and 84.5 ± 1.15 respectively), or on ewe body condition score at P100, P120 and P141 (pooled means ± standard error, 3.1 ± 0.07, 3.1 ± 0.05 and 3.1 ± 0.04).

Lamb body size measurements

Ewe iodine supplementation had no effect ($P > 0.1$) on lamb birth weight, crown-rump length or thoracic-girth-circumference (Table 20). Triplet-born lambs had lighter birth weights and smaller thoracic-girth-circumference than twin-born lambs ($P < 0.001$). Differences in thoracic-girth-circumferences however, were explained by lamb birth weight. For every one kg increase in lamb birth weight, crown-rump-length and thoracic-girth-circumference increased by 2.3 ±0.64 cm and 2.5 ± 0.32 cm, respectively.

***Table 20** The effect of iodine supplementation (Non-supplemented, Supplemented) and lamb birth rank (Twin, Triplet) on lamb birth weight (kg), crown-rump length (cm) and thoracic-girth-circumference (cm)*

	n	Birth weight	Crown-rump length	Thoracic-girth circumference
Iodine supplementation				
Non-supplemented	54	4.3 ± 0.10	50.0 ± 0.68	37.8 ± 0.41
Supplemented	56	4.4 ± 0.10	50.0 ± 0.68	37.3 ± 0.42
P value		ns	ns	ns
Lamb birth rank				
Twin-born	50	4.8 ± 0.10	50.7 ± 0.71	39.1 ± 0.42
Triplet-born	60	3.9 ± 0.09	49.3 ± 0.65	36.1 ± 0.39
P value		< 0.001	ns	< 0.001

Data were presented as means ± standard errors; ns, non significant

Lamb core body temperature after birth

Ewe iodine supplementation had no effect ($P > 0.1$) on lamb rectal temperature within five minutes of birth, or at one, three, six and twelve hours of age (Table 21). Triplet-born lambs had lower ($P < 0.01$) rectal temperatures within 5 minutes of birth and at one hour of age, compared to twin-born lambs. Lamb birth rank had no effect on lamb rectal temperatures at three, six and 12 hours of age.

Table 21 *The effect of iodine supplementation (Non supplemented, Supplemented) and birth rank (Twin, Triplet) on lamb rectal temperature within five minutes of birth (ºC), and at one, three, six and 12 hour (hr) of age.*

	Lamb rectal temperature									
	n	Within 5 minutes of birth	n	One (hr)	n	Three (hr)	n	Six (hr)	n	12 (hr)
Iodine Supplementation										
Non supplemented	49	39.9 (39.2 40.4)	49	40.1 (39.4-40.5)	45	39.9 (39.5-40.2)	47	39.8 (39.5-40.2)	49	39.6 (39.2–39.8)
Supplemented	55	40.0 (39.3-40.7)	51	40.2 (39.7-40.7)	50	40.2 (39.9-40.3)	51	39.8 (39.4-40.2)	50	39.7 (39.3-40.0)
P value[1]		ns		ns		ns		ns		ns
Birth rank										
Twin-born	46	40.4 (39.7-40.7)	46	40.4 (39.9-40.7)	44	40.0 (39.8-40.3)	46	39.7 (39.4-40.2)	48	39.7 (39.4-39.9)
Triplet-born	58	39.6 (38.8-40.3)	54	40.0 (39.0-40.5)	51	40.0 (39.8-40.3)	52	39.8 (39.4-40.2)	51	39.6 (39.2-39.9)
P value[1]		< 0.001		< 0.01		ns		ns		ns

Date presented as median and 95% interquartile range; [1]Significance was determined by Kruskal Wallis test.

Lamb plasma glucose, GGT and IgG concentrations

Triplet-lambs born to iodine supplemented ewes had greater glucose and IgG concentrations at 24 to 36 hours of age than triplet-born lambs born to non-supplemented ewes ($P < 0.01$; Table 22). This relationship was not observed in twin-born lambs ($P > 0.1$). Within the iodine supplemented treatment group, triplet-born lambs tended to have greater IgG concentration than twin-born lambs ($P = 0.07$). Within the non-supplemented treatment group, twin-born lambs had a greater glucose concentration than triplet-born lambs ($P < 0.05$). Ewe iodine supplementation and lamb birth rank had no effect on plasma GGT concentrations at 24 to 36 hours of age ($P > 0.1$).

Table 22 *The effect of iodine supplementation (Non-supplemented, Supplemented) and lamb birth rank (Twin, Triplet) on lamb plasma glucose (mmol l^{-1}), gamma-glutamyl-transferase (GGT; IU l^{-1}) and immuno-globulin G (IgG; mg ml^{-1}) concentrations at 24 to 36 hours of age.*

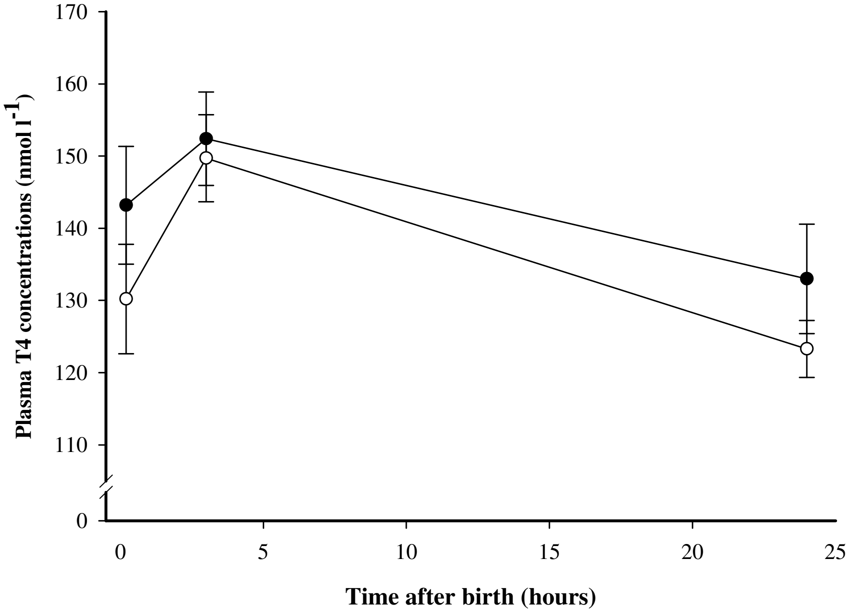
	n	Glucose	GGT	IgG
Iodine Supplementation				
Non-supplemented	27	4.9 ± 0.28	2248 ± 305.1	48.2 ± 5.05
Supplemented	30	5.4 ± 0.27	2339 ± 293.6	51.3 ± 4.87
P value		ns	ns	ns
Birth Rank				
Twin	28	5.4 ± 0.28	2350 ± 305.1	49.3 ± 4.96
Triplet	29	4.9 ± 0.27	2737 ± 293.6	50.2 ± 4.97
P value		ns	ns	ns
Interactions				
Non-supplemented				
Twin	14	5.5 ± 0.39^{a}	-	56.2 ± 7.01^{ab}
Triplet	13	4.3 ± 0.41^{b}	-	40.3 ± 7.27^{b}
Supplemented				
Twin	14	5.3 ± 0.39^{a}	-	42.2 ± 7.01^{ab}
Triplet	16	5.5 ± 0.37^{a}	-	60.2 ± 6.77^{a}
P value		< 0.01		< 0.01

Data were presented as means ± standard errors; means ± standard errors within interactions with differing superscripts are significantly ($P < 0.05$) different; ns, non significant

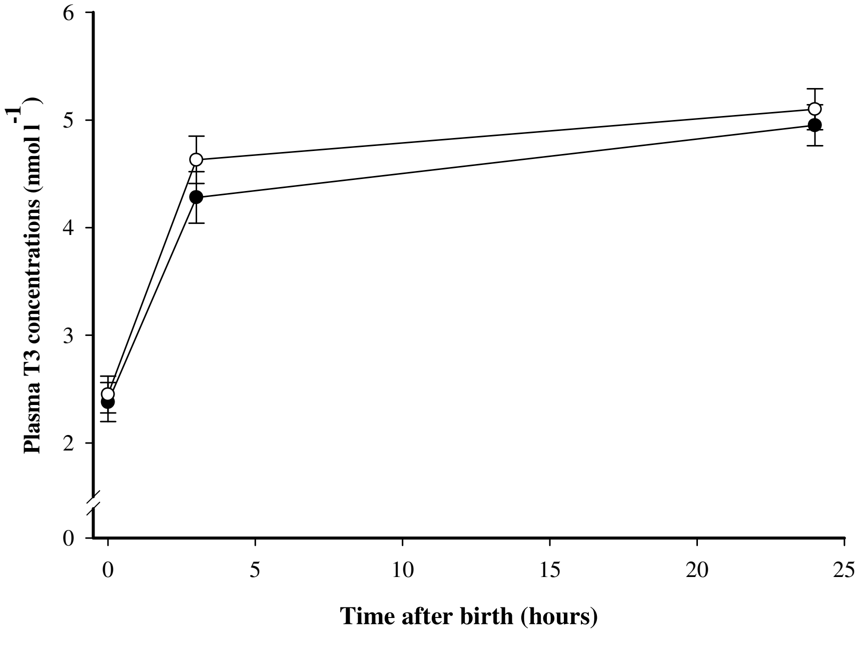
Lamb plasma thyroid hormone concentrations

Ewe iodine supplementation had no effect ($P > 0.1$) on newborn lamb plasma T_4 and T_3 concentrations within five minutes of birth, and three and 24 to 36 hours of age (Figure 8).

***Figure 8** The effect of iodine supplementation (Non-supplemented (●, n = 25) and Supplemented (○, n = 29)) on lamb plasma thyroxine (T_4; nmol l^{-1}) and tri-iodothyronine (T_3; nmol l^{-1}) concentrations within five minutes of birth, at three hours and 24 to 36 hours of age*



Data were presented as means ± standard errors



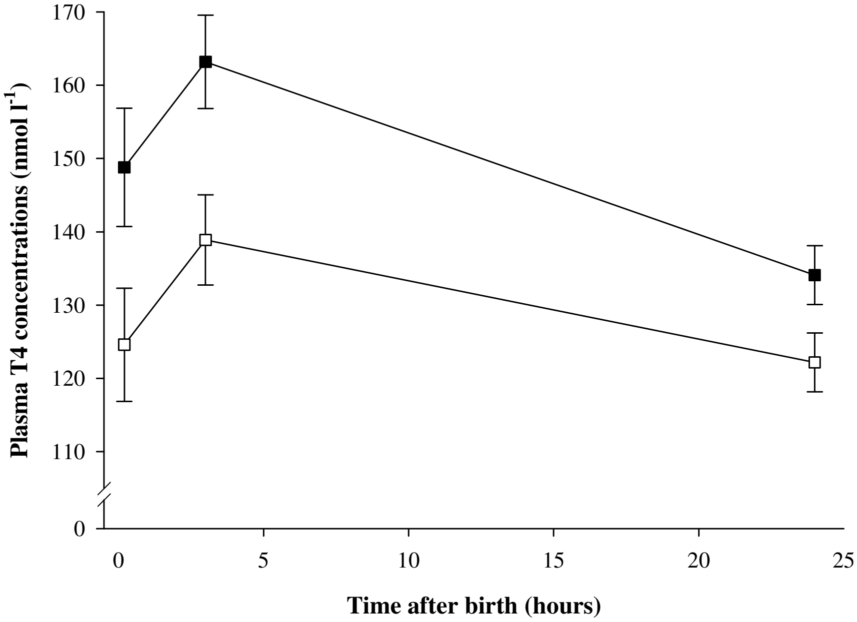
Data were presented as means ± standard errors

Over time, plasma T_4 concentrations of lambs born to non-supplemented ewes did not differ ($P > 0.1$) between birth and three hours of age, they did however, decrease ($P < 0.05$) from three hours of age to 24 hours of age. Plasma T_4 concentrations of lambs born to supplemented ewes increased ($P < 0.05$) from birth until three hours of age, and then decreased ($P < 0.01$) from three hours of age to 24 hours of age. Plasma T_3 concentrations of lambs born to both non-supplemented and supplemented ewes increased ($P < 0.001$) from birth until three hours of age. From three hours of age until 24 hours of age, plasma T_3 concentrations increased ($P < 0.05$) for lambs born to non-supplemented ewes, and stayed the same ($P > 0.1$) for lambs born to supplemented ewes.

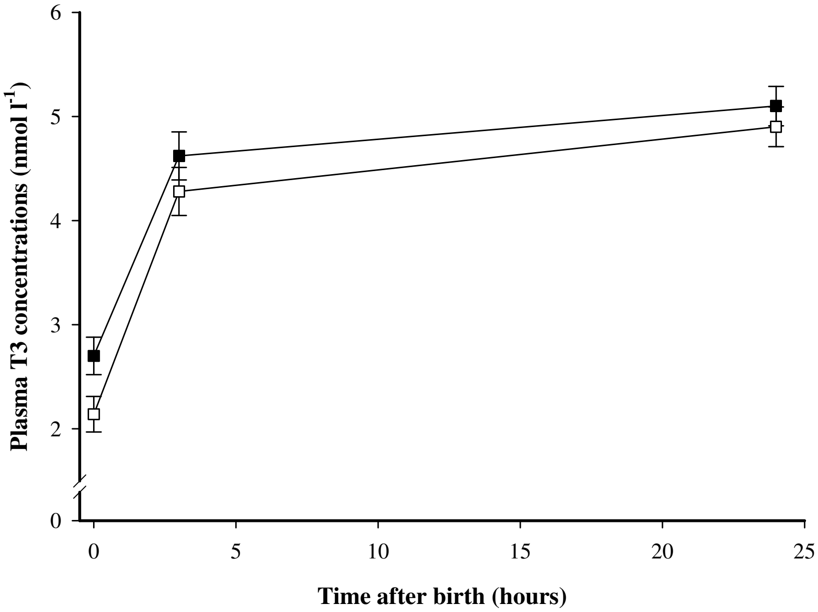
Twin-born lambs had greater ($P < 0.05$) plasma T_4 concentrations within five minutes of birth, three hours of age, and 24 hours of age then triplet-born lambs (Figure 9). After adjustment for lamb birth weight however, lamb birth rank had no effect on plasma T_4 concentrations. For every one kg increase in lamb birth weight, plasma T_4 concentrations increased by 10.9 ± 3.31 nmol l^{-1}. Twin-born lambs had greater plasma T_3 concentrations than triplet-born lambs within five minutes of birth ($P < 0.05$). Lamb birth rank had no effect ($P > 0.1$) on plasma T_3 concentrations at three hours of age, and at 24 hours of age. Lamb birth weight had no effect ($P > 0.1$) on plasma T_3 concentrations.

Over time, plasma T_4 concentrations of twin- and triplet-born lambs did not differ ($P > 0.1$) between birth and three hours of age, but did decrease ($P < 0.05$) from three hours of age until 24 hours of age. Plasma T_3 concentrations of twin- and triplet-born lambs increased ($P < 0.05$) from birth until three hours of age. From three hours of age until 24 hours of age, plasma T_3 concentrations did not differ ($P > 0.1$) for twin-born lambs, but increased for triplet-born lambs. Ewe iodine supplementation and lamb birth rank had no effect on plasma T_3: T_4 ratio within five minutes of birth, three hours of age or at 24 to 36 hours of age ($P > 0.1$; data not shown).

Figure 9 *The effect of lamb birth rank (twin (■, n = 25) and triplet (□, n = 29) on lamb plasma thyroxine (T_4) and plasma tri-iodothyronine (T_3) concentrations within five minutes of birth (nmol l^{-1}), and at three hours and 24 to 36 hours of age.*



Data were presented as means ± standard errors



Data were presented as means ± standard errors

Base heat production, maximum heat production and rate to reach maximum heat production

Iodine supplementation had a positive effect on the base heat production on a per lamb basis (W lamb^{-1}) and had no effect on the maximum heat production on a per lamb basis (Table 23). Triplet-born lambs tended to ($P = 0.08$) have a lower base heat production on a per lamb basis and had ($P < 0.01$) a lower maximum heat production on a per lamb basis than twin-born lambs. A one unit increase in plasma GGT concentration was associated ($P < 0.01$) with an increase in the maximum heat production on a per lamb basis, at a rate of 0.01 (± 0.002 (se)) W. After adjustment for lamb birth weight, lamb birth rank no longer had an effect on the base and maximum heat production on a per lamb basis. For every one kg increase in lamb birth weight, the base and maximum heat production on a per lamb basis increased by 6.46 (± 2.04 (se)) W, and 10.5 (± 3.49 (se)) W, respectively.

Newborn lambs born to iodine supplemented ewes had a greater base heat production on a per kg of birth weight basis (W kg^{-1}) than lambs born to non-supplemented ewes ($P < 0.05$; Table 23). Iodine supplementation did not effect maximum heat production on a per kg of birth weight basis, or the rate to reach maximum heat production (W kg^{-1} min^{-1}). The base heat production on a per kg of birth weight basis tended (P=0.08) to be greater in triplet-born lambs than twin-born lambs. Maximum heat production on a per kg of birth weight basis, and the rate to reach maximum heat production did not differ ($P > 0.05$) between twin- and triplet-born lambs. A one unit increase in plasma GGT concentration was found to be associated ($P < 0.01$) with an increase in the maximum heat production on a per kg of birth weight basis at a rate of 0.01 (± 0.003 (se)) W kg^{-1}. Lamb birth weight had no effect on the base and maximum heat production on a per kg of birth weight basis.

