The effect of maternal nutrition during mid- to late-pregnancy on ewe and lamb behaviour and the association with lamb survival

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ABSTRACT

Lambing percentage in New Zealand has increased by almost 30% in the last 20 years. This increase is associated with a greater percentage of twin- and triplet-born lambs which have lower survival rates than singletons. The behaviour of the ewe and her lambs has been associated with lamb survival, however, relevant data on the effect of ewe mid-pregnancy body condition score (BCS) and nutrition on ewe and lamb behaviour under New Zealand pastoral farming conditions is scarce. This research included seven experiments investigating the effects of feeding ewes, with a BCS of 2.0 to 3.0 at mid-pregnancy, either *ad libitum* or only sufficient to meet pregnancy maintenance requirements from mid- to very late-pregnancy, on ewe and lamb behaviour at 3 to 24 hours after birth. The association between behaviour and lamb survival was also investigated. Observations on ewe and lamb behavioural were conducted at tagging (3 to 18 hours after birth) and in a triangle pen test at approximately 12 or 24 hours after birth.

The effects of ewe mid-pregnancy BCS and feeding on behaviour were somewhat inconsistent across experiments, possibly due to variations in the timing and length of feeding treatments. Feeding ewes *ad libitum* in comparison to pregnancy maintenance requirements did not consistently improve the maternal behaviour score (MBS) of the ewe. This is not surprising as neither of the feeding treatment groups were nutritionally restricting. There was some evidence to suggest that lambs born to ewes offered the pregnancy maintenance diet exhibited a greater need, possibly due to a weaker ewe-lamb bond than lambs born to ewes on the *ad lib* treatment. This need was characterised in twins, in chapter four, by greater low-pitched bleating rates and

decreased time to contact, suck and follow the dam. Similar, but inconsistent results were reported in other chapters. Further, when investigating the relationship between behaviour and survival, it was found that twin-born lambs with the greater need (followed their dam more quickly) were more likely to die. The opposite relationship was found in triplet-born lambs, which may be a reflection of greater competition for milk within triplet-litters compared to twin-litters. Thus, in both twin- and triplet-born lambs following behaviour is an indicator of mortality. The practical use of this behaviour as a tool to predict lamb survival is limited.

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Looking back three years and I cannot believe I am almost at the end of my PhD journey. The last three years have consisted of a lot of hard work, sleepless nights and more than a little frustration. But they have also been fun, allowed me to grow in many ways and been incredibly rewarding. Would I do it again? No way! Do I regret a single day? Absolutely not!

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“Begin at the beginning," the King said, very gravely, "and go on till you come to the end: then stop.”

– Lewis Carroll, *Alice in Wonderland*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION AND NOTATION

Abbreviations

Ad lib *Ad libitum*

BCS Body condition score

MBS Maternal behaviour score

DM Dry matter

ha Hectare

CI Confidence interval

Notation

P*n* The *nth* day after start of breeding, with P0 being the first day that the ram was introduced to the ewes in the experiments.

L*n* The *nth* day of lambing, with L0 being the mean day of lambing for the ewes considered.

G*n* The generation of sheep used in the study. G0 being the first generation in chapter six to be exposed to feeding treatments, G1 being their progeny and G2 being the progeny of the G1.

CHAPTER 1

Review of literature

Introduction

Lamb mortality is of concern to the New Zealand sheep industry from both an economic and an animal welfare perspective (Mellor and Stafford 2004). Lamb mortality percentages range from 5% to 40% within individual flocks (Hight and Jury 1969; Dalton et al. 1980; Kenyon et al. 2002; Kerslake et al. 2005; Young et al. 2010), with the majority of lamb deaths occurring the first three days of life (Hight and Jury 1969; Dalton et al. 1980; Nicoll et al. 1999; Nowak et al. 2000; Dutra et al. 2007; Hinch and Brien 2014). The two main causes of pre-weaning lamb death are dystocia and starvation/exposure. Dystocia is one of the main contributors to lamb mortality (Hight and Jury 1969; Brien et al. 2010). Starvation/exposure is the other predominant cause of lamb death and in New Zealand accounts for approximately 30% of lamb losses (McCutcheon et al. 1981; Kerslake et al. 2005). There are a number of factors affecting lamb mortality, such as ewe breed and parity and the sex and birth weight of lambs (Hight and Jury 1969; Dalton et al. 1980; Alexander et al. 1983; Woolliams et al. 1983; Scales et al. 1986; Oldham et al. 2011; Hinch and Brien 2014; Paganoni et al. 2014). Additionally, studies have shown that lamb mortality rates are greater in multiple-born lambs compared with singletons (Rohloff et al. 1982; Hinch et al. 1983; Alexander et al. 1990; Nicoll et al. 1999; Oldham et al. 2011).

Under pastoral grazing conditions, improved levels of nutrition in late pregnancy and at lambing have been reported to have desirable effects on ewe and lamb behaviours, such as standing, sucking and following (Everett-Hincks et al. 2005). In addition, nutritional restriction of ewes during pregnancy has been shown to adversely affect their maternal behaviour and the behaviour and survival of their lambs (Corner et al. 2010; Kenyon et al. 2011; Thomson & Thomson; 1949).

Few studies have investigated whether the body condition score (BCS) of ewes influences the behaviour of the ewe and her lambs. Dwyer et al. (2003) reported that ewes that lost condition during pregnancy, as indicated by a reduction in back fat, produced lambs that were slower to stand and suck immediately after birth. To date, no studies have investigated the effects of ewe BCS in mid-pregnancy on ewe and lamb behaviour at tagging, 3 to 18 hours after birth under extensive pastoral conditions. Furthermore, there is a lack of research investigating the potential relationship between ewe and lamb behaviour at tagging and subsequent lamb survival.

Postnatal ewe and lamb behaviour

The maternal behaviour of the ewe play an important role in the survival of the neonatal lamb since the neonate is fully dependent on its dam for nutrition and guidance (Parker and Nicol 1993; Nowak et al. 2000; Poindron 2005). Appropriate maternal behaviours include; grooming and maintaining close contact with the lamb, low-pitched bleating, lamb recognition and cooperation with the lambs sucking attempts (Dwyer and Lawrence 2005).

Grooming

Shortly after giving birth the ewe stands and begins to groom her newborn(s) (Lynch and Alexander 1977). The ewe is strongly attracted to the amniotic fluids which encourages the ewe to vigorously lick the lamb (Levy and Poindron 1987). Grooming by the dam stimulates respiration and assists drying the lambs which aids in maintaining normal body temperature (McGlone and Stobart 1986). Grooming aids the ewe in the establishment of maternal recognition of her offspring (Alexander 1988; Nowak et al. 2008). Ewes that spend less time grooming their lamb(s) at birth have been reported to graze further away from their lamb(s) throughout lactation, when compared with ewes that had greater grooming times (Dwyer and Lawrence 1999). The act of grooming by the dam also stimulates the lamb to stand and orientate itself towards the udder (Alexander and Williams 1964; Dwyer and Lawrence 1998). Dwyer and Lawrence (1998) stated that the primary reason for maternal behaviours immediately post birth is to assist the neonate to suck. Ewe-lamb bond formation which facilitates mutual recognition and acceptance also results in the ewe facilitating the lamb to suckle. Sucking itself also plays an important role in the reinforcing mutual recognition (Nowak et al. 1997). Dwyer (2008) suggested that lambs that received less grooming had a weaker attachment to their dam which could have been the reason for a lower frequency of suckling in the neonatal period.

Vocalisation

While grooming, the ewe and her lamb typically emits deep, rumbling, low-pitched bleats which are thought to facilitate bonding between the ewe and her lamb (Kiley 1972; Shillito 1972; Nowak 1996; Dwyer and Lawrence 1998; Sèbe et al. 2007). High-pitched or “distress bleats”, on the other hand, are usually emitted by the ewe and lamb when they are separated from each other as a recall but can also indicate frustration or the level of need of the lamb (Kiley 1972; Shillito 1972; Poindron et al. 1994; Weary and Fraser 1995; Dwyer et al. 1998). Low-pitched bleats are typically emitted with a closed mouth while high-pitched bleats are emitted with an open mouth (Dwyer et al. 1998). When measuring the frequency of high- and low-pitched bleats emitted by the ewe and lambs after birth, it was found that both parties emitted mostly low-pitched bleats during the first 24 hours after birth. A mix of bleats were emitted on day six after birth and more high-pitched bleats were emitted by the ewe and lambs 15 days after birth (Sèbe et al. 2007).

Time spent at the birth site

The amount of time the dam spends with her lambs at the birth site helps develop the ewe-lamb bond and is believed to be particularly important for ewes with multiple lambs (Nowak 1996). It has been reported that the ability of the ewe to discriminate and bond with her whole litter improves the longer the ewe spends at the birth site (Alexander et al. 1983). Alexander et al. (1983) reported that as the time spent on the birth site increased, from zero to over four hours, the proportion of ewes that became permanently separated from their twin lambs decreased dramatically from almost 80% to approximately 5%. Husbandry practices that allow the ewe to remain undisturbed on the birth site for longer periods should be encouraged (Fisher and Mellor 2002).

Lamb behaviour

The behaviour of the newborn lamb is important to stimulate and reinforce maternal behaviors (Nowak 1990; Dwyer et al. 1999). The behaviour of the lamb is also essential in the ewe-lamb bonding process (O'Connor and Lawrence 1992). Minutes after being born the lamb raises and shakes its head, turns onto its sternum and bleats. Most lambs stand within 30 minutes of birth, will begin to search for the udder of the ewe and subsequently suck from the teat (Nowak et al. 2008). Lamb behaviours associated with survival are discussed later in the thesis and include; bleating, maintenance of close contact with the ewe, suckling and ewe recognition (Nowak 1996; Dwyer and Lawrence 2005). Appropriate expression of behaviours by the lamb is critical because maternal behaviours decline rapidly if the newborn lamb responses are weak (Alexander et al. 1990; Dwyer et al. 1999).

Summary

Mutual recognition between the ewe and the lamb and a strong bond are important for lamb survival (Nowak 1996). Impaired ewe-lamb bonding may result in decreased suckling and thus lead to dehydration, hypothermia and contribute to increased lamb mortality (McCutcheon et al. 1981; Slee and Springbett 1986; Dwyer 2008).

Techniques for measuring behaviour

Studies examining lamb survival have explored numerous aspects of both maternal and lamb behaviour. These behaviours could be measured either separately or as a lamb – dam unit because it is acknowledged that the behaviour of the ewe affects the behaviour of the lamb and vice versa (Dwyer 2003). An example of this is given by Lynch et al. (1992) who reported that increased tactile, visual, auditory and oral stimuli provided by the mother decreased the time taken for lambs to stand and suck. Further, if the ewe does not begin to clean the lambs, or does not emit low-pitched bleats lambs may remain still for a longer period of time post birth (Lynch et al. 1992). Therefore, measuring lamb behaviours without considering the behavior of the ewe has limitations and this will be discussed in chapter 7.

Ewe Behaviour Score

O’Connor et al. (1985) developed a method for assessing the maternal behaviour of ewes at 24 to 48 hours after birth. The maternal behaviour score (MBS) ranks the behavioural reaction of a ewe to a shepherd handling and tagging her lamb(s) on a scale from one to five (Table 1.1). The MBS is considered an indicator of the strength of the ewe-lamb bond with higher scores indicating a stronger bond and superior maternal behaviour (O'Connor 1996). Even though initially developed to be used at 24 to 48 hours after birth, numerous ewe and lamb studies have used this method of assessing maternal behavior as soon as 12 hours after birth (Parker and Nicol 1993; Everett-Hincks et al. 2005b; Corner et al. 2010). Everett-Hincks et al. (2005b) reported a positive relationship between lamb survival and ewe MBS. However, this relationship was not tested in the other studies mentioned.

Table 1.1 Description of the Maternal Behaviour Score (MBS) (O'Connor et al., 1985).

Description	Score
Ewe flees at the approach of the shepherd, shows no interest in lambs and does not return.	1
Ewe retreats further than 10 metres but comes back to her lamb as shepherd leaves them.	2
Ewe retreats to such a distance that tag identification is difficult (5 to 10 metres).	3
Ewe retreats but stays within 5 metres.	4
Ewe stays close to the shepherd during handling of her lambs.	5

A similar method of scoring maternal behaviour (Table 1.2) was developed by Holst (1987). Ewe behaviour during parturition as well as for several hours after was observed and each ewe was assigned a score. The effect of behaviour on lamb survival was not tested.

Table 1.2. Description of ewe maternal behaviour scoring system (Holst, 1987).

Description	Score
Little or no interest in lamb(s); leaves birth-site upon walking.	1
Slow to stand post-parturition (>20 minutes); little interest in lamb(s); easily distracted.	2
Stands and grooms lamb(s) within 5 minutes of birth; maintains interest in lamb(s) while feeding on/near birth site; unconcerned if only one of two lambs follow.	3
Stands and grooms lamb(s) within 3 minutes of birth; hinders lamb(s) attempts to stand by continuous grooming; aids in the lamb(s) teat-seeking by correct orientation while grooming.	4
Stands and grooms lamb(s) within 1 minute of birth; aids in the lambs teat-seeking by correct orientation while grooming; tends entire litter; long duration (> 4 hours) at birth-site.	5

A second maternal behaviour score three days after birth, based on the ewes' response to the shepherd slowly carrying the lambs out of a pen, has also been developed (Dwyer et al. 2003). The scale ranged from one (no concern, does not follow lambs from the pen) to four (follows lambs closely and leaves pen, Dwyer et al. 2003). The effects of ewe behaviour on lamb survival were again not tested.

Von Borstel et al. (2011) also compared maternal behaviour of ewes by removing day-old lambs from the lambing pen for 10 minutes. The lambs were held by a shepherd at a distance of five metres from the dam and the behaviour of the ewe was observed continuously during this time. The ewe was subsequently given a score between one and three depending on her behaviour (Table 1.3).

Table 1.3. Scoring of maternal behaviour and vocalisation test (Von Borstel et al. 2011).

Behaviour of the ewe at and during separation	Score
Ewe does not show any agitation	1
Ewe shows agitation and seeks for her offspring	2
Ewe shows agitation, climbs the fence of her box and is aggressive towards the handling person	3

Ewe vocalisation

Recording vocalisations between the ewe and lamb has been used frequently in the study of ewe and lamb behavior (Dwyer et al. 1998; Everett-Hincks et al. 2007; Sèbe et al. 2007; Corner et al. 2010). The frequency and type of bleat (low- or high-pitch) are generally recorded during a set timeframe after the birth.

Early post-partum vocalization by the dam consists mainly of low-pitched bleats and is most frequent during the first three hours following birth (Dwyer et al. 1998; Sèbe et al. 2007). Ewes emit low-pitched bleats or ‘rumbles’ almost exclusively to her neonates (Shillito 1972). These low-pitched bleats are thought to provide reassurance to the newborn lambs (Shillito 1972) and are involved in the ewe-lamb bonding process (Nowak 1990). High-pitched bleats have been described as a "protest" bleat in some circumstances (Kiley 1972), but are also emitted when the ewe is separated from their lambs (Shillito 1972). Dwyer et al. (1998) stated that although some ewes were observed to reject their lambs, and this was accompanied by high-pitched vocalisation, this type of bleat was seldom made when the lambs were with the ewe. Lambs and ewes become more frequently separated as the lambs becomes more active, hence the increase in high-pitched vocalisation may be a reflection of the regularity by which lambs become separated from their dams (Dwyer et al. 1998).

During the maternal behaviour testing conducted by Von Borstel et al. (2011), as described previously, the vocalisation of the ewe was also recorded. The ewe was given a score, between one and three, depending on the level of vocalisation that occurred during separation from her lambs (Table 1.4).

Even though ewe and lamb vocalisation has frequently been recorded, few studies have investigated the relationship between vocalisation and lamb survival (Dwyer 2003).

Table 1.4. Scoring of maternal behaviour and vocalisation test (Von Borstel et al. 2011)

Vocalisations of the ewe at and during separation	Score
No vocalisation of the ewe	1
Vocalisation, interrupted by calm phases	2
Vocalisation is continuous and of high intensity	3

Temperament testing

Temperament can be defined as the way in which an individual reacts to a challenging or novel situation (Réale et al., 2000). Temperament of the ewe has been used when attempting to predict maternal ability or lamb survival (Murphy et al. 1994; Kilgour and Szantar-Coddington 1995; Corner et al. 2005; Plush et al. 2011), however, the results vary significantly between studies. Common temperament tests include the 'box test' (Murphy et al. 1994; Plush et al. 2011) in which the ewe is enclosed in a small box (1.5 m x 1.5 m x 1.5 m in Murphy et al. 1994 and 1.5 m x 0.7 m x 1.5 m in Plush et al. 2011) for a short period of time (often less than a minute). While in the box the movement and vocalisation of the ewe is recorded. Murphy et al. (1994) tested ewes at weaning and then again at 2.5 years but found no effects of ewe temperament on lamb survival. Plush et al. (2011) only tested lambs at weaning and stated that lambs that were found to be more agitated had progeny with greater survival.

Another commonly used test is the 'arena test' (Murphy et al. 1994; Kilgour and Szantar-Coddington 1995; Corner et al. 2005). Although variations of the arena test exist, generally the ewe is placed in one end of an arena with a pen of sheep at the other end and a person seated in front of them. The floor is usually marked into square zones and the position and behaviour of the sheep is recorded for about 10 minutes. A similar test, an 'open field test' can also be conducted in the paddock (Kilgour and Szantar-Coddington 1995). Murphy et al. (1994) found no link between the arena test and lamb survival and Corner et al. (2005) found no link between maternal behavior score and the arena test.

Ewe-lamb bonding and recognition of offspring

The strength of the ewe-lamb bond and the ability of the ewe to discriminate her offspring from an alien lamb have also been repeatedly measured (Shillito and Alexander 1975; Hinch et al. 1990; Cloete et al. 2005). The lamb is separated from its dam and tethered or placed in a holding pen, often adjacent to other lambs of a similar age. The ewe is then released and the time required for the ewe to reunite and remain in contact with her lamb is recorded. Several variations of this test can be found in the literature.

Another approach has been to deprive the dam of one or more of her senses in order to determine how this impacts on her ability to discriminate between her lamb and an alien lamb and to better understand what senses are critical in lamb discrimination (Alexander 1977; Alexander and Shillito 1977; Alexander and Shillito 1978a; Keller et al. 2003). Alexander (1977) studied the roles of auditory and visual cues in the mutual discrimination of Merino sheep. The auditory and visual cues of both ewes and lambs were altered by blackening them and/or muting them. The study concluded that both visual and auditory clues are important in mutual discrimination; however, visual clues have the greatest influence. Alexander and Shillito (1977) found that ewes also use close-range olfactory cues, particularly from the tail region of the lamb, when discriminating their offspring from alien lambs. The relationship between the ability of the ewe to recognise and reunite with her lamb and lamb survival has not been tested.

Time of the lamb to perform developmental behaviours

Alexander (1988) stated that lambs that stand shortly after birth, suck soon after standing and remained in close proximity to the ewe are more likely to develop an exclusive bond with the dam than lambs that take longer to exhibit these behaviours. The behavioural progression by the neonate lamb can be measured by the length of time it takes for the lamb to initially perform these behaviours, e.g. the duration from expulsion to standing or to sucking (Owens et al. 1985; Cloete 1993; Dwyer et al. 2005). Dwyer (2003) also measured the average amount of time the lambs spent exhibiting certain behaviours such as sucking, lying down or playing during the first three days of life (Table 1.5). The relationship between lamb neonatal behaviours and lamb survival will be discussed on page 34.

Table 1.5. Postnatal lamb behaviours (Dwyer, 2003).

Behaviour	Definition
Shakes head	Lamb raises and shakes head.
To knees	Lamb rolls onto chest, gathers legs under itself and pushed front half of the body up off the ground.
Attempts to stand	Lamb supports body weight on at least one foot.
Stands	Lamb stands unsupported on all four feet for > 5 seconds.
Reaches udder	Lamb approaches ewe and nudges her in the udder region.
Unsuccessful suck	Lamb places head under ewe in contact with the udder but either fails to grasp the teat or releases it without sucking.
Sucks	Lamb holds teat in its mouth and appears to be sucking with appropriate mouth and head movements, may be tail-wagging, remains in this position for > 5 seconds.

Lamb vigour

Lamb vigour has been measured in a number of behavioral studies, however, an absolute definition of lamb vigour is often lacking (O'Connor and Lawrence 1992). Lamb vigour has previously been defined as the time taken to perform certain behaviours immediately after birth such as sucking (Dwyer et al. 2005), general alertness of the lamb (Nash et al. 1996) and growth rate to weaning (Wassmuth et al. 2001). Others have used the amount of time the lamb spent playing or in close proximity to its dam, during the first 72 hours after birth, as a measure of vigour (Dwyer 2003). These definitions have been used alone or in combination, although in some studies 'vigour' has not been defined (Alexander et al. 1959).

Although, the definitions of lamb vigour vary significantly between studies subjective assessments are often used, such as the use of the terms poor, fair or good (Alexander et al. 1959; Owens et al. 1985; Pfister et al. 2006a). Holst (1987) developed a numerical scoring system using a scale from one to five to describe lamb vigour (Table 1.6). The lamb is observed during several hours starting from parturition and allocated a score for vigour between one and five. A more recent vigour scoring system has been developed by Brien et al. (2009) whereby the scoring takes place while the lambs are being restrained by a shepherd and for 30 seconds after release (Table 1.7). The lambs are scored within the first 24 hours after birth and the age is noted. A number of studies have reported on a relationship between lamb vigour and survival and this will be further discussed on page 34.

Table 1.6. Description of lamb vigour scores (Holst, 1987).

Description	Score
Doesn't stand for at least 40 min; little or no teat-seeking drive.	1
Attempts to stand after 30 min; low teat-seeking drive and tendency to follow ewe.	2
Shakes head within 30 sec; attempts to stand within 15 min; seeking teat within 10 min of standing: follows ewe but distracted by other moving objects.	3
Attempts to stand within 10 min of birth; seeking teat within 5 min of standing; strong tendency to follow ewe.	4
Attempts to stand within 5 min of birth; follows ewe closely.	5

Table 1.7. Description of lamb vigour scores (Brien et al., 2009).

Description	Score
Constant struggle - bleat in response to ewe - on release reaches ewe quickly and follows.	1
Regular struggle while held - moves to the ewe on release - bleating common.	2
Some struggle - walking in direction of ewe bleats but no contact - may bleat.	3
Some struggle - attempts to walk but aimless - no apparent response to ewe bleats.	4
Little movement when held - lies on release	5

A previous study have also made the interpretation that lambs that were quicker at exhibiting certain behaviours, such as standing and moving towards dam, after tagging were not more vigorous but more ‘needy’ (Corner et al. 2010). The activity level of the lamb has in the previously mentioned studies been labelled both need and vigour. ’Vigour’ suggests abundance of energy and resources while ‘need’ suggests that the lamb is missing a vital resource; such as food. Factors beyond the behaviour of the lamb may have to be taken into consideration when attempting to interpret whether a lamb is more vigorous or whether it has an unmet need that prompt the seeming increase in vigour.

Lamb vocalisation

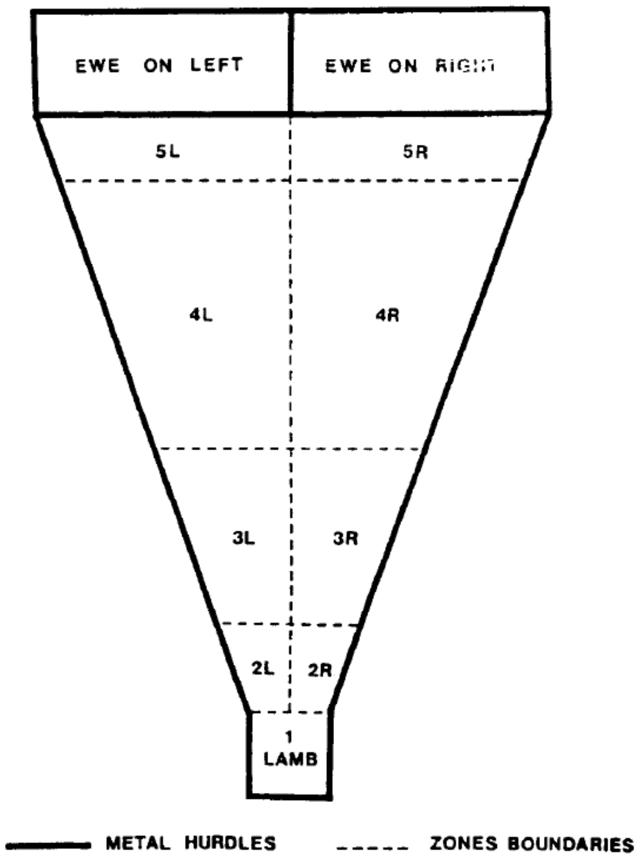
Vocalization of the lamb is often recorded when assessing behaviour. In the same manner as ewe vocalization, the frequency and type of bleat emitted by the lamb is generally measured during a set period of times and may give an indication of the strength of the ewe-lamb bond (Dwyer and Lawrence 1998; Dwyer et al. 1999; Everett-Hincks et al. 2004; Sèbe et al. 2007; Corner et al. 2010). Low-pitched vocalization by the neonate appears to both stimulate the ewe and facilitate bonding (Pollard 1992) and are almost exclusively emitted between the ewe and her lambs in the first 24 hours (Dwyer et al 1998). High-pitched bleating rates may indicate the quality and quantity of maternal care provided to the lamb; and high rates of bleating by lambs in the neonatal period can indicate inadequate suckling (Dwyer et al. 1998). Lamb high- and low-pitched bleats are also believed to act as a recognition signal for the ewe and reinforce a strong ewe-lamb bond (Nowak 1990; 1996). Nowak (1990) reported that lamb bleating rates in the first 30 minutes after birth are highly varied between individuals. Although, the numbers of bleats were positively related to the time the lamb spent next to its dam at 12 hours of age (Nowak 1990). When measuring the frequency of high- and low-pitched bleats emitted by the ewe and lambs after birth, it was found that both parties emitted mostly low-pitched bleats during the first 24 hours after birth. A mix of bleats were emitted on day six after birth and more high-pitched bleats were emitted by the ewe and lambs 15 days after birth (Sèbe et al. 2007).

Lamb behaviour and recognition of dam

A variety of lamb behavioral tests have been conducted (Alexander and Shillito 1978b; Nowak et al. 1989; Cloete et al. 2005; Pfister et al. 2006b; Sèbe et al. 2007).

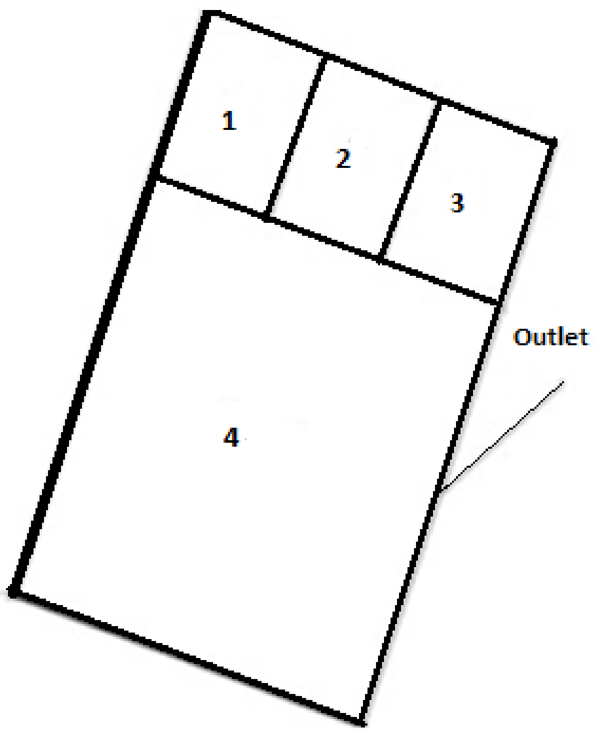
Several authors have examined the ability of the neonate lamb to discriminate its mother from alien ewes at six to 72 hours after birth (Nowak et al. 1989; Nowak and Lindsay 1992; Sèbe et al. 2007). Nowak et al. (1989) and Nowak and Lindsay (1992) used a triangular testing pen (Fig. 1.1) with the dam placed in a small pen at the base of the triangle next to an alien ewe that had also recently lambed. The lamb was then released from the apex of the triangle and observed for five minutes; the time spent in each of the areas of the triangle was recorded. Twin-born lambs that survived beyond seven days of life had spent significantly more time with either ewe as well as proportionately more time with their dam, during the triangle test, than twin-born lambs that died within seven days of birth (Nowak and Lindsay 1992).

Figure 1.1 Testing pen (Nowak et al. 1989).



An alternative test was conducted by Cloete et al. (2005) who also measured the ability of the lambs to re-unite with their dam and recognise her among two other ewes at three days of age. Similar to the triangle test conducted by Nowak et al. (1989) and Nowak and Lindsay (1992), the lamb was released into a square pen (5.4 m x 5.4 m, area 4, Fig. 1.2) and the time required for the lamb to reunite with its dam was measured. The dam was held in a smaller holding pen (1.35 m x 1.35 m, area 1, 2 or 3, Fig. 1.2) on the far side of the larger lamb pen, together with two alien ewes that had lambed at a similar time (Cloete et al. 2005). The authors subsequently measured the ability of the lamb to follow its dam when both were chased by a shepherd over a distance of 10 metres. However, the effect of behaviour on lamb survival was not investigated.

Figure 1.2. Testing pen (based on Cloete et al. 2005).



In addition, Pfister et al. (2006b) tested the cognitive ability of lambs by placing one, or several, barriers for lambs to navigate around in order to reach their dams. These tests were conducted between 12 hours and six days of age. The effect of behaviour on lamb survival was not tested.

The mentioned testing procedures all differ in their design, execution, breeds of sheep and age of lamb. It is therefore difficult to reliably compare the results between the studies.

Are the various behaviours measured interrelated?

The relationship between ewe post-natal behaviours and other ewe and lamb traits

Ewe MBS has been reported to be related to lamb neonatal progression, particularly to the time it takes for the lamb to reach the udder and begin to suck (Parker and Nicol 1993). Lambs born to ewes with a greater MBS progress more quickly through early neonatal behaviours than those whose dams have a low MBS. Parker and Nicol (1993) showed that lambs born to ewes with a MBS of four and five located the udder in 30% less time than lambs born to ewes with a MBS of three. In addition Corner et al. (2010) showed that ewe MBS was negatively correlated with the latency of the lamb to make contact with its dam (Table 1.8). Murphy et al. (1994) reported a negative correlation between the total time the ewe spent licking her lambs and her box test score. There was, however, a positive correlation between MBS and the score from the 'box test' (Murphy et al. 1994). Similar results were reported by Plush et al. (2011) where MBS was also positively correlated with the score from the 'box test'.

The MBS was designed by O'Conner et al (1985) to be determined at 24-48 hours after birth, however in the studies by Parker and Nicol (1993), Corner et al. (2010) and Plush et al. (2011) MBS was recorded around 12-24 hours. Disturbing the ewe and her lambs too close to birth could jeopardize the formation of the ewe-lamb bond (Fisher and Mellor 2002). However, in the study by Murphy et al. (1994) MBS was recorded immediately after birth. Nevertheless, the temperament of the ewe and her MBS, regardless if recorded immediately after birth or at 24 hours, appear to be related (Parker and Nicol 1993; Murphy et al. 1994; Corner et al. 2010; Plush et al. 2011).

Table 1.8. Correlations of post-tagging ewe and lamb behaviours (from Corner et al. 2010).

	1. Ewe weight	2. Lamb birth weight	3. Ewe MBS	4. High bleats	5. Low bleats	6. Lamb bleats	7. Time to bleat	8. Time to stand	9 .Time to contact	10. Time to suck	11. Time to move	12. Time to follow
1. Ewe weight (P130)	-		0.17**	-0.19**		-0.20**			-0.20**		-0.21*	
2. Lamb birth weight	267	-				-0.16^{+}						
3. Ewe MBS	265	265	-	-0.29***					-0.36***			
4. Ewe high bleats	266	266	265	-		0.16**			0.58***	0.20*		-0.13*
5. Ewe low bleats	267	267	265	266	-						0.29**	
6. Lamb bleats	263	263	261	262	263	-	-0.14*	-0.12^{+}	0.15*			
7. Lamb time to bleat	239	239	237	238	239	235	-	0.56***	0.19**	0.24*	0.25*	0.16*
8. Lamb time to stand	240	240	238	239	240	236	222	-	0.40***	0.21*	0.31**	0.16*
9 .Lamb time to contact	238	238	236	237	238	234	219	222	-	0.32**	0.20*	
10. Lamb time to suck	89	89	89	89	89	89	84	89	88	-		0.27*
11. Lamb time to move	108	108	107	108	108	106	98	106	104	28	-	
12. Lamb time to follow	267	105	265	266	267	263	239	240	238	89	108	-

MBS= maternal behaviour score.

Correlation coefficients and P-values are presented above the diagonal and number of animals included below.

Blank cells indicate non-significant correlations (P>0.1); +P<0.1, *P<0.05, **P<0.001 and ***P<0.0001.

O'Connor et al. (1996) reported that there was a positive correlation between the frequency of ewe bleats and ewe MBS over a 14 week period between birth and weaning. Murphy et al. (1994) reported a weak negative relationship between ewe maternal behaviour and the number of high-pitched bleats emitted by the ewe. A negative correlation between ewe MBS and the number of ewe high-pitched bleats at tagging were reported by Everett-Hincks et al. (2005a; 2007). Further a positive correlation between ewe MBS and ewe low-pitched bleats was reported by Everett-Hincks et al. (2007). Corner et al. (2010) reported that the MBS of the ewe was negatively correlated with the number of ewe high-pitched distress bleats that were emitted by the ewe. Ewe high-pitched bleats were also negatively correlated with the time required for the lamb to follow its mother (Corner et al. 2010). They further found that the number of ewe high-pitched bleats was positively correlated with: the number of total lamb bleats, the time required for the lamb to make contact with ewe and the time to suck from the dam.

The ewe's grooming behaviour and reaction to sucking attempts appear to be related to vocalisation of the newborn lamb (Dwyer et al. 1999). As previously mentioned, lamb vocalisation has been suggested to indicate the need of the lamb, and may thus elicit increased maternal attention (Dwyer et al. 1999). Conversely, Dwyer (1999) stated that the number of low-pitched bleats emitted by the ewe is correlated with positive maternal behaviours such as grooming.

Vocal activity of the lamb and discrimination of the ewe has also been found to be linked. Nowak (1990) reported that lambs which emitted a higher number of bleats are more stimulating to their mothers. The dam will thus respond with increased maternal care and stimuli, each stimulus reinforcing the ability of the ewe and the lamb to recognise each other.

It has further been suggested that a vigorous lamb is better at discriminating its dam from an alien ewe and reaches her more quickly during a pen test than a less vigorous lamb (Nowak et al. 1989). Nowak et al. (1997) found that lambs that were prevented from sucking during the first six hours after birth were significantly worse in discriminating their dams during a separation test, when compared to lambs that had free access to suck. A vigorous lamb is likely to have had more opportunity to suck and interact with the ewe, and hence have learnt the mother’s features better than a non-vigorous lamb (Nowak et al. 1997).

Lamb neo-natal behaviours with lamb traits

Many neonatal behaviours expressed at 12 to 24 hours after birth are inter-related (Corner et al. 2010; Dodd et al. 2012). Corner et al. (2010; Table 1.8) showed that the number of lamb bleats was negatively correlated with the latency of the lamb to bleat at 12 to 24 hours after birth. The time it took for the lambs to bleat was found to be positively correlated with: time to stand, make contact, suck, move and follow their dam at 12 to 24 hours after birth (Corner et al. 2010). Corner et al. (2010) also reported a positive relationship between the latency for the lamb to make contact with the dam and latency to suck and move, also at 12 to 24 hours after birth.

Nowak (1990) showed a positive correlation between the number of lamb bleats emitted at birth and the time it subsequently spent with its dam during a triangle test at 12 hours. This indicate that lambs that showed higher bleating at birth were more likely to successfully discriminate their mothers from alien ewes, compared with those that have low bleating rates (Nowak 1990).

Even though a number of studies report on behavioural correlations many inconsistencies exist. A possible reason for these inconsistencies may be the breed of sheep, the external environment and the timing of the behavioural observations in relation to birth.

Factors affecting ewe and lamb behaviour and lamb survival

Lamb mortality percentages range between 5% and 40% for individual New Zealand flocks (Hight and Jury 1969; Dalton et al. 1980; Kenyon et al. 2002; Kerslake et al. 2005; Young et al. 2010). A number of studies have shown that lamb mortality is greater in multiple-born lambs compared with singleton lambs (Table 1.9). New Zealand studies have reported lamb losses to weaning for twins and triplets of 14% to 20% and 28% to 56%, respectively (Hight and Jury 1969; Dalton et al. 1980; Scales et al. 1986; Kenyon et al. 2002; Morris and Kenyon 2004; Morel et al. 2008; Morel et al. 2009).

Dystocia, as identified through post-mortem examination, has been cited as a main cause of lamb mortality and accounted for 55% of lamb deaths based on an on-farm study in 2003 and 2004 in New Zealand (Kerslake et al. 2005). Starvation/exposure was the other large contributor to lamb mortality and under New Zealand conditions accounted for approximately 30% of lamb losses (McCutcheon et al. 1981; Kerslake et al. 2005). The pathological signs of starvation/exposure include evidence of hypothermia accompanied by the absence of evidence of feeding (Haughey 1991). Dystocia can also in some cases be a precursor to starvation/exposure (McCutcheon et al. 1981). Other causes of neonatal lamb death include disease, predation, placental insufficiency and congenital malformations (Hight and Jury 1969; Lynch et al. 1992; Sargison 1997; Dwyer et al. 2005; Kerslake et al. 2005; Dutra et al. 2007).

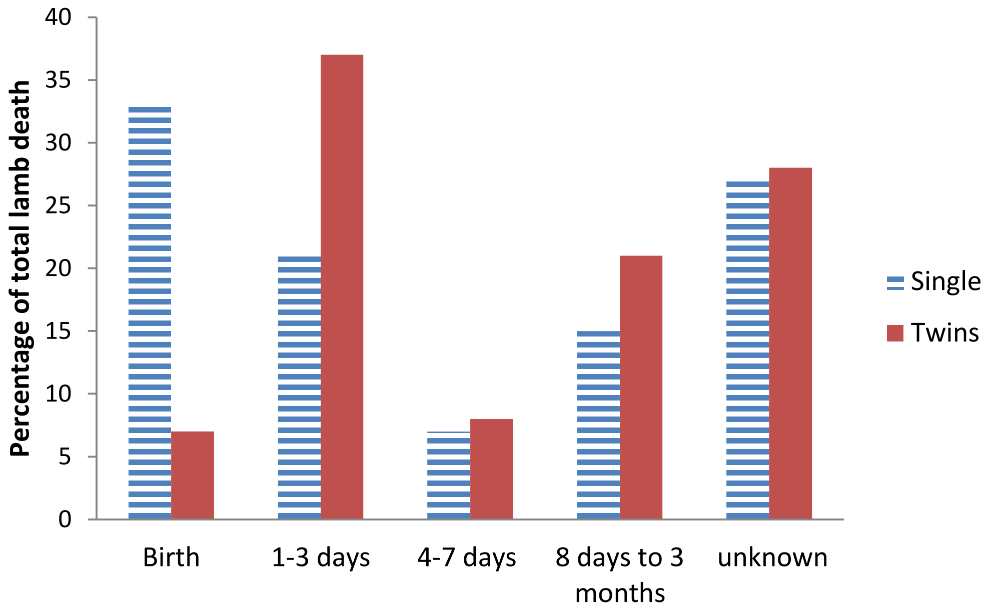
Table 1.9. Mortality rates (%) to weaning for single-, twin-, triplet- and quadruplet-born lambs.

	Litter size			
Author	Singles	Twins	Triplets	Quadruplets
Hight and Jury (1970)	21.0	16.0		
Dalton et al. (1980)	17.0	26.0		
Rohloff et al. (1982)	9.0	7.0	18.0	
Johnson et al. (1982)	15.7	20.7		
Hinch et al. (1983)				
Flock A	11.5	24.4	49.7	57.1
Flock B	7.1	17.6	34.9	43.3
Hinch et al. (1986)	11.7	17.0	40.3	55.4
Scales et al. (1986)	14.1	14.7	33.0	
Holst (1987)		20.8		
Knight et al. (1988)	15.0	19.0		
Alexander et al. (1990)	7.0	11.0	26.0	
Nicoll et al. (1999)	12.0	21.0	43.0	
Kleeman et al (1991)	12-13	29-38		
Nowak and Lindsay (1992)		13.2		
Holst et al. (2002)	11.3	20.8	46.2	
Kenyon et al. (2002)	17.4	19.7	40.7	
Morris and Kenyon (2004)		14.0	32.0	
Thomson et al. (2004)	10.0	12.0	24.0	
Kerslake et al. (2005)	14.0	16.0	29.0	
Kleemann and Walker (2005b)		43.8		
Kleemann and Walker (2005)	16.6			
Fowler et al. (2007)		31.5		
Oldham et al. (2011)				
Study 1	16.5	40.5		
Study 2	10.7	37.9		

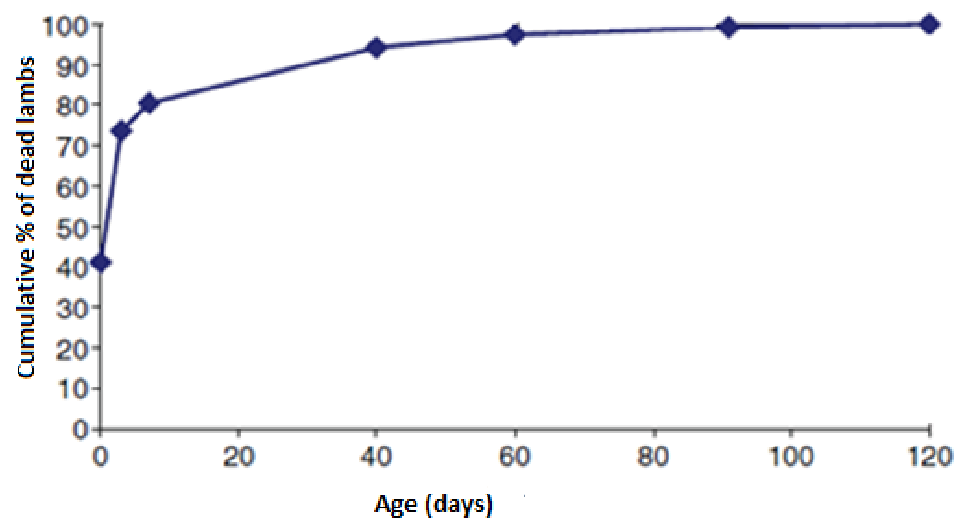
The average age at death varies markedly (Fig. 1.3a and b); lambs dying of dystocia generally die within the first 24 hours after birth while lambs dying of starvation/exposure often die between three and six days after birth (Hinch et al. 1986). Lambs that died between 24 hours and three days could have died of either cause. Even though large inconsistencies in reported lamb deaths occur, they are generally most common during the first three days after birth (Hight and Jury 1969; Nicoll et al. 1999; Nowak et al. 2000; Dutra et al. 2007; Hinch and Brien 2014). Of total lamb deaths to weaning, approximately 50% (Hight and Jury 1969), 70% (Dwyer et al. 2003), 80% (Hinch et al. 1985; Hinch et al. 1986; Binns et al. 2002) and 99% (Young et al. 2010) have been reported to have died within the first three days after birth.

Figure 1.3a & b. The proportions of lamb loss from birth to weaning (a) Nowak et al. 2000; b) Hinch and Brien, 2014).

a)



b)



Maternal nutrition during pregnancy

In pastoral based production systems mild chronic undernutrition is more likely to be encountered than acute undernutrition or starvation (Mellor, 1983). The feeding requirements for maintenance in the first two trimesters of pregnancy are similar to levels required for non-pregnancy ewes, but in the third trimester metabolisable energy (ME) requirements increase by 50-100% in order to support the exponential growth of the fetus (Geenty and Rattray, 1987). The feeding requirements of pregnant ewes are dependent on the ewes live weight and the number of foetuses. A month prior to lambing ewes bearing singleton, twin and triplet foetuses require an additional 2.6, 4.6 and 6.5 MJ ME per day, respectively (Geenty and Rattray, 1987). Therefore, twin- and triplet-bearing ewes should be offered pasture masses of approximately 1200 kg DM/ha during mid- to late-pregnancy to achieve optimal lamb birth weight.

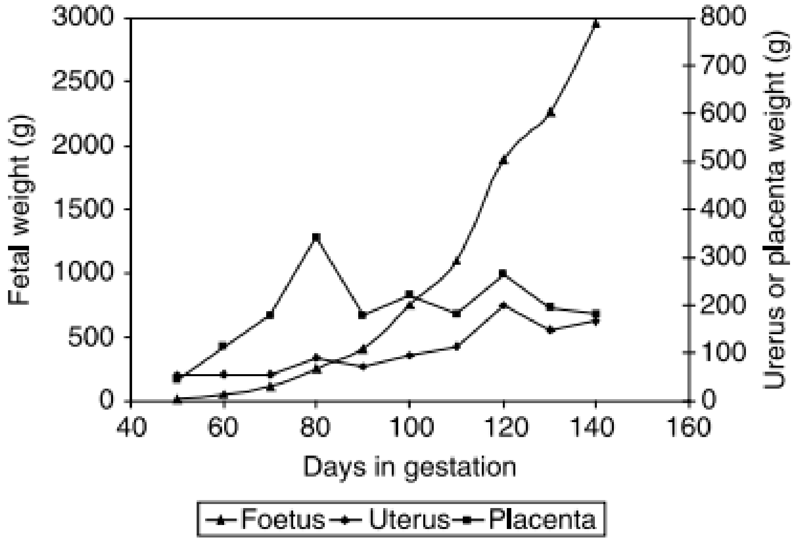
This section is not intended to provide an extensive discussion on the physiological mechanisms of ewe nutrition or BCS during pregnancy, for a more in-depth review see Mellor (1983) and Robinson et al (1977; 1999). The emphasis of this section will be on the main physiological effects of maternal nutrition and BCS on the ewe and lamb, and the subsequent effect on behaviour, as this is the main aim of this thesis. Other effects of maternal nutrition and BCS, such as those on production traits, are briefly covered throughout the thesis but for more in-depth reviews see (Kenyon et al. 2012; Kenyon et al. 2014).

The effect of maternal nutrition during pregnancy

In the pregnant ewe, undernutrition can result in altered plasma concentrations of metabolites such as glucose, NEFA and β-OHB (Chandler et al., 1985; O'Doherty and Crosby, 1998). Feeding pregnant mammals above or below pregnancy maintenance requirements can result in changes in steroid somatotrophic axis and thyroid hormones (Breier, 1999; Renaville et al. 2002; Vonnahme et al. 2013). These endocrine changes have been linked with the capacity of the placenta to transport nutrients to the fetus (Fowden et al. 2006). Undernutrition of the pregnant ewe causes maternal hypoglycaemia, reduced umbilical and uterine uptake of glucose, reduced glucose transfer capacity and a decreased demand for maternal glucose by fetal, placental and uterine tissues (Beuman and Currie, 1980;Leury et al. 1990), all of which can have a direct effect on fetal growth and performance (Lemley et al. 2014).

Insufficient ewe nutrition during pregnancy may lead to retarded placental and fetal growth and low birth weights (Mellor 1983; Symonds et al. 2006; Kenyon 2008; Belkacemi et al. 2010). It could also lead to reduced mammary development (Wallace 1948; Charismiadou et al. 2000) resulting in delayed onset of lactation and lower milk secretion (Mellor et al. 1987; Meyer et al. 2011), reduced colostrum yield (Mellor and Murray 1985; Meyer et al. 2011) and total milk production (Wallace 1948; Bizelis et al. 2000), and subsequently increased lamb mortality due to starvation and exposure (Alexander et al. 1984). The physiological effects on the ewe and fetus of feeding above pregnancy maintenance requirements during pregnancy are not well documented.

Figure 1.4 Increase in weight of fetus, placenta and uterus during gestation in sheep (Gootwine et al. 2007).



Placenta size

Lamb birth weights are influenced by maternal nutrition and placental size (Reynolds and Redmer, 1995; Redmer et al. 2004). The placenta transports nutrients to the fetus and eliminates waste products from the fetal circulation (Kelly, 1992). The delivery of nutrients to the fetus is determined by the size of the placenta, functional capability, blood flow rate and the concentration gradient between fetal and maternal circulation (Kelly, 1992). In sheep, placental growth proceeds rapidly between day 40 and day 80 of pregnancy (Gootwine et al. 2007; Figure 1.4) and this is a critical period of sensitivity to maternal nutrition (Robinson et al. 1999). The impact of nutrition on placental growth differs according to the body condition and live weight of the ewe at breeding; light ewes have smaller placentas while ewes in good condition may have enhanced placental growth in response to nutrient restriction (Kelly, 1992; McCrabb et al. 1992; Robinson et al. 1999). Placental insufficiency can lead to low birth weights (discussed in detail on page 33), hypoxia and metabolic immaturity. Hypoxia may develop due to insufficient circulation of respiratory gases through the placenta and it compromises the lambs' ability to thermoregulate (Mellor, 1988). Metabolic immaturity refers to lambs that are not adequately developed to cope with the extra-uterine environment (Mellor, 1988; Greenwood et al. 2002). Theirs vital organs, such as heart, lungs and kidneys may not be fully developed (Greenwood and Bell, 2003) and they often have raised urea levels in the blood stream due to reliance on protein metabolism for energy supply instead of carbohydrate metabolism (Thomson et al. 2004).

Fetal growth

Fetal growth follows an exponential pattern (Figure 1.4), with a rapid increase in fetal weight in mid- to late-pregnancy (Ferrell, 1991). At the time of maximum placental size (approximately day 90 of pregnancy) the fetus has only gained 10% of its potential birth weight, thus 90% of fetal growth occurs after this time (Rattray et al 1974; Redmer et al. 2004). In early pregnancy (breeding to day 50 of pregnancy) the increase in fetal weight is minor and the energy requirements are low (Robinson et al. 1999). Previous studies have shown that ewe undernutrition during this period had little effect of on fetal weights (Davies et al. 1981; Clarke et al. 1998; Oliver et al. 2005). In mid- to-late-pregnancy (day 64 to 115 of pregnancy), ewe undernutrition causes inconsistent effects on fetal growth (Morris and Kenyon, 2004; Rattray et al. 1974; Morris et al. 1993; Davies et al. 1981). The effects of undernutrition may fluctuate due to level and length of restriction and phase of pregnancy (Redmer et al. 2004). The condition and live weight of the ewes may also affect fetal weights (Clarke et al. 1997). In late-pregnancy (day 90 of pregnancy and parturition) placental development is complete and fetal growth is rapid (Figure 1.4). Severe malnutrition of the ewe in the final 50 days of pregnancy can reduce fetal growth by up to 70% (Mellor, 1983). The effects of low lamb birth weights on behaviour and survival are further discussed on page 35.