The proportion of lambs to reach maximum heat production during calorimetry

Ewe iodine supplementation and lamb birth rank had no effect ($P > 0.1$) on the proportion of lambs reaching maximum heat production during 88 minutes of cold stress (92.8%, 85.2%, 89.4%, and 89.8% for supplemented, non-supplemented, twin-born, and triplet-born lambs, respectively).

Table 23 *The effect of iodine supplementation (Non-supplemented, Supplemented) and birth rank (Twin, Triplet) on lamb base heat production (W and W kg^{-1}), maximum heat production (W and W kg^{-1}) and the rate to reach maximum heat production at 24 to 36 hours of age (W kg^{-1}min^{-1}).*

		Base heat production			Maximal heat production		Rate to reach maximal heat production
		W	W kg^{-1}		W	W kg^{-1}	W kg^{-1}min^{-1}
Iodine supplementation							
Non supplemented	27	34.8 ± 2.13	8.3 ± 0.45	23	63.7 ± 3.68	14.8 ± 0.64	0.23 ± 0.019
Supplemented	31	42.5 ± 1.99	9.8 ± 0.42	29	71.7 ± 3.54	15.4 ± 0.61	0.21 ± 0.018
P value		< 0.05	< 0.05		ns	ns	ns
Birth rank							
Twin-born	28	41.2 ± 2.09	8.4 ± 0.44	25	76.6 ± 3.68	15.3 ± 0.64	0.21 ± 0.019
Triplet-born	30	36.1 ± 2.03	9.8 ± 0.43	27	58.7 ± 3.55	15.3 ± 0.62	0.22 ± 0.019
P value		0.08	0.08		< 0.01	ns	ns

Data is presented as means ±.standard errors; ns, non significant

Plasma glucose and NEFA concentrations before and after calorimetry

Lambs born to iodine supplemented ewes had greater plasma glucose concentrations after calorimetry than lambs born to non-supplemented ewes (9.4 ± 0.40 and 8.3 ± 0.40 mmol l^{-1}, respectively; $P < 0.05$). Lamb birth rank had no effect ($P > 0.1$) on lamb plasma glucose concentrations before or after calorimetry, or in the change of glucose from before until after calorimetry. Iodine supplementation and lamb birth rank had no effect ($P > 0.1$) on plasma NEFA concentrations before or after calorimetry, or in the change of NEFA from before until after calorimetry (1.0 ± 0.09 (pooled mean ± standard error), 1.3 ± 0.12, and 0.3 ± 0.16 mmol l^{-1}, respectively).

Correlations between lamb birth weight, thyroid hormones, plasma metabolites and maximum heat production on a per lamb and per kg of birth weight basis.

Lamb birth weight, plasma T_4 concentrations within five minutes of birth, plasma T_3 concentrations at 3 hours and 24 to 36 hours of age, plasma GGT concentrations at 24 to 36 hours of age, plasma glucose concentrations before calorimetry were positively ($P < 0.05$) correlated with maximum heat production on a per lamb basis (Table 24).

Lamb plasma T_3 concentrations at 24 to 36 hours of age, plasma GGT concentrations at 24 to 36 hours of age and plasma glucose and NEFA concentrations before calorimetry were positively correlated ($P < 0.05$) with maximum heat production on a per kg of birth weight basis.

Table 24 *Correlations between lamb birth weight, thyroid hormones, plasma metabolites and maximum heat production on a per lamb (W lamb^{-1}) and per kg of birth weight basis (W kg^{-1}).*

		Maximum heat production	
Variables	n	W lamb^{-1}	W kg^{-1}
Lamb birth weight (kg)	110	0.58 ***	-
T_4 concentrations within 5min (nmol l^{-1})	54	0.39 **	-
T_3 conc. within 5min (nmol l^{-1})	54	-	-
T_4 conc. at 3 hrs (nmol l^{-1})	56	-	-
T_3 conc. at 3 hrs (nmol l^{-1})	56	0.28 *	-
T_4 conc. at 24-36 hrs (nmol l^{-1})	56	-	-
T_3 conc. at 24-36 hrs (nmol l^{-1})	56	0.43 ***	0.41 **
GGT conc. at 24-36hrs (IU l^{-1})	57	0.28 *	0.32 *
IgG conc. at 24-36hrs (mg ml^{-1})	57	-	-
Glucose conc. before calorimetry (mmol l^{-1})	58	0.44 ***	0.48 ***
NEFA conc. before calorimetry (mmol l^{-1})	58	-	0.43 **

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

T_4, thyroxine, T_3, tri-iodothyronine; GGT, Gamma-glutamyl-transferase; IgG, immunogobulinG, NEFA. Non-esterfied fatty acid

Discussion

The aim of the present study was to determine if maternal iodine supplementation had a positive effect on twin- and triplet-born lamb thyroid hormone concentration, rectal temperature and heat production at 24 to 36 hours post birth. Based on the serum iodine status of newborn calves, and the thyroid-weight birth weight ratio of newborn lambs, McCoy *et al* (1997) and Knowles and Grace (2007) suggested that maternal plasma iodine concentrations of approximately 40 µg l^{-1}, which are similar to the maternal plasma iodine concentrations of the non-supplemented treatment group in the present study, were inadequate. Maternal iodine concentrations of 65 µg l^{-1}, which are similar to the maternal plasma iodine concentrations of the supplemented treatment group, are considered adequate.

Positive associations between maternal iodine plasma concentrations and thyroid hormone concentrations of the newborn lamb (Andrewartha *et al* 1980; Rose *et al* 2007), and between thyroid hormone concentrations of the newborn lamb and its' thermoregulation have previously been reported (Alexander 1970; Caple and Nugent 1983; Polk *et al* 1987; Symonds *et al* 1995). In the present study however, maternal iodine supplementation had no effect on the thyroid hormone concentration of the lamb within the first 24 to 36 hours of life, rectal temperature from birth to 12 hours of age, or the maximum amount of heat produced at 24 to 36 hours of age on a W lamb^{-1} or W kg^{-1} of birth weight basis. Given that previous studies have reported a positive association between maternal iodine supplementation and plasma T_4 concentrations (Andrewartha *et al* 1980; Rose *et al* 2007), and given that maternal iodine concentrations were different between treatment groups, it is unclear why differences in lamb plasma thyroid concentrations were not observed. Lamb plasma T_4 concentrations within five minutes of birth, and lamb plasma T_3 concentrations at three hours of age and at 24 to 36 hours of age were found to be positively correlated with either maximum heat production on a W lamb^{-1} or on a W kg^{-1} of birth weight basis. The inability of maternal iodine supplementation to alter lamb plasma thyroid concentrations may therefore explain the lack of differences between treatment groups for lamb heat production. This finding also supports previous work (Alexander 1970; Caple and Nugent 1983; Polk *et al* 1987; Symonds *et al* 1995), which has shown that improvements in thyroid hormone concentrations does have a positive effect on lamb heat production.

In support of previous studies, triplet-born lamb birth weights were lighter than twin-born lamb birth weights, and triplet-born lamb plasma T_4 and T_3 concentrations after birth and rectal temperatures within five minutes of birth and at one hour of age were lower than twin-born lambs (Alexander 1962a; Dwyer and Morgan 2006; Darwish and El-Bahr 2007; Stafford *et al* 2007). These results, combined with the positive correlation between plasma thyroid hormone concentrations and maximum heat production on a W lamb^{-1} or W kg^{-1} of birth weight basis, suggests that triplet-born lambs may be more susceptible to a hypothermic death during periods of cold stress than twin-born lambs.

Maternal iodine supplementation had a positive effect on the plasma IgG concentrations of triplet-born lambs at 24 to 36 hours of age, which, in this study, were used as indicator of colostrum intake (Maden *et al* 2003; Kenyon *et al* 2005b). Maternal iodine supplementation has been previously been reported as having a positive effect on the production of milk (Odjakova 1999; Angelow *et al* 2004), which in turn, may have a positive effect on the intake of colostrum by the lamb and therefore its survival. Caution needs to be taken here however, as: a) these positive effects were only seen in triplet-born lambs and not twin-born lambs, b) IgG uptake is not only influenced by colostrum intake, but also by the amount of IgG in the milk and the efficiency of colostrum absorption, neither of which were measured in this experiment (Boland *et al* 2004; Boland *et al* 2005), and c) recent research has clearly shown that increasing the level of iodine in the diet of ewes in late pregnancy is associated with reduced immunoglobulin uptake (Boland *et al* 2004; Boland *et al* 2005; Rose *et al* 2007), not increased uptake. Differences between the present study and the multitude of studies which have shown a reduction in IgG uptake, could be the amount of iodine and/or mode of iodine delivery. Because IgG uptake is important for diseases resistance in early life, further investigation into the effect of different modes of iodine administration and the effects this may have on the colostrum uptake of the lamb, merit further investigation.

Conclusion

Under the conditions of the present experiment maternal iodine supplementation had no effect on twin- and triplet-born lamb thyroid hormone production, rectal temperature and maximum heat production. Maternal iodine supplementation did however, have a positive effect on plasma IgG concentrations in triplet-born lambs, which may indicate an increase in colostrum uptake. Independent of maternal iodine treatment, plasma T_4 concentrations within five minutes of birth, and plasma T_3 concentrations at three and 24 to 36 hours of age were found to be positively correlated with maximum heat production on a per lamb or per kg of birth weight basis. This indicates that if maternal iodine supplementation had been successful in increasing plasma T_4 or T_3 concentrations, twin- and triplet-born lamb heat production may have been improved. Overall, triplet-born lambs were found to be lighter, have lower rectal temperatures within the first hour of birth and lower plasma T_4 and T_3 concentrations than twin-born lambs.

CHAPTER 6

Does the physiological status of lambs within a twin- and triplet-born litter differ during the first 24 hours of life?

Related Publication;

Based on a publication in Animal Production Science

Kerslake JI, Kenyon PR, Morris ST, Stafford KJ, Morel PCH (2010) Does the physiological status of lambs within a twin- and triplet-born litter differ during the first 24 hours of life? *Journal of Animal Production,* **50,** 522-527

Abstract

This study examined the physical and physiological differences between lambs within twin- (heavy and light) and triplet-born (heavy, medium and light) litters from birth until 24 to 36 hours of age. In 2005 and 2006, the parturition of 75 twin- and 62 triplet-bearing Romney ewes were observed. After parturition lamb blood samples were taken within five minutes of birth, at three, 12 and 24 hours post-birth and rectal temperature were measured within five minutes of birth, at one, three, six and 12 hours post-birth. Lamb birth weight, crown-rump length and thoracic-girth circumference were measured at three hours of age. Plasma lactate, thyroxine and tri-iodothyronine concentrations within five minutes of birth were the same between the heaviest-twin- and lightest-twin-born and heaviest-triplet-born lambs ($P > 0.05$). The lightest- and medium triplet-born lambs however, tended to have greater ($P = 0.09$) lactate concentrations and lower (P=0.08) plasma thyroxine concentrations within five minutes of birth. The lightest- and medium triplet-born lambs also had lower ($P < 0.05$) plasma tri-iodothyronine concentrations with five minutes of birth than all twin-born lambs and the heaviest-triplet-born lamb. All of these characteristics are known to have a negative impact on the ability of the lamb to maintain its body temperature after birth. These results may offer some explanation as to why triplet-born lambs have a greater mortality rate than twin-born lambs, and why the lightest-triplet-born lambs have a greater mortality rate within a litter.

Introduction

Litter size, and weight of lamb produced at weaning are both important productive traits of sheep bred for meat production. While selection for increased ewe fecundity has successfully resulted in an improvement in the number of lambs being born (Anon 2008), greater mortality rates of lambs born within large litters (Thomson *et al* 2004; Everett-Hincks *et al* 2005b; Kerslake *et al* 2005) has limited progress and requires further investigation.

Lamb birth weight is a well known risk factor for lamb mortality (Christley *et al* 2003; Everett-Hincks and Dodds 2008). Recent research indicates that within twin- and triplet-born litters, the lightest lambs have the greatest risk of death when compared to its heaviest sibling/s (Morel *et al* 2008; Morel *et al* 2009). While lamb birth weight plays a significant role in lower survival rates, it is not necessarily the only contributing factor. Compared to twin-born lambs, triplet-born lambs have been shown to have lighter birth weights (Morris and Kenyon 2004; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008), lower rectal temperatures (Chapter 2 and 5, Dwyer and Morgan 2006; Stafford *et al* 2007), lower plasma fructose, tri- iodothyronine (T_3), thyroxine (T_4) concentrations, and greater plasma lactate concentrations within five minutes of birth (Stafford *et al* 2007). These physiological characteristics have been used as indices for placental insufficiency, acute intra-partum hypoxemia and inadequate thermogenesis after birth, resulting in poor lamb survival during the post-natal period (Barlow *et al* 1987; Mellor 1988; Mellor and Stafford 2004). Studies comparing physiological characteristics between birth ranks however, have often failed to examine variation within litters. This knowledge might help explain why the lightest lamb within a twin- or triplet-born litter has a greater mortality when compared to its heavier sibling/s.

The aim of this experiment was therefore to examine the physical and physiological differences between lambs within twin- and triplet-born litters from birth until 24 hours of age.

Materials and Methods

This chapter is an analysis of the physical and physiological differences of lambs within twin- and triplet-born litters. This chapter uses original data and some data used in chapters two and five.

Experimental design

In 2005 and 2006 the parturition of 75 twin- and 62 triplet-bearing Romney ewes were observed. In 2005, 40 twin- and 60 triplet-bearing ewes were grazed on *ad-libitum* ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture throughout pregnancy. In 2006, 30 twin- and 30 triplet-bearing Romney ewes were injected intramuscularly in the anterior half of the neck with 1.5 ml of iodised peanut oil (Flexidine®, (26% w/w of iodine bound to ethyl esters of unsaturated fatty acids in oil), Bomac Laboratories Ltd, Auckland, New Zealand) 35 days prior to the start of mating (Supplemented). Another 30 twin- and 30 triplet-bearing Romney ewes were not injected (Non-supplemented). All ewes were grazed as one mob until pregnancy day 68 (P68). At P68, 14 twin- and 16 triplet-bearing ewes were randomly selected from each supplemented and non-supplemented treatment group. These ewes were grazed on kale (*Brassica oleracea)* from P68 until P120, and then on pasture until parturition. The remaining twin- and triplet-bearing ewes were grazed on *ad-libitum* ryegrass and white clover until parturition.

In 2005 and 2006, all ewes which had been walked around twice daily for one week prior to lambing and habituated to the presence of humans, were moved into a 2.5 ha paddock on P145 and were supervised continually for ten days. Seventy five twin- (In 2005, 22; In 2006, 53) and 62 triplet-bearing ewes (In 2005, 18; In 2006, 44) which showed signs of parturition were moved quietly to a temporary pen (5 x 3 m) located in the front of their paddock. The ewes remained in these pens during parturition and ewes and lamb were released from the pen 24 hours after the last lamb was born. All ewes had access to herbage and water within the pen. The remaining ewes gave birth in the paddock with no observations recorded and no measurements taken.

Ewes which were penned gave birth with minimal disturbance, but within five minutes of the birth of each lamb it was tagged and its birth-rank recorded. Also at this time, two 5 mL blood samples were collected by jugular venepuncture (Lithium heparin and potassium oxalate vacutainer, Becton Dickson Vacutainer Systems, USA) and the rectal temperature was measured. The rectal temperature of each lamb was also recorded at one, three, and 12 hours of age. At three hours of age the lamb was weighed, the sex determined and crown-rump length and thoracic-girth circumference measured. Surface area (SA) of the lamb was calculated using the mathematical formula for the total SA of a cylinder,

$$SA\left(cm^2\right)=2\pi r h+2\pi r^2$$

where r is the thoracic girth circumference of the lamb divided by 2π , h is the crown rump length of the lamb.

An additional 5 mL blood sample was collected by jugular venepuncture (Lithium heparin vacutainer, Becton Dickson Vacutainer Systems, USA) at 24 hours of age. To minimize the disruption to the ewe/lamb bond, all blood samples were collected and rectal temperatures taken within the pen. This allowed continuous contact between the ewe and lambs during sampling. The weight and rectal temperature were measured and blood samples from parturition to 24 hours of age were attempted to be taken from 46 twin- and 54 triplet-born lambs in 2005, and 106 twin- and 132 triplet-born lambs in 2006.

These lambing experiments were undertaken at Massey University on Keeble farm, Palmerston North, New Zealand, from 4th September until 10th September in 2005, and 6 September until 16 September in 2006. In 2005 the weather conditions consisted of an average wind speed of 1.74 m s^{-1}, total rainfall of 0 mm, average temp of 11.7ºC (max = 18.0ºC, min = 5.4ºC), and an average wind chill of 919.0 kj $m^{2\ -1}$ h^{-1}. In 2006 the weather condition consisted of an average wind speed of 3.87 m s^{-1}, total rainfall of 0.01 mm, average temp of 10.3ºC (max=12.3ºC, min=7.25ºC), and an average wind chill of 997.1 Kj $m^{2\ -1}h^{-1}$. Massey University Animal Ethics Committee approved these experiments.