Maternal physiology

Maternal behaviour is established during parturition through the release of hormones in the ewe. In the ewe estrogen priming and oxytocin release causing uterine contractions are essential for the establishment of maternal behaviour, while progesterone and prolactin are less important (Dunn, 2013). Plasma oestradiol concentration in late-pregnancy has been shown to correlate with the expression of maternal behaviours (Shipka & Ford, 1991; Dwyer et al. 1999) and progesterone is also involved in the control of maternal behaviour, mainly by reducing aggressive behaviours (Kendrick & Keverne, 1991). Oestradiol and progesterone function mainly to promote synthesis of oxytocin and b-endorphin messenger ribonucleic acid (mRNA) in brain regions linked with the expression of maternal behaviour (Broad et al. 1993) and increase synthesis of oxtocinergic receptors in the limbic areas of the brain (Insel, 1990).

Maternal nutrition can affect maternal behaviours directly through acting on the hormonal regulators of maternal behaviour or by, for example, the competing motivations of hunger and the desire to express maternal behaviours (Dwyer et al. 2003). Dwyer et al. (2003) compared ewes that were fed a level that were approximately equal to the estimated energy requirements for a gravid 50 kg ewe (H) and ewes which received approximately 65% of the energy requirements (L). This level of nutritional restriction was intended to mimic what ewes may experience during a moderately poor winter in Europe. In addition to behavioural measurements, ewe plasma progesterone and oestradiol concentrations were measured throughout pregnancy. Plasma progesterone was raised in both groups at week 6 of pregnancy and increased in L ewes until week 18. This increase was smaller in H ewes resulting in a significantly higher progesterone concentration in L ewes at week 14 which was maintained for the remainder of the pregnancy (Dwyer et al. 2003). Pre-conception oestradiol concentration did not differ between ewes of either nutrition group until mid-gestation (week 14 and 16) when L ewes had significantly higher plasma oestradiol than H ewes (Dwyer et al. 2003). Dwyer et al. (2003) reported that the change in progesterone and oestradiol concentrations affected the expression of maternal care directly through a reduction in grooming behaviour, and indirectly by lengthening the birth process.

Relevant literature on the physiological and behavioural effects of feeding ewes above-pregnancy-maintenance requirements during pregnancy are lacking. It is known, however, that feeding levels above-pregnancy-maintenance can cause an endocrinological effect, even when ewe live weight did not differ. Vonnahme et al. (2013) compared ewes fed 60% (RES), 100% (CON) and 140% (EXC) of pregnancy requirements. They reported that on day 131, EXC ewes had increased insulin and T3 concentrations compared to RES and CON ewes. By mid-pregnancy RES ewes had increased circulating progesterone compared to EXC and CON. Throughout pregnancy EXC had greater cortisol concentrations than RES and CON ewes, which did not differ

(Vonnahme et al. 2013). The effect of these changes on behaviour was not measured in the study by Vonnahme et al. (2013).

A number of studies have investigated the effects of *ad libitum* grazing, compared with levels designed to meet pregnancy-maintenance requirement on production traits such as lamb live weight and survival (Kenyon et al. 2011, 2012a, 2012b, 2013; Corner et al. 2015). Overall, the results from those studies suggest that, from a production point of view, there are no clear advantages in offering twin- and triplet-bearing ewes of body condition 2.0 to 3.0 in mid-pregnancy above-pregnancy-maintenance feeding levels in mid-to late-pregnancy. These studies did not, however, investigate the effects of ewe BCS and nutrition on ewe and lamb behaviour. The effects of maternal nutrition on ewe behaviours will be discussed further on page 40.

The effects of birth weight on ewe and lamb behaviour and lamb survival

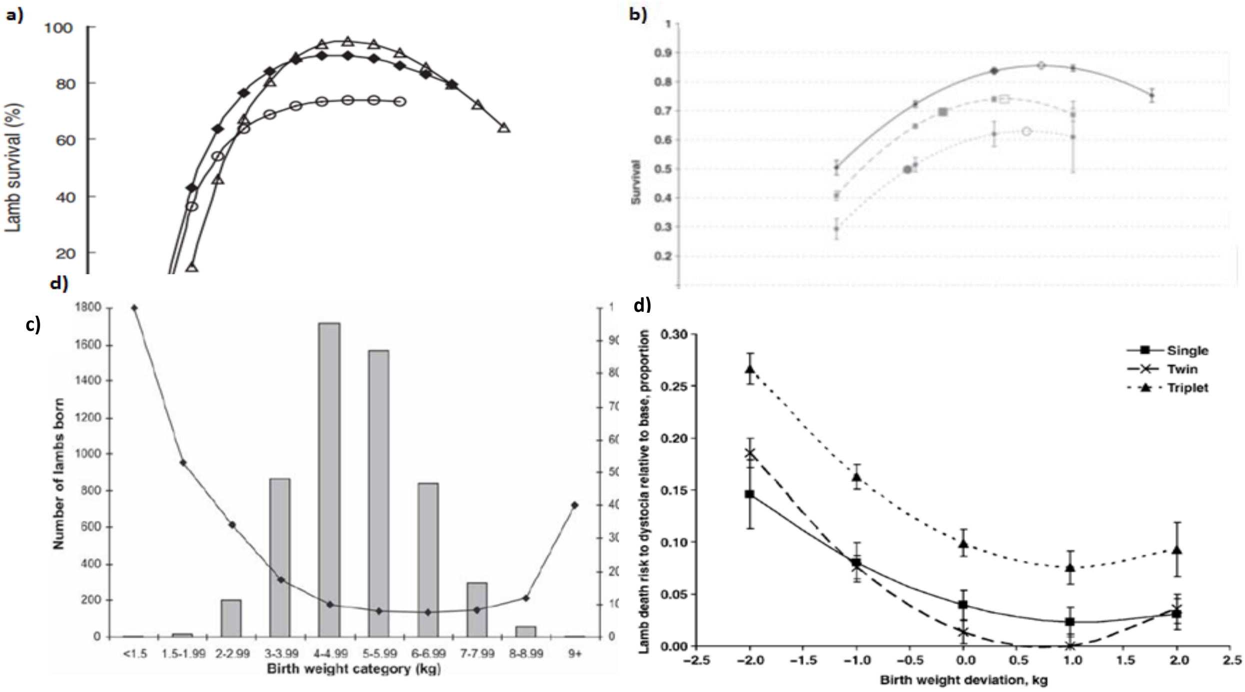
Lamb birth weight is the single largest driver of lamb deaths during the first three days of life (Dalton et al. 1980; Donnelly 1984; Scales et al. 1986; Paganoni et al. 2014). Lamb birth weight has a curvilinear relationship with survival (Fig. 1.5) such that across studies, lambs within the birth weight range of 4.5 to 6.5 kg have the greatest chance of survival (Dalton et al. 1980; Fogarty et al. 1992; Oldham et al. 2011; Hinch and Brien 2014; Paganoni et al. 2014). In addition, within multiple litters, survival of the lamb is dependent on its birth weight relative to the birth weight of its littermates (Morel et al. 2008; Morel et al. 2009).

An inadequate supply of nutrients, either as a result of maternal undernutrition during pregnancy and/or failure of the placenta to deliver nutrition can result in low birth weight lambs (Mellor et al 1983; Robinson and McDonald 1989; Redmer et al. 2004; Gootwine et al. 2007). Lambs of lighter birth weights have a greater surface-area-to-birth-weight ratio than lambs of heavier birth weights (Slee 1977; Alexander 1979) and are born with less energy reserves (Alexander 1962). Lighter lambs will lose more heat to the environment than heavier lambs, making it more susceptible to excessive heat loss and hypothermia. Even if a lighter lamb produces enough heat to counter the heat being lost it is still at a disadvantage. This is because, compared to heavier lambs, lighter lambs have a higher critical temperature below which it must generate heat to maintain a homeothermic status (Slee, 1977). This puts a greater demand on the lamb's energy reserves and a lighter lamb will thus exhaust its energy reserves faster than a heavier lamb (Eales et al., 1980). Colostrum is essential for heat production (Clarke et al. 1997), especially when body reserves approach depletion (Mellor and Cockburn, 1986) and hence appropriate ewe and lamb behaviours that facilitate bonding and suckling are vital for survival (Atroshi and Österberg 1979; O'Connor et al. 1985; Shillito-Walser 1978; Poindron et al. 1980).

Because of their lighter birth weights, triplet-born lambs are more likely to lose heat to their environment and have less energy reserves than single- and twin-born lambs. This offers some explanation to why the starvation/exposure syndrome is more commonly reported in multiple-litters (McCutcheon et al. 2003).

Several studies have shown that maternal undernutrition can affect lamb behaviour indirectly. Low maternal nutrition can result in reduced lamb birth weight which has been found to affect the behaviour and survival of the neonate lamb (Owens et al. 1985; Cloete 1993; Dwyer 2003; Hernandez et al. 2009; Corner et al. 2010). Low lamb birth weight has, for example, been linked with a reduced ability to recognise the dam from an alien ewe at 12 hours after birth (Nowak et al. 1987). Everett-Hincks (2005) showed that increasing pasture sward heights for ewes with triplet lambs increased the litter birth weight and improved lamb survival to tagging. The effects of birthweight on lamb behaviour may actually be an effect of litter size, and the subsequent proportion of time the ewe interacts with each lamb (Hernandez et al. 1992).

Figure 1.5. The relationship between lamb survival (%) and birth weight (kg) shown for a number of studies. A) The percentage lamb survival for single (Δ), twin (♦), and multiple (o) lambs (Holst et al. 2002). B) The percentage lamb survival for single –, twin ---- and triplet····· lambs (Hatcher et al. 2009). C) The mortality percentage represented by the u-shaped line for a mix of single-, twin- and triplet-born lambs. The number of lambs in different weight categories are also represented by vertical bars (Thomson et al. 2004) D) Lamb death risk to dystocia relative to base proportion is given for single –, twin ---- and triplet····· (Everett-Hincks and Dodds 2008).



The effect of ewe nutrition on ewe maternal behaviours

Thomson and Thomson (1949) showed that ewes fed on a lower nutritional plane were slower in cleaning their lambs and allowing them to suck. They further showed that severe under-nourishment during pregnancy depressed maternal behaviour and increased lamb mortality rates in both singletons and twin-born lambs. Muñoz et al. (2009) reported that low nutrition during early pregnancy increased the duration and frequency of ewe ‘withdrawing from their lamb’ (ewe moves back directly away from the lamb at her head) in the first 30 minutes after birth compared with ewes on a medium or high nutrition. Putu et al. (1988) found an increased number of desertations of twin-born lambs by Merino ewes that had been on a low nutrition plane in the last six weeks of pregnancy compared with well-fed ewes. Further, Dwyer et al. (2003) reported that ewes fed 65% of the control diet displayed more aggressive behaviour and spent less time grooming their lambs than well-fed ewes. Furthermore, Lindsay et al. (1990) reported that the time spent on the birth site increased when twin-bearing ewes were on a higher plane of nutrition during late-pregnancy than when on a low plane of nutrition. Based on the results from these studies it can be concluded that ewe undernutrition during pregnancy may have harmful consequences for the expression of maternal behaviour (Dwyer et al. 2003).

Corner et al. (2010) reported that ewes offered 4 cm sward height in both early- and late-pregnancy emitted more low-pitched bleats (61%) to their young than ewes offered 2 cm in early- and late-pregnancy (41%) or 4 cm in early- and 2 cm in late-pregnancy (39%). Additionally, Everett-Hincks et al. (2005) showed that within 12 hours after birth, triplet-bearing ewes offered 2 cm sward height during pregnancy emitted more high-pitched bleats, were at a greater distance from their litter and were less likely to make contact with the litter than ewes grazing higher sward heights.

The effect of nutrition on ewe MBS is still uncertain. Holst (1987) and Mulvaney et al. (2008) found that supplementary feeding during pregnancy did not affect maternal behaviour. These results were similar to those of Corner et al. (2010) and Everett-Hincks et al. (2005a) who concluded that a slight under nutrition (2 cm sward height) in early- and late-pregnancy had no effect on ewe MBS, thus contradicting results previously reported by Dwyer et al. (2003) and Corner et al. (2006). Many aspects of these studies varied, not only in the external environment, but also in breed of sheep and the age and maternal experience of the ewe, possibly causing the differences between studies. Few studies conducted under extensive pastoral farming conditions, such as those in New Zealand, have found a link between nutrition during pregnancy and ewe maternal behaviour (Everett-Hincks et al. 2005a; Corner et al. 2010). Furthermore, very few studies appear to have investigated the effect of *ad libitum* feeding during pregnancy on MBS (Everett-Hincks et al. 2005a).

The effect of ewe nutrition on lamb neonatal behaviours

There is evidence to suggest that the nutrition of the ewe during pregnancy affects the behaviour of the newborn lamb. Both Thomson and Thomson (1949) and Moore et al. (1986) reported after a subjective assessment, that lambs from underfed ewes lacked vitality and vigour. In addition, lambs born to ewes offered lower feeding levels in mid- and late-pregnancy (2-2) showed greater need (in terms of a greater bleating frequency) than lambs offered high feeding levels in early and/or late pregnancy (4-2, 4-4), in the study conducted by Corner et al. (2010).

Muñoz et al. (2008) showed that lambs born to ewes offered pregnancy maintenance feeding levels during mid-pregnancy tended to be quicker at attempting to stand than lambs from ewes offered above pregnancy maintenance feeding levels. They also stood more frequently, spent more time standing and tended to be quicker at attempting to suck from their dam. Early pregnancy diet had no effect on lamb behaviour; however, lambs born to ewes offered below pregnancy maintenance feeding levels during early-pregnancy had higher survival rates than lambs born to ewes offered pregnancy maintenance levels or above. Mid-pregnancy feeding levels did not affect survival.

Similarly, Muñoz et al. (2009) reported that lambs born to ewes offered 60% of predicted metabolisable energy (ME) requirements for pregnancy maintenance during mid-pregnancy showed a greater frequency of udder seeking behaviour than lambs born to ewes offered above pregnancy maintenance feeding levels. They also showed a greater frequency and duration of sucking compared with the lambs from ewes offered above pregnancy maintenance feeding levels. No differences were observed between the treatment groups in the latency to perform lamb neonatal behaviours; and lamb behaviours were not affected by ewe nutritional treatments during early pregnancy (Muñoz et al. 2009).

Conversely, Everett-Hincks et al. (2005a) reported that ewe nutrition during late-pregnancy had a greater effect on lamb behaviour than on ewe behaviour and that triplet-born lambs were more affected than twin-born lambs. Triplet-born lambs born to ewes offered 2 cm sward height (approximately 800 kg DM/ha which is below pregnancy maintenance levels for triplet-bearing ewes [Rattray et al., 1987]) were less likely to stand, locate the dam's udder and follow their dam compared to those whose dams were offered > 2cm sward heights, they were also more likely to bleat before making contact with their dam. There are a number of differences between the study by Everett-Hincks et al. (2005a) and those by Muñoz et al. (2008; 2009) such as breed, rearing rank and husbandry procedures which could all contribute to the contradictory results.

Few studies, both in New Zealand and otherwise, have investigated the effects of *ad libitum* compared to pregnancy maintenance feeding levels on lamb behaviours.

The effect of ewe body condition score (BCS) on ewe and lamb behaviour

Few studies have investigated whether ewe body condition score (Jefferies 1961) affects ewe and lamb post-partum behaviour and survival.

In late-pregnancy, the nutritional demand for the multiple-bearing ewe is significantly increased (Nicol & Brookes 2007). If the ewe cannot meet the increased nutritional demand via intake, she must utilise body reserves. Therefore, it may be expected that the impact of BCS on fetal growth and lamb birth weight would be greatest in late-pregnancy (Kenyon et al., 2014). In the majority of previous studies, ewe BCS has had little effect on lamb birth weight or survival, possibly due to the large variation in the timing of BCS measurement, the number of fetuses per ewe and maternal nutrition. For a full review see Kenyon et al. (2014).

It has been shown that a change in ewe BCS during pregnancy can affect the behaviour of neonatal lambs (Dwyer 2003). Dwyer et al. (2003) showed that ewes which lost body condition during pregnancy, as indicated by a reduction in back fat, produced lambs that were slower to stand and suck during the neonatal period than lambs born to ewes that did not lose body condition. The study also found that lambs born to ewes that lost a large amount of condition during pregnancy were lighter at birth than those born to ewes that did not lose body condition.

Several studies have reported that ewe BCS can affect lamb survival (Al-Sabbagh et al. 1995; Kenyon et al. 2011; Kenyon et al. 2012; Kenyon et al. 2013; Kenyon et al. 2014), although, studies are often inconsistent. It is possible that some of the effects of ewe BCS on lamb survival are driven by changes in ewe and lamb behaviour.

The effects of litter size on ewe and lamb behaviour

It has been suggested that litter size stimulates maternal ability. O'Connor et al. (1985) reported that there was a positive relationship between MBS and litter size. This relationship has also been found by others (Lambe et al. 2001; Corner et al. 2005; Everett-Hincks et al. 2005b; Ekiz et al. 2007), however, other authors have found no relationship (Dwyer and Lawrence 1998; Corner et al. 2010). The reason for these inconsistent results is not yet clear.

Ewe and lamb high-pitched bleats, i.e. 'distress bleats', have also been shown to be positively correlated with litter size. Ewes rearing twin- or triplet-born lambs emitted more high-pitched bleats in the neonatal period than ewes with singletons (Pollard 1992; Dwyer et al. 1998). Pollard (1992) argued that this greater number of bleats was associated with an increased likelihood for ewes with twins and triplets being separated from their offspring. Twin-born lambs have also been found to bleat more frequently in the neonatal period than singletons, possibly due to the ewe having to divide her attention between her offspring (Hernandez et al. 1992).

The duration of intense grooming of individual lambs after birth for twin- and triplet-born lambs is less than for singletons (Alexander et al. 1990; Dwyer and Lawrence 1998). Alexander et al. (1990) reported that as a result of the dam spending less time grooming each multiple-born lamb the formation of the ewe-lamb bond was weaker. They hypothesised that the time the ewe spent grooming and in close contact with her lambs was directly related to her ability to discriminate her lambs.

Separation of the lamb from its dam is more common in multiple-born lambs than in singletons during the first days following birth (Alexander et al. 1983), thus resulting in fewer opportunities to nurse and to learn maternal features. Nowak and Lindsay (1992) suggested this may be a reason for a delayed ability of multiple-born lambs to discriminate their mother in a triangle test compared with singletons.

The effects of parity on ewe behaviour

Primiparous ewes may have a more difficult parturition (Wallace 1949), more birth injuries (Woolliams et al. 1983) and poorer maternal behaviour (Wallace 1949) than multiparous ewes. Poor maternal behaviour in primiparous ewes is well established (O'Connor et al. 1992; Alexander et al. 1993; Dwyer 2003; Ekiz et al. 2007; Otal et al. 2009; Mulvaney 2011). The ability of the ewe to display the appropriate maternal behaviour is influenced by experience (Poindron 2005) with improvement shown between the first and second birthing event.

A study by Poindron (2005) reported that primiparous ewes showed nearly 50% more behavioural problems compared with multiparous ewes. Primiparous ewes have been found to display less and delayed physical contact (Poindron and Neindre 1980; Alexander et al. 1993; Otal et al. 2009) and grooming behaviours towards their young (Owens et al. 1985; O'Connor et al. 1992; Ekiz et al. 2007) than multiparous ewes. They are also more likely to move away from their lambs and circle to avoid sucking attempts by their lambs (O'Connor et al. 1992; Alexander et al. 1993; Dwyer and Lawrence 2000) resulting in increased rejection rates (Dwyer and Lawrence 2000; Otal et al. 2009) compared with mature ewes. Alexander (1993a) reported primiparous ewes were more likely than mature ewes not to groom or show any interest in their lambs. Further, Mulvaney et al. (2011) reported that MBS was lower in primiparous ewes compared with mature multiparous ewes. Mulvaney et al. (2011) also reported that one-year-old ewes emitted a greater number of high-pitched ‘distress’ bleats than mature ewes.

As a result of these negative behavioural traits primiparous ewes tended to have weaker attachments to their lambs often resulting in rejection of the lamb (O'Connor et al. 1992; Dwyer et al. 1998; Dwyer and Lawrence 2000; Dwyer 2003; Otal et al. 2009). Kendrick et al. (1991) reported that primiparous ewes were less accurate in discriminating their lambs from alien lambs at four to six hours after birth. Primiparous ewes have also been shown to be separated more often from their lambs than multiparous ewes (Alexander et al. 1984).

The effects of parity on lamb behaviour and survival

Alexander (1993) reported that parity had no effect on neonatal lamb behaviour. A number of studies, however, have reported conflicting evidence. In contrast, Dwyer (2003) reported a negative correlation between parity and the time taken for lambs to perform all neonatal righting and standing behaviours with the exception of time to raise and shake head. Lambs from primiparous ewes were especially slow to stand and suck. Dwyer (2003) also stated that lambs born to primiparous ewes exhibited less frequent play behaviour compared with those born to multiparous ewes. Primiparous ewes have been shown to display aggression towards their lambs and exhibit non-cooperative sucking behaviours more frequently than multiparous ewes (Alexander et al. 1993; Dwyer and Lawrence 2000). This may help explain the slow neonatal progress of these lambs as neonatal lamb behaviour is known to be affected by ewe behaviour (Dwyer et al. 2003).

Frequency of lamb vocalisation has also been found to be affected by ewe parity. Dwyer et al. (1998) reported that lambs born to primiparous ewes bleated more frequently than lambs born to multiparous ewes. They suggested that the greater bleating rates from lambs born to primiparous ewes may indicate a lack of sucking, since their time to suck successfully was prolonged (O'Connor et al. 1992). Mulvaney (2011) reported that singleton lambs born to primiparous one year old ewes emitted a greater number of bleats and were slower to make contact with their dam, than those born to mature multiparous ewes within 24 hours of birth.

A number of studies have suggested that lamb mortality is greater for primiparous compared with multiparous ewes (Muñoz et al. 2009; Morel et al. 2010; Mulvaney 2011). There is, however, conflicting evidence as neither Alexander et al. (1993), Ekiz et al. (2006) nor Mulvaney (2010) reported any effects of parity on lamb survival. Corner et al. (2013) compared the performance of primiparous versus multiparous ewes. In a study of singleton bearing ewes, lamb survival was lower in lambs born to primiparous ewes. In a second study, which included both singleton and twin bearing ewes, lamb survival was only lower in twin lambs born to primiparous ewes.

The effects of breed on ewe and lamb behaviour

Previous studies indicate that ewe breed and experience, rather than age, affect maternal care in ewes. Previous research has shown that there are differences between breeds in the ability of the lambs to identify their dam (Nowak and Lindsay 1990), lamb vigour (Stevens et al. 1984), time to stand and suck after birth (Cloete et al. 2002) and vocalisation (von Borstel et al. 2011). Breed differences in ewe behaviour has also been reported.

The New Zealand Romney breed, when compared with Merinos, had lower rates of twin-separation in primiparous ewes when lambed under similar conditions (Alexander et al. 1983). A study conducted by Dwyer and Lawrence (1998) found that Suffolk ewes showed poorer maternal behaviour compared with Blackface ewes. Suffolk ewes were more likely to abandon their lamb and to show aggression towards it, whereas Blackface ewes were more cooperative to lamb sucking attempts and spent more time grooming their lambs. Blackface lambs were also more active in all aspects of neonatal behaviour and made more sucking attempts (Dwyer and Lawrence 1998; Dwyer 2003). Dwyer and Buenger (2012) stated that breeds selected narrowly for greater productivity were more likely to experience poor lamb vigour. The more selected breeds of sheep, such as the Merino, were thus believed to be inferior mothers to the more wild-type breeds, such as Bighorn (Dwyer and Lawrence 1998).

The effects of maternal stressors on the lamb

Stress has been defined as “the biological response elicited when an individual perceives a threat to its homeostasis” (Moberg, 2000). Prenatal stress has been defined by Braastad (1998) as “stress experienced by the pregnancy mother which affects the development of the offspring”. Prenatal stressors can alter fetal growth and birth weights (Mellado et al. 2000; Morris et al. 1999) and cause abnormal endocrine and behavioural responses to a stressor (Lay et al. 1997; Roussel et al. 2005). Sheep may be exposed to a variety of stressors including disease, injury, poor nutrition and hypo/hyperthermia (Dwyer and Bornett, 2004). The focus of this thesis is to examine the effects of ewe mid-pregnancy BCS and nutrition on ewe and lamb behaviours, therefore the potential outputs of the other stressors will not be reviewed.

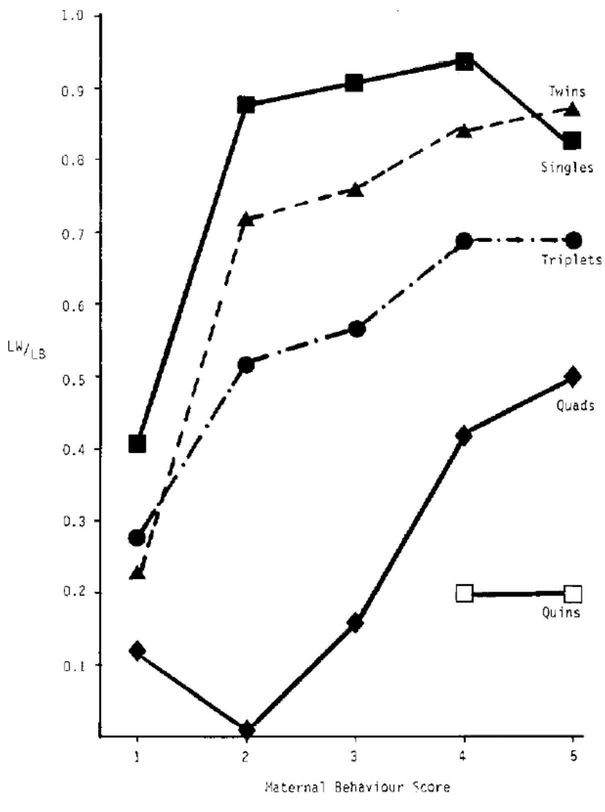
Does ewe and lamb behaviour affect lamb survival?

Ewe maternal behaviour and temperament

O'Connor (1985) identified a positive relationship between ewe MBS and lamb survival (Fig 1.6). Similar results were presented by a number of other authors reporting that an increase in MBS resulted in higher lamb survival rates (Lambe et al. 2001; Everett-Hincks et al. 2005b; Plush et al. 2011). Similarly Murphy et al. (1994) found a positive relationship between the amount of time the ewe spent licking her lamb during the first hour after birth and lamb survival.

Research investigating the relationship between ewe vocalisation and lamb survival appear to be lacking.

Figure 1.6. Relationship between lamb survival LW/LB (lambs weaned/lambs born) and ewe MBS (O'Connor et al. 1985).



Lamb vigour and its effects on lamb survival

Lamb vigour has frequently been hypothesised to influence lamb survival (Alexander et al. 1959; Wassmuth et al. 2001). The time required for the lamb to progress through a series of behaviours immediately after birth, such as stand and suck, have been used to assess the likelihood of lamb survival in intensive farming conditions such as those in Europe (Dwyer 2003; Dwyer et al. 2003). More vigorous lambs have been shown to have higher survivability (Dwyer and Buenger 2012). Cloete (1993) found that lambs that died before weaning were slower to stand and tended to be slower at progressing from standing to suckling than lambs that survived until weaning. It has been suggested, however, that behavioural development might not provide an accurate reflection of lamb survival under more extensive conditions, i.e. large flocks managed under pastoral conditions such as those experienced in New Zealand and Australia (Lindsay et al. 1990). The relationship between lamb high- and low-pitched bleats and survival under pastoral farming conditions is also lacking. Further research in this area is needed.

Conclusion

Appropriate ewe and lamb behaviours are vital for the formation of a strong ewe-lamb bond and may affect lamb survival. These bonds are established in the first few days of the lambs' life, however, the majority of lamb mortalities also occur during this time. Research has shown that the behaviour of the ewe and the lamb during this time may affect lamb survival in intensive sheep management systems. Little research, however, exists on the effects of feeding ewes above pregnancy maintenance levels and ewe mid-pregnancy BCS on twin- and triplet-born lamb behaviour and survival under extensive pastoral conditions.

A majority of previous studies have examined neonatal lamb and ewe behaviour directly following birth and their effect on lamb survival. The nature of extensive pastoral farming conditions in New Zealand make conducting observations immediately after birth impractical. In addition, disturbance during this period can have negative consequences for ewe-lamb bonding, which could lead to the ewe rejecting the lamb (Fisher and Mellor 2002). Therefore, farmers will wait a few hours after before interacting with the ewe and her lambs. Therefore, the experiments in this study observed lamb and ewe behaviour at tagging, approximately 3 to 18 h after birth and during the triangle test at 12 or 24 h after birth. Hatcher et al. (2009) reported that of all the 28% of lambs that died before weaning, 6% died at or within 24 h of birth, 14% died within the first week of life, 3% died between 7 and 30 days of age and 8% between day 30 and weaning. Thus knowledge of behavioural parameters related to survival of these lambs is of interest.

Objective and aims

The overall aims were to determine

1. whether feeding twin- and triplet-bearing ewes above pregnancy maintenance requirements affected the behaviour of the ewe and her lambs.
2. if mid-pregnancy ewe BCS over the range of BCS 2.0 to 3.0 affected ewe and lamb behaviour and thus whether it is worth farmers manipulating ewe BCS .
3. which ewe and lamb behaviours affect lamb survival so that researchers can focus on these behaviours and farmers may use this information to identify lambs at risk of pre-weaning mortality

All experiments were conducted over a five year period on Massey University's Keeble Farm, 5 km south of Palmerston North, New Zealand (40°S, 175°E) with approval of the Massey University Animal Ethics Committee. Publications arising from each experiment are indicated on the title page of the respective chapter.

The objectives for this thesis were to examine:

- the effect of ewe nutrition during pregnancy on the behaviour of twin-bearing ewes and their lambs at tagging and at 12 to 24 hours after birth in a triangle pen test (Chapter 2 and 4).

- the effect of ewe nutrition during pregnancy on the behaviour of triplet-bearing ewes and their lambs at tagging and at 12 to 24 hours after birth in a triangle pen test (Chapter 3).

- the effect of maternal BCS during pregnancy on the behaviour of twin- and triplet bearing ewes and their lambs at tagging and at 12 to 24 hours after birth in a triangle pen test (Chapter 2, 3 and 4).

- whether ewe nutrition during pregnancy has a developmental programming effect on ewe and lamb behaviour at tagging (Chapter 5).

- maternal and neonatal behaviours at tagging and at 12 to 24 hours after birth in a triangle pen test and the relationship between behaviour and with survival of twin- and triplet-born lambs (Chapter 6).

CHAPTER 2

The effect of ewe nutrition and body condition score during late-pregnancy on the behaviour of twin-bearing ewes and their lambs

Abstract

This chapter describes the effect of maternal nutrition and body condition score (BCS) during mid- to late-pregnancy on the behaviour of twin-bearing ewes and their lambs, at tagging, in two experiments conducted under extensive pastoral conditions. The experiments included ewes with BCS of 2.0, 2.5 or 3.0 (as measured in mid-pregnancy) that were offered either medium or *ad libitum* (*ad lib*) feeding from day 112 until day 136 of pregnancy for experiment one and from day 128 until day 141 of pregnancy for experiment two. The time taken for lambs to stand, contact dam, suck from dam and follow their dam was recorded at tagging, three to 18 hours after birth. The number of high- and low-pitched bleats emitted by the ewe and lambs were recorded as well as maternal behaviour score for the ewe (MBS). In experiment one, a subset of lambs were also tested for their ability to identify their dam at 12h or 24h of age in a triangle test. In experiment one, a greater percentage of lambs born to BCS 2.0 ewes made contact with their dam, at 5 seconds after observations begun, than those born to BCS 2.5 ewes ($P < 0.05$) but they did not differ from BCS 3.0 ewes ($P > 0.05$). Further, a greater proportion of lambs born to ewes in the medium treatment made contact with their dam at 2 minutes after observations begun, than those born to *ad lib* ewes ($P < 0.05$). In experiment two, a greater proportion of lambs born to the *ad lib* ewes made contact at 30 seconds, compared to lambs born to medium ewes ($P < 0.05$). The number of high- and low-pitched bleats emitted by ewes and lambs in both experiments showed an interaction of ewe feeding treatment and BCS. There was, however, no clear pattern identified between studies. In the triangle pen test, at 24h of age, lambs spent more time walking and more time with their dam compared with lambs tested at 12h of age (P

< 0.05). These results indicate that lambs tested at 24h of age had developed a stronger bond with the ewe and had increased ability to identify her than at 12h of age. The majority of lamb paddock and triangle pen test behaviours were not affected by ewe BCS at mid pregnancy and feeding treatment or were not consistently expressed between studies. These experiments show no overall advantage of feeding ewes *ad libitum* during mid to late-pregnancy in terms of ewe or lamb behaviours at tagging.

Introduction

The average lambing percentage in New Zealand has increased from approximately 100% in 1990 to 128% in 2013 (Anon. 2013). This increase is associated with a greater percentage of twin- and triplet-litters (Amer et al. 1999) which have greater mortality rates than singles (Hight and Jury 1970; Dalton et al. 1980; Hinch et al. 1983; Kenyon et al. 2002; Kerslake et al. 2005). Lamb mortality is of concern to the New Zealand sheep industry from both an economic and an animal welfare perspective (Mellor and Stafford 2004). A better understanding of the factors involved in multiple-born lamb mortality could improve ewe flock productivity through the development of strategies to reduce lamb mortality.

Ewe and lamb behaviours are known to affect lamb survival (Alexander et al. 1959; Murphy et al. 1994; Lambe et al. 2001; Plush et al. 2011). Appropriate maternal and lamb behaviour at parturition facilitates lamb survival (Atroshi and Österberg 1979; O'Connor et al. 1985; Nowak 1996). The formation of a ewe-lamb bond is the result of a rapid learning process by both the ewe and her lambs (Shillito-Walser 1978). Poindron et al. (1980) suggested that the behaviour of the newborn lamb also influences the behaviour of the ewe and Everett-Hincks et al. (2005) recommended that ewe and lamb behaviours should be investigated conjointly when studying the environmental conditions that influence the development of an effective ewe-lamb bond.

Previous studies have shown that ewe feeding levels during pregnancy can impact maternal behaviours including lamb cleaning (Dwyer et al. 2003), making and maintaining contact with the lamb (Putu et al. 1988; Everett-Hincks et al. 2005) and mothering ability (Thomson and Thomson 1949; Corner et al. 2006). In addition, lamb neonatal behaviours such as the time to stand after birth, time to follow dam (Everett-Hincks et al. 2005) and udder seeking (Muñoz et al. 2009) can be affected by maternal nutrition. To date studies examining the impact of ewe nutrition on ewe and lamb behaviour have been focussed on the potential consequences of undernutrition. There is a lack of information on the potential advantages or otherwise of above-pregnancy-maintenance nutrition on ewe and lamb behaviour.

It is possible that the variation in the results observed between previous nutritional studies could be explained by variation in the body condition score (BCS) of the ewe during pregnancy. Dwyer (2003) reported that ewes that lost condition during pregnancy, as indicated by a reduction in back fat depth, produced lambs that were slower to stand and suck than lambs from ewes that did not lose condition. In general, however, there appears to be a lack of research examining the effect of ewe BCS on maternal behaviour and lamb behaviour. Therefore, this study investigated the effect of both mid-pregnancy BCS and maternal nutrition during late-pregnancy on the behaviour of twin-bearing ewes and their newborn lambs under extensive pastoral conditions.

Materials and methods

Two experiments were conducted during consecutive years on Massey University's Keeble Farm, 5 km south of Palmerston North, New Zealand (40°S, 175°E), with the approval of the Massey University Animal Ethics Committee. The effects of ewe maternal nutrition and BCS during pregnancy on the liveweight of the ewe and her lambs to weaning have been previously reported (Kenyon et al. 2012a; Corner-Thomas et al. 2015a), however, the effects on the behaviour of the ewe and lambs were not reported. Briefly, these experiments compared the effects of *ad libitum* (*ad lib*) feeding with feeding levels that met only pregnancy-maintenance requirements (medium). In both experiments, total liveweight gain of ewes in the feeding period indicated that the desired nutritional conditions were met (Rattray et al. 1974; Kenyon et al. 2012a; Corner-Thomas et al. 2015a).

The aim of this analysis was to explore the behaviours of the ewe and her twin-born lambs. Only complete sets of twin-born lambs (where both lambs were alive at investigation) and their dams were included in the analysis due to the potential variation in behaviour of a single lamb as opposed to a complete set.

Experiment one (2010)

Background

The experiment included 154 twin-bearing Romney ewes (3 to 5 years of age) mated over a 17-day period, and their lambs (n=308). These ewes were a subset of 185 ewes utilised in the study by Kenyon et al. (2012a). The experiment began 89 days after the start of breeding (P89), when mean live weight of ewes was 64.9 ± 0.42 kg. From P89 to P111 the pre- and post-grazing covers were 1273±27 and 885±26 kg DM/ha, respectively (Kenyon et al. 2012a). At P112, ewes were randomly allocated to either a 'medium' or '*ad lib'* feeding treatment until P136. Each feeding treatment included ewes with body condition scores of 2.0, 2.5 and 3.0 as measured on P89 (scale of one to five, one=emaciated, five=obese; Jefferies, 1961; Kenyon et al. 2014). Ewe BCS 2.0 and 2.5 during pregnancy are commonly seen in New Zealand flocks (Kenyon et al. 2014) but are below the recommended BCS of 3.0 (Sheep and Beef Code of Welfare, 2010). The ewe sub-groups contained the following number of ewes: *ad lib* BCS2.0 n=21, *ad lib* BCS2.5 n=28, *ad lib* BCS3.0 n=25, medium BCS2.0 n=27, medium BCS2.5 n=25 and medium BCS3.0 n=26.

The aim of the medium feeding treatment was to restrict ewe intake by offering pre-grazing herbage masses below 1200 kg DM/ha and post-grazing masses less than 1000 kg DM/ha (Kenyon et al. 2012a). The *ad lib* feeding treatment ensured post-grazing herbage masses did not fall below 1200 kg DM/ha. In the period between P112 and P136, the herbage masses of the *ad lib* feeding treatment were greater than the medium treatment for both mean pre-grazing (2005 ± 71 kg DM/ha versus 1272 ± 68 kg DM/ha) and post-grazing (1424 ± 76 kg DM/ha versus 855 ± 81 kg DM/ha. Kenyon et al.

2012a). Previous studies have indicated that ewe intakes were not restricted when ewes were offered herbage masses of greater than 1200 kg DM/ha (Morris and Kenyon 2004; Kenyon et al. 2011; Kenyon et al. 2012a; Kenyon et al. 2012b).

At P136 the feeding treatments were completed and the ewes were grazed as one group with average pre- and post-grazing herbage masses of 1913 ± 141 kg and 1434 ± 141 kg DM/ha, respectively. At P142, ewes were randomly allocated to paddocks for lambing with mean herbage masses of 1885 ± 80 kg DM/ha and herbage mean masses during lactation were 1368 ± 29 kg DM/ha (Kenyon et al. 2012a). All ewes lambed over a 20-day period and the experiment was completed at weaning (91 days after the midpoint of lambing).

Animal and behavioural measurements

Live weight and BCS of ewes were recorded at P112, P136 and P142. During the lambing period ewes were inspected twice daily at 8 am and 4 pm. Lambs were tagged once their coat was dry and the lamb was mobile (at approximately 3-18 hours of age). During tagging all lambs were identified to their dam, ear-tagged and had their weight, birth-rank and sex recorded. Immediately following tagging, the two lambs were placed together, lying on the ground while the observers moved approximately 10 metres away. The lambs were all released at the same time and this was considered to be 'time zero'. The observers then recorded the individual behaviours of the lamb and ewe during the next five minutes. Behavioural observations were conducted by teams of three and the teams remained consistent for the duration of the study.

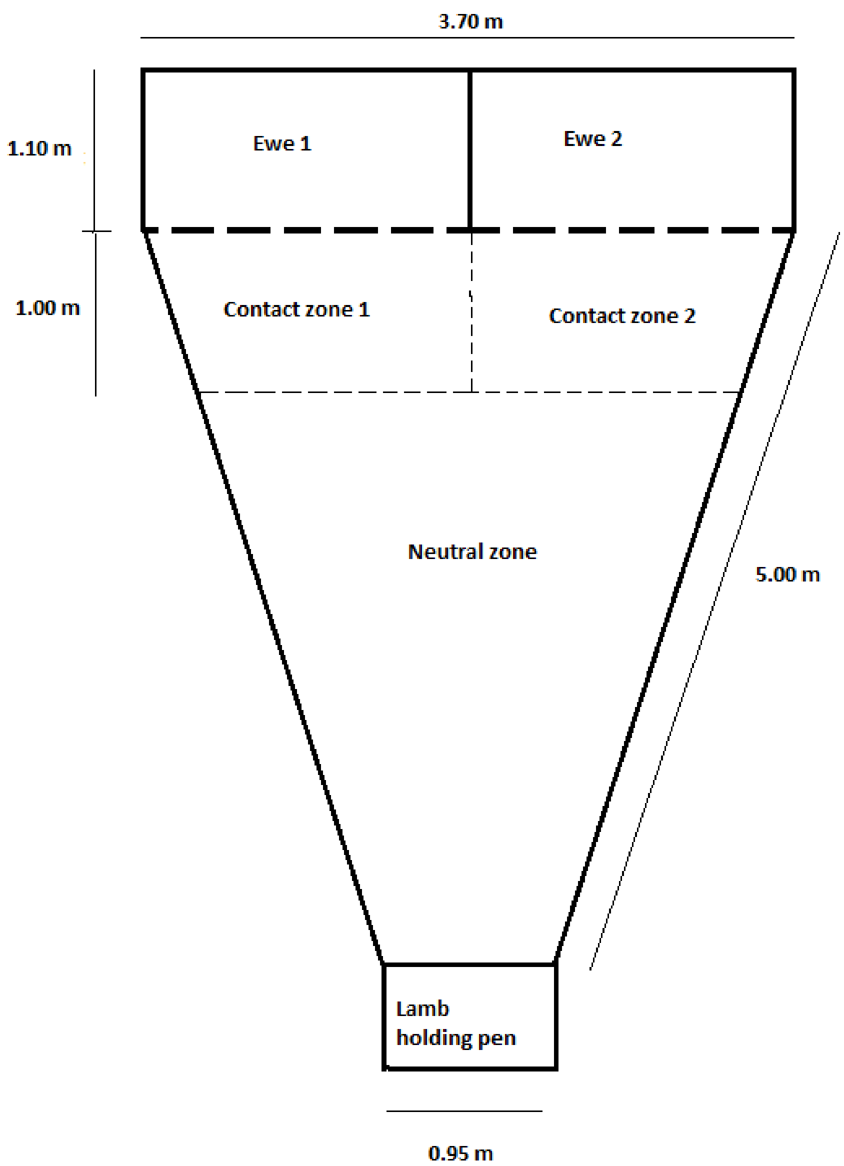
The behaviours recorded included time required for the lamb to stand and fully support itself on all four legs for at least five seconds, the time required for the lamb and ewe to make contact (lamb is within 0.5 metres of the ewe [Corner et al., 2005; Everett-Hincks et al., 2005]) and the time required for the lamb to follow the ewe (time from 'time zero' until ewe and lamb moved at least five metres away from their first contact point [Everett-Hincks et al., 2005]) was recorded. The time until the lamb successfully sucked from the dam's teat (lamb held teat in its mouth and appeared to be sucking with for at least five seconds) was noted. The total number of low-pitched bleats (bleats involving little mouth movement) and high-pitched bleats (bleats involving full mouth movement) emitted by each lamb during the five minute observation period were also counted.

A maternal behaviour score (MBS), as described by O'Connor et al. (1985), was recorded for each ewe. The MBS was assessed on a five-point scale based on the distance the ewe moved away from her lambs while the lambs were being tagged (one = at the approach of the shepherd ewe flees and does not return, five = ewe stays within one metre of the shepherd and lamb and touched either the shepherd or the lambs at least once). High- and low-pitched bleats emitted by the ewe were counted separately during the five-minute observation period.

Triangle test

A random subset of lambs (n=190, 95 complete sets of twins) were subjected to a triangle pen test, similar to that described by Nowak et al. (1987; 1989). The pen was of a triangular shape fenced by one metre high solid walls (3.7 x 6.1 m., Fig. 2.1). Adjacent to the narrow end of the triangle pen was a lamb holding pen. At the wider end of the triangle pen were two pens (1.85 x 1.1 m) side by side, in which ewes were placed. The triangle pen itself was divided into three zones with lines drawn on the ground, a neutral zone (the area of the triangle that was more than one metre from either ewe pen), and two ewe contact zones adjacent to the ewe pens (Fig. 2.1).

Figure 2.1 Diagram of the lamb triangle testing pen area showing dimensions of the area, layout and zones. Based on that by Nowaket al. (1987).



Testing occurred between 1pm and 3pm daily. As a result, lambs were tested at either approximately 12 (n=130) or 24 (n=60) hours of age depending on when they were born and tagged. The dam of the lamb being tested was placed randomly in one of the two ewe pens, with an 'alien' ewe which had lambed at a similar time placed in the other pen. Each lamb was tested individually and was placed, standing, in the waiting pen facing the two ewes. Once the lamb was let out into the neutral zone the lamb could see both ewes through a mesh gate. All other lambs, including the sibling of the lamb being tested and those of the alien ewe, were kept approximately five metres away so that the ewes could hear them but not see them. Each lamb was only tested once to avoid any possible learning effects (Shillito and Alexander 1975; Nowak et al. 1989).

Each triangle test was conducted for five minutes. The total amount of time that the lamb spent standing, sitting and walking was recorded. The time the lamb spent in the contact zone next to their dam, the time spent in the contact zone next to the alien ewe and the number of high- and low-pitched bleats emitted by the lamb was also recorded. The behavioural measurements were recorded at 10 second intervals. The lamb was considered to be attracted to any ewe if it reached the contact zone. Further, the lamb was considered to be preferentially attracted to a particular ewe if at least 2/3 of the time spent in the contact area was in the zone adjacent to that ewe (Nowak et al. 1987). There were three possible outcomes of this preference test; *correct choice*; the lamb entered the contact zone and spent at least 2/3 of this time beside its dam; *incorrect choice*; the lamb entered the contact zone and spent at least 2/3 of this time beside the alien ewe; and *no choice*; the lamb spent more than 1/3 but less than 2/3 of its time with each ewe (Nowak et al. 1987).

Experiment two (2011)

Background

In study two a similar procedure to that undertaken in experiment one was carried out with 120 twin-bearing Romney ewes (3 to 5 years old) mated over 17 days and their lambs (n=240). The experiment began 92 days after the start of the breeding (P92), when ewes weighed an average of 74.8 ± 0.5 kg. Ewes with body condition scores 2.0, 2.5 and 3.0 were included as in experiment one. In the period between P92 and P127 pre- and post-grazing herbage masses were 1080.3 ± 22.8 kg DM/ha and 795.3 ± 24.5 kg DM/ha (Corner-Thomas et al. 2015a), respectively. At P128, ewes were allocated to either a medium or *ad lib* feeding treatment until P141. The various sub-groups contained the following number of ewes; *ad lib* BCS2.0 n=15, *ad lib* BCS2.5 n=23, *ad lib* BCS3.0 n=18, medium BCS2.0 n=23, medium BCS2.5 n=19 and medium BCS3.0 n=22.

Between P128 and P141 herbage masses of the *ad lib* treatment were greater than the medium treatment both the pre-grazing (1784.2 ± 69.6 vs. 1069.5 ± 69.6kg DM/ha) and post-grazing (1333.4 ± 33.7 vs. 800.5 ± 30.2kg DM/ha. Corner-Thomas et al. 2015). At P142, the feeding treatments ended and the ewes were grazed together with average herbage masses of 1928 ± 50.5kg DM/ha. At P146 ewes were randomly allocated to paddocks for lambing. The mean herbage mass was 1410.1 ± 31.2 kg DM/ha during lactation (Corner-Thomas et al. 2015a). All ewes lambed over a 20-day period and the study was completed 91 days after the midpoint of lambing.

Animal and behavioural measurements

At, P113, P128 and P142 the live weights and BCS of the ewes were recorded. The behaviour of the ewes and lambs recorded at tagging and within five minutes of tagging were as described in experiment one. No lamb triangle test was conducted during experiment two.

Statistical Analysis

Statistical analyses were conducted using SAS v. 9.3 (SAS Institute Inc., Cary, 2011, NC, USA). Each experiment was analysed separately due to the differences in the timing of feeding treatments.

The effect of ewe feeding treatment and BCS on ewe live weight and BCS

Live weight and BCS of the ewes were analysed using a mixed model allowing for repeated measures that included the fixed effects of ewe feeding treatment (medium vs. *ad lib*), BCS group (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction and the random effect of ewe. The model also included days to lambing as a covariate which was the number of days between P140 and the date the ewe lambed.

The effect of ewe feeding treatment and BCS on lamb vocalisation

The percentage of lambs that emitted at least one high-pitched bleat and the percentage of lambs that emitted at least one low-pitched bleat was analysed using a generalised model based on a binomial distribution and a logit transformation. The number of bleats emitted was analysed using a generalised model based on a Poisson distribution. The models included the fixed effects of feeding treatment (medium and *ad lib*), BCS group (BCS 2.0, BCS 2.5 and BCS 3.0) and their two-way interaction. Lamb bleating behaviour in the triangle pen test also included the fixed effects of age of lamb at testing (12 or 24h) and the interaction of ewe feeding treatment with age of lamb and ewe BCS with age of lamb. The results are presented as the back-transformed means with 95% confidence interval in brackets.

The effect of ewe feeding treatment and BCS on lamb behaviours

The time required for lambs to stand, make contact with their dam, suck from their dam and follow her when she moved away during the paddock observations was tested for normality using the Kolmogorov-Smirnov test. All data were not normally distributed and could not be normalised. The time to exhibit a behaviour was analysed using survival analysis and using a Kruskal-Wallis test for comparisons of the median value. Survival curves were obtained using Kaplan-Meyer estimates. The effect of ewe feeding treatment and BCS group was tested in separate models. Lambs that did not perform a particular behaviour within the five minute observation period were censored at 301 seconds. Both the Wilcoxon p-value and the log-rank p-value are presented in the results. In general, the Wilcoxon test tends to be more powerful in detecting differences early in time while the log-rank test tends to be sensitive to distributional differences later in time (Martinez and Naranjo 2010).