Lamb plasma samples

At collection all blood samples were immediately placed on ice until centrifugation (15 min, 1000 *g*). The plasma was then separated and stored at -20 °C. In 2005, blood samples collected from lambs of 44 twin- and 54 triplet-born lambs were analysed. In 2006, a random sample of blood samples from lambs of 46 twin- and 66 triplet-born lambs were analysed.

Glucose concentrations were determined using a hexokinase assay (Roche Diagnostics Ltd, Switzerland), fructose using an enzymatic assay (R Biopharm, Darmstadt, Germany) and lactate using a lactate oxidase/peroxidase assay (Roche Diagnostics Ltd, Switzerland). Lamb plasma T_4, and T_3 concentrations were analysed by a radioimmunoassay diagnostic kit (Coat-A-Count, Diagnostic Products Corporation, CA, USA). Plasma gamma-glutamyl-transferase (GGT) concentrations were analysed using a Roche kit (Roche Diagnostics Ltd, Mannheim, Germany) and plasma immuno-globulin G (IgG) concentrations were analysed using a standard direct ELISA assay.

Statistical analysis

Data from both years were combined. The datasets for metabolite and hormone concentrations and rectal temperatures contained some missing values due to the following reasons: a) if the lamb was dead at birth blood samples and rectal temperatures were not measured, b) blood samples that were not obtained after two attempts were not collected, and c) blood samples and rectal temperatures that were not taken within 10 minutes of the required time were not included in the analyses, and d) if the rectal temperature of a lamb fell below 36°C during the observation period, no further measurements were taken and the lamb was removed from the remainder of the study.

Within twin- and triplet-born litters, lambs were categorised from lightest to heaviest (relative live weight within litter; triplet heavy, triplet medium, triplet light, twin heavy and twin light).

Plasma glucose and fructose concentrations within five minutes of birth, and plasma GGT concentrations were not normally distributed. To achieve normal distribution plasma glucose and fructose concentrations were transformed using the $\log_{10}$ function. Lamb rectal temperatures from within five minutes of birth until 12 hours of age were also not normally distributed. Transformations were unable to normalise this data. Lamb rectal temperatures from birth until 12 hours of age were therefore presented as median and inter-quartile ranges. The non-parametric test of Kuskal-Wallis was used to determine the significance of lamb birth rank and relative weight within litter.

A general linear model (PROC GLM, SAS, 2003) was used to identify any significant differences between treatments, and between and within birth ranks for lamb physical measurements (birth weight, surface-area-to-birth-weight ratio, crown-rump length and thoracic-girth circumference), and lamb physiological measurements (plasma glucose, fructose, lactate, T_4, T_3 concentrations within five minutes of birth, and plasma glucose, GGT and IgG concentrations at 24 to 36 hours of age). These models contained the fixed effects of maternal iodine supplementation (non-supplemented, supplemented), ewe nutrition (pasture, kale), lamb birth rank (twin, triplet), lamb sex (ram, ewe), and their interactions. Birth weight category (heavy, middle, light) was nested within lamb birth rank (twin, triplet), along with its interactions. Lamb birth weight was fitted as a covariate for the following lamb physical measurements, crown-rump length and thoracic-girth circumference, and all physiological measurements. This identified if the dependent variables were influenced more by lamb birth rank, relative live weight within litter or lamb birth weight. The interactions or covariates only remained in the general linear model if significant ($P < 0.05$).

Results

Lamb birth weight and body size

Within a litter of twins, the lightest-twin-born lambs were on average 600 g lighter than the heaviest-twin-born lambs ($P < 0.001$; Table 25). The lightest-twin-born lambs also had greater ($P < 0.001$) surface-area-to-birth-weight ratio and smaller ($P < 0.001$) thoracic-girth-circumference than the heaviest-twin-born lambs. Within a litter of triplets, the lightest-triplet-born lambs were on average 600 g lighter than the medium-triplet-born lambs and 1.1 kg lighter than the heaviest-triplet-born lambs ($P < 0.001$). The lightest-triplet-born lambs also had a greater ($P < 0.001$) surface-area-to-birth-weight ratio, and a smaller ($P < 0.001$) thoracic-girth-circumference than the medium- and heaviest-triplet-born lambs. Within twin- and triplet-born litters, the heaviest-twin-born lambs had a greater ($P < 0.001$) birth weight, smaller ($P < 0.001$) surface-area-to-birth-weight ratio, and greater ($P < 0.001$) thoracic-girth circumference than all triplet-born lambs. The birth weight, surface-area-to-birth-weight ratio and thoracic-girth circumference of the lightest-twin-born lambs and the heaviest-triplet-born lambs did not differ ($P > 0.05$).

After adjustment for lamb birth weight, birth rank and relative weight within litter had no effect on crown-rump length or thoracic-girth circumference, where a one kg increase in lamb birth weight was associated ($P < 0.001$) with a 2.9 (± 0.41 (se)) cm and a 2.2 (± 0.23 (se)) cm increase in crown-rump length and thoracic-girth circumference, respectively.

Table 25 *The effect of birth rank (Twin, Triplet), and relative live weight within a litter (Twin Heavy, Twin Light, Triplet Heavy, Triplet Medium, Triplet Light) on lamb birth weight (kg), surface-area-to-birth weight ratio (SA:Bw; cm^2 kg^{-1}), crown-rump length (cm) and thoracic-girth circumference (cm).*

	n	Birth weight	SA : Bw	Crown-rump length	Thoracic-girth circumferen
Birth Rank					
Twin	150	4.9 ± 0.06^{b}	150.2 ± 1.81^{a}	51.3 ± 0.42	39.8 ± 0.25^{b}
Triplet	186	4.0 ± 0.06^{a}	163.7 ± 1.72^{b}	49.0 ± 0.42	36.9 ± 0.25^{a}
P value		< 0.001	< 0.001	ns	< 0.001
Relative live weight within a litter					
Twin					
Heavy	75	5.2 ± 0.08^{d}	145.4 ± 2.39^{a}	51.6 ± 0.55	40.6 ± 0.34^{d}
Light	75	4.6 ± 0.08^{c}	155.0 ± 2.39^{b}	51.0 ± 0.55	39.0 ± 0.34^{c}
Triplet					
Heavy	62	4.5 ± 0.09^{c}	154.0 ± 2.60^{b}	49.8 ± 0.66	38.4 ± 0.36^{c}
Medium	62	4.0 ± 0.09^{b}	163.0 ± 2.60^{c}	49.5 ± 0.67	37.1 ± 0.37^{b}
Light	62	3.4 ± 0.09^{a}	174.0 ± 2.70^{d}	47.6 ± 0.67	35.1 ± 0.38^{a}
P value		< 0.001	< 0.001	ns	< 0.001

Values are presented as means ± standard errors

Differing superscripts within columns of main and nested effects indicate significant differences ($P < 0.05$).

Lamb plasma concentrations within five minutes of birth

Lamb birth rank and relative live weight within a litter had no effect on plasma glucose or fructose concentrations (Table 26). Relative live weight within a litter tended to have an effect on plasma lactate ($P = 0.09$) and T_4 concentrations ($P = 0.08$) and had a significant effect ($P < 0.05$) on plasma T_3 concentrations. Within a litter of twins, plasma lactate, T_4 and T_3 concentrations did not differ ($P > 0.05$) between the heaviest- and lightest-twin-born lambs. Within a litter of triplets, the heaviest-triplet-born lambs had smaller ($P < 0.05$)

plasma lactate concentrations, and greater ($P < 0.05$) plasma T_4 and T_3 concentrations than the medium- and lightest-triplet-born lambs. Within twin- and triplet-born litters, plasma lactate, T_4 and T_3 concentrations between the heaviest- and lightest-twin-born lambs and the heaviest-triplet-born lambs did not differ ($P > 0.05$).

After adjustment for lamb birth weight, birth rank and relative weight within litter had no effect on plasma lactate concentrations, where a one kg increase in lamb birth weight was associated ($P < 0.05$) with a 1.0 (± 0.44 (se)) mmol l^{-1} decrease in plasma lactate concentrations. Lamb birth weight was not associated ($P > 0.05$) with plasma glucose, fructose, T_4 and T_3 concentrations.

Lamb rectal temperatures within five minutes of birth until 12 hours of age

Within five minutes of birth and at one hour of age, lamb rectal temperatures differed ($P < 0.001$) between twin- and triplet-born lambs (Table 27). Within a twin-born litter, the heavier lambs had a greater ($P < 0.01$) rectal temperature at one hour of age than the lighter lambs. Within a triplet-born litter, lamb rectal temperature differed ($P < 0.05$) within five minutes of birth and at one hour of age.

Lamb plasma concentrations at 24 to 36 hours of age

At 24 to 36 hours of age there were no differences in plasma glucose, GGT and IgG concentrations between, and within twin- and triplet-born litters (data not shown). Lamb birth weight was not associated ($P > 0.05$) with plasma glucose, GGT and IgG concentrations.

Table 26 *The effect of birth rank (Twin, Triplet) and relative live weight within a litter (Twin Heavy, Twin Light, Triplet Heavy, Triplet Medium, Triplet Light) on lamb plasma glucose (mmol* l^{-1}*), fructose (mmol* l^{-1}*), lactate (mmol* l^{-1}*), thyroxine (T4 nmol* l^{-1}*) and tri-iodothyronine (T3: nmol* l^{-1}*) concentrations within five minutes of birth.*

	n	Glucose[1]	n	Fructose[1]	n	Lactate	n	T4	n	T3
Birth Rank										
Twin	79	0.94 ± 0.07 (2.5)	86	0.98 ± 0.08 (2.6)	87	7.05 ± 0.52^{a}	83	151.3 ± 5.77^{b}	83	2.8 ± 0.14^{b}
Triplet	88	0.85 ± 0.06 (2.3)	88	0.91 ± 0.07 (2.3)	100	8.67 ± 0.45^{b}	110	132.5 ± 4.95^{a}	99	2.2 ± 0.13^{a}
P value		ns		ns		< 0.01		< 0.01		< 0.001
Relative live weight within a litter										
Twin										
Heavy	39	0.90 ± 0.09 (2.4)	43	0.93 ± 0.09 (2.5)	43	7.05 ± 0.64^{a}	42	154.0 ± 7.19^{b}	42	2.9 ± 0.18^{b}
Light	40	0.98 ± 0.09 (2.6)	43	1.04 ± 0.09 (2.7)	44	7.15 ± 0.64^{a}	41	148.6 ± 7.26^{b}	41	2.7 ± 0.18^{b}
Triplet										
Heavy	34	0.85 ± 0.09 (2.1)	34	0.97 ± 0.09 (2.4)	38	7.41 ± 0.64^{a}	37	146.6 ± 7.11^{b}	37	2.5 ± 0.17^{b}
Medium	29	0.90 ± 0.09 (2.5)	29	0.97 ± 0.09 (2.5)	33	9.35 ± 0.69^{b}	34	125.9 ± 7.38^{a}	34	1.9 ± 0.18^{a}
Light	25	0.80 ± 0.11 (2.2)	25	0.78 ± 0.11 (2.2)	29	9.23 ± 0.72^{b}	28	124.9 ± 8.13^{a}	28	2.0 ± 0.20^{a}
P value		ns		ns		0.09		0.08		< 0.05

Differing superscripts within columns of main and nested effects indicate significant differences ($P < 0.05$).

[1] Data was log-transformed to achieve normal distribution. Transformed data with standard errors presented with back-transformed means in parenthesis. ns, non significant

Table 27 *The effect of birth rank (Twin, Triplet) and relative live weight within a litter (Twin-Heavy, Twin-Light. Triplet-Heavy, Triplet-Medium, Triplet Light) on lamb rectal temperature within five minutes of birth (°C), one, three and 12 hours of age (Median (Lower confidence interval – Upper confidence interval).*

	Rectal body temperature							
	n	Within five minutes	n	1hr	n	3hr	n	12hr
Birth rank								
Twin	135	40.2 (39.4 – 40.6)	131	40.3 (39.8 – 40.6)	131	39.9 (39.6 – 40.2)	135	39.6 (39.3 – 39.9)
Triplet	155	39.7 (39.0 – 40.3)	151	40.0 (39.4 - 40.4)	151	39.9 (39.5 – 40.3)	151	39.7 (39.3 – 39.9)
P value[1]		< 0.001		< 0.001		ns		ns
Relative live weight within a litter								
Twin								
Heavy	67	40.2 (39.6 – 40.6)	65	40.4 (40.0 – 40.7)	66	40.0 (39.6 – 40.2)	67	39.6 (39.2 – 39.8)
Light	68	40.2 (39.3 – 40.6)	66	40.1 (39.6 – 40.5)	65	39.8 (39.6 – 40.3)	68	39.6 (39.3 – 39.9)
P Value[1]		ns		< 0.01		ns		ns
Triplet								
Heavy	53	40.0 (39.5 – 40.5)	52	40.2 (39.9 – 40.5)	53	40.0 (39.6 – 40.3)	52	39.7 (39.5 – 39.9)
Medium	53	39.8 (38.9 – 40.3)	51	39.9 (39.5 – 40.4)	51	40.0 (39.6 – 40.3)	51	39.7 (39.3 – 39.9)
Light	49	39.3 (38.8 – 40.1)	48	39.8 (39.0 – 40.4)	47	39.9 (39.5 – 40.3)	48	39.6 (39.4 – 39.9)
P value[1]		< 0.05		< 0.05		ns		ns

[1] Significance was determined by Kruskal Wallis test

Discussion

The aim of the present study was to examine the physical and physiological differences between lambs within twin- and triplet-born litters from birth until 24 hours of age.

In agreement with previous studies, triplet-born lambs had greater lactate and lower plasma thyroid hormone concentrations within five minutes of birth than twin-born lambs (Stafford *et al* 2007; Chapter 2). Within five minutes of birth, the two lightest triplet-born lambs tended to have greater plasma lactate concentrations than the heaviest-triplet-born lambs, heaviest-twin-born lambs and the lightest-twin-born lambs. These results are in agreement with Stafford *et al* (2007) who showed that the lightest-triplet-born lamb had greater plasma lactate concentrations than the heaviest triplet-born lamb. Greater plasma lactate concentrations after birth can indicate signs of hypoxia (Mellor 1988), which is a physiological characteristic resulting from placental insufficiency or acute intra-partum hypoxia (Barlow *et al* 1987). Both placental insufficiency (Dwyer *et al* 2005; Stafford *et al* 2007) and birthing difficulties (Kerslake *et al* 2005), which can result in acute intra-partum hypoxia (Mellor and Stafford 2004), have previously been reported in triplet-born lambs. These events are known to impair heat production and progressive post-natal behaviours such as suckling (Eales *et al* 1982; Mellor and Stafford 2004).

In addition to greater plasma lactate concentrations, the two lightest triplet-born lambs also had lower plasma T_4 and T_3 concentrations when compared to the heaviest-triplet-born lambs, heaviest-twin-born lambs and the lightest-twin-born lambs. These results are also in agreement with Stafford *et al* (2007) who showed that the lightest-triplet-born lamb had smaller plasma T_4 concentrations that the heaviest triplet-born lamb. Lower plasma thyroid hormone concentrations have been found to be associated with poor thermoregulation, as both thyroid hormones, T_4 and T_3, are important stimulants for the onset of heat production after birth (Symonds 1995; Schermer *et al* 1996). The lower plasma thyroid hormone concentrations of the lightest-triplet-born lambs may therefore result in a lower ability to generate heat influencing the ability of the lamb to maintain its body temperature (Dwyer and Morgan 2006). In this experiment, this is further supported by the fact that lightest triplet-born lambs had a lower rectal temperature within five minutes of birth than their heavier siblings.

Within five minutes of birth, lamb birth rank and size within litter had no effect on plasma glucose and fructose concentrations, suggesting that endogenous glucose production was similar between twin- and triplet-born lambs, and within twin- and triplet-born litters. These results are in agreement with others (Barlow *et al* 1987; Stafford *et al* 2007), who showed no difference in plasma glucose concentrations between twin- and triplet-born lambs and within twin- and triplet-born litters. They are also in disagreement with others, who showed plasma fructose concentrations are different between twin- and triplet-born lambs and within triplet-born litters. The lack of difference observed in plasma glucose and fructose concentrations in this study suggest that both twin- and triplet-born lambs are able to produce a similar amount of glucose, an important energy source of non shivering thermogenesis (Mellor and Cockburn 1986), which is crucial for survival after birth.

Because newborn lamb survival is reliant on the ability of the lamb to produce heat, in this study, the greater plasma lactate concentrations and lower plasma thyroid hormone concentrations of the lightest-triplet-born lambs suggest they have a greater potential of succumbing to hypothermia than the heaviest-triplet-born lambs and all twin-born lambs. These physiological characteristics may offer some explanation as to why triplet-born lambs on average have a greater mortality rate than twin-born lambs (Thomson *et al* 2004; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008), and in turn, why the lightest-triplet-born lambs have greater mortality rates within a litter (Morel *et al* 2008). Because of the lack of differences between twin-born lambs, these physiological characteristics do not explain the greater mortality rates observed in the lightest-twin-born lamb (Morel *et al* 2009).