Time to exhibit a behaviour was further analysed using the non-parametric Kruskal-Wallis test. The effect of ewe feeding treatments and BCS group were tested in separate models and the analysis contained all lambs including those that did not show the behaviour during the observation period. The lamb was given a value of 301 for a variable if that particular behaviour variable was not shown. The association between feeding treatment or BCS and the behaviour variable were investigated using the Wilcoxon Test. If ewe BCS was found to be significant, the Wilcoxon two-sample *post hoc* test was then carried out. Wilcoxon two-sample tests were used together with a Bonferroni adjustment to reduce the chance of Type 1 errors (Field et al. 2010).

The effect of ewe feeding treatment and BCS on the percentage of lambs to perform a behaviour during the triangle test

The percentage of lambs that stood, sat, walked, spent time with their dam or with the alien ewe was analysed using a generalised model based on a binomial distribution and logit transformation. The models included the fixed effects of age of lamb at testing (12 or 24h), feeding treatment and BCS group of the ewe and their two-way interactions. The results are presented as the back-transformed means with 95% confidence interval in brackets.

The effect of ewe feeding treatment and BCS on time spent exhibiting a behaviour during the triangle test

The distribution of the time the lamb spent standing, sitting, walking, spent with their dam or with the alien ewe were tested for normality using the Kolmogorov-Smirnov test. The distributions were not normal and could not be normalised using transformations. These behaviours were analysed using non-parametric Kruskal-Wallis tests. The individual models included ewe feeding treatment or BCS group, however, the interaction could not be analysed using non-parametric testing. In the event of a significant result, a Wilcoxon two-sample *post hoc* test was carried out. Each behaviour was analysed separately.

The effect of ewe feeding treatment and BCS on the outcome of the triangle test

The outcome of the preference test, i.e. whether the lambs made a 'correct choice', an 'incorrect choice' or made 'no choice' (correct choice =1, incorrect choice =2 and no choice = 3), was analysed using a generalised model based on a Poisson distribution. Sex of lamb, age of lamb at testing, feeding treatment and BCS group of the ewe were included as fixed effects and the interaction of ewe feeding treatment with BCS, ewe feeding treatment with age of lamb and ewe BCS with age of lamb.

The effect of ewe feeding treatment and BCS on ewe maternal behaviour score

Ewe MBS was analysed using a generalised model based on a Poisson distribution that contained the fixed effects of ewe feeding treatment, BCS group and their interaction. The results are presented as the back-transformed means with 95% confidence interval in brackets.

Results

The effect of ewe feeding treatment and BCS on ewe weight and BCS

Experiment one

The live weight for all three BCS groups differed at P112, P136 and P142 ($P < 0.05$, Table 2.1) such that ewes within the BCS 2.0 group were the lightest and ewes within the BCS 3.0 the heaviest. The live weight of the ewes in the two feeding treatments differed only at P136 (the end of the feeding period) such that ewes offered the *ad lib* treatment were heavier than the ewes offered the medium treatment (P < 0.05).

The BCS for all BCS groups also differed at P112, P136 and P142 (P < 0.05, Table 2.1). The mean BCS of the ewes for the two feeding treatments differed only at P136, when ewes offered the *ad lib* feeding treatment had greater body condition scores than ewes offered the medium feeding treatment (P < 0.05).

Table 2.1 Experiment one; the effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0 as on P89) on ewe liveweight (kg) and BCS at P112, P136 and P142 (means ± SE).

	n	P 112	P 136	P 142
			Ewe liveweight	
Feeding treatment				
medium	80	75.6 ± 0.60	81.5^{a} ± 0.67	85.2 ± 0.66
ad lib	74	75.0 ± 0.62	85.8^{b} ± 0.69	86.9 ± 0.69
BCS group				
BCS 2.0	50	71.7^{a} ± 0.75	79.2^{a} ± 0.83	82.1^{a} ± 0.82
BCS 2.5	53	74.9^{b} ± 0.74	84.3^{b} ± 0.82	86.5^{b} ± 0.81
BSC 3.0	51	79.2^{c} ± 0.74	87.5^{c} ± 0.82	89.7^{c} ± 0.81
			Ewe BCS	
Feeding treatment				
medium	80	2.5 ± 0.04	2.6^{a} ± 0.05	2.6 ± 0.04
ad lib	74	2.4 ± 0.05	2.8^{b} ± 0.04	2.7 ± 0.05
BCS group				
BCS 2.0	50	2.2^{a} ± 0.05	2.4^{a} ± 0.05	2.3^{a} ± 0.06
BCS 2.5	53	2.3^{b} ± 0.05	2.6^{b} ± 0.05	2.6^{b} ± 0.06
BSC 3.0	51	3.0^{c} ± 0.05	2.8^{c} ± 0.05	2.9^{c} ± 0.06

[abc] Different superscripts within columns and main effects indicate values that significantly differ (P < 0.05).

Experiment two

Ewe live weight did not differ between feeding treatments at any time point (P > 0.05, Table 2.2). At P113, P128 and P142 ewes within the BCS 2.0 group were lighter (P < 0.05) than the other two groups which did not differ (P > 0.05).

For ewe BCS at P113, P128 and P142 there was a significant interaction between feeding treatment and BCS group. Within the *ad lib* feeding treatment, at P113, BCS group 2.0 had a lower BCS than and the BCS group 2.5 (P < 0.05, Table 2.2) which in turn had a lower BCS than the BCS 3.0 group (P < 0.05). Within the medium feeding treatment, however, the BCS 2.0 group had a lower BCS than the BCS 3.0 group (P < 0.05) but not the BCS 2.5 group (P > 0.05). At P128 and P142, within the *ad lib* feeding treatment, BCS group 2.0 had a lower mean BCS than BCS group 2.5 and 3.0 (P < 0.05). Within the medium feeding treatment the BCS 3.0 group had greater BCS than the two other BCS groups (P < 0.05) which did not differ from each other (P > 0.05).

Table 2.2 Experiment two; the effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0 as on P92) and their interaction on ewe liveweight (kg) and BCS at P113, P128 and P142 (means ± SE).

	n	P 113	P 128	P 142
Feeding treatment			<u>*Live weight*</u>	
medium	64	73.6 ± 0.81	79.3 ± 0.82	85.6 ± 0.92
ad lib	56	73.5 ± 0.88	79.0 ± 0.88	85.0 ± 0.99
BCS group				
BCS 2.0	38	70.8^{a} ± 1.10	75.7^{a} ± 1.10	81.9^{a} ± 1.20
BCS 2.5	42	73.5^{b} ± 1.01	79.8^{b} ± 1.01	86.0^{b} ± 1.13
BSC 3.0	40	76.3^{b} ± 1.04	81.9^{b} ± 1.04	87.8^{b} ± 1.16
Feeding treatment			<u>*Ewe BCS*</u>	
medium	64	2.5 ± 0.04	2.5 ± 0.05	2.5 ± 0.05
ad lib	56	2.4 ± 0.04	2.4 ± 0.05	2.5 ± 0.05
BCS group				
BCS 2.0	38	2.2^{a} ± 0.06	2.1^{a} ± 0.05	2.1^{a} ± 0.06
BCS 2.5	42	2.6^{b} ± 0.06	2.4^{b} ± 0.05	2.4^{b} ± 0.06
BSC 3.0	40	2.8^{c} ± 0.06	2.8^{c} ± 0.05	2.7^{c} ± 0.06
Feeding treatment x BCS				
medium BCS 2.0	23	2.2^{ab} ± 0.07	2.2^{ab} ± 0.09	2.2^{a} ± 0.09
medium BCS2.5	19	2.4^{b} ± 0.08	2.3^{bc} ± 0.10	2.4^{ab} ± 0.10
medium BCS 3.0	22	2.6^{c} ± 0.09	2.6^{e} ± 0.09	2.6^{c} ± 0.09
ad lib BCS 2.0	15	2.0^{a} ± 0.09	2.0^{ac} ± 0.11	2.1^{a} ± 0.11
ad lib BCS 2.5	23	2.4^{b} ± 0.09	2.5^{de} ± 0.09	2.6^{bc} ± 0.09
ad lib BCS 3.0	18	2.9^{c} ± 0.08	2.8^{e} ± 0.10	2.8^{c} ± 0.10

[abc] Different superscripts within columns and effects indicate values that significantly differ (P < 0.05).

The effect of ewe feeding treatment and BCS on lamb paddock behaviours

Experiment one - vocalisation

There was no interaction on the percentage of lambs that emitted high- and low-pitched bleats (P > 0.05). A greater percentage of lambs born to ewes in the medium treatment emitted high-pitched bleats compared with lambs born to ewes in the *ad lib* treatment (P < 0.05, Table 2.3a). The percentage of low-pitched bleats were not affected by ewe BCS group or feeding treatment (P > 0.05)

The number of high-pitched bleats showed an interaction between ewe feeding treatment and BCS such that within the BCS 2.5 group lambs born to ewes in the *ad lib* treatment emitted more bleats than those in the medium treatment but within the BCS 3.0 group lambs born to ewes in the *ad lib* treatment emitted fewer bleats than those in the medium treatment (P < 0.05, Table 2.3a). No difference was found within the BCS 2.0 group (P > 0.05).

The number of low-pitched bleats also showed an interaction between ewe feeding treatment and BCS, such that in the BCS 2.0 group lambs born to ewes in the *ad lib* treatment emitted more low-pitched bleats than those in the medium treatment (P < 0.05, Table 2.3a). No difference was found within the BCS 2.5 and 3.0 groups (P > 0.05).

Table 2.1a. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction on the percentage (95% CI) of lambs that emitted high- and low-pitched bleats and on the total number of high- and low-pitched bleats in the paddock.

a)	n	High-pitched bleat		Low-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Experiment 1					
Feeding treatment					
medium	160	90.2[b] (84.5-93.9)	13.7 (13.1-14.3)	80.6 (73.7-86.0)	4.2 (3.9-4.5)
ad lib	148	78.9[a] (71.7-84.7)	14.3 (13.6-14.9)	74.3 (66.6-80.6)	4.5 (4.2-7.9)
BCS group					
BCS 2.0	100	87.8 (79.9-92.8)	14.4 (13.7-15.2)	80.6 (71.8-87.1)	4.7[b] (4.3-5.2)
BCS 2.5	106	87.0 (79.2-92.2)	13.7 (12.9-14.4)	76.8 (67.8-83.8)	4.0[a] (3.6-4.4)
BSC 3.0	102	80.8 (71.9-87.3)	13.9 (13.1-14.7)	75.2 (66.0-82.5)	4.4[ab] (1.0-4.8)
Feeding x BCS group[3]					
medium BCS 2.0	54	—	14.3[b] (13.3-15.4)	—	4.1[ab] (3.6-4.7)
medium BCS2.5	50	—	12.3[a] (11.3-13.3)	—	4.4[ab] (3.8-5.0)
medium BCS 3.0	52	—	14.4[b] (13.4-15.5)	—	4.1[ab] (3.6-4.7)
ad lib BCS 2.0	42	—	14.5[b] (13.3-15.7)	—	5.5[c] (4.9-6.3)
ad lib BCS 2.5	56	—	15.0[b] (13.9-16.1)	—	3.7[a] (3.2-4.2)
ad lib BCS 3.0	50	—	13.2[a] (12.1-14.3)	—	4.6[b] (4.0-5.2)

[1]The percentage of lambs that bleated are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2] The number of high- and low-pitched bleats are presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within columns and effects indicate values that significantly differ ($P < 0.05$).

Table 2.2 b. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction on the percentage (95% CI) of lambs that emitted high- and low-pitched bleats and on the total number of high- and low-pitched bleats in the paddock.

b)	n	High-pitched bleat		Low-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Experiment 2					
Feeding treatment					
medium	128	95.5[b]	15.3[b]	90.7	5.9[a]
		(90.3-98.0)	(14.6-16.0)	(84.2-94.6)	(5.5-6.4)
ad lib	112	86.9[a]	14.0[a]	84.1	7.4[b]
		(78.6-92.2)	(13.3-14.7)	(76.0-89.9)	(7.0-7.9)
BCS group					
BCS 2.0	76	96.2[b]	15.3[b]	89.2	6.4[a]
		(88.6-98.8)	(14.4-16.2)	(79.8-94.5)	(5.8-7.0)
BCS 2.5	84	93.0[b]	16.5[b]	87.6	6.3[a]
		(85.4-96.8)	(15.6-17.3)	(78.7-93.1)	(5.8-6.9)
BSC 3.0	80	83.2[a]	12.5[a]	86.3	7.3[b]
		(73.1-90.1)	(11.7-13.3)	(76.8-92.3)	(6.7-8.0)
Feeding x BCS group[3]					
medium BCS 2.0	46	97.8[b]	13.7[b]	—	8.6[d]
		(86.1-99.7)	(12.6-14.8)		(7.7-9.3)
medium BCS2.5	38	94.7[b]	16.9[cd]	—	5.3[b]
		(81.3-98.7)	(15.6-18.3)		(4.6-6.1)
medium BCS 3.0	44	90.9[b]	15.6[c]	—	8.3[d]
		(78.2-96.6)	(14.5-16.8)		(7.5-9.2)
ad lib BCS 2.0	30	93.3[b]	18.1[d]	—	3.7[a]
		(76.9-98.3)	(16.6-19.6)		(3.0-4.4)
ad lib BCS 2.5	26	89.1[ab]	16.0[c]	—	7.1[c]
		(76.4-95.4)	(14.9-17.2)		(6.4-8.0)
ad lib BCS 3.0	36	72.2[a]	8.8[a]	—	6.3[bc]
		(55.6-84.4)	(7.9-9.8)		(5.6-7.2)

[1]The percentage of lambs that bleated are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2] The number of high- and low-pitched bleats are presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within columns and effects indicate values that significantly differ ($P < 0.05$).

Experiment two - vocalisation

There was an interaction between ewe feeding treatment and BCS for the percentage of lambs that emitted high-pitched bleats. In the BCS 3.0 group, fewer lambs born to ewes in the *ad lib* feeding treatment emitted high-pitched bleats than those in the medium feeding treatment (P < 0.05, Table 2.3b), but no differences were observed in the BCS 2.0 and 2.5 groups (P > 0.05). The number of high-pitched bleats emitted also showed an interaction between ewe feeding treatment and BCS, such that in the BCS 3.0 group lambs born to ewes in the *ad lib* feeding treatment emitted fewer high-pitched bleats than those in the medium feeding treatment (P < 0.05), no differences were observed in the BCS 2.0 and 2.5 groups (P > 0.05).

The percentage of low-pitched bleats were not affected by ewe BCS group or feeding treatment (P > 0.05). The number of low-pitched bleats emitted showed an interaction between ewe feeding treatment and BCS, such that in the BCS 2.0 and 3.0 groups lambs born to ewes in the *ad lib* feeding treatment emitted fewer low-pitched bleats than those in the medium feeding treatment (P < 0.05, Table 2.3b). The reverse effect was seen in the BCS 2.5 group with more bleats emitted by lambs born to ewes in the *ad lib* than the medium feeding treatment (P < 0.05).

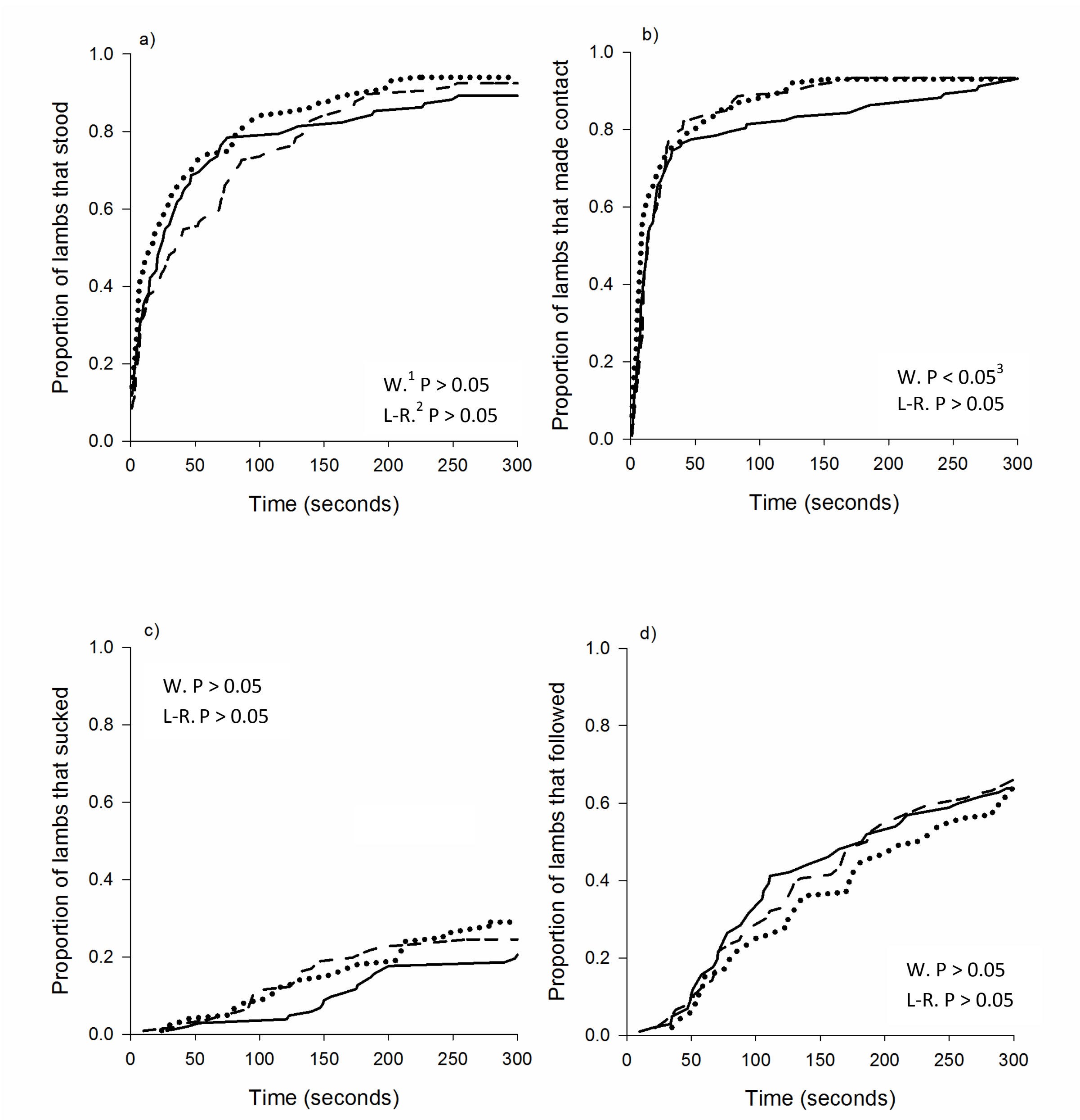
Experiment one – paddock behaviours

Results from the survival analysis showed that the time for lambs to make contact with their dam during the observation period differed between lambs born to ewes in the BCS 2.0 and the BCS 2.5 group (Wilcoxon P < 0.05, Fig. 2.2b), but they did not differ from lambs born to ewes in the BCS 3.0 group ($P > 0.05$). Five seconds after observations began 31% of lambs born to ewes in the BCS 2.0 group had made contact with their dam, compared with 14% in the BCS 2.5 group. Furthermore, the median time to make contact was less for lambs born to ewes in the BCS 2.0 group than lambs born to ewes in the BCS 2.5 group but not the BCS 3.0 group ($P < 0.05$, Table 2.4).

The survival analysis showed that the time for the lambs to make contact with their dam during the observation period differed between feeding treatments (Log-rank $P < 0.05$, Fig. 2.3b). Two minutes after observations began 83% of lambs born to ewes in the *ad lib* treatment had made contact with their dam, compared with 91% in the medium treatment ($P < 0.05$). The median time to make contact did not differ between feeding treatments ($P > 0.05$).

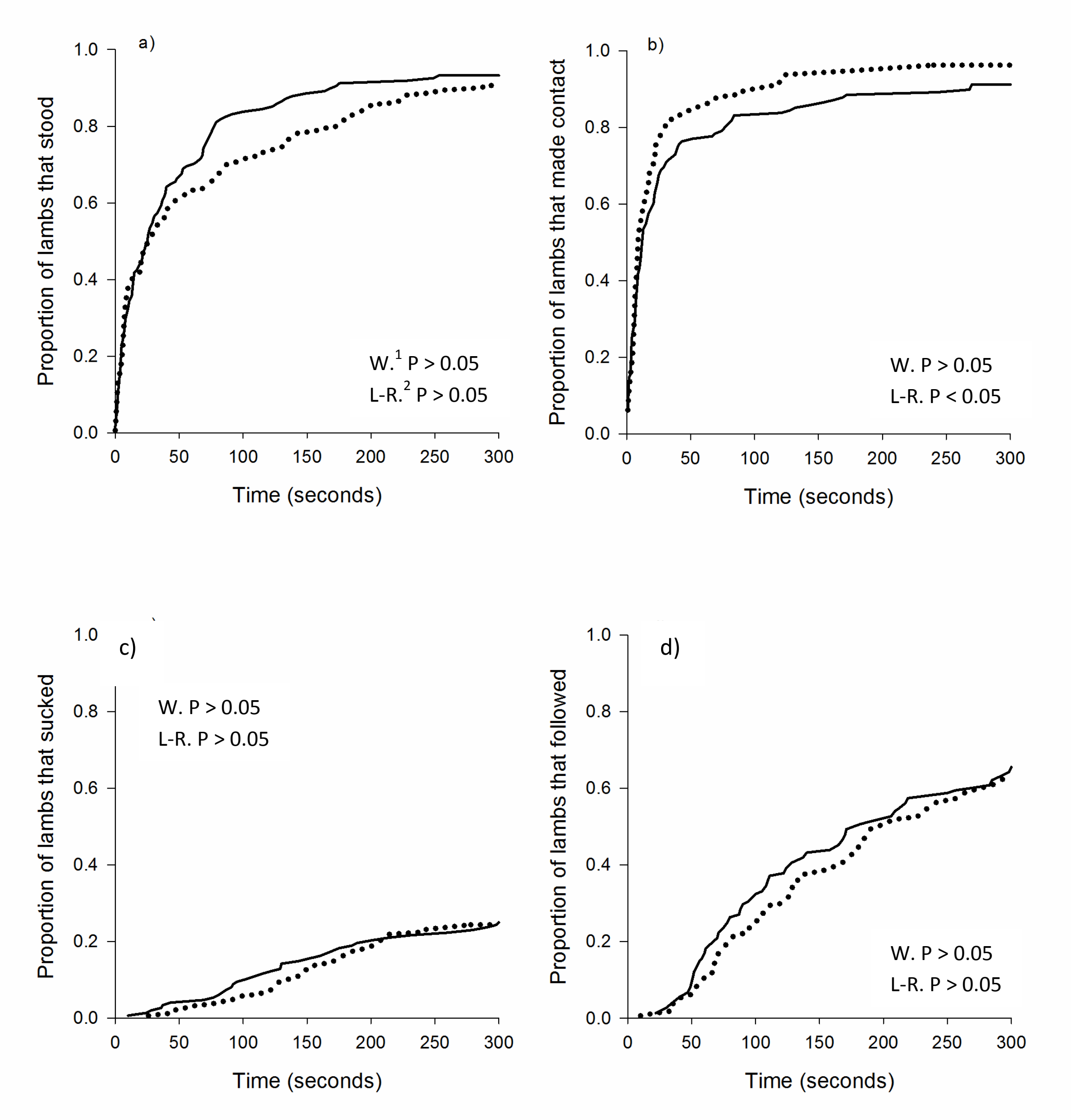
Survival analysis showed no effect of ewe feeding treatment and BCS on the time for the lambs to stand, suck or follow their dam during the observation period ($P > 0.05$, Fig. 2.2 and 2.3a, c, d). There were also no differences in the median time to exhibit these behaviours ($P > 0.05$, Table 2.4).

Figure 1.2 Experiment one; the effect of ewe BCS group (BCS 2.0 ....., BCS 2.5 - - - and BCS 3.0 —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value. [3]The Wilcoxon, but not the Log-rank P-value show a difference between lambs born to ewes in the BCS 2.0 and the BCS 2.5 group ($P < 0.05$, Fig. 2.2b), but neither differ from lambs born to ewes in the BCS 3.0 group ($P > 0.05$).

Figure 2.2. Experiment one; the effect of ewe feeding treatments (medium ….. and *ad lib* —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

Table 2.4. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) on the back-transformed median time (seconds) required for the lambs exhibit the various behaviours.

	n	Stand	Contact	Suck	Follow
Experiment 1					
Feeding treatment					
medium	160	26.5	10.0	301.0	200.0
ad lib	148	25.5	13.5	301.0	182.0
BCS group					
BCS 2.0	100	17.5	9.0^{a}	301.0	227.5
BCS 2.5	106	38.5	14.0^{b}	301.0	187.0
BSC 3.0	102	24.5	13.0^{ab}	301.0	184.00
Experiment 2					
Feeding treatment					
medium	128	25.0	22.5	301.0	165.0
ad lib	112	30.0	16.0	301.0	211.0
BCS group					
BCS 2.0	76	24.5	17.0	301.0	167.0
BCS 2.5	84	27.5	25.0	301.0	109.0
BSC 3.0	80	32.0	17.0	301.0	240.0

^{abc} Different superscripts within columns and main effects indicate values that significantly differ (P < 0.05).

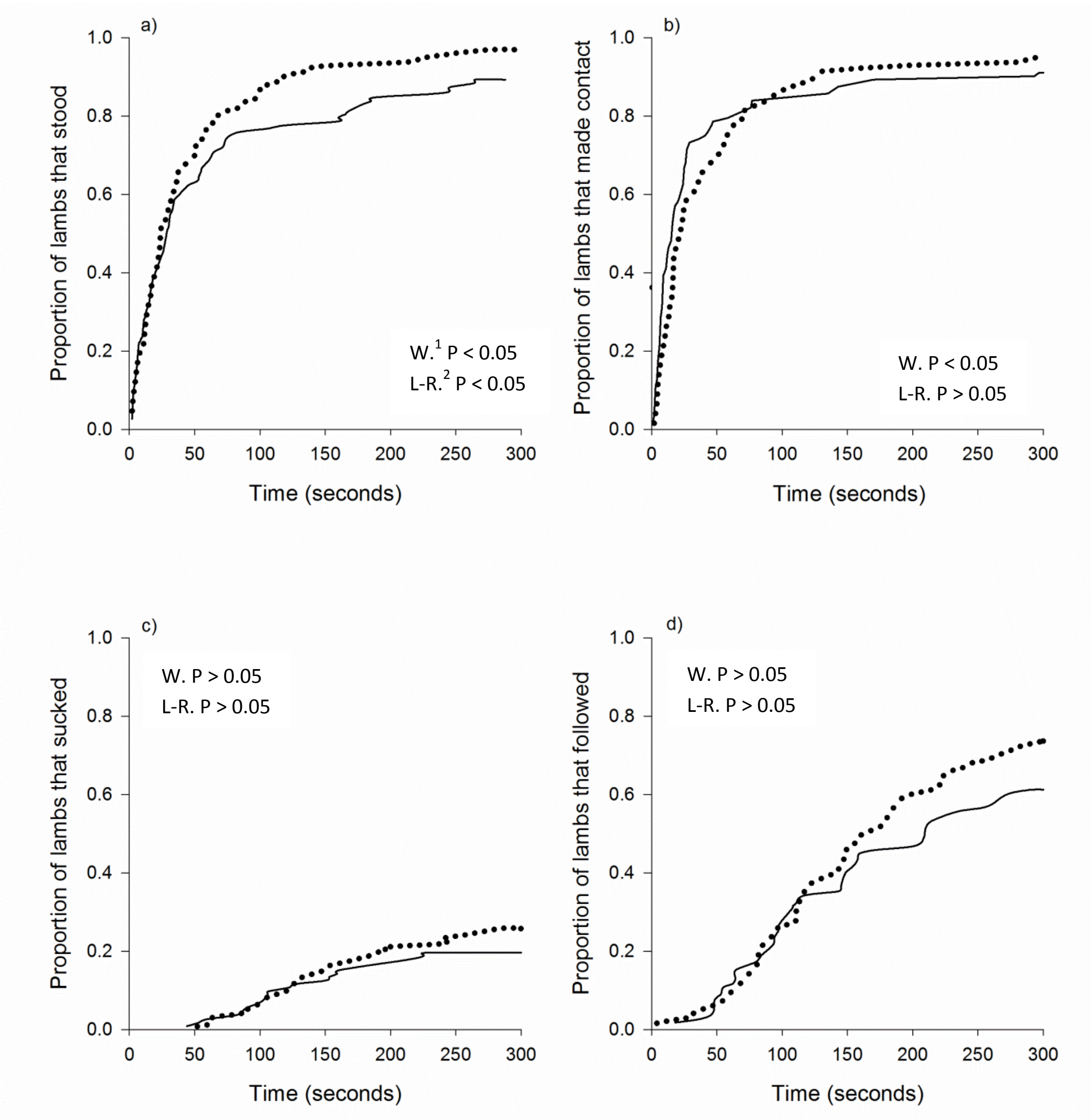
Experiment two – paddock behaviours

Survival analysis showed that the time to stand during the observation period differed between lambs born to ewes in the medium and the *ad lib* treatments (Wilcoxon and Log-rank P < 0.05, Fig. 2.4a). Two minutes after the start of the observation period 89% of lambs born to ewes in the medium treatment had stood, compared with 76% of lambs born to ewes in the *ad lib* treatment, however, the median time to stand did not differ (P > 0.05, Table 2.4).

In experiment two, the survival analysis showed that time to contact their dam during the observation period differed between lambs born to ewes in the medium and the *ad lib* treatments (Wilcoxon P < 0.05, Fig. 2.4b). Thirty seconds after the start of the observation period 59% of lambs born to ewes in the medium treatment had made contact with their dam, compared with 73% of lambs born to ewes in the *ad lib* treatment, however, the median time to contact did not differ (P > 0.05, Table 2.4).

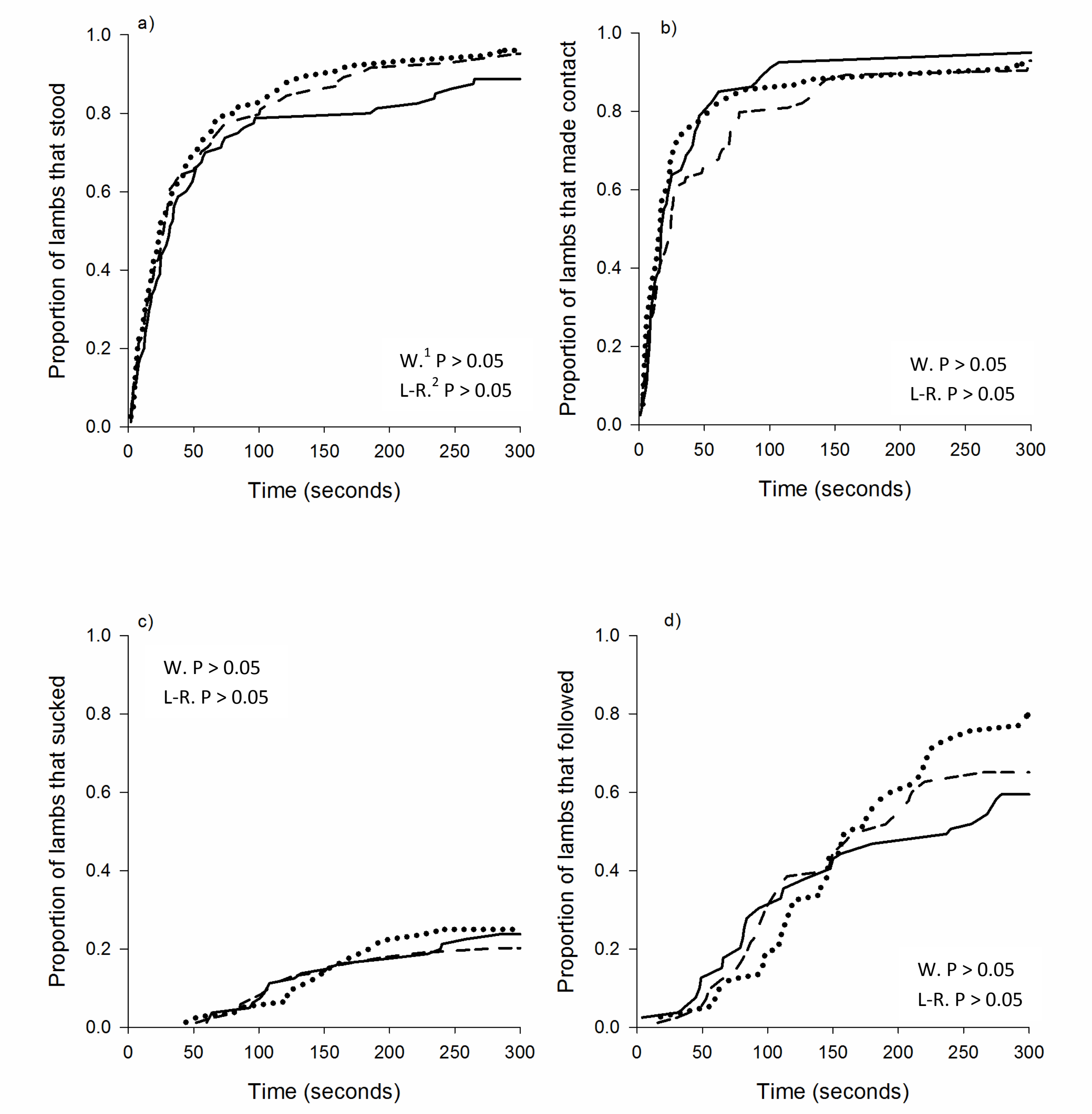
In experiment two, the survival analysis showed no effect of ewe feeding treatment on the lambs' time to suck or follow their dam during the observation period (P > 0.05, Fig. 2.4 c & d). The median times to suck or follow their dam were also not affected by ewe feeding treatment (P > 0.05, Table 2.4). The survival analysis showed no effect of ewe BCS on time to stand, contact, suck or follow their dam over the observation period (P > 0.05, Fig. 2.5a, b, c, d). The median times to stand, contact, suck and follow were also not affected by ewe BCS (P > 0.05, Table 2.4).

Figure 2.4. Experiment two; the effect of ewe feeding treatments (medium ….. and *ad lib* —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

Figure 2.5. Experiment two; the effect of ewe BCS group (BCS 2.0 ....., BCS 2.5 - - - and BCS 3.0—) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

The effect of ewe feeding treatment and BCS on ewe behaviour

Maternal behaviour score (MBS) of the ewe was not affected by feeding treatment or BCS group of the ewe in either experiment (mean MBS for experiment one and two 2.8 [2.5-3.3] and 2.7 [2.5-3.2], respectively, P > 0.05).

Experiment one

A greater percentage of ewes in the medium feeding treatment emitted high- and low-pitched bleats than the ewes in the *ad lib* treatment P < 0.05, Table 5a). A greater percentage of ewes in the BCS 2.5 group emitted high-pitched bleats than ewes in the BCS 2.0 group (P < 0.05, Table 2.5a) but neither differed from the BCS 3.0 group (P > 0.05). Ewe BCS did not affect the percentage of ewes that emitted low-pitched bleats (P > 0.05). There were no interactions on the percentage of ewes that emitted high- and low-pitched bleats (P > 0.05).

In experiment one, the number of high-pitched bleats emitted showed an interaction between ewe feeding treatment and BCS. Within the BCS 2.0 and 2.5 groups ewes in the medium feeding treatments emitted more high-pitched bleats than ewes in the *ad lib* feeding treatment (P < 0.05, Table 2.5a), this effect was not observed within the BCS 3.0 group (P > 0.05). The number of low-pitched bleats emitted showed an interaction between ewe feeding treatment and BCS such that, within the BCS 2.5 and 3.0 groups ewes in the *ad lib* feeding treatments emitted more low-pitched bleats than ewes in the medium feeding treatment ($P < 0.05$), this effect was reversed within the BCS 2.0 group ($P < 0.05$).

Table 2.5 a & b. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction on the percentage (95% CI) of ewes that emitted high- and low-pitched bleats and on the total number of high- and low-pitched bleats in paddock.

a)	n	High-pitched bleat		Low-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Experiment 1					
Feeding treatment					
medium	80	86.9[b]	9.5[b]	95.0[b]	15.2[a]
		(77.4-92.8)	(9.0-10.0)	(87.5-98.1)	(14.6-15.8)
ad lib	74	80.0[a]	7.9[a]	81.8[a]	17.7[b]
		(69.2-87.7)	(7.4-8.3)	(71.5-88.9)	(17.0-18.4)
BCS group					
BCS 2.0	50	70.8[a]	6.7[a]	89.6	14.3[a]
		(57.0-81.6)	(6.2-7.2)	(77.7-95.5)	(13.5-15.0)
BCS 2.5	53	90.9[b]	8.8[b]	90.8	15.2[a]
		(79.8-96.2)	(8.3-9.4)	(79.7-96.2)	(14.5-16.0)
BCS 3.0	51	84.9[ab]	11.0[c]	90.31	20.2[b]
		(72.5-92.3)	(10.4-11.7)	(78.8-95.9)	(19.4-21.1)
Feeding x BCS group[3]					
medium BCS 2.0	27	—	8.1[b]	—	16.1[b]
			(7.3-8.9)		(15.1-17.2)
medium BCS2.5	25	—	10.2[c]	—	11.8[a]
			(9.3-11.2)		(10.8-12.7)
medium BCS 3.0	26	—	11.0[c]	—	17.8[b]
			(10.1-11.9)		(16.7-19.0)
ad lib BCS 2.0	21	—	5.1[a]	—	11.4[a]
			(4.4-5.9)		(10.5-12.5)
ad lib BCS 2.5	28	—	7.6[b]	—	18.5[c]
			(7.0-8.4)		(17.4-19.7)
ad lib BCS 3.0	25	—	11.3[c]	—	22.8[c]
			(10.3-12.3)		(16.7-19.0)

b)	n	High-pitched bleat		Low-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Experiment 2					
Feeding treatment					
medium	64	95.4[b]	8.0[b]	100[5]	28.0[a]
		(86.5-98.5)	(7.6-8.6)		(27.2-29.0)
ad lib	56	82.5[a]	7.1[a]	100[5]	33.6[b]
		(70.0-90.5)	(6.6-7.6)		(32.5-34.7)
BCS group					
BCS 2.0	38	92.6	6.4[b]	100[5]	38.5[c]
		(78.5-97.7)	(6.4-7.6)		(37.2-40.0)
BCS 2.5	42	88.8	10.5[c]	100[5]	24.9[a]
		(74.4-95.6)	(9.8-11.2)		(23.8-25.9)
BCS 3.0	40	90.6	5.9[a]	100[5]	30.1[b]
		(77.4-95.4)	(5.3-6.4)		(29.0-31.4)
Feeding x BCS group[3]					
medium BCS 2.0	23	—	7.5[b]	—	35.4[c]
			(6.7-8.3)		(33.7-37.1)
medium BCS2.5	19	—	9.5[c]	—	19.4[a]
			(8.5-10.5)		(18.1-20.9)
medium BCS 3.0	22	—	7.6[b]	—	30.3[b]
			(6.9-8.5)		(28.7-91.9)
ad lib BCS 2.0	15	—	6.4[b]	—	41.9[d]
			(5.6-7.4)		(39.7-44.3)
ad lib BCS 2.5	13	—	11.3[d]	—	29.9[b]
			(10.4-12.3)		(28.4-31.5)
ad lib BCS 3.0	18	—	3.8[a]	—	29.7[b]
			(3.2-4.5)		(27.9-31.5)

[1]The percentage of ewes that bleated are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2] The number of high- and low-pitched bleats are presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within columns and effects indicate values that significantly differ ($P < 0.05$).

Experiment two

In experiment two, a greater percentage of ewes in the medium treatment emitted high-pitched bleats than ewes in the *ad lib* treatment (P < 0.05, Table 2.5b). The percentage of ewes that emitted low-pitched bleats did not differ between ewe feeding treatment or BCS group (P < 0.05).

The number of high-pitched bleats emitted showed an interaction between ewe feeding treatment and BCS such that, within the BCS 2.5 group ewes in the medium feeding treatment emitted fewer high-pitched bleats than ewes in the *ad lib* treatment (P < 0.05, Table 2.5b), this effect was reversed within the BCS 3.0 group (P < 0.05) and no effect was seen within the BCS 2.0 group (P > 0.05).

In experiment two, the number of low-pitched bleats emitted showed an interaction between ewe feeding treatment and BCS such that, within the BCS 2.0 and 2.5 groups ewes in the medium feeding treatment emitted fewer low-pitched bleats than ewes in the *ad lib* treatment (P < 0.05, Table 2.5b), but no differences were observed within the BCS 3.0 group (P > 0.05).

The effect of ewe feeding treatment and BCS on lamb triangle pen test

Lambs born to ewes in the medium feeding treatment spent a greater time in the contact zone than lambs born to ewes in the *ad lib* treatment ($P < 0.05$, Table 2.6). The percentage of lambs that spent time in the contact zone was not affected by ewe BCS or age at testing ($P > 0.05$). Lambs tested at approximately 24 hours spent a greater time in the contact zone next to their dam and more time walking compared with lambs tested at approximately 12 hours ($P < 0.05$, Table 2.6 and 2.7).

The percentage of lambs that sat during the triangle pen test showed an interaction of ewe feeding treatment and BCS such that within the BCS 2.5 group a lesser percentage of lambs born to ewes offered the *ad lib* treatment sat compared with lambs born to ewes offered the medium treatment ($P < 0.05$), however, this effect was not observed within the BCS 2.0 and 3.0 groups ($P > 0.05$, Table 2.7b). There was also an interaction of ewe BCS and age of the lamb on the percentage of lambs that sat, such that within the BCS 2.5 group a smaller percentage of lambs tested at 24 hours sat compared with lambs tested at 12 hours ($P < 0.05$), this effect was not observed within the BCS 2.0 and 3.0 groups ($P > 0.05$, Table 2.7b).

Table 2.6. Experiment one; the effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and lamb age (12h and 24h) at testing on the percentage (95% CI) of lambs, and the median time (seconds) they spent with the dam, the alien ewe and in the contact zone (for those that expressed the behaviour) in the triangle pen test.

	n	Spent time with dam		Spent time with alien ewe		Spent time in contact zone	
		Percentage[1]	Time[2]	Percentage[1]	Time[2]	Percentage[1]	Time[2]
Feeding treatment							
medium	104	81.1 (72.3-87.5)	200	76.9 (67.7-84.0)	120	93.9 (87.1-97.2)	280.0[b]
ad lib	86	88.3 (79.6-93.6)	195	74.5 (64.2-82.6)	85	95.8 (88.9-98.5)	270.0[a]
BCS group							
BCS 2.0	68	83.6 (72.8-90.7)	220	77.7 (66.2-86.1)	110	95.9 (87.6-98.7)	280
BCS 2.5	60	84.0 (72.4-91.3)	195	71.6 (59.0-81.6)	90	90.3 (79.7-95.6)	280
BSC 3.0	62	87.3 (76.5-93.6)	180	77.5 (65.5-86.2)	110	96.8 (88.1-99.2)	280
Age							
12h	130	83.9 (76.5-89.2)	180.0[a]	76.9 (68.9-83.4)	110	93.1 (87.2-96.4)	280
24h	60	85.0 (73.6-92.0)	240.0[b]	73.3 (60.8-82.9)	80	96.7 (87.6-99.2)	280

[1]The percentage of lambs that spent time with dam, spent time with alien and spent time in contact zone are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]Time for those that expressed the behaviour is presented as the back-transformed median time in seconds. [abc] Different superscripts within columns and effects indicate values that significantly differ ($P < 0.05$).

Table 2.7a. Experiment one; the effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and lamb age (12h and 24h) at testing on the percentage (95% CI) of lambs, and the median time (seconds) they spent walking and sitting (for those that expressed the behaviour) in the triangle pen test.

	n		Walked		Sat	
		Time standing[1]	Percentage[2]	Time[3]	Percentage[2]	Time[3]
Feeding treatment						
medium	104	240	94.3 (87.7-97.4)	55	35.0 (26.4-44.7)	25
ad lib	86	230	97.7 (91.3-99.4)	40	34.4 (25.1-45.1)	50
BCS group						
BCS 2.0	68	240	96.4 (88.3-98.9)	50	30.8 (21.0-42.8)	20
BCS 2.5	60	230	97.1) (88.4-99.3	60	33.3 (22.6-46.1)	25
BSC 3.0	62	220	96.0 (86.5-98.6)	40	40.3 (28.9-52.9)	40
Age						
12h	130	240	96.2 (91.1-98.4)	40.0^{b}	35.4 (27.7-44.0)	35
24h	60	230	95.0 (85.6-98.4)	60.0^{a}	33.3 (22.6-46.1)	20

[1] All lambs were standing at the beginning of the triangle test. Standing is presented as the back-transformed median time in seconds. [2]The percentages of lambs that walked and sat are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [3] Time for those that expressed the behaviour is presented as the median time in seconds. [abc] Different superscripts within columns and effects indicate values that significantly differ (P < 0.05

Table 2.7b. Experiment one; the effect of the two-way interaction of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and lamb age (12h and 24h) at testing on the percentage (95% CI) of lambs, and the median time (seconds) spent walking and sitting (for those that expressed the behaviour) in the triangle pen test.

	n	Walked			Sat	
		Time standing[1]	Percentage[1]	Time[1]	Percentage[2]	Time[1]
Feeding x BCS[3]						
medium BCS 2.0	42	-	-	-	25.2[ab] (12.9-43.4)	-
medium BCS2.5	32	-	-	-	37.8[b] (21.4-57.6)	-
medium BCS 3.0	30	-	-	-	45.3[b] (28.2-63.6)	-
ad lib BCS 2.0	26	-	-	-	46.0[b] (28.3-64.9)	-
ad lib BCS 2.5	28	-	-	-	11.9[a] (3.8-31.7)	-
ad lib BCS 3.0	32	-	-	-	37.5[b] (20.4-68.5)	-
BCS group x age[3]						
BCS 2.0 12h	48	-	-	-	31.3[ab] (18.8-47.3)	-
BCS 2.5 12h	38	-	-	-	43.6[b] (28.5-60.1)	-
BSC 3.0 12h	44	-	-	-	36.1[b] (23.2-51.4)	-
BCS 2.0 24h	20	-	-	-	38.7[b] (20.1-61.3)	-
BCS 2.5 24h	22	-	-	-	9.5[a] (2.5-30.2)	-
BSC 3.0 24h	18	-	-	-	46.8[b] (24.9-70.0)	-

[1] Non-significant interactions are not presented. [2]The percentages of lambs that sat are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not presented. [abc] Different superscripts within columns and effects indicate values that significantly differ ($P < 0.05$).

There were no effects of ewe feeding treatment or BCS group on the percentage of lambs that spent time with their dam or the alien ewe (Table 2.6), walked, sat (table 2.7 a&b) or reached the contact zone and the dam (P > 0.05, Table 2.8, Fig. 2.6 and 2.7a, b, c). There were also no effects of ewe feeding treatment or BCS group on the time they spent with the alien ewes or the time the lambs spent standing, walking, sitting, the time taken to reach the contact zone or the dam (P > 0.05, Table 2.6, 2.7 and 2.8, Fig. 2.6 and 2.7a, b, c).

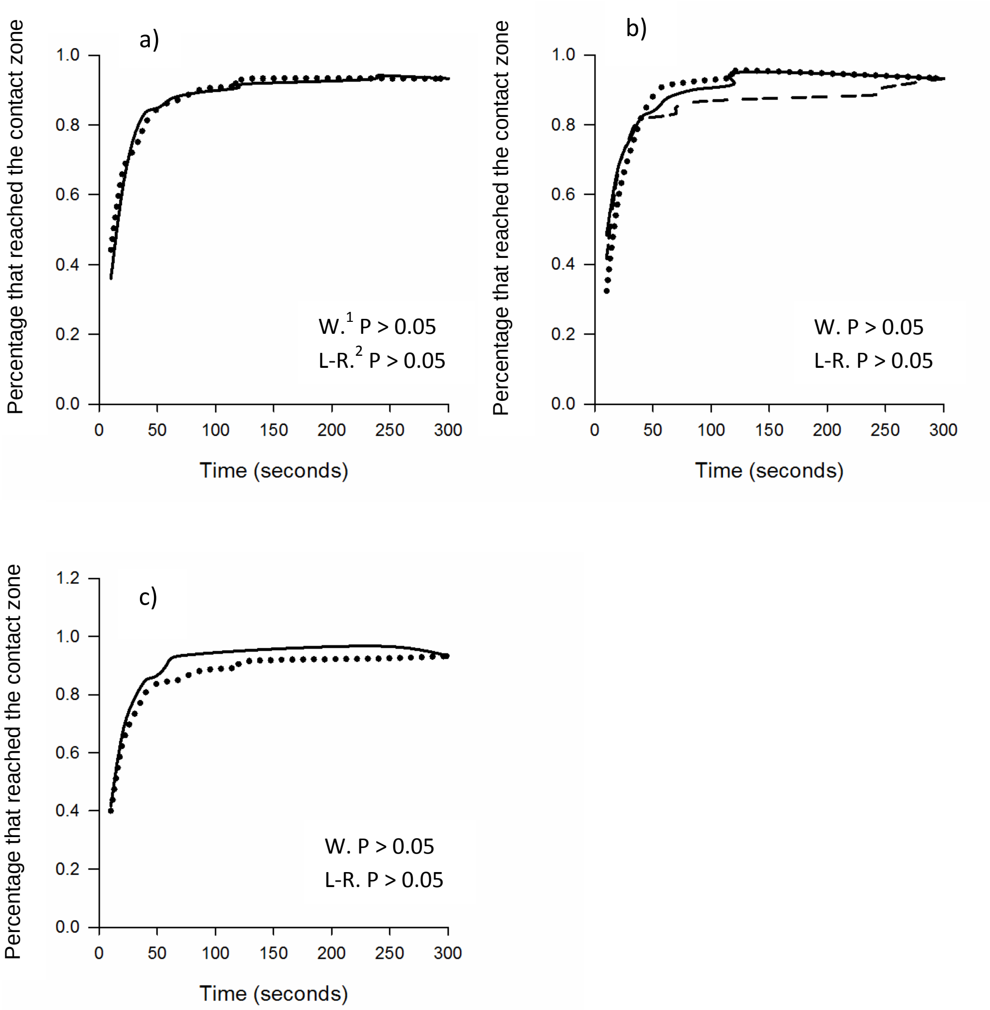
The outcome of the preference test, as part of the triangle pen test, was not affected by ewe feeding treatment, BCS group or age of lamb (P > 0.05, data not shown). In total, 55% of lambs made the 'correct choice', 19% made the 'incorrect choice' and 26% made 'no choice'.