The ability to thermoregulate effectively after birth is not only dependent on producing heat, but also reducing heat loss. Lighter lambs have a greater surface-area-to-birth-weight ratio when compared to heavier lambs and therefore have a greater potential to lose heat to the environment (Alexander 1962c; Mount and Stephens 1970). Compared to the heaviest lambs within a twin- or triplet-born litter, the lightest lambs are at a greater risk of dying from excessive heat loss during cold conditions. Again these results may offer some explanation as to why triplet-born lambs on average have a greater mortality rate than twin-born lambs (Thomson *et al* 2004; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008), and in turn, why the lightest-triplet-born lambs have greater mortality rates within a litter (Morel *et al* 2008). In addition, it may also offer some explanation as to why the lightest-twin-born lambs have greater mortality rates within a litter (Morel *et al* 2009).

After adjusting all physiological parameters for lamb birth weight, lamb birth rank and relative size within litter had no effect on plasma lactate concentrations within five minutes of birth. This indicates that it is the actual live weight of the lamb that affects plasma lactate concentrations after birth. Increasing the birth weight of the lightest lambs within a triplet-born litter may therefore have a positive effect on plasma lactate concentrations and potentially remove any negative effects lactate may be having on lamb heat production. Lamb birth weight had no effect on plasma T_4 and T_3 concentrations after birth. This indicates that independent of lamb birth weight, the lightest-triplet-born lambs have greater plasma T_4 and T_3 concentrations than the heaviest-triplet-born lamb and the all twin-born lambs. Because plasma thyroid hormones have a positive effect on lamb heat production (Symonds 1995; Schermer *et al* 1996), these findings suggest that the relative size of a lamb within a litter may have an important impact on the ability of the lamb to produce heat.

The early consumption of colostrum can compensate for any energy reserve deficits that may have occurred during the prenatal period and is of crucial importance for the lamb to sustain heat production from 24 hours of age (Mellor and Cockburn 1986). Indices of colostrum intake, such as plasma glucose, GGT and IgG concentrations indicated that there were no differences in colostrum intake between twin- and triplet-born litters, which are in agreement with Kenyon *et al* (2005b) and Halliday (1971). Our results also suggested that there were no differences in colostrum intake within twin- and triplet-born litters, which is in disagreement with Kenyon et al (2005). The present results suggest that that survival differences between twin- and triplet-born lambs and within twin- and triplet-born litters are unlikely to be due to differences in colostrum intake. Differences between the present study and that of Kenyon et al (2005) could be due to a number of factors which affect colostrum production, such as ewe nutrition (Mellor and Murray 1985a; Banchero *et al* 2006), and colostrum intake, such as lamb behaviour and competitiveness within litters (Nowak 1996).

Conclusion

The physical and physiological impairments observed in the lightest triplet-born lambs provides some evidence as to why triplet-born lambs have a greater mortality rate than twin-born lambs, and why the lightest-triplet-born lambs have a greater mortality rate within a litter. While physiological characteristics were the same between twin-born lambs and offer no explanation for greater mortality rates of the lightest-twin-born lamb, the lightest-twin-born lambs do have a greater surface-area-to-birth-weight ratio than its heavier siblings, making it more susceptible to excessive heat loss. This may offer some explanation for the mortality differences observed within twin-born litters.

CHAPTER 7

Do twin- and triplet-born lambs within a litter produce different amounts of heat during severe cold stress?

Related Publication;

Based on a brief communication published in the Proceedings of the New Zealand Society of Animal Production

Kerslake JI, Kenyon PR, Morris ST, Stafford KJ, Morel PCH (2010) Do twin- and triplet-born lambs within a litter produce different amounts of heat during severe cold stress? New Zealand Society of Animal Production **70** *in press*

Abstract

This experiment investigated if lambs within twin- and triplet-born litters produced different amounts of heat during severe cold stress at 24 to 36 hours of age. Forty six twin- and 45 triplet-born lambs had their live weight and body size measured at birth and at 24 to 36 hours of age had their maximum heat production measured by indirect open-circuit calorimetry. Blood samples were collected before and after calorimetry measurements. The lightest-lamb within a twin- or triplet-born litter had a greater ($P < 0.001$) surface-area-to-birth-weight ratio than their heavier-sibling/s suggesting that the lightest-lamb within a twin- or triplet-born litter would lose a greater amount of heat to the environment than their heavier-sibling/s. The lightest-lamb within a triplet-born litter produced less heat during severe cold stress than all twin-born lambs and their heavier-siblings ($P < 0.01$). Once adjusted for lamb birth weight however, all twin- and triplet-born lambs produced similar amounts of heat suggesting that lamb birth rank and the relative size of the lamb within the litter had no effect on maximum heat production above and beyond that of lamb birth weight. Overall the findings suggest that compared to twin-born lambs and the heaviest-siblings within a triplet-born litter, the lightest-triplet-born lamb loses the most heat to the environment and produces the least amount of heat during severe cold exposure. These findings highlight the importance of achieving greater birth weights and ensuring greater colostrum intakes for adequate heat production after birth, especially for the lightest-triplet-born lamb. They also may offer some explanation as to why the lightest-triplet-born lambs have a greater mortality rate to weaning than their heavier-counterparts.

Introduction

An increase in the number of twin- and triplet-born lambs has the potential to increase ewe output. The greater mortality rates observed in twin- and triplet-born lambs however (Thomson *et al* 2004; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008), can limit ewe performance. Recent research has shown that within twin- and triplet-born litters, the lightest-twin- and triplet-born lambs have the greatest risk of mortality (Morel *et al* 2008; Morel *et al* 2009).

In this thesis, chapter six showed that within a litter of twins and triplets, the medium- and lightest-triplet-born lambs have the greatest surface-area-to-birth-weight ratio, lowest rectal temperature, greatest plasma lactate concentration, and lowest plasma thyroid hormone concentration within five minutes of birth. These findings are in agreement with Stafford *et al* (2007). Because surface-area-to-birth-weight ratio is associated with heat conservation (Mount and Stephens 1970), greater plasma lactate concentrations are associated with inhibited heat production (Eales and Small 1980b; Eales and Small 1985), and greater plasma thyroid hormone concentrations are associated with greater heat production (Cabello and Levieux 1981; Polk *et al* 1987; Symonds 1995), the lightest-, and medium-triplet-born lamb may have insufficient thermoregulatory capabilities when compared to twin-born lambs and their heavier sibling. This may offer some explanation as to why the lightest- and medium triplet-born lambs within a litter have a greater mortality rate when compared to its heaviest sibling, and why on average, triplet-born lambs have a greater mortality rate than twin-born lambs.

While heat production differences between twin- and triplet-born litters have been investigated in chapters four and five, heat production differences within litters has not been examined. The aim of this experiment was therefore to determine if lambs within twin or triplet-born litter produce different amounts of heat during cold stress at 24 to 36 hours of age.

Materials and Methods

This chapter is an analysis of heat production differences of lambs within twin- and triplet-born litters. This chapter uses original data and some data from chapter four and five.

Experimental design

The experimental design of the study was a 2 x 2 x 2 factorial, which included two litter sizes (Twin- and Triplet-born lambs), two maternal iodine supplementation treatments (Supplemented and Non-supplemented) and two ewe nutritional treatments (Pasture and Kale).

From mating until pregnancy day 68 (P68), 1150 mixed-age Romney crossed ewes were grazed on *ad-libitum* ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* pasture. At P68, 30 twin- and 30 triplet-bearing ewes were randomly selected from a mob of ewes (n=575) which had been injected intramuscularly in the anterior half of the neck with 1.5 ml ewe^{-1} of iodised peanut oil (Flexidine® (26% w/w of iodine bound to ethyl esters of unsaturated fatty acids in oil), Bomac Laboratories Ltd, Auckland, New Zealand) 35 days prior to the start of mating (Supplemented). Another 30 twin- and 30 triplet-bearing ewes were randomly selected from a mob of ewes (n=575) which were not injected (Non-supplemented). 14 twin- and 16 triplet-bearing ewes were randomly selected from each supplemented and non-supplemented treatment group. These ewes were grazed on kale from P68 until P120, and then on *ad-libitum* ryegrass and white clover pasture until parturition. The remaining twin- and triplet-bearing ewes were grazed on *ad-libitum* ryegrass and white clover pasture until parturition.

This experiment was conducted at Massey University Keeble farm, Palmerston North, New Zealand (lat. 40º, long. 175º, elevation: 44 m) from 8 February 2006 until 16 September 2006. Weather conditions during the lambing period consisted of an average wind speed of 3.87 m s^{-1}, total rainfall of 0.01 mm, average temp of 10.3ºC (max=12.3ºC, min= 7.2ºC), and an average wind chill of 997.1 Kj m$^{2\ -1}$ h^{-1}.

Animal measurements

Within 12 hours of birth, lambs were identified to their dam, tagged and their sex and birth rank determined. In addition, lamb birth weight, crown rump length and thoracic-girth circumference was recorded. Surface area (SA) of the lamb was calculated using the mathematical formula for the total SA of a cylinder,

$$SA\left(cm^2\right)=2\pi r h+2\pi r^2$$

where r is the thoracic-girth circumference of the lamb divided by 2π , h is the crown-rump length of the lamb.

At 24 to 36 hours of age, 18 twin- (Supplemented, n = 2; Non-supplemented, n = 16) and 34 triplet-born (Supplemented, n = 17; Non-supplemented, n = 17) lambs born to ewes grazing kale followed by pasture, and 28 twin- (Supplemented, n = 14; Non-supplemented, n = 14) and 30 triplet-born (Supplemented, n = 17; Non-supplemented, n = 13) lambs born to ewe grazing pasture only were randomly selected to determine their maximum heat production (summit metabolism) by indirect open-circuit calorimetry. Between 24 and 36 hours old a 5 ml blood sample was collected by jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA). An average cold stress index (CSI) within the first 24 to 36 hours of life was calculated using the following equation (Donnelly 1984),

$$CSI\,(kJ/m^2/h) = (11.7 + 3.1W^{0.5})\,x\,(40 - T) + 481 + 418(1 - e^{-0.04R})$$

where, W is the mean wind speed (m s^{-1}), T is the mean temperature (ºC), and R is the mean rainfall (mm).

A 10 ml blood sample was taken from the lamb via jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA) immediately prior to the lamb being placed in a crate within an indirect open circuit calorimeter (McCutcheon *et al* 1983a). The lamb's head was placed in a plastic hood, which was sealed around the neck with a collar. Air was drawn into and from the hood for measurement of oxygen consumption. A temperature probe was inserted in the rectum of the lamb for measurement of body temperature. The lamb was exposed to an environmental temperature that ranged from 6–8°C, and was allowed to acclimatize to these conditions for a total of 12 min. This period of time is referred to as the adjustment period. During this time rectal temperature and oxygen consumption measurements were taken over three successive 4 min periods to obtain a stabilized metabolic rate so a base heat production could be calculated. At the end of 12 min, lambs were exposed to wet and windy conditions to induce maximum heat production (summit metabolism). This period of time is referred to as the onset of severe cold stress. Artificial chilled rain (1°C) was applied through sprinklers at a standardized rate of (1.08 l min^{-1}) and cold air was passed over the lamb by a fan positioned behind the animal at a rate of 1.0 m s^{-1}. After 20 min cold air was passed over the lamb at a rate of 1.5 m s^{-1}, and after another 20 min at a rate of 2.0 m s^{-1}. The rate of cold air then stayed constant for the remaining period of time. Rectal temperature and oxygen consumption measurements were taken at 4 min intervals for 88 min or until the lamb reached maximum heat production, whichever occurred first. Maximum heat production was assumed to have been met when the rectal temperature of lamb declined at the rate of 1°C / 20 min and there was no further increase in the consumption rate of oxygen (Alexander 1962c). To facilitate heat loss and to encourage heat production to reach a maximum, all lambs with a birth weight above 4 kg had the wool removed from their back and sides leaving a wool depth of 3 mm. Maximum heat production in Watts (W) was calculated from oxygen consumption using the following formula (Revell *et al* 2002):

$$Maximal\ heat\ \ production(W) = oxygen\ consumption\,(l/h) \times 20.46/3.6$$

where it was assumed that 20.46 kJ of heat is produced per litre of oxygen consumed (McLean 1972). Division of oxygen consumption by 3.6 converts from kJ/h to W.

After the lamb reached maximum heat production or after 88 min in the calorimeter, the lamb was removed, dried and a 5 ml blood sample collected via jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA). Lambs were then placed in a heated room for 1 h before being returned to their dams.

Lamb plasma samples

All blood samples were placed on ice until they were centrifuged at 1000 *g* for 15 minutes. The plasma was removed and frozen at -20ºC. Lamb plasma samples were analysed for gamma-glutamyl-transferase (GGT) (Roche, diagnostics Ltd, Mannheim, Germany), glucose (hexokinase method, Roche Diagnostics Ltd, Switzerland) and NEFA (Sigma, Illinois, USA).

Statistical analysis

Within twin- and triplet-born litters, lambs were categorised from lightest to heaviest (relative weight within litter; triplet heavy, triplet medium, triplet light, twin heavy and twin light).

A general linear model (PROC GLM, SAS, 2003) was used to identify any significant differences between treatments, and between and within birth ranks for lamb physical measurements (birth weight, surface-area-to-birth-weight ratio, crown-rump length and thoracic-girth circumference), lamb heat production measurements (base heat production on a per lamb basis, base heat production on per kg of birth weight basis, maximum heat production on a per lamb basis, maximum heat production on per kg of birth weight basis), and plasma concentrations before and after calorimetry (plasma GGT concentrations at 24 to 36 hours of age, and plasma glucose and NEFA concentrations before, after and change in concentrations from before until after calorimetry). These models contained the fixed effects of maternal iodine supplementation (Non-supplemented, Supplemented), ewe nutrition (Pasture, Kale), lamb birth rank (Twin, Triplet), and their interactions. Birth weight category (Heavy, Medium, Light) was nested within birth rank (Twin, Triplet), along with its interactions. The average cold stress index within the first 24 hours of the lamb's life, the initial rectal temperature of the lamb before calorimetry, and plasma GGT concentrations at 24 to 36 hours of age were all fitted as covariates for the lamb heat production dependent variables. Lamb birth weight and weight within litter were fitted as a covariate for lamb physical measurements, crown-rump length and thoracic-girth circumference, all lamb heat production measurements, and all plasma concentrations at 24 to 36 hours of age. This identified if the dependent variables were influenced more by lamb birth rank, lamb birth weight or birth weight within litter. The interactions or covariates only remained in the general linear model if significant ($P<0.05$).

Results

Maternal iodine supplementation and ewe nutrition

Maternal iodine supplementation, ewe nutrition and their potential interactions had no effect on lamb physical measurements (birth weight, surface-area-to-birth-weight ratio, crown-rump length and thoracic-girth circumference), lamb heat production measurements (base heat production on a per lamb basis, base heat production on per kg of birth weight basis, maximum heat production on a per lamb basis, maximum heat production on per kg of birth weight basis), or plasma concentrations before and after calorimetry (plasma GGT concentrations at 24 to 36 hours of age, and plasma glucose and NEFA concentrations before, after and change in concentrations from before until after calorimetry) (data not shown). For the remainder of this paper only the differences between birth ranks and within twin- and triplet litters will be presented.

Lamb birth weight and body dimensions

Twin-born lambs had a heavier ($P < 0.001$) birth weight and a smaller ($P < 0.001$) surface-area-to-birth-weight ratio than triplet-born lambs. Twin-born lambs also had a greater thoracic-girth circumference ($P < 0.001$) than triplet-born lambs (Table 28). Within a litter of twins, the lightest-twin-born lamb was on average 700 g lighter ($P < 0.001$) than the heaviest-twin-born lamb. The lightest-twin-born lamb also had a greater ($P < 0.01$) surface-area-to-birth-weight ratio, and a smaller ($P < 0.001$) thoracic-girth-circumference than the heaviest-twin-born lamb. Within a litter of triplets, the lightest-triplet-born lamb was on average 400 g and 900 g lighter ($P < 0.001$) than the medium- and heaviest-triplet-born lamb. The lightest-triplet-born lambs had greater ($P < 0.01$) surface-area-to-birth-weight ratio, and a smaller ($P < 0.05$) thoracic-girth-circumference than the medium- and heaviest-triplet-born lambs. Within litters of twins and triplets, the heaviest-twin-born lamb had heavier ($P < 0.001$) birth weight, smaller ($P < 0.01$) surface-area-to-birth-weight-ratio, and greater ($P < 0.001$) thoracic-girth circumference than all triplet-born lambs. The birth weight, surface-area-to-birth-weight-ratio, and thoracic-girth circumference of the lightest-twin-born lamb and heaviest-triplet-born lamb did not differ ($P > 0.1$).

After adjustment for lamb birth weight however, birth rank and relative size within litter had no effect on crown-rump length or thoracic-girth circumference, where a one kg increase in lamb birth weight was associated ($P < 0.001$) with a 2.8 (± 0.56 (se)) cm and a 2.3 (± 0.32 (se)) cm increase in crown-rump length and thoracic-girth circumference, respectively.

Table 28 *The effect of birth rank (Twin, Triplet) and relative size within litter (Twin Heavy, Twin Light, Triplet Heavy, Triplet Medium, Triplet Light) on lamb birth weight (kg), surface-area-to-birth-weight ratio (cm^2 kg^{-1}), crown-rump length (cm) and thoracic-girth-circumference (cm)*

	n	Birth weight	SA : Bw	Crown-rump length	Thoracic-girth circumference
Birth Rank					
Twin	96	4.8 ± 0.06	150.6 ± 1.97	50.9 ± 0.47	39.3 ±0.29
Triplet	138	3.9 ± 0.06	167.2 ± 1.94	49.4 ± 0.46	36.6 ± 0.28
P Value		0.001	0.001	ns	0.001
Relative size within litter					
Twin					
Heavy	48	5.1 ± 0.08^d	145.2 ± 2.75^a	50.9 ± 0.66	40.1 ± 0.40^d
Light	48	4.5 ± 0.09^c	155.9 ± 2.82^b	50.8 ± 0.67	38.4 ± 0.41^c
Triplet					
Heavy	46	4.4 ± 0.10^c	155.7 ± 3.30^b	50.1 ± 0.79	37.8 ± 0.48^c
Medium	46	4.0 ± 0.10^b	166.2 ± 3.30^c	49.9 ± 0.79	36.8 ± 0.48^b
Light	46	3.4 ± 0.11^a	179.7 ± 3.47^d	48.2 ± 0.83	35.3 ± 0.50^a
P Value		0.001	0.001	ns	0.001

Data were presented as means ± standard errors; ns, non-significant

Differing superscripts within columns of relative size within litter indicate significant differences ($P < 0.05$).

Lamb heat production

Triplet-born lambs produced the same amount ($P > 0.1$) of base heat on a per lamb basis as twin-born lambs (Table 29). Within a litter of twins, the lightest- and heaviest-twin-born lamb produced the same ($P > 0.1$) amount of base heat on a per lamb basis. Within a litter of triplets, the lightest-triplet-born and medium-triplet-born lamb produced less ($P < 0.05$) base heat on a per lamb basis than the heaviest-triplet-born lamb. Within litters of twins and triplets, the heaviest-twin-born lambs and heaviest-triplet-born lambs produced a greater (*P*

< 0.001) amount of base heat on a per lamb basis than the medium- and lightest-triplet-born lambs. Between birth ranks and within litters of twins and triplets there were no significant differences in the base heat production on a per kg of birth weight basis ($P > 0.1$).

Triplet-born lambs produced less ($P < 0.05$) maximum heat on a per lamb basis than twin-born lambs (Table 29). Within litters of twins, the lightest-twin-born lamb produced the same amount ($P < 0.1$) of maximum heat on a per lamb basis. Within litter of triplets, the lightest-triplet-born lamb produced less ($P < 0.05$) maximum heat on a per lamb basis than the heaviest-triplet-born and medium-triplet-born lamb. Within litters of twins and triplets, the heaviest-twin-born, medium-twin-born, heaviest-triplet-born and medium-triplet-born lamb produced a greater ($P < 0.05$) amount of maximum heat on a per lamb basis than the lightest-triplet-born lambs. Between birth ranks and within litters of twins and triplets, there were no significant differences in the maximum heat production on a per kg of birth weight basis, or the rate to reach maximum heat production ($P > 0.1$).

After adjustment for lamb birth weight, lamb birth rank and size within litter had no effect on the base or maximum heat production per lamb, the base or maximum heat production on per kg of birth weight basis, and the rate to reach maximum heat production (pooled means ± std dev were 49.9 ± 18.3 W, 63.8 ± 21.7 W, 11.6 ± 3.01 W/kg, 15.2 ± 3.86 W/kg, and 0.15 ± 0.08 W/kg/min, respectively). Lamb birth weight had a positive effect on the base and maximum heat production on a per lamb basis. A one kg increase in lamb birth weight was associated with a 13.1 (± 2.74 (se)) W increase in base heat production ($P < 0.001$) and a 12.4 (± 3.81(se)) W increase in maximum heat production ($P < 0.001$).

Table 29 *The effect of birth rank (Twin, Triplet) and relative size within litter (Twin-Heavy, Twin-Light, Triplet-Heavy, Triplet-Medium, Triplet-Light) on lamb base heat production (W lamb^{-1}), base heat production on a per kg of birth weight basis (W kg^{-1}), maximum heat production (W lamb^{-1}), maximum heat production on a per kg of birth weight basis (W kg^{-1}), and rate to reach maximum heat production (W kg^{-1}min^{-1}).*

	Base heat production			Maximal heat production			Rate to reach MHP
	n	W	W kg^{-1}	n	W	W kg^{-1}	W kg^{-1} min^{-1}
Birth Rank							
Twin	46	41.6 ± 2.10	8.82± 0.43	35	71.0 ± 3.83	15.2 ± 0.72	0.16 ± 0.014
Triplet	64	38.0 ± 1.77	9.66 ± 0.36	56	61.2 ± 2.94	15.4 ± 0.56	0.15 ± 0.013
P Value		ns	ns		0.05	ns	ns
Relative size within litter							
Twin							
Heavy	24	44.1 ± 2.85^{b}	8.78 ± 0.59	18	76.7 ± 5.22^{b}	15.3 ± 0.98	0.17 ± 0.020
Light	22	39.0 ± 2.98^{ab}	8.86 ± 0.61	17	65.2 ± 5.33^{b}	15.2 ± 1.00	0.15 ± 0.020
Triplet							
Heavy	25	45.5 ± 2.74^{b}	10.1 ± 0.56	22	72.6 ± 4.58^{b}	16.0 ± 0.87	0.20 ± 0.023
Medium	24	35.6 ± 2.80^{a}	9.14 ± 0.58	20	64.2 ± 4.83^{b}	16.1 ± 0.92	0.13 ± 0.022
Light	15	32.5 ± 3.55^{a}	9.76 ± 0.73	14	46.8 ± 5.75^{a}	14.1 ± 1.09	0.13 ± 0.022
P Value		0.05	ns		0.01	ns	ns

Data is presented at means ± standard errors; ns, non significant;

Differing superscripts within relative size within litter indicate significant differences ($P < 0.05$).

Plasma GGT, glucose and NEFA concentrations

There were no differences between birth ranks of within litters of twins and triplets in plasma GGT concentrations at 24 to 36 hours of age (pooled means ± std dev for GGT concentrations were 2208.7 ± 1419.1 IU l^{-1}, and plasma glucose and NEFA concentrations before or after calorimetry, or in the change from before until after calorimetry (pooled means ± std dev for glucose concentrations were 6.0 ± 1.92 mmol l^{-1}, 8.8 ± 2.46 mmol l^{-1} and 2.9 ± 2.7 mmol l^{-1}, respectively, and pooled means ± std dev for NEFA concentrations were 0.9 ± 0.59 mmol l^{-1}, 1.3 ± 0.79 mmol l^{-1}, 0.4 ± 1.02 mmol l^{-1}, respectively). Birth weight had no effect on plasma GGT concentrations at 24 to 36 hours of age, and plasma glucose or NEFA concentration before or after calorimetry.

Discussion

The heaviest-twin, lightest-twin, heaviest-triplet-, and medium-triplet-born lamb produced a greater amount of heat on a per lamb basis then the lightest-triplet-born lamb. Once adjusted for lamb birth weight however, all twin- and triplet-born lambs within a litter produced the same amount of heat on a per lamb basis. This indicates that within litters of twins or triplets, the relative size of the lamb has no effect on heat production at 24 to 36 hours of age. Instead, it is the weight of the lamb that drives heat production. These findings are in disagreement with Stott and Slee (1987), who suggested that greater metabolic rates were a characteristic of litter size and not simply a reflexion of lamb birth weight. These findings are however, in agreement with those of Alexander (1962c), who showed that the summit metabolism on a per kg of birth weight basis was the same in light and heavy lambs. These results combined suggest that increasing the birth weights of twin- or triplet-born lambs would have a positive effect on lamb heat production.

The ability to thermoregulate effectively after birth is not only dependent on heat production, but also heat loss. The lightest lamb/s within a litter of twins or triplets had a greater surface-area-to-birth-weight ratio than their heaviest sibling suggesting that the lightest twin- and -triplet-born lambs would lose a greater amount of heat to the environment than their heaviest sibling (Alexander 1962c). This would also mean that the lightest lambs within twin- and triplet-born litters, would have a greater lower critical temperature, below which the lamb must generate heat to maintain homoeothermic status (Slee 1977). This would place a greater demand on energy reserves of the lightest lambs within twin- and triplet-born litters, causing a greater rate of depletion when compared to their heavier sibling (Eales *et al* 1980). These results may help explain why the lightest lambs within litters of twins and triplets have a greater mortality rate than their heavier sibling (Morel *et al* 2008; Morel *et al* 2009).

Because hypothermia is associated with excessive heat loss, limited energy reserves and/or failure to replenish energy reserves through colostrum intake (Stott and Slee 1985), it would be advantageous for the lightest-twin or triplet-born lamb to have a greater amount of energy reserves, and to consume sufficient amounts of colostrum after birth to sustain high rates of heat production (Mellor and Murray 1986). Previous research has shown however, that compared to heavy-weight lambs, light-weight lambs have less energy reserves (Alexander 1962a), and are slower at standing and successfully sucking from their mother after birth (Dwyer and Morgan 2006). In the present study, plasma glucose concentrations before the onset of calorimetry and plasma GGT concentrations, an indicator of colostrum uptake (Tessman *et al* 1997; Maden *et al* 2003), were the same within litters of twins and triplets indicating that the lightest twin- or triplet-born lambs were not disadvantaged during the uptake of colostrum. Further work in this field however, has shown large variation in plasma glucose and GGT concentrations within twin- and triplet-born lamb litters, especially between lambs born in a triplet-litter (Kenyon *et al* 2005b). While the positive relationship between colostrum intake and the ability to produce heat and maintain body temperature is well established (Eales and Small 1981), further research into the factors that cause variation within twin- and triplet-born litters in colostrum uptake, and potentially heat production, would be of interest.

Overall the results from this experiment suggest that the lightest lamb/s within a twin- and triplet-born litter would need to produce a greater amount of heat to maintain its body temperature in the same cold environment as its heavier sibling. As shown in this experiment however, the lightest-twin- and –triplet-born lambs within a twin- or triplet-born litter produce less heat during cold stress than their heaviest sibling. These factors combined suggest that the lightest-triplet-born lambs would be more susceptible to a hypothermic death (McCutcheon *et al* 1981), followed by the medium-triplet-born lambs, which in turn would be followed by the heaviest-triplet-born lamb and the lightest-twin-born lamb, then by the heaviest-twin-born lamb. This knowledge may provides some explanation as to why on average triplet-born lambs have a greater mortality rate than twin-born lambs and why the lightest-twin- and triplet-born lambs within a litter have a greater mortality rate (Morel *et al* 2008; Morel *et al* 2009).

Conclusion

The lightest-triplet-born lamb within twin and triplet-born litter not only produces less heat, but also likely to lose a greater amount of heat during severe cold stress than twin-born lambs and their heavier siblings. This is a reflection of lamb birth weight, and not its relative size within its litter. Ensuring greater lamb birth weights within triplet-born lamb litters will improve the thermoregulatory capabilities of the lamb and potentially survival.

CHAPTER 8

Physical and physiological factors associated with twin- and triplet-born lamb thermoregulation at 24 to 36 hours of age

Abstract

This study attempted to identify physical and physiological factors of twin- and triplet born lambs within the first 24 to 36 hours of life which are associated with a) maximum heat production on a per lamb basis (W $lamb^{-1}$), and a per kg of birth weight basis (W kg^{-1}), and b) the likelihood of a lamb maintaining its body temperature during 88 minutes of severe cold stress. The parturition of 46 twin- and 45 triplet-born Romney lambs were observed and lamb blood samples were taken within five minutes of birth, at three, 12 and 24 to 36 hours post-birth. Lamb birth weight, crown-rump length and thoracic-girth circumference were measured at three hours of age and their maximum heat production measured at 24 to 36 hours of age by indirect open-circuit calorimetry. Blood samples were collected from lambs directly before and after calorimetry measurements. Multiple regression analyses revealed that a) lamb birth weight accounted for 34 percent of the variation in maximum heat production on a per lamb basis ($P < 0.001$), whereas, inclusion of plasma non-esterified fatty acids (NEFA; $P < 0.01$), tri-iodothyronine (T_3; $P < 0.05$) and glucose concentrations at 24 to 36 hours of age ($P < 0.05$) increased the accountability to 59 percent, b) plasma NEFA concentrations at 24 to 36 hours of age accounted for 15 percent of the variation in maximum heat production on a per kg of birth weight basis ($P < 0.01$), whereas, inclusion of plasma T_3 at 24 to 36 hours of age increased the accountability to 22 percent ($P < 0.05$), and c) that for every one nmol l^{-1} increase ($P < 0.01$) in plasma T_3 concentrations at three hours of age or one mmol l^{-1} increase ($P < 0.05$) in plasma fructose concentrations within five minutes of birth, twin- and triplet-born lambs were 1.8 times more likely and 1.4 times less likely to maintain their body temperature during severe cold stress, respectively. These results suggest that having greater lamb birth weights, plasma thyroid hormone, glucose and NEFA concentrations at 24 to 36 hours of age and lower plasma fructose concentrations within five minutes of birth is beneficial for twin- and triplet-born lamb thermoregulation during periods of cold stress. Practical means of increasing these factors could increase the ability of the lamb to produce heat and could be beneficial for lamb heat production. There was also a lot of variation unaccounted for in the models, and therefore further investigation into the factors which affect heat production would be of benefit.

Introduction

In New Zealand there has been a particular focus on increasing the litter size of the ewe (Anon 2008) to improve lamb production and increase on-farm profit. The lower survival rates of triplet-born lambs (Nicoll *et al* 1999; Kerslake *et al* 2005; Everett-Hincks and Dodds 2008) however, has limited the potential production of increasing ewe litter size, and in turn, has created both an economic (Amer *et al* 1999) and potential welfare (Mellor and Stafford 2004; Dwyer 2008b) concern for the New Zealand sheep industry.

The results of chapter two to seven in this thesis have consistently indicated that triplet-born lambs have lighter birth weights and lower plasma thyroid hormone concentrations when compared to twin-born lambs. These results are in agreement with those of Barlow *et al* (1987) and Stafford *et al* (2007). The lighter birth weights and lower plasma thyroid hormone concentrations of triplet-born lambs suggest that they have a greater capacity to lose heat and a reduced capacity to produce heat when compared to twin-born lambs. The observed lower rectal temperature of triplet-born lambs at birth in chapters two, five and six which is supported in the literature by Barlow *et al* (1987), Stafford *et al* (2007) and Dwyer and Morgan (2006), and the lower heat production on a per lamb basis in chapter five and seven, support this statement. Identification of physical and physiological factors which are associated with the lambs' ability to produce heat and/or remain homoeothermic during a cold stress event will provide useful information on which factors should be targeted to improve the thermoregulation capability of the triplet-born lamb. Practical on-farm management strategies, which could improve the physical and physiological impairments identified, could then be used to improve triplet-born lamb survival.

The aim of this study is therefore to identify physical and physiological factors associated with: a) maximum heat production on a per lamb basis (W lamb^{-1}) and per kg of birth weight basis (W kg^{-1}), and b) the likelihood of a lamb maintaining its body temperature during a cold stress event.

Materials and Methods

This chapter is an analysis of physical and physiological factors which influence neonatal lamb heat production at 24 to 36 hours of age and their ability to remain homoeothermic during a cold stress event. This chapter uses the same data from chapter 7, where full details of experimental design and animal measurements are presented in chapter 7.

Briefly, the experimental design of the study was a 2 x 2 x 2 factorial, which included two litter sizes (Twin and Triplet), two maternal iodine supplementation treatments (Supplemented and Non-supplemented) and two ewe nutritional treatments (Pasture and Kale).

Eleven hundred and fifty mixed-aged Romney crossed ewes were grazed on *ad-libitum* ryegrass *(Lolium perenne)* and white clover pasture *(Trifolium repens)* from pregnancy scanning until pregnancy day 68 (P68). At P68, 30 twin- and 30 triplet-bearing ewes which had been supplemented with iodine were randomly selected from a mob of ewes (n=575). In addition, 30 twin- and 30 triplet-bearing ewes which has not been supplemented were randomly selected from a mob of ewes (n=575). Fourteen twin- and 16 triplet-bearing ewes were randomly selected from each of the supplemented and non-supplemented treatment groups and grazed on kale from P68 until P120, and then on *ad-libitum* ryegrass and white clover pasture until parturition. The remaining twin- and triplet-bearing ewes were grazed on *ad-libitum* ryegrass and white clover pasture from P68 until parturition.