Table 2.8. Experiment one; the effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and lamb age (12h and 24h) at testing on the percentage (95% CI) and the median time (seconds) required for lambs to reach their dam and the contact zone (for those that expressed the behaviour) in the triangle pen test.

	n	Reached dam		Reached contact zone	
		Percentage[1]	Time[2]	Percentage[1]	Time[2]
Feeding treatment					
medium	104	80.9 (72.2-87.4)	40	93.5 (86.8-97.0)	20
ad lib	86	87.2 (78.3-92.8)	30	94.6 (87.3-97.8)	20
BCS group					
BCS 2.0	68	83.4 (72.5-90.5)	30	95.7 (87.3-98.6)	20
BCS 2.5	60	83.8 (72.2-91.1)	40	90.1 (79.6-95.5)	20
BSC 3.0	62	85.6 (74.5-92.4)	40	95.2 (86.1-98.4)	20
Age					
12 h	130	83.1 (75.6-88.6)	40	92.3 (86.3-95.8)	20
24 h	60	85.0 (73.6-92.0)	30	96.7 (87.6-99.2)	20

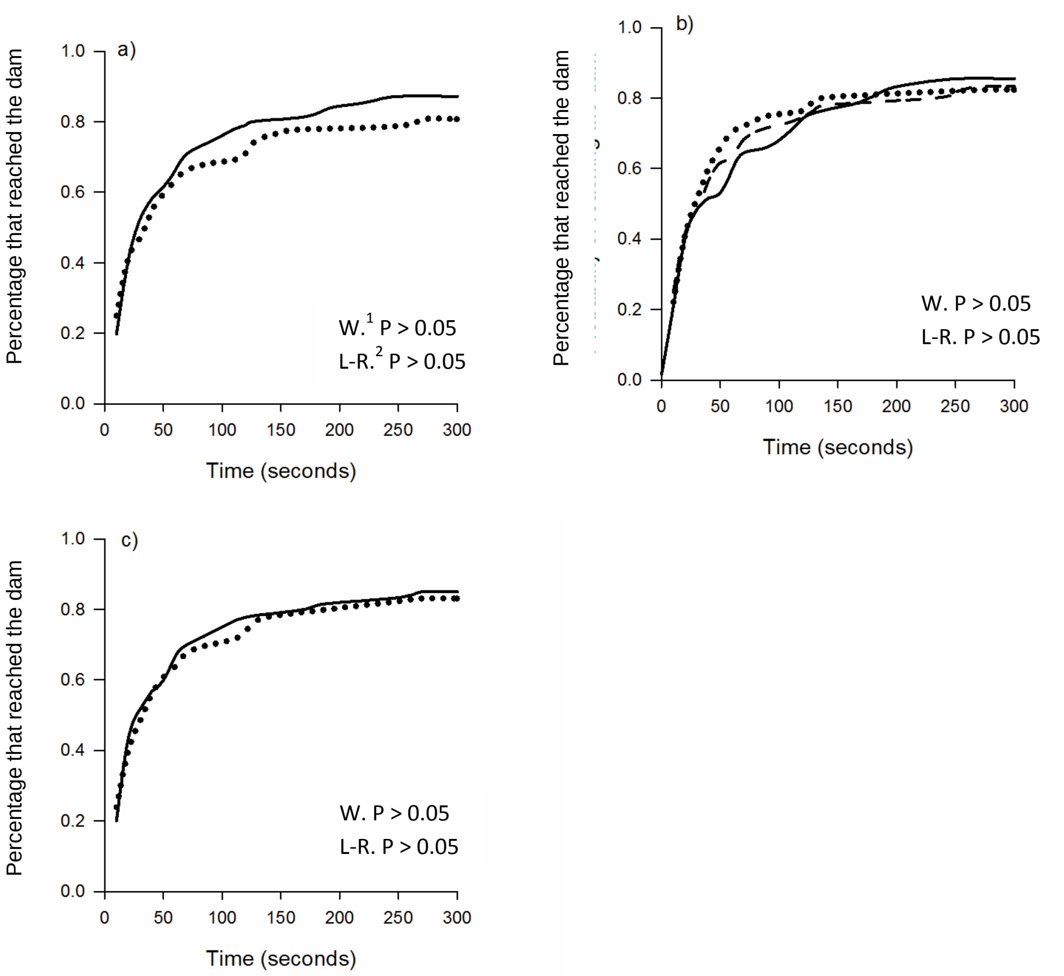
[1]The percentages of lambs that reached their dam and the contact zone are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2] The time for those that expressed the behaviour is presented as the back-transformed median time in seconds. [abc] Different superscripts within columns and effects indicate values that significantly differ (P < 0.05).

Figure 2.6. Experiment one; the effect of ewe a) feeding treatments (medium ….. and *ad lib* —), b) ewe BCS (BCS 2.0 ….., BCS 2.5 - - - and BCS 3.0 —) and c) age of lamb (12h ….. and 24h —) on the percentage of lambs that reached the contact zone in the triangle test. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

Figure 2.7. Experiment one; the effect of ewe a) feeding treatments (medium ….. and *ad lib* —), b) ewe BCS (BCS 2.0 ….., BCS 2.5 - - - and BCS 3.0 —) and c) age of lamb (12h ….. and 24h —) on the percentage of lambs that reached their dam in the triangle pen test. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

The percentage of lambs that emitted high- or low-pitched bleats was no affected by ewe feeding treatment or BCS on (P > 0.05, Table 2.9a & b).

There was an interaction of ewe feeding treatment and BCS on the number of high-pitched bleats emitted, such that in the BCS 2.0 and 3.0 groups lambs born to ewes offered the medium treatment emitted fewer high-pitched bleats than those born to ewes offered the *ad lib* treatment (P < 0.05, Table 2.9a), yet the opposite effect was observed for lambs born to ewes in the BCS 2.5 group (P < 0.05). The number of high-pitched bleats also showed an interaction of ewe BCS and age of lamb such that within the BCS 3.0 group lambs tested at 12 hours emitted more high-pitched bleats than at 24 hours (P < 0.05), while within the other two BCS groups no difference was observed (P > 0.05).

The number of low-pitched bleats showed an interaction of ewe feeding treatment and BCS such that in the BCS 2.5 and 3.0 groups lamb born to ewes in the medium feeding treatment emitted fewer low-pitched bleats than in the *ad lib* treatment (P < 0.05, Table 2.9b). In the BCS 2.0 group, however, lambs born to ewes in the medium treatment emitted more low-pitched bleats than those in the *ad lib* treatment (P < 0.05).

There was also an interaction of ewe feeding treatment and age of lamb such that within the *ad lib* treatment lambs tested at 24 hours of age emitted fewer low-pitched bleats than those tested at 12 hours of age (P < 0.05, Table 2.9b). Within the medium treatment the number of bleats did not differ between age of lambs (P > 0.05). The number of low-pitched bleats showed an interaction of ewe BCS and age of lamb such that, within the BCS 2.0 and 3.0 groups lambs tested at 12 hours of age emitted more low-pitched bleats than at 24 hours of age (P < 0.05), while within the BCS 2.5 group the number of bleats did not differ (P > 0.05).

Table 2.9 a. Experiment one; The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0), lamb age (12h and 24h) at testing and their interaction on the percentage (95% CI) of lambs that emitted low- and high-pitched bleats, and the total number of bleats in the triangle pen test.

	n	High-pitched bleat		Low-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Feeding treatment					
medium	104	100	32.7[a] (31.6-33.8)	65.3 (55.6-74.0)	3.8[a] (6.4-7.6)
ad lib	86	100	34.5[b] (33.3-35.8)	76.7 (66.5-84.4)	7.0[b] (3.4-4.2)
BCS group					
BCS 2.0	68	98.8 (91.0-99.9)	35.7[b] (34.3-37.2)	65 (52.8-75.5)	4.6[a] (4.2-5.2)
BCS 2.5	60	95.6 (85.7-98.7)	36.7[b] (35.2-38.2)	72.4 (59.8-82.2)	4.0[a] (3.5-4.5)
BSC 3.0	62	100	29.0[a] (27.7-30.4)	76.0) (63.8-85.1	7.2[b] (6.6-7.9)
Age					
12h	130	98.5 (94.1-99.6)	34.3 (33.3-35.3)	66.2 (57.6-73.8)	5.9 (5.5-6.4)
24h	60	96.7 (87.6-99.2)	32.5 (31.1-34.0)	78.3 (66.2-87.0)	4.3 (3.8-4.9)
Feeding x BCS group[3]					
medium BCS 2.0	42	-	32.1[b] (30.5-33.9)	-	5.0[c] (4.4-5.7)
medium BCS2.5	32	-	40.3[c] (38.1-42.6)	-	2.5[a] (2.0-3.1)
medium BCS 3.0	30	-	27.1[a] (25.3-29.0)	-	3.5[b] (2.9-4.3)
ad lib BCS 2.0	26	-	41.0[c] (38.6-43.5)	-	3.8[b] (3.1-4.6)
ad lib BCS 2.5	28	-	32.6[b] (30.5-34.8)	-	5.9[c] (5.1-6.9)
ad lib BCS 3.0	32	-	30.8[b] (28.9-32.8)	-	11.4[d] (10.3-12.7)

[1] The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2] The number of high- and low-pitched bleats are presented as the mean value (with the 95% confidence limit in parenthesis). [3] Non-significant interactions are not presented. [abc] Different superscripts within columns and effects indicate values that significantly differ (P<0.05).

Table 2.9b. Experiment one; The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0), lamb age (12h and 24h) at testing and their interaction on the percentage (95% CI) of lambs that emitted low- and high-pitched bleats, and the total number of bleats in the triangle pen test.

	n	High-pitched bleat		Low-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Feeding treatment x age					
medium 12h	72	-	-	-	4.1[ab] (3.6-4.5)
ad lib 12h	58	-	-	-	8.2[d] (7.5-9.0)
medium 24h	32	-	-	-	3.3[a] (2.7-4.0)
ad lib 24h	28	-	-	-	5.5[c] (4.7-6.4)
BCS group x age[3]					
BCS 2.0 12h	48	-	36.4[c] (34.7-38.1)	-	4.9[bc] (4.3-5.6)
BCS 2.5 12h	38	-	36.6[c] (34.7-38.6)	-	4.1[ab] (3.5-4.8)
BSC 3.0 12h	44	-	30.1[b] (28.5-31.8)	-	8.5[d] (7.7-9.5)
BCS 2.0 24h	20	-	33.5[c] (31.0-36.1)	-	3.6[a] (2.9-4.5)
BCS 2.5 24h	22	-	36.7[c] (34.3-39.3)	-	4.1[ab] (3.3-5.0)
BSC 3.0 24h	18	-	26.3[a] (24.1-28.8)	-	5.3[c] (4.4-6.5)

[1] The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2] The number of high- and low-pitched bleats are presented as the mean value (with the 95% confidence limit in parenthesis). [3] Non-significant interactions are not presented. [abc] Different superscripts within columns and effects indicate values that significantly differ (P<0.05).

Discussion

The aim of these experiments was to investigate the effects of ewe feeding treatment and BCS in late-pregnancy on the behaviour of twin-bearing ewes and their lambs. The pre- and post-grazing masses in both experiments indicate that nutritional conditions of the feeding treatments differed. In addition, differing BCS in mid-pregnancy were investigated to determine the effect of BCS on behaviour and if it modified the effects of nutrition on ewe and lamb behaviour.

Effects of ewe feeding treatment and BCS on ewe and lamb vocalisation were present in both experiments. A greater percentage of ewes in both experiments offered the medium feeding treatment emitted high-pitched bleats compared to those offered the *ad lib* treatment. High-pitched or 'distress bleats' are usually emitted by the ewe and lamb when they are separated from each other (Poindron et al. 1994; Dwyer et al. 1998). In the present study the number of lamb high-pitched bleats showed an interaction of ewe feeding treatment and BCS, such that within the BCS 3.0 group lambs born to ewes in the *ad lib* treatment emitted fewer bleats than lambs born to ewes in the medium treatment. The same effect was observed for ewe high-pitched bleating in experiment two, but not one. Ewe and lamb low-pitched bleats facilitate the establishment of the ewe-lamb bond and are also considered 'care-giver' bleats which are emitted exclusively between the ewe and her lamb (Shillito 1972; Nowak 1990; 1996; Dwyer et al. 1998). A greater number of low-pitched bleats were emitted by ewes offered the medium feeding treatment within the BCS 3.0 group in experiment two, and by their lambs in experiment one and two. Furthermore, in experiment one more lambs born to ewes on the medium treatment had contacted their dam at 2 minutes and in experiment two stood at 2 minutes, compared to lambs born to ewes on the *ad lib* treatment. Combined these results may suggest that lambs born to the medium ewes bleated more and exhibited these behaviours quicker as a result of their dams increased bleating behaviour.

Lamb behaviour in the triangle pen test was only tested in experiment one. The number of lamb low-pitched bleats emitted in the triangle pen test showed significant interactions between age of lamb at testing and ewe feeding treatment and age of lamb at testing and ewe BCS, however, the number of bleats in each group were small and hence the biological effect may be weak. The number of lamb low-pitched bleats within the *ad lib* feeding treatment increased as BCS increased, however this effect was not seen among the medium feeding treatment. Even though significantly different, the number of lamb low-pitch bleats for the different BCS groups within the medium feeding treatment only differed by one or two bleats, suggesting that the biological effect may be small. In the triangle pen test, the number of lamb high-pitched bleats only differed between the age of lamb at testing within the ewe BCS 3.0 group, suggesting that age had a larger effect on lambs born to these ewes compared to those in the BCS 2.0 and 2.5 groups. The inconsistent interaction effects on bleating behaviour may be due to the narrow BCS range used in this study. However, this study wanted to investigate this range as majority of farmers in New Zealand keep their ewes between BCS 2.0 and 3.0 during pregnancy and lambing as production has been shown to drop if kept below 2.0 and plateau above 3.0 (Kenyon et al. 2014).

Nowak et al. (1989) reported that lambs subjected to the triangle test at 24 hours were better able to identify their dam than 12 hour old lambs. The findings of the present study indicated that the older lambs spent more time walking and more the time in the zone next to their dam which is in agreement with Nowak et al. (1989). Presumably older lambs would have spent more time with their dam, and have therefore had more time to learn her characteristics, strengthening the ewe-lamb bond and may possibly be physically stronger than younger lambs. Since lamb behaviours progress with age (Nowak et al. 2008) It is likely that a similar age effect could have been observed among the behaviours recorded at tagging if age information had been recorded.

Nowak (1996) reported that improved maternal nutrition during pregnancy may result in improved maternal behaviours. Likewise, Dwyer et al. (2003) and Thomson and Thomson (1949) reported that restricted feeding of ewes during pregnancy resulted in the ewes being more likely to exhibit poor maternal behaviour and have lower MBS compared with well-fed ewes. However, in the present study no effect of ewe feeding treatment or BCS was found for ewe MBS. This may be due to the ewes not being nutritionally restricted during the present experiments (Rattray et al., 1974). These results thereby support results by Everett-Hincks et al. (2005) and Corner et al. (2010), who studied the effects of similar feeding levels as in the current study. Results therefore indicate that offering twin-bearing ewes of BCS 2.0 to 3.0 feeding levels above pregnancy requirements is not a viable management tool to increase ewe MBS.

The majority of lamb behaviours at tagging (e.g. suck and follow) and in the triangle pen test (e.g. time spent with dam or alien, time to reach dam or contact zone) were unaffected. This is likely explained by ewes being offered feeding levels designed to meet pregnancy requirements or above. Furthermore, ewe BCS and live weights only differed very briefly in experiment one. Likewise, in experiment two there were no differences in ewe live weights between the BCS 2.5 and 3.0 group at P128 and ewe BCS only differed between ewes offered the medium treatment and those offered the *ad lib* treatments within the BCS 2.5 group. The physiological effects of the feeding treatments may hence not have been strong enough to induce a behavioural effect on the ewes or their lambs. Likewise, the difference between BCS 2.0. 2.5 and 3.0 may not be large enough to induce behavioural differences. These results are supported by the lack of an effect of ewe feeding treatment on lamb survival, stated in the original studies by Corner-Thomas et al. (2015a) and Kenyon et al. (2012a). The inconsistent effects between experiments may also be due to the variation in the timing of the treatments and in the total number of treatment days. The feeding treatments lasted for 24 days in mid-pregnancy in experiment one and only 15 days in late-pregnancy in experiment two.

Conclusion

There was little effect of ewe mid-pregnancy BCS or feeding treatment on ewe or lamb behaviours at 3-24 hours after birth. This is likely explained by ewes being offered feeding levels of pregnancy-maintenance requirements or above and minimal differences in ewe live weight and BCS between treatment groups. Feeding ewes of BCS

2.0 to 3.0 *ad libitum* during mid- to late-pregnancy did not appear to provide any advantage to ewe and lamb behaviours that could influence lamb survival.

Implications

The current chapter showed little effect of ewe feeding treatment and BCS group on the behaviour of twin-bearing ewes and their lambs. However, previous research indicates that the effects of ewe nutrition and BCS on ewe and lamb behaviour may be greater in triplet-bearing ewes and their lambs than in twin- and single-bearing ewes due to the greater nutritional demands during pregnancy of the former (Nicol and Brookes 2007). The next chapter explores the effects of ewe feeding and BCS on triplet-bearing ewes and their progeny.

CHAPTER 3

The effect of ewe nutrition and body condition score during pregnancy on the behaviour of triplet-bearing ewes and their lambs

Abstract

In this chapter, the effect of ewe body condition score (BCS) and nutrition during mid- to late-pregnancy on the behaviour of triplet-bearing ewes and their lambs in three experiments is described. The experiments included ewes with a BCS of 2.0, 2.5 or 3.0 in mid-pregnancy that were offered either medium or *ad libitum* (*ad lib*) feeding in mid- to late-pregnancy (from day 93 until 114 of pregnancy for experiment one, from day 115 until 136 for experiment two and from day 128 until 141 for experiment three). The time taken for lambs to stand, contact dam, suck from dam and follow dam was recorded at tagging, three to 18 hours after birth. The number of high- and low-pitched bleats emitted by the ewe and lambs were recorded, as well as a maternal behaviour score for the ewe (MBS). A subset of lambs from experiment one and three were exposed to a triangle pen test at approximately 12 or 24 hours of age. In experiment three, but not one or two, ewes in the BCS 2.0 group had poorer MBS than ewes in the BCS 2.5 or 3.0 groups ($P < 0.05$). In experiment three, but not one or two, ewes in the BCS 2.0 group had poorer MBS than ewes in the BCS 2.5 or 3.0 groups ($P < 0.05$). In all three experiments, within the BCS 2.0 group lambs born to ewes in medium feeding treatment emitted more low-pitched bleats than lambs in the *ad lib* treatment ($P < 0.05$), perhaps to re-enforce the bond with their dam. The effects of ewe nutrition and BCS on the majority of ewe and lamb behaviours were, however, either lacking or inconsistent across experiments. This may have been due to variations in ewe age and parity as well as feeding treatment length and timing between the experiments. There is, however, some evidence to suggest that lambs in the BCS 2.0 group born to ewes offered the medium treatments experienced a weaker ewe-lamb bond. Further research is nevertheless required before a robust conclusion can be made.

Introduction

The increase in the average lambing percentage in New Zealand over the last 20 years (Anon. 2013) has resulted in an increase in the number of triplet-born lambs (Amer et al. 1999). Triplet-born lambs have lower survival rates than both single- and twin-born lambs (Hight and Jury 1970; Dalton et al. 1980; Hinch et al. 1983; Kenyon et al. 2002; Kerslake et al. 2005), even when birth weight is accounted for (Oldham et al. 2011). Lamb losses are of concern to the New Zealand sheep industry from both an animal welfare and a financial perspective (Mellor and Stafford 2004).

At birth, triplet-born lambs are lighter (Dwyer et al., 2003) and have lower plasma thyroid hormones than singles and twins (Barlow et al., 1987) and hence they may have a limited ability to thermoregulate effectively. Colostrum is essential for heat production (Clarke et al., 1997), especially when body reserves approach depletion (Mellor and Cockburn, 1986), and appropriate ewe and lamb behaviours that facilitate bonding and suckling are vital for lamb survival (Atroshi and Österberg 1979; O'Connor et al. 1985; Shillito-Walser 1978; Poindron et al. 1980). Compared to twin-born lambs, triplet-born lambs have been reported to take longer to stand and suck (Dwyer et al., 2005; Everett-Hincks et al., 2005). As there is significant competition for milk within triplet-litters (Treacher 1978; 1983; Snowder and Glimp 1991) triplet-born lambs may be more at risk of receiving inadequate levels of colostrum compared to single- or twin-born lambs. Improving triplet-bearing ewe nutrition during mid- to-late pregnancy could have a positive impact on fetal development (Stevens et al., 1990), energy reserves (Slee 1987) and colostrum intake through increased colostrum production (Murphy et al., 1996).

Information on the likely advantages or otherwise of above-pregnancy-maintenance feeding on triplet-bearing ewe and lamb behaviour is lacking.

It is possible that the inconsistent results observed between previous nutritional studies (Putu et al. 1988; Everett-Hincks et al. 2005; Corner et al. 2006) of pregnant ewes could be explained by differences in the body condition score (BCS) of ewes. It has been suggested that the BCS of the ewe during pregnancy affects the behaviour and survival of her lambs (Dwyer 2003; Kleemann et al. 2006; Kenyon et al. 2011; Kenyon et al. 2014), but only one study has reported on the effect of ewe BCS on lamb behaviour. In 2003, Dwyer reported that ewes that lost body condition during pregnancy, as indicated by a reduction in back fat, produced offspring that were slower to stand and suck after birth. It appears that no studies have examined the effect of both ewe mid-pregnancy BCS and nutrition during mid- to late-pregnancy on the behaviour of triplet-bearing ewes and their lambs managed under extensive pastoral conditions. In chapter two it was observed that ewe nutrition and BCS had minimal effect on the twin-bearing ewe and her lambs. It is, however, possible that the effects of mid-pregnancy BCS and mid- to late-pregnancy nutrition on ewe and lamb behaviour may be greater in triplet-bearing ewes and their progeny than in twin- and single-bearing ewes due to the greater nutritional demands during a triplet pregnancy (Nicol and Brookes 2007).

The majority of studies which examined neonatal lamb and ewe behaviour have observed behaviours immediately after birth. The nature of extensive pastoral farming conditions in New Zealand make such observations difficult, and disturbance of the ewe and lamb during this period can have negative consequences for ewe-lamb bonding, which could lead to ewes rejecting their lambs (Fisher and Mellor 2002). The earliest a farmer is likely to handle the ewe and ear-tag her lambs are around 12 hours after birth. Therefore this study investigated the effect of ewe nutrition and BCS during mid- to late-pregnancy on the behaviour of triplet-bearing ewes and their lambs at tagging under extensive pastoral conditions.

Materials and methods

Three separate experiments were conducted during consecutive years, 2009 (Kenyon et al. 2011), 2010 (Kenyon et al. 2012b) and 2011 (Kenyon et al. 2013) on Massey University's Keeble Farm, 5 km south of Palmerston North, New Zealand (40°S, 175°E) with approval of the Massey University Animal Ethics Committee. The effects of ewe feeding treatment and BCS on the liveweight of the ewe and lamb, and lamb survival have previously been reported (Kenyon et al. 2011; 2012b; 2013), however, the effects on the behaviour of triplet-bearing ewes and their lambs was not reported.

Briefly, the feeding treatments in mid- to late-pregnancy in these all experiments compared the effects of *ad libitum* feeding to a feeding level which meet pregnancy-maintenance requirements, to determine whether there was any advantage of the former. In all three experiments, total ewe liveweight gain in the feeding period indicated the desired nutritional conditions were met (Rattray et al. 1974; Kenyon et al. 2011; Kenyon et al. 2012b; Kenyon et al. 2013).

The aim of this analysis was to explore the behaviours of a ewe and her triplet-born lambs. The behaviour of a triplet-bearing lamb (or triplet-bearing ewe), with only one or no siblings may be different from that of a complete set. Therefore, only complete sets of triplet lambs (where all three lambs were alive at tagging) and their dams were included in the present analysis (Corner et al. 2010).

Experiment one

Background

This experiment included 88 primiparous triplet-bearing Romney ewes (2 years of age) and their lambs (n=264). These ewes were a subset of 144 ewes included in the study of Kenyon et al. (2011). The ewes were managed under commercial conditions until day 92 of pregnancy (P92, mean ewe live weight 67.1 ± 0.60 kg). At P93 ewes were randomly allocated to either a 'medium' (designed to meet pregnancy-maintenance requirements) or '*ad libitum'* (*ad lib)* feeding treatment for 21 days (P93-P114). Pre- and post-grazing masses achieved were 1133 ± 62 and 921 ± 44 kg DM/ha and 2012 ± 71 and 1541 ± 71 kg DM/ha, respectively for the medium and the *ad lib* treatment (Kenyon et al. 2011). Previous studies have indicated that ewe intake is not restricted above pasture masses of 1200 kg DM/ha (Morris and Kenyon 2004; Kenyon et al. 2011; Kenyon et al. 2012a; Kenyon et al. 2012b). Ewes with BCS of ≤2.0, 2.5 and ≥3.0 (as measured on P92) were randomly allocated to each feeding treatment (scale of one to five, one=emaciated, five= obese; Jefferies, 1961; Kenyon et al. 2014). The ewe sub-groups contained the following number of ewes; *ad lib* BCS≤2.0 n=21, *ad lib* BCS2.5 n=13, *ad* *lib* BCS≥3.0 n=9, medium BCS≤2.0 n=14, medium BCS2.5 n=15 and medium BCS≥3.0 n=16.