Fifty three twin- and 44 triplet-bearing ewes which showed signs of parturition were moved quietly to a temporary pen (5 x 3 m) located in the front of their paddock. Ewes which were penned gave birth with minimal disturbance, but within five minutes of the birth of each lamb, the lamb was tagged and its birth rank recorded. Also at this time, two 5 ml blood samples were collected by jugular venepuncture (Lithium heparin and potassium oxalate vacutainer, Becton Dickson Vacutainer Systems, USA). At three hours of age the lamb was weighed, its sex determined and crown-rump length and thoracic-girth circumference measured. An additional 5 ml blood sample was collected by jugular venepuncture (Lithium heparin vacutainer, Becton Dickson Vacutainer Systems, USA) at 24 hours of age. At 24 to 36 hours of age, 18 twin- (Supplemented, n = 2; Non-supplemented, n = 16) and 34 triplet-born (Supplemented, n = 17; Non-Supplemented, n = 17) lambs born to ewes which grazed kale followed by pasture, and 28 twin- (Supplemented, n = 14; Non-supplemented, n = 14) and 30 triplet-born (Supplemented, n = 17; Non-supplemented, n = 13) lambs born to ewes which grazed pasture only were randomly selected to determine their maximum heat production (summit metabolism) by indirect open-circuit calorimetry. Maximum heat production was assumed to have been met when the rectal temperature of lamb declined at the rate of 1°C / 20 min and there was no further increase in the consumption rate of oxygen (Alexander 1962c). To facilitate heat loss and to encourage heat production to reach a maximum, all lambs with a birth weight above 4 kg had the wool removed from their back and sides leaving a wool depth of 3 mm. Maximum heat production was calculated from oxygen consumption using the following formula (Revell *et al* 2002):

$$Maximum\ heat\ \ production(W) = oxygen\ consumption\,(l/h) \times 20.46/3.6$$

where it was assumed that 20.46 kJ of heat is produced per litre of oxygen consumed (McLean 1972). Division of oxygen consumption by 3.6 converts from kJ h^{-1} to W.

After the lamb reached maximum heat production or after 88 min in the calorimeter, the lamb was removed, dried and a 5 ml blood sample collected via jugular venepuncture (Lithium heparin, Becton Dickson Vacutainer Systems, USA). Lambs were then placed in a heated room for 1 h before being returned to their dams.

Lamb plasma samples

All blood samples were placed on ice until they were centrifuged at 1000 *g* for 15 minutes. The plasma was removed and frozen at -20ºC. Only the blood samples from lambs whose heat production was measured via indirect calorimetry were analysed. Lamb plasma samples were analysed for glucose (hexokinase assay, Roche Diagnostics Ltd, Switzerland), fructose (Enzymatic assay, Biopharm, Darmstadt, Germany), lactate (lactate oxidase/peroxidase assay, Roche Diagnostics Ltd, Switzerland), thyroxine (T_4) and tri-iodothyronine (T_3) (Radioimmunoassay diagnostic kit, Coat-A-Count, Diagnostic Products Corporation, CA, USA), gamma-glutamyl-transferase (GGT) (Roche, diagnostics Ltd, Mannheim, Germany), NEFA (Sigma, Illinois, USA), and Immuno-globulin (IgG) (Standard direct ELISA assay).

Statistical analysis

Univariate and multivariate analyses were conducted to identify which physical (birth weight, surface-area-to-birth-weight ratio) and physiological factors within 24 to 36 hours of age (plasma glucose, fructose, lactate, T_4, T_3 concentrations within five minutes of birth, plasma T_4, T_3 and creatine kinase concentrations at three hours of age, plasma GGT and IgG concentrations at 24 hours of age, plasma glucose, NEFA, T_4 and T_3 concentrations directly before calorimetry, and plasma glucose and NEFA concentrations directly after calorimetry) were associated with maximum heat production on a per lamb basis (W lamb^{-1}) and on a per kg of birth weight basis (W kg^{-1}), and with the likelihood of a lamb maintaining its body temperature during a cold stress event. Of the 46 twin- and 64 triplet-born lambs that had their heat production measured, 35 twin- and 56 triplet-born lambs reached maximum heat production within 88 minutes. Eleven twin- and eight triplet-born lambs did not reach maximum heat production within 88 minutes.

The datasets for metabolite and hormone concentrations contained some missing values due to the following reasons: a) if the lamb was dead at birth blood samples and rectal temperatures were not measured, b) blood samples that were not obtained after two attempts were not collected, c) blood samples and rectal temperatures that were not taken within 10 minutes of the required time were not included in the analyses, and d) if the rectal temperature of a lamb fell below 36°C during the observation period, no further measurements were taken and the lamb was removed from the remainder of the study.

A general linear model (PROC GLM, SAS, 2003) was first used to identify if maternal iodine supplementation, ewe nutrition, lamb birth rank and their potential interactions had any effect on lamb physical and physiological factors within the first 24 to 36 hours of life. Because there were no significant effects ($P > 0.1$) of maternal iodine supplementation, ewe nutrition and their potential interactions on the physical and physiological factors measured, only the differences between birth ranks were analysed further.

Univariate analysis of factors associated with maximum heat production

A general linear model (PROC GLM, SAS, 2003) was used to identify any significant associations ($P < 0.1$) between each physical and physiological variable and maximum heat production on a per lamb basis (W lamb^{-1}) and per kg of birth weight basis (W kg^{-1}). An ANCOVA was also used to identify any slope differences between lamb birth ranks for any significant associations found.

Multivariate analysis of factors associated with maximum heat production

All significant variables found from the univariate analysis above were entered into a multiple linear regression model (PROC REG, SAS, 2003). A forward selection process was employed for the development of the model. If the variables were statistically significant at $P < 0.05$ they remained in the model. The final model was checked for multicolinarity using the tolerance inflation factor.

Univariate analysis of factors associated with the ability to maintain body temperature

A logistic regression model (PROC LOGISTIC, SAS, 2003) was used to identify and significant associations with the likelihood of a lamb maintaining its body temperature during a cold stress event. Unadjusted odds ratios and 95% confidence intervals were produced by entering every physical and physiological variable one at a time into the model. Any variable with a wald statistic P value < 0.1 was retained for inclusion in the multivariate model.

Multivariate analysis of factors associated with the ability to maintain body temperature

All significant variables found from the univariate analysis mentioned above were entered into a multiple logistic regression model (PROC LOGISTIC, SAS, 2003). A backward stepwise selection process was employed for development of the model. At each step, the variable with the highest Wald statistic P value was omitted and the model re-calculated. This was continued until the final main-effects model only included variables that were significant, using a threshold P value for inclusion of 0.05. Biological plausible two-way interactions were then added one at a time to the model and retained only if they were associated with a P value < 0.05.

Correlations

Correlations between physical and physiological factors were analysed using the Pearson correlation coefficient (PROC CORR, SAS, 2003).

Results

Maternal iodine supplementation and ewe nutrition

Maternal iodine supplementation, ewe nutrition and their potential interactions had no ($P >$

0.1) effect on lamb physical and physiological measurements analysed in this analysis (data not shown). For the remainder of this paper only the differences between birth ranks will be presented.

Factors associated with lamb heat production on a per lamb basis

Univariate analysis

An increase in birth weight, plasma T_3 and T_4 concentrations within five minutes of birth and at three hours of age, plasma IgG, NEFA, T_4 and T_3 concentrations at 24 to 36 hours of age had a positive effect ($P < 0.1$) on maximum heat production on a per lamb basis (Table 30). No slope differences ($P > 0.1$) were found between twin- and triplet-born lambs for any significant associations found (data not shown).

Table 30 *Univariate analysis of the physical and physiological factors which had an affect (P < 0.1) on maximum heat production on a per lamb basis (W lamb^{-1}) at 24 to 36 hours of age.*

Variable	n	Intercept	Slope ± SE	*P* Value	R^2 (%)
Birth weight (kg)	110	-12.0	18.5 ± 2.31	< 0.001	37.3
Glucose @24-36 h of age (mmol l^{-1})	104	34.2	5.6 ± 1.24	< 0.001	16.8
T_3 @24-36 h of age (nmol l^{-1})	103	31.7	7.1 ± 1.86	< 0.001	12.6
T_4 @24-36 h of age (nmol l^{-1})	104	35.6	0.2 ± 0.06	< 0.001	11.8
NEFA @24-36 h of age (mmol l^{-1})	103	55.7	12.4 ± 3.60	< 0.001	10.6
T_4 @ 3 hours of age (nmol l^{-1})	100	41.4	0.16 ± 0.05	< 0.01	8.0
T_4 @ 5 min of birth (nmol l^{-1})	101	46.1	0.15 ± 2.73	< 0.01	7.0
T_3 @ 3 hours of age (nmol l^{-1})	100	50.2	3.94 ± 1.66	< 0.05	5.5
IgG @ 24-36 hours of age (mg ml^{-1})	104	60.5	0.16 ± 0.09	< 0.1	3.4
T_3 @ 5 min of birth (nmol l^{-1})	101	57.4	4.57 ± 2.46	< 0.1	3.4

T_3, tri-iodothyronine; T_4, thyroxine; NEFA, Non esterified fatty acids; IgG, immunoglobulin G

The linear regression equations are of the form y = intercept + slope x variable

Multivariate analysis

The final multiple regression analysis revealed that lamb birth weight accounted for 34 percent of the variation in maximum heat production on a per lamb basis, whereas, inclusion of plasma NEFA, T_3 and glucose concentrations before calorimetry increased the accountability to 59 percent (Table 31).

Table 31 *Multivariate analysis of the physical and physiological factors which had an affect on ($P < 0.05$) maximum heat production on a per lamb basis (W lamb^{-1}) at 24 to 36 hours of age.*

Variable	n	Slope	*P* Value	Partial R^2 (%)	Model R^2 (%)
Intercept		-12.0 ± 12.4	< 0.001		
Birth weight (kg)	84	18.3 ± 2.36	< 0.001	34	34
NEFA @24-36 h of age (mmol l^{-1})	84	10.4 ± 3.39	< 0.01	5	39
T_3 @24-36 h of age (nmol l^{-1})	84	5.09 ± 2.08	< 0.05	4	43
Glucose @24-36 h of age (mmol l^{-1})	84	2.66 ± 1.27	< 0.05	16	59

T_3, tri-iodothyronine; NEFA, Non esterified fatty acids

The multiple regression equation are of the form y = -12.0 + 18.3(birth weight) + 10.4(NEFA before calorimetry) + 5.09(T_3 before calorimetry) + 2.66(glucose before calorimetry).

Factors associated with lamb heat production on a per kg of birth weight basis

Univariate analysis

An increase in the surface-area-to-birth-weight ratio of the lamb had a negative effect ($P < 0.1$) on maximum heat production on a per kg of birth weight basis (Table 32), where an increase in plasma IgG, glucose, NEFA, T_4 and T_3 concentrations before calorimetry had a positive effect ($P < 0.1$) on maximum heat production on a per kg of birth weight basis. No slope differences ($P > 0.1$) were found between twin- and triplet-born lambs for any significant associations found.

Table 32 *Univariate analysis of the physical and physiological factors which had an effect ($P < 0.1$) on maximum heat production on a per kg of birth weight basis (W kg^{-1}) at 24 to 36 hours of age.*

Variable	n	Intercept	Slope ± SE	*P* Value	R^2 (%)
NEFA @24-36 h of age (mmol l^{-1})	103	13.1	0.07 ± 0.59	< 0.001	14.7
Glucose @24-36 h of age (mmol l^{-1})	104	10.6	0.83 ± 0.21	< 0.001	13.1
T_3 @24-36 h of age (nmol l^{-1})	103	10.4	1.00 ± 0.32	< 0.01	9.1
T_4 @24-36 h of age (nmol l^{-1})	104	12.0	0.03 ± 0.01	< 0.02	5.1
Surface-area-to-birth-weight ratio	110	21.2	-0.53 ± 0.02	< 0.05	3.8
IgG @ 24-36 hours of age (mg ml^{-1})	104	14.3	0.03 ± 0.01	< 0.08	3.0

T_3, tri-iodothyronine; T_4, thyroxine; NEFA, Non esterified fatty acids; IgG, immunoglobulin G

The linear regression equations are of the form y = intercept + slope (variable).

Multivariate analysis

The final multiple regression analysis revealed that plasma NEFA concentrations before calorimetry accounted for 15 percent of the variation in maximum heat production on a per kg of birth weight basis, whereas, inclusion of plasma T_3 before calorimetry increased the accountability to 22 percent (Table 33).

Table 33 *Multivariate analysis of the physical and physiological factors which affect ($P < 0.05$) maximum heat production on a per kg of birth weight basis (W kg^{-1}) at 24 to 36 hours of age.*

Variable	n	Slope	*P* Value	Partial R^2 (%)	Model R^2 (%)
Intercept		11.6 ± 3.30	< 0.001		
NEFA @24-36 h of age (mmol l^{-1})	101	1.89 ± 0.61	< 0.01	14.8	14.8
T_3 @24-36 h of age (nmol l^{-1})	101	0.63 ± 0.34	< 0.05	6.6	21.5

T_3, tri-iodothyronine; NEFA, Non-esterified fatty acids.

The multiple regression equation are of the form y = 11.6 + 1.89(NEFA before calorimetry) + 0.63(T_3 before calorimetry).

Factors associated with the ability of the lamb to maintain its body temperature during severe cold stress

Univariate analysis

An increase in lamb birth weight, plasma T_4 concentrations within five minutes of birth, and plasma T_4 and T_3 concentrations at three hours of age had a positive influence ($P < 0.1$) on the ability of twin- and triplet-born lambs to maintain their body temperature during severe cold stress (Table 34). An increase in plasma fructose or lactate concentrations within five minutes of birth had a negative effect ($P < 0.1$) on the ability of the lamb to maintain its body temperature during severe cold stress. No slope differences were found between birth ranks for any of the significant associations found (data not shown).

Table 34 *Unadjusted odds ratios (OR) and 95% confidence intervals (CI) for twin- and triplet-born lamb physical and physiological factors at birth and at three hours of age which were associated (P < 0.1) with the likelihood of maintaining their body temperature during a cold stress event.*

Variable	Maintained	Not maintained	*P* Value	OR (95% CI)
	n	n		
Birth weight (kg)	15	76	< 0.01	1.7 (0.93-3.21)
T_3 @ 3 hours of age (nmol l^{-1})	13	70	< 0.01	1.7 (1.15-2.64)
T_4 @ 3 hours of age (nmol l^{-1})	13	70	0.02	1.0 (1.00-1.03)
Fructose @ 5 min of birth (mmol l^{-1})	13	69	0.03	0.7 (0.45-0.97)
T_4 @ 5 min of birth (nmol l^{-1})	13	69	0.06	1.0 (1.00-1.03)
Lactate @ 5 min of birth (mmol l^{-1})	14	69	0.07	0.8 (0.69-1.01)

T_3, tri-iodothyronine; T_4: thyroxine; OR, odds ratio; CI, confidence interval

Multivariate analysis

The final multivariate results indicated that for every one nmol l^{-1} increase in plasma T_3 concentrations at three hours of age, twin- and triplet-born lambs were 1.8 times more likely to maintain their body temperature during severe cold stress (Table 35). For every one mmol l^{-1} increase in plasma fructose concentrations within five minutes of birth, twin- and triplet-born lambs were 1.4 times more likely not to maintain their body temperature during severe cold stress.

Table 35 *Multivariate logistic regression analysis of twin- and triplet-born lamb physical and physiological factors which are associated with maintaining body temperature during a cold stress event.*

Variable	Maintained	Not maintained	*P* Value	OR (95% CI)
	n	n		
T_3 @ 3hr of age (nmol l^{-1})	16	79	0.01	1.8 (1.15-2.76)
Fructose @ five min of birth (mmol l^{-1})	16	79	0.05	0.6 (0.49-1.00)

T_3: tri-iodothyronine, T_4: thyroxine; OR, odds ratio; CI, confidence interval

Correlations

Significant correlations ($P < 0.05$) found between physical and physiological factors are presented in table 36. Lamb birth weight was negatively correlated to plasma lactate concentrations within five minutes of birth ($R = -0.24$), positively correlated with plasma thyroxine concentrations within five minutes of birth, and at three and 24 hours of age (R = 0.37, 0.37, 0.24, respectively), and positively correlated with plasma tri-iodothyronine within five minutes of birth and at three hours of age (R = 0.29 and 0.34, respectively). Plasma fructose concentrations within five minutes of birth were positively correlated with plasma lactate concentrations within five minutes of birth ($R = 0.45$). Plasma lactate concentrations within five minutes of birth were negatively correlated with plasma T_4 and T_3 concentrations within five minutes of birth and three hours of birth (R = -0.33, -0.42 and -0.29, -0.23, respectively). Plasma IgG concentrations at 24 to 36 hours of age were positively correlated with plasma GGT ($R = 0.47$), glucose ($R = 0.40$) and NEFA ($R = 0.22$) concentrations at 24 to 36 hours of age. Plasma GGT and glucose concentrations were also positively correlated with plasma T_3 concentrations at 24 to 36 hours of age (R = 0.31 and 0.49, respectively).

Table 36 *Correlations of lamb birth weight, plasma metabolites and hormones within 5 minutes of birth (5), 3(3) and 24 (24) hours of age with correlations coefficients and P values presented above the diagonal and number of animals included below*

	Bw	Gl(5)	Fr(5)	La(5)	T4(5)	T3(5)	T4(3)	T3(3)	T4(24)	T3(24)	Ig(24)	Gg(24	Gl(24)	Ne(24)
Bw	-			-0.24	0.37	0.29	0.37	0.34	0.24					
Gl(5)	95	-	0.75	0.48					031	0.22				
Fr(5)	101	94	-	0.45										
La(5)	102	93	99	-	-0.33	-0.42	-0.29	-0.23			-0.20			
T4(5)	101	94	101	99	-	0.29	0.81	0.39	0.52	0.12				0.25
T3(5)	101	94	101	99	101	-	0.33	0.49		0.30			0.32	
T4(3)	100	89	95	96	95	95	-	0.49	0.57					
T3(3)	100	89	95	96	95	95	100	-	0.40	0.39				
T4(24)	104	93	99	100	99	99	98	98	-	0.58			0.19	
T3 (24)	103	92	98	99	98	98	97	97	103	-		0.31	0.49	
IgG(24)	104	93	99	100	99	99	98	98	104	103	-	0.47	0.40	0.22
Gg(24)	75	71	73	74	73	73	72	72	73	73	73	-	0.42	
Gl(24)	104	93	99	100	99	99	98	98	103	102	103	73	-	0.36
Ne(24)	103	92	98	99	98	98	97	97	102	101	102	72	103	-

Bw, birth weight; Gl, Glucose, Fr, Fructose; La, Lactate. T4, thyroxine, T3, tri-iodothyronine, Ig, mmunogloblulin, Gg, Gamma-glutyl-tranferase, Ne; Non-esterified fatty acids

Blank cells indicate non-significant correlations (*P* > 0.05), filled cells indicate significant correlations (*P* < 0.05)

Discussion

This analysis attempted to identify physical and physiological factors within the first 24 to 36 hours of life which were associated with a) maximum heat production on a per lamb and per kg of birth weight basis and b) the likelihood of a lamb maintaining its body temperature during a cold stress event. This will provide useful information on which factors should be targeted to improve the thermoregulation capability of the triplet-born lamb.

Lamb birth weight was positively associated with maximum heat production on a per lamb basis. The positive association between lamb birth weight and heat production (Alexander 1962c; Chapter four), the negative association between birth weight and surface-area-to-birth-weight ratio, and the positive association between surface-area-to-birth-weight ratio and heat loss are already well documented (Alexander 1962c; Slee 1977; Alexander 1979; McCutcheon *et al* 1983b). Practical means of increasing lamb birth weights, and thus reducing the surface-area-to-birth-weight ratios should therefore have positive effects on the ability of the lamb to produce and conserve heat. Increasing lamb birth weights, through such practical means as improving maternal nutrition during pregnancy (Robinson *et al* 1999, chapter three) and mid pregnancy shearing (Kenyon *et al* 2006), should improve the lambs' chances of survival during a cold stress event.

Physiological factors which were associated with maximum heat production on a per lamb basis include an increase in plasma glucose, NEFA and T_3 concentrations directly before the onset of cold stress. The latter of these factors were also associated with maximum heat production on a per kg of birth weight basis. Glucose and NEFA are both important energy sources for the production of heat (Eales and Small 1981; Mellor and Cockburn 1986), where greater concentrations of plasma glucose have previously been identified as one of the most important determinants for high rates of heat production in the newborn lamb (Eales and Small 1986). Plasma T_3 concentrations are also important for production of heat, where T_3 increases the expression and activation of UCP-1 (Dauncey 1990; Symonds *et al* 2000), the proton channel found in brown adipose tissue which is responsible for heat production (Nedergaard *et al* 2001). In addition, associations between low plasma thyroid hormone concentrations and low rectal temperatures and increased susceptibility to hypothermia have previously been reported (Caple and Nugent 1983). Having greater plasma concentrations of T_3, glucose and NEFA directly before cold stress, should therefore have a positive effect on maximum heat production and consequently the ability of the lamb to thermoregulate during cold stress. In this analysis, plasma GGT and/or plasma IgG concentrations at 24 to 36 hours of age, which were both measured as indicators of colostrum intake (Maden *et al* 2003; Kenyon *et al* 2005b), were found to be positively correlated with plasma glucose, NEFA and T_3 concentrations directly before cold stress. One potential practical way of increasing plasma T_3, glucose and NEFA concentrations may therefore be to ensure that the newborn lambs has been well fed and has access to a plentiful colostrum and/or milk supply. Previous experiments have shown that the intake of colostrum can increase the plasma concentrations of glucose and NEFA, which in turn can increase summit metabolism by 17-20% (Eales and Small 1980a; Eales and Small 1981). In addition, feeding 20-50 ml of milk to cold-exposed lambs can increase the plasma concentrations of T_3, colonic temperature and the thermogenic activity of brown adipose tissue (Symonds 1995; Gate *et al* 1999). The intake of colostrum is not only important for providing energy for heat production but also for developing a bond between the mother and the young (Nowak *et al* 1997) and sustaining teat-seeking behaviours (Alexander and Williams 1966). Another potential practical approach to increase lamb plasma thyroid hormone concentrations are to ensure that maternal plasma concentrations of iodine and selenium are of a sufficient status. Maternal iodine and selenium deficiency during late pregnancy have both been shown to decrease plasma T_4 or T_3 concentrations of the newborn (Caple and Nugent 1983; Arthur *et al* 1999). Supplementing ewes with iodine and/or selenium in late pregnancy has shown mixed results however, with maternal iodine and/or selenium supplementation having both a positive (Andrewartha *et al* 1980; Donald *et al* 1994; Rock *et al* 2001; Rose *et al* 2007) and negative (Boland *et al* 2008) effect on plasma T_4 and/or T_3 concentrations of the newborn lamb. In chapter five of this thesis, supplementing ewes with iodine had no effect. Differences between studies may be due to amount and route of delivery for iodine and selenium supplementation.

Plasma fructose concentrations within five minutes of birth and plasma T_3 concentrations at three hours of age were the main physiological factors found to be associated with the likelihood of a lamb maintaining its body temperature during severe cold stress. While an increase in plasma T_3 concentrations increased the likelihood of the lamb maintaining its body temperature during severe cold stress, an increase in plasma fructose concentrations decreased the likelihood. Because plasma fructose concentrations have previously been suggested as being an important energy source for the newborn lamb (Barlow *et al* 1987; Stafford *et al* 2007), it is unclear why an increase in plasma fructose concentrations would have a negative impact on the ability of the lamb to maintain its body temperature. In the present analysis however, plasma fructose concentrations were found to be positively correlated with plasma lactate concentrations, which in turn, were negatively correlated with thyroid hormones. While only plasma thyroid hormone concentrations were found to be positively correlated in the present analysis, both greater plasma lactate concentrations and lower plasma thyroid hormones have been previously reported as having a negative impact on lamb heat production (Eales and Small 1980b; Eales and Small 1985; Cabello and Levieux 1981; Polk *et al* 1987; Symonds 1995).

Overall these results suggest that greater lamb birth weights, plasma thyroid hormone, glucose and NEFA concentrations and lower plasma fructose concentrations should help improve lamb thermoregulation during periods of cold stress. These physical and physiological factors however only accounted for 59 and 23 percent of maximum heat production on a per lamb basis and on a per kg of birth weight basis, respectively. While practical means of improving these physical and physiological factors in the newborn lamb may offer some improvements in newborn lamb thermoregulation, further research is also needed to explain the large amount of unaccounted variation in heat production.

DISCUSSION

Overview

The greater mortality rates observed in triplet-born lambs is an economic and potential welfare concern for the New Zealand sheep industry. While there has been a substantial amount of research on the physical and physiological factors which affect lamb survival, there has been a limited research looking at why lambs from a larger litter have greater mortality rates than lambs from a smaller litter.

The objectives of this thesis were therefore to identify physical and physiological differences between lambs of different birth ranks, and to use this information to identify practical on-farm management strategies which could improve triplet-born lamb survival.

The results of this thesis consistently indicated that triplet-born lambs had lighter birth weights and lower plasma thyroid hormone concentrations when compared to twin-born lambs. This suggested that compared to twin-born lambs, triplet-born lambs may have a greater capacity to lose heat and a reduced capacity to produce heat. The observations of lower rectal temperatures at birth and the lower heat production on a per lamb basis support this statement. Two practical on-farm management strategies which were trialled in this thesis to improve triplet-born lamb thermoregulation were offering concentrate supplement during late pregnancy to improve lamb birth weight, and maternal iodine supplementation to improve lamb thyroid hormone concentrations. While offering concentrate showed positive effects, such as increasing lamb birth weight, colostrum uptake and triplet-born lamb heat production on a per kg of birth weight basis, the results were either inconsistent across experiments or between birth ranks suggesting additional work is required to determine the repeatability and cost effectiveness of these findings. Maternal iodine supplementation offered no benefits in terms of lamb birth weight, plasma thyroid hormone concentrations or lamb heat production. Further investigations within twin- and triplet-born litters identified that the lightest- and medium-triplet-born lambs had greater plasma lactate and lower plasma thyroid hormone concentrations than the heaviest-triplet-born lambs and the heaviest- and lightest-twin-born lambs. This suggested that compared to all twin-born lambs and their heavier sibling, the medium- and lightest-triplet-born lambs may have a greater capacity to lose heat and a reduced capacity to produce heat after birth. Within twin- and triplet-born litters, the lightest-triplet-born lambs were observed to have the lowest heat production on a per lamb basis. Overall these investigations suggest that the lightest lambs of the triplet-born litter have the greatest susceptibility to hypothermia during a cold stress event. Multiple regression analyses identified that lamb birth weight, thyroid hormone concentrations, glucose and non-esterified fatty acids (NEFA) concentrations are positively associated with maximum heat production. On-farm management strategies which could improve these physical and physiological factors may offer some improvement in lamb heat production and the survival rates of triplet-born lambs.

This thesis has identified and confirmed that triplet-born lambs, especially the lightest-triplet-born lambs, are physically and physiologically different to twin-born lambs. It has also identified that these lambs produce less heat on a per lamb basis. Multiple regression analyses also revealed which physical and physiological factors should be targeted by future on-farm management strategies to improve lamb heat production. While this thesis did not successfully identify on-farm management strategies to improve triplet-born lamb survival, it should provide valuable information for future direction and investigation.

Summary of experimental chapters and conclusions drawn

The first experiment in this thesis confirmed triplet-born lambs had lighter birth weights and greater surface-area-to-birth weight ratios than single- and twin-born lambs. It also identified that triplet-born lambs had lower rectal temperatures and lower plasma glucose, fructose and thyroid hormone concentrations within five minutes of birth than single- and twin-born lambs. These results combined suggested that triplet-born lambs were likely to lose a greater amount of heat to the environment than twin-born lambs during a cold stress event. They also indicated that compared to single- and twin-born lambs, triplet-born lambs may have less energy and/or stimulatory factors for heat production. Overall, the results suggest that triplet-born lambs may have inadequate thermoregulatory capabilities when compared to single- and twin-born lambs.

Based on the results of chapter two it was hypothesised that on-farm management strategies which could improve triplet-born lamb birth weights may have a positive effect on the amount of heat the lamb loses to the environment and the amount of heat it can produce. In addition, interventions which result in improvements of physiological impairments, such as low plasma glucose, fructose and thyroid hormone concentrations, may have a positive effect on the thermoregulation capabilities of triplet-born lambs.

Improving the nutrient intake of the ewe during late pregnancy is one management strategy which can have a positive effect on lamb birth weight. Because triplet-bearing ewes appear to be unable to consume enough ryegrass and white clover in late pregnancy to meet the energy requirements of their fetuses, it was hypothesised that offering concentrate during this time would improve the nutrient intake of the ewe and consequently improve the growth and development of all three fetuses. Offering concentrate had a positive effect on lamb birth weights in chapter three, but had no effect in chapter four. Production differences between chapters may have arisen from different substitution rates of pasture for concentrate on different quality pastures and/or differences in the live weight and/or body condition of the ewes. Unfortunately herbage and concentrate intakes were not measured in both chapters three and four, and so this theory can only hypothesised.

Chapter four showed that offering concentrate supplement had no effect on the maximum amount of heat a twin- or triplet-born lamb produced on a per lamb basis, but had a positive effect on the maximum amount of heat a triplet-born lamb produced on a per kg of birth weight basis. The same positive effect on a per kg of birth weight basis was not observed in twin-born lambs. While this may suggest that the endothermic mechanisms of the triplet-born lamb were more sensitive to ewe nutrition during late pregnancy, the exact reasons for the differences observed were not identified in this thesis. Both twin- and triplet-born lamb birth weights were found to be positively associated with maximum heat production on a per lamb basis, but only triplet-born lamb birth weights were found to be positively associated with maximum heat production on a per kg of birth weight basis. The same positive association was not observed with twin-born lamb birth weights. Because of their lighter birth weights, triplet-born lambs have the potential to lose a greater amount of heat to the environment and are likely to have less energy reserves than single- and twin-born lamb. The efficient use of energy reserves to sustain high rates of heat production during cold stress would therefore be of particular importance for the triplet-born lamb.

Based on the findings in chapter three and four, offering concentrate during late pregnancy may be a management option for farmers to improve triplet-born lamb birth weight and heat production. To be economical however, concentrate supplementation must result in production differences that cover the expense of the concentrate and the associated labour and equipment required. An in depth study looking at the substitution rates of pasture for concentrate on *ad-libitum* pastures of different qualities, and the effects this has on lamb production needs to be undertaken before advice can be given.

While offering concentrate had no effect on plasma immunoglobulin G (IgG) concentrations in chapter three, offering concentrate tended to have a positive effect on ewe udder size in chapter three and had a positive effect on plasma gamma-glutamyl-transferase (GGT) concentrations of twin- and triplet-born lambs in chapter three and four. These findings tentatively suggested that offering concentrate to ewes in late pregnancy may have a positive effect on the intake of colostrum by the lamb. However, because colostrum intake in the first 24 hours is influenced both by colostrum availability and progressive lamb behaviours, such as standing and actively seeking the udder, there is uncertainty on which of these factors would have been positively affected by offering concentrate. Because the uptake of colostrum is important for sustainable heat production, development of immunity to fight infections and establishment of a strong mother-young bond, further research into the effects of offering concentrate on colostrum production, lamb behaviour and lamb survival is warranted.

The focus of chapter five was to determine if maternal iodine supplementation would have a positive effect on the newborn lamb thyroid hormone concentration, rectal temperatures and heat production. Despite maternal iodine supplementation having a positive effect on maternal iodine concentrations, there was no effect on lamb plasma thyroxine (T_4) or tri-iodothyronine (T_3) concentrations within the first 24 hours of life, lamb rectal temperature or lamb heat production. It was noted however, that plasma thyroid hormone concentrations were correlated with the ability of the lamb to produce heat. It is therefore reasonable to assume that if maternal iodine supplementation had been successful in improving plasma thyroid hormone concentrations then it may have been successful in improving lamb heat production.

On-farm management strategies of offering concentrate to twin- and triplet-bearing ewes in late pregnancy only offered moderate gains and maternal iodine supplementation appeared to offer no advantage in terms of improving thyroid hormone concentrations and heat production of the newborn lamb. Independent of maternal treatments, a number of physical and physiological differences between twin- and triplet-born lambs were still apparent.

Chapters two to seven reconfirmed that triplet-born lambs had lighter birth weights, greater surface-area-to-birth-weight ratio, lower plasma T_4 and T_3 concentrations within five minutes of birth, and lower rectal temperatures within five minutes of birth than twin-born lambs. Additional physiological differences identified were that triplet-born lambs had lower plasma T_4 concentrations at three hours of age in chapter five and at 24 hours of age in chapter two and five. Within twin- and triplet-born litters in chapter six, the heaviest- and lightest-twin-born lamb and heaviest-triplet-born lamb had greater plasma lactate concentrations within five minutes of birth and lower plasma T_4 and T_3 concentrations within five minutes of birth than the medium- and lightest-triplet-born lambs. Because lamb birth rank and relative size within a litter are highly correlated with lamb birth weight, all models throughout this thesis were run with and without lamb birth weight as a covariate to determine if physiological differences observed were due to a birth rank or relative size within litter effect or a birth weight effect. The differences between birth ranks in plasma T_4 concentrations within the first 24 hours and the differences between the relative sizes within twin- and triplet-born litters in plasma lactate concentrations were explained more by lamb birth weight than lamb birth rank or relative size within litter. In contrast, the differences between birth ranks in plasma T_3 concentrations within five minutes of birth, and the differences between relative sizes within twin- and triplet-born litters in plasma T_4 and T_3 concentrations were explained more by lamb birth rank and relative size within litter than lamb birth weight. This means that compared to twin-born lambs, triplet-born lambs, especially the lightest- and medium-triplet-born lambs, are not only physically impaired due to their light birth weights, but also physiologically impaired due to their lower plasma thyroid hormone concentrations. These findings suggested that in terms of lamb heat production, the triplet-born lamb, especially the lightest- and medium-triplet-born lamb, may have inadequate heat production when compared to twin-born lambs. Chapters four and seven concluded that on average triplet-born lambs, especially the lightest-triplet-born lamb, produced less maximum heat on a per lamb basis than twin-born lambs. In chapter four, five and seven there were no differences in lamb heat production on a per kg of birth weight basis between or within twin- and triplet-born lambs. The differences observed between and within birth ranks in maximum heat production on a per lamb basis were explained more by lamb birth weight than birth rank or relative size within litter.

Overall these findings suggest that triplet-born lambs, especially the lightest-triplet-born lamb, not only have a greater capacity to lose heat but also have a reduced capacity to produce heat when compared to twin-born lambs. This may offer some explanation as to why triplet-born lambs, especially the lightest-triplet-born lamb, have greater mortality rates than twin-born lambs.

Multiple regression analyses in chapter 8 showed that lamb birth weight, plasma glucose, NEFA and T_3 concentrations before calorimetry were positively associated with maximum heat production on a per lamb basis, and that plasma NEFA and T_3 concentrations before calorimetry were positively associated with maximum heat production on a per kg of birth weight basis. These results suggest that practical on-farm management strategies which increase lamb birth weights, and improve plasma T_3, NEFA or glucose concentrations could offer improvements in triplet-born lamb heat production and therefore triplet-born lamb survival.

Potential limitations of this thesis

The outcome of lamb survival is dependent on complex interactions between the ewe, lamb and the environment. Large numbers of lambs are therefore required to accurately determine any effect on lamb survival. In this thesis ewe numbers were insufficient to look at the overall impact that on-farm management strategies may have on lamb survival. With a power of 80%, group sizes of 90 are required to detect survival differences of 20% between twin- and triplet-born lambs. For this reason, this thesis mainly looked at indicators which may improve lamb survival, such as lamb birth weight, thyroid hormone concentration, heat production, and colostrum intake. The overall impact of offering concentrate during late pregnancy or maternal iodine supplementation on twin- and triplet-born lamb survival were not able to be identified in this thesis.

It would have been useful to measure ewe herbage and concentrate intakes in chapters three, and ewe concentrate intakes in chapter four. This would have allowed assessment of the variability in concentrate intakes, substitution rates of pasture for concentrate and production responses of individual ewes. If substitution of pasture for concentrate is too high, or individual intakes of concentrate are too variable between ewes, the overall production response of offering concentrate to a group of ewes can be found to be small and uneconomic. Having a better understanding of the diet selection, total nutrient intake and production response of each ewe may have offered some explanation as to why offering concentrate showed inconsistent effects across years. In addition, it would have helped with the accurate assessment of the amount of feed consumed and therefore the cost effectiveness of offering concentrate with a 6 cm sward of herbage.

The inability to control the environmental temperature and colostrum intake of the lamb before their heat production measurements at 24 to 36 hours of age was another limitation in this thesis. Cold environmental temperatures and insufficient intakes of colostrum within the first 24 to 36 hours of life will have a negative impact on the amount of energy available from energy reserves, such as glycogen in the liver and muscle, and lipids from brown adipose tissue (Mellor and Cockburn 1986). The amount of energy available for maximum heat production at 24 to 36 hours of age is therefore likely to vary between individual lambs, with exhaustion of energy reserves having a negative impact on the ability of the lamb to produce maximum heat (Mellor and Cockburn 1986). Environmental temperature and colostrum intake were attempted to be controlled for by adjusting heat production models for plasma GGT concentrations, an indicator of colostrum intake, and the average cold stress index of the lamb within the first 24 hours of its life. However, with environmental temperatures ranging from 7.2°C to 12.3°C during the lambing period, and colostrum intake potentially ranging from 0 to 250 ml per kg of body weight, previous research has shown that energy reserve exhaustion can occur as early as 10 to 26 hours of age (Mellor and Cockburn 1986). To ensure that energy reserve availability did not have a negative effect on maximum heat production, it may have been better to measure maximum heat production at an earlier age. Because environmental temperatures and the intake of colostrum are not controlled for in a normal farm environment, from a practical point of view, these results are still considered relevant.

In chapter eight of this thesis, the physical and physiological factors which were found to be associated with maximum heat production on a per lamb basis and per kg of birth weight only accounted for 59 and 21% of the variation, respectively. Heat production is dependent on the both non-shivering and shivering thermogenesis (Alexander and Williams 1968; Ribeiro *et al* 2001; Silva 2001) and the availability and mobilisation of energy from energy reserves, such as glycogen and brown fat, and colostrum (Mellor and Cockburn 1986). Because the release of noradrenaline, cortisol and thyroid hormones act independently and/or in synergism to mobilise energy substrates such as carbohydrates and fats (Silva 1995), in hind site, noradrenaline and cortisol concentrations should have been measured alongside plasma thyroid hormones. In addition, measurements of respiratory quotients during indirect calorimetry, by measuring carbon dioxide content alongside oxygen consumption, would have allowed estimations of the relative rates of carbohydrate and lipid use (Mellor and Cockburn 1986). Measurements of these additional factors may have helped explained a greater amount of variation observed in lamb heat production in chapter six of this thesis.

To ensure that heavier lambs lost enough heat to reach maximum heat production during the 88 minute period in the indirect calorimeter, lambs which were heavier than 4 kg had their wool clipped from their back to a depth of 3 mm. Because the rate to reach maximum heat production is dependent on both the base and maximum heat production, clipping the lambs would have been an important source of variation for the rate to reach maximum heat production. In hind site, clipped (yes or no) should have been fitted as fixed effect in both base heat production during the adjustment period and rate to reach maximum heat production models to try to take into account this variation.

Because selenium-dependent enzymes, type 1 iodothyronine deiodinases, are responsible for the conversion of the biologically inactive thyroid hormone T_4 to the biologically active thyroid hormone T_3 (Robinson *et al* 2002), it would have been useful to measure selenium concentrations throughout this thesis to ensure that lower circulating plasma T_3 concentrations of triplet-born lambs were not a result of selenium deficiency (Arthur *et al* 1991). While selenium concentrations were not measured in this thesis, selenium deficiencies have previously been characterised by greater plasma T_4 and lower T_3 concentrations (Donald *et al* 1994). Throughout the thesis, plasma T_4 to T_3 ratios did not differ between maternal treatments or lamb birth ranks suggesting selenium deficiency was not an issue.

Where to from here?

While this thesis did not identify practical on-farm management strategies which could improve triplet-born lamb thermoregulation, multiple regression analyses did identify that an improvement in lamb birth weight, plasma thyroid hormone concentrations, plasma glucose and NEFA concentrations may increase the ability of the lamb to produce heat. The multiple regression analyses also showed that there was a lot of unexplained variation between individual lambs. Further investigations into practical on-farm management strategies which could improve the factors identified above may offer some improvements in triplet-born lamb survival rates. In addition, further investigations into factors associated with lamb heat production may provide further useful information on which factors should be targeted to improve triplet-born lamb thermoregulation.

Improving ewe nutrition during late pregnancy to improve lamb birth weight

Further investigations into why offering concentrate to twin- and triplet-bearing ewes grazing a 6cm sward height had an inconsistent effect on lamb birth weight could help extend the concentrate supplementation work carried out in this thesis. By using alkanes to measure herbage intakes (Dove and Mayes 2005) and labelling the concentrate supplement with ytterbium acetate or lithium chloride to measure concentrate intakes (Holst *et al* 1994; Holst *et al* 1996b), we could have a better understanding of diet selection, substitution rate of pasture for concentrate, total nutrient intake and the production response of individual twin- and triplet-bearing ewes grazing a 6 cm sward height. This information could then be used to help identify specific management strategies to improve the production response of twin- and triplet-bearing ewes and their lambs. Because pasture growth can be limited during winter months in some regions of New Zealand (Matthews *et al* 2000), additional investigations into the effects of offering concentrate to ewes whose pasture intakes are restricted would also be of value. A 2 x 2 x 2 factorial experimental design which includes two litter sizes (twin- and triplet-bearing ewes) two pasture sward heights (2 cm and 6 cm) and two nutritional treatments (no concentrate supplementation and concentrate supplementation from pregnancy day 100 until parturition) could be used to identify which ewes are most responsive to concentrate supplementation when grazing either a 6 cm or 2 cm sward height and to help identify why. Once we have a better understanding of which ewes under which pastoral conditions are likely to have the biggest production response to make the practice cost effective, further investigations into identifying the optimal type of concentrate to offer during pregnancy, and time and duration of offering concentrates could help reduce the costs of this practice further.

Another strategy for improving triplet-born lamb birth weight may be to graze triplet-bearing ewes on greater quality herbages. The use of greater quality herbages in early lactation, such as plaintain, has recently been shown to be beneficial in terms of twin-rearing ewe milk production and twin-born lamb growth (Judson *et al* 2009). Because these positive effects are likely to have resulted from an improvement in the nutrient intake of the twin-rearing ewes, grazing triplet-bearing ewes on greater quality herbages may also offer an improvement in triplet-bearing ewe nutrient intake, which in turn, may have a positive effect on triplet-born lamb birth weight. Similar to the concentrate experiment mentioned above, a 2 x 2 factorial experimental design which includes two litter sizes (twin- and triplet-bearing ewes) two pastures of different qualities (ryegrass and white clover and plaintain from pregnancy day 100 until parturition) could be used to identify any positive effects on lamb birth weight. Additional production effects which could be looked at during this experiment would include colostrum production, colostrum intake within the first 24 hours, lamb growth and survival.

Improving the intake of colostrum to increase plasma thyroid hormones and/or glucose and NEFA concentrations

Previous research has shown that colostrum intake has a positive effect on lamb plasma T_3 concentrations (Symonds 1995; Gate *et al* 1999) and plasma glucose and NEFA concentrations (Eales and Small 1980a; Eales and Small 1981). Colostrum intake is vital for sustainable heat production and survival (Nowak and Poindron 2006). Further investigation into factors which effect colostrum intake, such as the amount of colostrum produced, and the competitive sucking behaviour of triplet-born lambs within a litter would therefore be of interest.

While plasma GGT concentrations of twin- and triplet-born lambs in this thesis suggest that colostrum intake did not differ between and within twin- and triplet-born litters, other experiments have reported the opposite with wide variations of colostrum intakes being reported (Halliday 1974; Hunter *et al* 1977; Hinch 1989; Kenyon *et al* 2005b). In chapter 2 of this thesis, twin- and triplet-bearing ewes had plasma progesterone concentrations which suggest that colostrum let-down was delayed (Hartmann *et al* 1973). Australian research has shown that offering high energy supplement in the last two weeks of pregnancy to ewes receiving Lucerne hay has a positive effect on single- and twin-bearing ewe plasma concentrations of progesterone and their capacity to produce colostrum (Banchero *et al* 2004b). A 2 x 2 x 2 factorial experimental design which includes two litter sizes (twin- and triplet-bearing ewes) two pasture sward heights (2 cm and 6 cm) and two nutritional treatments (no concentrate supplementation and concentrate supplementation from two weeks before parturition) could be used to measure progesterone concentrations before and after birth and to quantify the amount and quality of colostrum available to a triplet-born litter at one, six and 18 hours after birth. This experimental design would also inform us if offering concentrate on either a 6 cm or 2 cm sward height offers any benefits in terms of colostrum let-down, and colostrum and milk production.

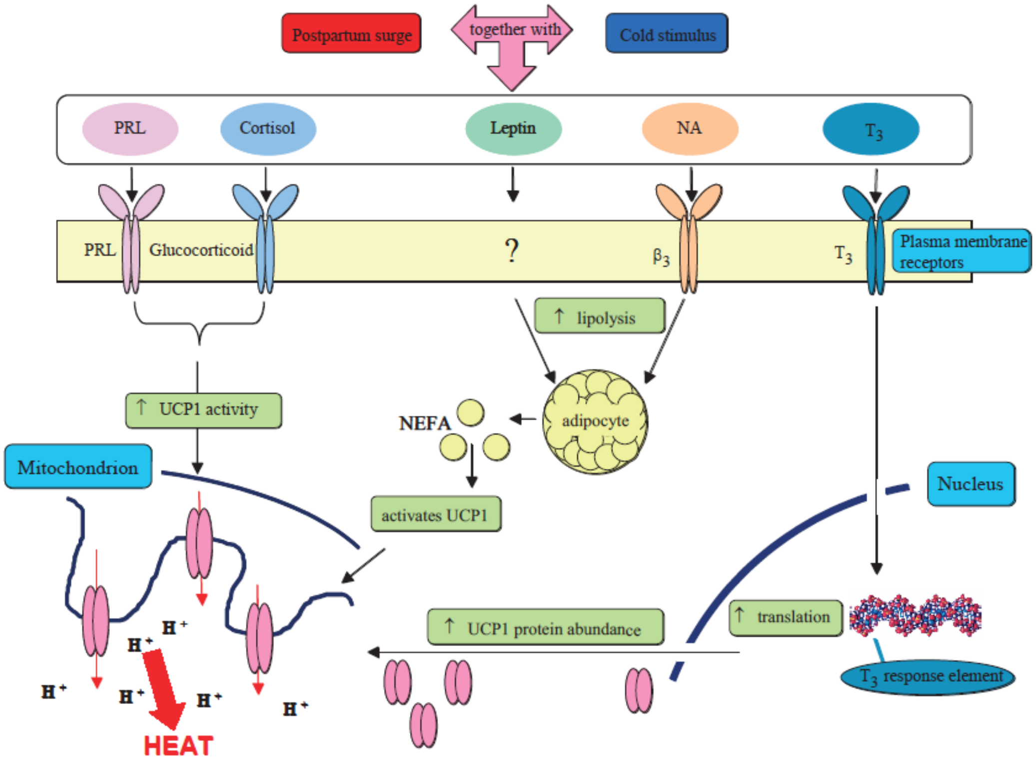
Using the same experimental design above, a second experiment which observed the time, frequency and duration of sucking bouts of individual lambs within a triplet-born litter from birth to 24 hours of age could be used to identify any competitive sucking behaviours within the first 24 hours which may have a positive impact on colostrum intake of the lamb and whether one lamb, for example the lightest lamb, is consistently excluded. Again, this experimental design would also inform us if offering concentrate on either a 6 cm or 2 cm sward height offers any benefits in terms of competitive sucking behaviour.

Overall both of these experiments would help clarify if differences in colostrum intakes within litters are a result of colostrum availability or competitive sucking behaviours within litters, and whether improving ewe nutrition in late pregnancy by offering concentrate in the last two weeks of pregnancy offers any benefit in terms of colostrum production, competitive sucking behaviours and consequently colostrum intake.

Improving the understanding of what effects lamb heat production

Overall the results from this thesis concluded that triplet-born lambs are physically and physiologically impaired due to their light birth weights and low plasma T_3 concentrations within five minutes of birth. This thesis also concluded that triplet-born lambs have lower rectal temperatures than twin-born lambs within five minutes of birth which may indicate that they have inadequate thermoregulation directly after birth. The cortisol and T_3 surge before birth, along with other hormones and receptors shown in figure 10, are responsible for the expression and activation of uncoupling protein one (UCP-1), the mitochondrial protein responsible for the production of heat during non-shivering thermogenesis (Symonds *et al* 2003).

Figure 10 *The endocrine regulation of fetal adipose tissue maturation in preparation for life after birth. This figure is taken from Symonds et al (2003).*



PRL, prolactin; NA, noradrenaline; β_3, adrenergic receptor; NEFA, non-esterified fatty acids; T_3, tri-iodothyronine; UCP, uncoupling protein.

Examination of the literature has revealed that there has been little research done on the timing and size of cortisol and T_3 surge before birth in twin- and triplet-fetuses and the potential impact it may have on the expression and activation of UCP-1, and consequently the production of heat after birth. With twin- and triplet-bearing ewes being housed in an environmentally and nutritionally controlled environment, daily changes in plasma cortisol and T_3 concentrations of twin- and triplet-fetuses during the last three weeks of gestation could be measured by inserting catheters into the umbilical arteries and veins of the fetuses. At day 142 of gestation fetuses could be euthanized, peri-renal adipose tissue collected, and using immuno chemistry, the amount of glucocorticoid receptors, T_3 receptors, and UCP-1 in brown adipose tissue could be quantified. Associations found between plasma cortisol and T_3 concentrations before birth and UCP-1 may help explain further why the physical and physiological differences observed between and within twin- and triplet-born litters exist.

Further investigations into the effects that offering concentrate has on the amount and thermogenic efficiency of endogenous energy reserves at 24 to 36 hours of age may also help clarify the positive association observed in this thesis between offering concentrate in late pregnancy and triplet-born lamb heat production on a per kg of birth weight basis. Improvements in ewe nutrition during late pregnancy are known to have a positive effect on the amount of UCP-1 in brown adipose tissue (Budge *et al* 2003; Symonds *et al* 2003). By using a 2 x 2 x 2 factorial experimental design, which includes two litter sizes (twin- and triplet-bearing ewes) two pasture sward heights (2 cm and 6 cm) and two nutritional treatments (no concentrate supplementation and concentrate supplementation from pregnancy day 100 until parturition), and measuring the amount of UCP-1 in brown adipose tissue of euthanized lambs, the effect that ewe nutrition may have on the amount and thermogenic efficiency of brown adipose tissue could be quantified. Having a greater amount of endogenous energy reserves and/or with greater thermogenic efficiency could be of particular importance for triplet-born lambs, whose light birth weight and lower critical temperature requires them to utilise endogenous energy reserves earlier and consequently for longer periods than twin-born lambs.

Concluding remarks

This thesis identified that triplet-born lambs, especially the lightest-triplet-born lamb, has inadequate thermoregulatory capabilities when compared to twin-born lamb. While on-farm management strategies to improve triplet-born lamb survival were not identified in this thesis, it has provided valuable information on which physical and physiological factors should be targeted in the future research.

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