At P114 the ewe feeding treatments were merged, and the ewes were subsequently offered *ad libitum* feeding conditions until P141. At P142 ewes were moved to lambing paddock. During lambing and lactation the average grazing masses were 1869 ± 73 kg and 1834 ± 35 kg DM/ha, respectively (Kenyon et al. 2011). All ewes lambed over a 21-day period and the study was completed 70 days after the midpoint of lambing period.

Animal and behavioural measurements

Ewe BCS and live weights were recorded at P93, P113, and P142. During the lambing period ewes were inspected twice daily at 8 am and 4 pm. Ear-tagging and behavioural observations were conducted as outlined in chapter two (page 49).

Triangle pen test

A randomly chosen subset of lambs (n=150 i.e. 50 triplet sets) were used in a triangle pen test, similar to that described by Nowak et al. (1987; 1989). The testing pen (Fig. 2.1) and procedures were as outlined in chapter two (page 50).

Experiment two

Background

The study included 119 triplet-bearing Romney ewes (3 to 5 years of age) and their lambs (n=357). These ewes were a subset of 155 ewes used for a study by Kenyon et al. (2012b). At day 115 of pregnancy (P115) ewes were allocated to either a 'medium' or '*ad lib'* feeding treatment for 22 days (P115-P136). Pre- and post-grazing masses achieved were 1578 ± 47.3 kg and 895 ± 36.6 kg DM/ha and 1973 ± 47.3 kg and 1210 ± 34.9 kg DM/ha respectively, for the medium and the *ad lib* treatment (Kenyon et al. 2012b). Ewes with BCS of 2.0, 2.5 and 3.0 (as measured on P72) were randomly allocated to each feeding treatment (mean ewe live weight 68.6 ± 0.57kg at P90). The ewe sub-groups contained the following number of ewes; *ad lib* x BCS2.0 n=17, *ad lib* x BCS2.5 n=21, *ad lib* x BCS3.0 n=23, medium x BCS2.0 n=16, medium x BCS2.5 n=23 and medium x BCS3.0 n=19.

At P136, ewe feeding treatments were merged and the ewes offered *ad libitum* feeding conditions until P141. At P142 ewes were moved to lambing paddocks with mean pasture masses of 1577 ± 30.9 kg DM/ha, and during lactation mean pasture masses of 1295 ± 25.9 kg DM/ha. All ewes lambed over a 20-day period and the study was completed 91 days after the midpoint of the lambing period.

Animal and behavioural measurements

At P115, P136 and P142 ewe BCS and live weights were recorded. The behaviour of the ewes and lambs recorded at tagging and within five minutes of tagging were as described in chapter two. No triangle test was conducted during this experiment.

Experiment three

Background

This study included 71 triplet-bearing Romney ewes (3 to 5 years of age) and their lambs (n=213). These ewes were a subset of 197 ewes used for a study by Kenyon et al. (2013). At P128 ewes were allocated to either a 'medium' or '*ad lib*' feeding treatment for 14 days (P128-P141). Pre- and post-grazing masses achieved were 1125 ± 58.5 and 807.9 ± 58.5 kg DM/ha and 1744 ± 64.1 and 1254 ± 61.1 kg DM/ha, respectively for the medium and the *ad lib* treatment (Kenyon et al. 2013). Ewes with BCS of 2.0, 2.5 and 3.0 (as measured on P72) were randomly allocated to each feeding treatment (mean ewe live weight 68.6 ± 0.48 kg at P83). The various sub-groups contained the following number of ewes; *ad lib* x BCS2.0 n=10, *ad lib* x BCS2.5 n=13, *ad lib* x BCS3.0 n=11, medium x BCS2.0 n=15, medium x BCS2.5 n=13 and medium x BCS3.0 n=9.

During P142 to P146 all ewes were grazed together as one mob and offered *ad libitum* feeding (Kenyon et al. 2013). The mean herbage mass at the start of lambing (P146) was 1531 ± 42.8 kg DM/ha with a mean herbage mass of 1574 ± 43.8 kg DM/ha during the lactation period (Kenyon et al. 2013). All ewes lambed over a 19-day period and the study was completed 80 days after the midpoint of the lambing period.

Animal and behavioural measurements

Ewe BCS and live weights were recorded at P114, P128 and P142. The behaviour of the ewes and lambs recorded at tagging and within five minutes of tagging were as described in chapter two. The triangle pen test was conducted in the same manner as in experiment two.

Statistical Analysis

All statistical analyses were conducted using SAS v. 9.3 (SAS Institute Inc., Cary, 2011, NC, USA). Each experiment was analysed separately due to the differences in the timing of the individual feeding treatments. The statistical analyses were conducted in the same manner as in chapter two, however, the tests used are also described below.

The effect of ewe feeding treatment and BCS on ewe live weight and BCS

Live weight and BCS of the ewes were analysed using a mixed model allowing for repeated measures that included the fixed effects of ewe feeding treatment (medium and *ad lib*), BCS group (BCS 2.0, BCS 2.5 and BCS 3.0) and their two-way interaction with ewe as a random effect. The model also included days to lambing as a covariate which was the number of days between P140 and the date the ewe lambed.

The effect of ewe feeding treatment and BCS on lamb vocalisation

The percentage of lambs that emitted at least one high-pitched bleat or one low-pitched bleat was analysed using a generalised model based on a binomial distribution and a logit transformation. The number of bleats emitted was analysed using a generalised model based on a Poisson distribution. The models included the fixed effects of feeding treatment and BCS group. The analysis of lamb bleating behaviour in the triangle pen test also included the fixed effects of age of lamb at testing (12 hours or 24 hours) and the interaction of ewe feeding treatment with age of lamb and ewe BCS with age of lamb. High- and low-pitched bleats were analysed separately.

The effect of ewe feeding treatment and BCS on lamb behaviours

The time required for the lambs to stand, make contact with their dam, suck from their dam and follow her when she moved away during the paddock observations was tested for normality using the Kolmogorov-Smirnov test. All data were not normally distributed and could not be normalised with transformations. The time to exhibit a behaviour was analysed using survival analysis and using a Kruskal-Wallis test for comparisons of the median value. Survival curves were obtained using Kaplan-Meyer estimates. Lambs that did not perform a particular behaviour within the 5-minute observation period were treated as censored data at 301 seconds. The effect of ewe feeding treatments and BCS group was tested in separate models. Both the Wilcoxon p-value and the log-rank p-value are presented in the results. In general, the Wilcoxon tends to be more powerful in detecting differences early in time, while log-rank tests tend to be sensitive to distributional differences later in time (Martinez and Naranjo 2010).

The median time to exhibit a behaviour was analysed using the non-parametric Kruskal-Wallis test. The effects of ewe feeding treatments or BCS group were tested in separate models and the analysis contained all animals including those that did not show the behaviour during the observation period. For both models the association between the fixed effects and the behaviour variable was investigated using the

Wilcoxon Test. Further, if BCS was found to be significant, the Wilcoxon two-sample *post hoc* test was carried out. Wilcoxon two-sample tests were used together with a Bonferroni adjustment to reduce the chance of Type 1 errors (Field et al. 2010).

The effect of ewe feeding treatment and BCS on the percentage of lambs to perform a behaviour during the triangle test

The percentage of lambs that stood, sat, walked, reached the contact zone, reached their dam, spent time with their dam or with the alien ewe was analysed using a generalised model based on a binomial distribution and logit transformation. The models included the fixed effects of age of lamb at testing (12 hours or 24 hours), feeding treatment and BCS group of the ewe and their two-way interactions.

The effect of ewe feeding treatment and BCS on time spent exhibiting a behaviour during the triangle test

The distribution of the time the lamb spent standing, sitting, walking, spent with their dam or with the alien ewe were tested for normality using the Kolmogorov-Smirnov test. The distributions were not normal and could not be normalised using transformations. These behaviours were analysed using the non-parametric Kruskal-Wallis tests. The individual models included ewe feeding treatment or BCS group, however, the interaction could not be analysed using non-parametric testing. In the event of a significant result, a Wilcoxon two-sample *post hoc* test with a Bonferroni correction was carried out. Each behaviour was analysed separately.

The effect of ewe feeding treatment and BCS on the outcome of the triangle test

The outcome of the preference test, i.e. whether the lambs made a 'correct choice', an 'incorrect choice' or made 'no choice' as described on page 52 (correct choice =1, incorrect choice =2 and no choice = 3), was analysed using a generalised model based on a Poisson distribution. Sex of lamb, age of lamb at testing, feeding treatment and BCS group of the ewe were included as fixed effects. In addition, the interaction of ewe feeding treatment with BCS, ewe feeding treatment with age of lamb and ewe BCS with age of lamb was included.

The effect of ewe feeding treatment and BCS on ewe maternal behaviour score

Ewe MBS was analysed using a generalised model based on a Poisson distribution containing the fixed effects of ewe feeding treatment, BCS group and their interaction.

Results

The effect of ewe feeding treatment and BCS on ewe weight and BCS

Experiment one

At P93 ewes in the BCS 2.0 group were lighter ($P < 0.05$, Table 3.1) than ewes in the BCS 2.5 and BCS 3.0 groups, which did not differ ($P > 0.05$). On P114 and P142, ewes in the BCS 2.0 group were lighter than ewes in the BCS 3.0 group ($P < 0.05$), but not ewes in the BCS 2.5 group ($P > 0.05$). At P114, ewes in the medium feeding treatment were lighter than ewes in the *ad lib* treatment ($P < 0.05$). There was no difference in live weight at P93 and P142 between feeding treatment groups ($P > 0.05$).

Ewes in the BCS 3.0 group had a greater BCS than ewes in the BCS 2.5 group, which in turn had greater BCS than the BCS 2.0 group at P93 ($P < 0.05$, Table 3.1). The BCS groups did not differ in BCS at P114, but at P142 ewes in the BCS 2.0 group had a lower BCS than ewes in the other two BCS groups ($P < 0.05$). The ewes within the medium treatment had a lower BCS at P114 and P142 ($P < 0.05$) than ewes in the *ad lib* treatment, but not at P93 ($P > 0.05$).

Table 3.1. Experiment one; the effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (2.0, BCS 2.5 and BCS 3.0 as measured on P92) on ewe liveweight (kg) and BCS at P93, P114 and P142 (means ± SE).

	n	P 93	P 114	P 142
			Ewe live weight	
Feeding treatment				
medium	43	67.8 ± 0.77	72.3[a] ± 0.90	86.7 ± 1.02
ad lib	45	67.1 ± 0.82	75.5[b] ± 0.96	86.9 ± 1.09
BCS group				
BCS 2.0	35	64.1[a] ± 0.89	71.3[a] ± 1.04	84.5[a] ± 1.17
BCS 2.5	28	68.5[b] ± 0.98	73.8[ab] ± 1.15	86.5[ab] ± 1.29
BSC 3.0	22	69.8[b] ± 1.07	76.7[b] ± 1.25	89.4[b] ± 1.40
			Ewe BCS	
Feeding treatment				
medium	43	2.5 ± 0.02	2.8[a] ± 0.06	3.0[a] ± 0.09
ad lib	45	2.5 ± 0.03	3.4[b] ± 0.07	3.3[b] ± 0.09
BCS group				
BCS 2.0	35	2.0[a] ± 0.03	3.1 ± 0.07	2.8[a] ± 0.01
BCS 2.5	28	2.5[b] ± 0.03	3.1 ± 0.08	3.2[b] ± 0.11
BSC 3.0	22	3.1[c] ± 0.09	3.3 ± 0.09	3.4[b] ± 0.13

[abc] Different superscripts within columns and effects indicate values that significantly differ (P < 0.05).

Experiment two

At P115 ewes in the BCS 2.0 group were lighter (P < 0.05) than ewes in the other two BCS groups, which did not differ (P > 0.05, Table 3.2). Ewe live weight did not differ between BCS groups on P136 (P > 0.05) or P142 (P > 0.05). Likewise, ewe live weight did not differ between feeding treatment groups on any of the days of measurement (P > 0.05).

The mean BCS differed between all BCS groups at P115, P136 and P142, with ewes in the BCS 2.0 group having the lowest BCS and ewes in the BCS 3.0 group the highest

BCS (P < 0.05, Table 3.2). At P115, the ewes in the medium treatment had a greater BCS than the ewes in the *ad lib* treatment (P < 0.05). On P136 and P142, however, the ewes in the medium treatment had a lower BCS than the ewes in the ad *lib* treatment (P < 0.05).

Table 3.2. Experiment two; the effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0 as measured on P72) and their interaction on ewe liveweight (kg) and BCS at P115, P136 and P142 (means ± SE).

	n	P 115	P 136	P 142
Feeding treatment			Ewe live weight	
medium	61	77.6 ± 0.84	86.2 ± 1.47	90.7 ± 1.50
ad lib	58	77.7 ± 0.82	89.3 ± 1.43	92.0 ± 1.47
BCS group				
BCS 2.0	33	74.1^{a} ± 1.10	85.7 ± 1.94	89.2 ± 1.99
BCS 2.5	44	78.7^{b} ± 0.96	89.8 ± 1.68	94.1 ± 1.72
BSC 3.0	42	80.1^{b} ± 0.98	87.8 ± 1.72	90.8 ± 1.77
Feeding treatment			Ewe BCS	
medium	61	2.6^{b} ± 0.03	2.4^{a} ± 0.06	2.5^{a} ± 0.06
ad lib	58	2.5^{a} ± 0.03	2.8^{b} ± 0.06	2.7^{b} ± 0.06
BCS group				
BCS 2.0	33	2.1^{a} ± 0.04	2.2^{a} ± 0.08	2.1^{a} ± 0.07
BCS 2.5	44	2.6^{b} ± 0.03	2.7^{b} ± 0.07	2.7^{b} ± 0.07
BSC 3.0	42	3.1^{c} ± 0.04	2.9^{c} ± 0.07	3.0^{c} ± 0.07

[abc] Different superscripts within columns and effects indicate values that significantly differ (P < 0.05).

Experiment three

Ewes in the BCS 3.0 group were heavier than ewes in the BCS 2.0 and 2.5 groups on all three measurement days (P114, P128 and P142, P < 0.05, Table 3.3). The ewes in the BCS 2.0 and 2.5 groups did not differ in live weight (P > 0.05) on any of the days. Ewes in the medium treatment were lighter at P142 than ewes in the *ad lib* treatment (P

< 0.05, Table 3), but there were no differences in live weight at P114 and P128 (P > 0.05).

Ewes in the BCS 3.0 group had a greater BCS than ewes in the BCS 2.0 and 2.5 groups on all three measurement days (P114, P128 and P142, P < 0.05, Table 3.3). Ewe BCS did not differ at any of the time points between the feeding treatments (P > 0.05).

Table 3.3. Experiment three; the effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0 as measured on P72) on ewe liveweight (kg) and BCS at P114, P128 and P142 (means ± SE).

	n	P 114	P 128	P 142
Feeding treatment			Ewe live weight	
medium	34	76.1 ± 0.82	81.4 ± 0.93	89.1[a] ± 1.24
ad lib	37	75.7 ± 0.82	80.7 ± 0.96	97.1[b] ± 1.27
BCS group				
BCS 2.0	25	72.2[a] ± 0.97	78.2[a] ± 1.14	90.9[a] ± 1.50
BCS 2.5	26	74.4[a] ± 0.94	79.8[a] ± 1.10	92.1[a] ± 1.46
BSC 3.0	20	81.1[b] ± 1.07	85.3[b] ± 1.25	96.5[b] ± 1.70
Feeding treatment			Ewe BCS	
medium	34	2.4 ± 0.06	2.6 ± 0.07	2.4 ± 0.07
ad lib	37	2.4 ± 0.06	2.5 ± 0.07	2.6 ± 0.07
BCS group				
BCS 2.0	25	2.2[a] ± 0.07	2.3[a] ± 0.08	2.3[a] ± 0.08
BCS 2.5	26	2.3[a] ± 0.07	2.5[a] ± 0.08	2.4[a] ± 0.08
BSC 3.0	20	2.8[b] ± 0.08	2.9[b] ± 0.09	2.8[b] ± 0.09

[abc] Different superscripts within columns and effects indicate values that significantly differ (P < 0.05).

The effect of ewe feeding treatment and BCS on lamb paddock behaviours

Experiment one –vocalisation

Ewe feeding treatment and BCS group did not affect the percentages of lambs that emitted high- or low- pitched bleats (P > 0.05, Table 3.4a).

The number of high-pitched bleats showed a significant interaction of ewe feeding treatment and BCS, such that in the BCS 2.0 and 3.0 groups lambs born to ewes in the *ad lib* treatment emitted more high-pitched bleats than lambs born to ewes in the medium treatment (P < 0.05). The opposite effect was observed within the BCS 2.5 group (P < 0.05).

The number of low-pitched bleats also showed a significant interaction of ewe feeding treatment and BCS such that within the BCS 2.0 and 3.0 groups lambs born to ewes in the medium treatment emitted more low-pitched bleats than lambs born to ewes in the *ad lib* treatment (P < 0.05). No differences were observed between ewe in the medium and the *ad lib* treatment within the BCS 2.5 group (P < 0.05).

Experiment two –vocalisation

Ewe feeding treatment and BCS group did not affect the percentage of lambs that emitted high- or low- pitched bleats (P > 0.05, Table 3.4b).

The number of high-pitched bleats showed a significant interaction of ewe feeding treatment and BCS, such that within the BCS 2.0 group lambs in the medium treatment emitted less bleats than lambs in the *ad lib* treatment ($P < 0.05$). Within the BCS 3.0 group lambs in the *ad lib* treatment emitted fewer bleats than lambs in the medium treatment ($P < 0.05$), whereas lambs within the BCS 2.5 group did not differ ($P > 0.05$).

The number of low-pitched bleats also varied among groups. Within the BCS 2.0 group lambs in the medium treatment emitted the more low-pitched bleats than lambs in the *ad lib* treatment ($P < 0.05$). The opposite effect was observed in the BCS 3.0 group ($P < 0.05$) and in the BCS 2.5 group the number of low-pitched bleats did not differ between treatments ($P > 0.05$).

Table 3.4 a. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and. BCS 3.0) and their interactions on the percentage (95% CI) of lambs that emitted high- and low-pitched bleats and on the total number of high- and low-pitched bleats in the paddock.

	n	High-pitched bleat		Low-pitched bleat	
		Percentage [1]	Number [2]	Percentage [1]	Number [2]
Experiment 1					
Feeding treatment					
medium	129	82.5 (74.7-88.3)	14.0 (13.3-14.6)	57.4 (48.5-65.9)	4.1^{b} (2.8-3.4)
ad lib	135	82.9 (75.6-88.4)	13.7 (13.0-14.3)	62.4 (53.9-70.2)	3.1^{a} (3.8-4.4)
BCS group					
BCS 2.0	105	81.0 (72.3-87.5)	15.1^{b} (14.3-15.8)	64.3 (54.7-73.0)	3.3^{a} (3.0-3.7)
BCS 2.5	84	84.5 (75.1-90.8)	13.7^{a} (12.9-14.5)	54.6 (43.9-64.9)	3.3^{a} (2.9-3.7)
BSC 3.0	75	82.6 (72.2-89.7)	12.8^{ab} (12.0-13.6)	60.7 (49.1-71.2)	4.2^{b} (3.8-4.7)
Feeding x BCS[3]					
medium BCS 2.0	42	–	13.6^{c} (12.5-14.8)	–	4.2^{cd} (3.7-4.9)
medium BCS 2.5	45	–	16.6^{d} (15.5-17.9)	–	3.0^{ab} (2.5-3.6)
medium BCS 3.0	48	–	11.7^{b} (10.8-12.7)	–	5.1^{d} (4.5-5.8)
ad lib BCS 2.0	63	–	16.0^{d} (15.0-17.0)	–	2.6^{a} (2.2-3.0)
ad lib BCS 2.5	39	–	10.3^{a} (9.3-11.3)	–	3.7^{bc} (3.1-4.3)
ad lib BCS 3.0	27	–	14.9^{cd} (13.5-16.4)	–	3.2^{ab} (2.6-4.0)

[1]The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within experiments, columns and effects indicate values that significantly differ (P < 0.05).

Table 3.4 b. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and. BCS 3.0) and their interactions on the percentage (95% CI) of lambs that emitted high- and low-pitched bleats and on the total number of high- and low-pitched bleats in the paddock.

	n	High-pitched bleat		Low-pitched bleat	
		Percentage [1]	Number [2]	Percentage [1]	Number [2]
Experiment 2					
Feeding treatment					
medium	183	91.6 (86.4-94.9)	17.9 (17.3-18.6)	57.2 (49.7-64.4)	2.4 (2.2-3.7)
ad lib	174	86.9 (81.2-91.1)	17.8 (17.2-18.5)	53.3 (46.0-60.5)	2.7 (2.4-2.9)
BCS group					
BCS 2.0	99	91.2 (83.8-95.4)	19.6[b] (18.7-20.5)	53.6 (43.8-63.2)	2.4 (2.2-2.8)
BCS 2.5	132	91.0 (84.8-94.9)	20.3[b] (19.5-21.1)	50.7 (42.2-59.1)	2.4 (2.2-2.7)
BSC 3.0	126	85.5 (78.2-90.6)	14.4[a] (13.7-15.0)	61.3 (52.5-69.4)	2.7 (2.5-3.0)
Feeding x BCS[3]					
medium BCS 2.0	48	–	18.2[c] (17.0-19.4)	–	3.0[cd] (2.5-3.5)
medium BCS2.5	69	–	20.8[d] (19.7-21.9)	–	2.3[ab] (2.0-2.7)
medium BCS 3.0	57	–	15.2[b] (14.2-16.2)	–	2.2[ab] (1.8-2.6)
ad lib BCS 2.0	51	–	21.0[d] (19.8-22.4)	–	2.0[a] (1.6-2.4)
ad lib BCS 2.5	63	–	19.8[cd] (18.7-20.9)	–	2.6[bc] (2.2-3.1)
ad lib BCS 3.0	69	–	13.7[a] (12.9-14.6)	–	3.2[d] (2.8-3.7)

[1]The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within experiments, columns and effects indicate values that significantly differ (P < 0.05).

Experiment three –vocalisation

A greater percentage of lambs born to ewes in the medium treatment emitted high-pitched bleats (P < 0.05, Table 3.4c) than lambs born to ewes in the *ad lib* treatment. A greater percentage of lambs born to ewes in the BCS 2.5 and 3.0 groups emitted high-pitched bleats than lambs born to ewes in the BCS 2.0 group (P < 0.05). A greater percentage of lambs born to ewes in the BCS 3.0 group emitted low-pitched bleats than lambs born to ewes in the other two BCS groups (P < 0.05).

Of those lambs that bleated, there was a significant interaction of ewe feeding treatment and BCS on the number of high-pitched bleats such that in the BCS 2.0 and 3.0 groups lambs in the medium treatment emitted more high-pitched bleats than lambs in the *ad lib* treatment (P < 0.05, Table 3.4c). There was no difference observed in the BCS 2.5 group (P > 0.05). The number of low-pitched bleats also showed a significant interaction of ewe feeding treatment and BCS such that within the BCS 2.0 group lambs in the medium treatment emitted more low-pitched bleats than lambs in the *ad lib* treatment (P < 0.05). There were no differences observed in the other two BCS groups (P > 0.05).

Table 3.4 c. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and. BCS 3.0) and their interactions on the percentage (95% CI) of lambs that emitted high- and low-pitched bleats and on the total number of high- and low-pitched bleats in the paddock.

	n	High-pitched bleat		Low-pitched bleat	
		Percentage [1]	Number [2]	Percentage [1]	Number [2]
Experiment 3					
Feeding treatment					
medium	103	89.5^{b} (82.2-94.0)	17.4^{b} (12.9-14.4)	68.8 (59.1-77.1)	3.7 (3.3-4.1)
ad lib	110	78.4^{a} (69.1-85.5)	13.6^{a} (16.6-18.2)	70.5 (60.6-78.7)	3.5 (3.2-3.9)
BCS group					
BCS 2.0	75	71.2^{a} (59.8-80.6)	15.3 (14.4-16.2)	60.2^{a} (48.7-70.6)	2.1^{a} (1.8-2.5)
BCS 2.5	78	89.1^{b} (80.1-94.4)	14.4 (14.6-16.3)	64.5^{a} (50.4-71.6)	3.4^{b} (3.0-3.8)
BSC 3.0	60	89.4^{b} (79.0-95.0)	15.5 (14.6-16.6)	83.3^{b} (71.6-90.8)	6.6^{c} (6.0-7.3)
Feeding x BCS[3]					
medium BCS 2.0	45	–	16.5^{d} (15.3-17.7)	–	2.6^{b} (2.2-3.1)
medium BCS2.5	39	–	15.7^{bc} (14.5-17.0)	–	3.2^{bc} (2.6-3.8)
medium BCS 3.0	27	–	21.3^{e} (19.6-23.1)	–	6.5^{d} (5.6-7.5)
ad lib BCS 2.0	30	–	14.1^{b} (12.9-15.5)	–	1.5^{a} (1.2-2.1)
ad lib BCS 2.5	39	–	15.4^{bc} (14.2-16.7)	–	3.6^{c} (3.0-4.2)
ad lib BCS 3.0	33	–	10.7^{a} (9.6-11.9)	–	6.6^{d} (5.8-7.6)

[1]The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within experiments, columns and effects indicate values that significantly differ ($P < 0.05$).

Experiment one –paddock behaviours

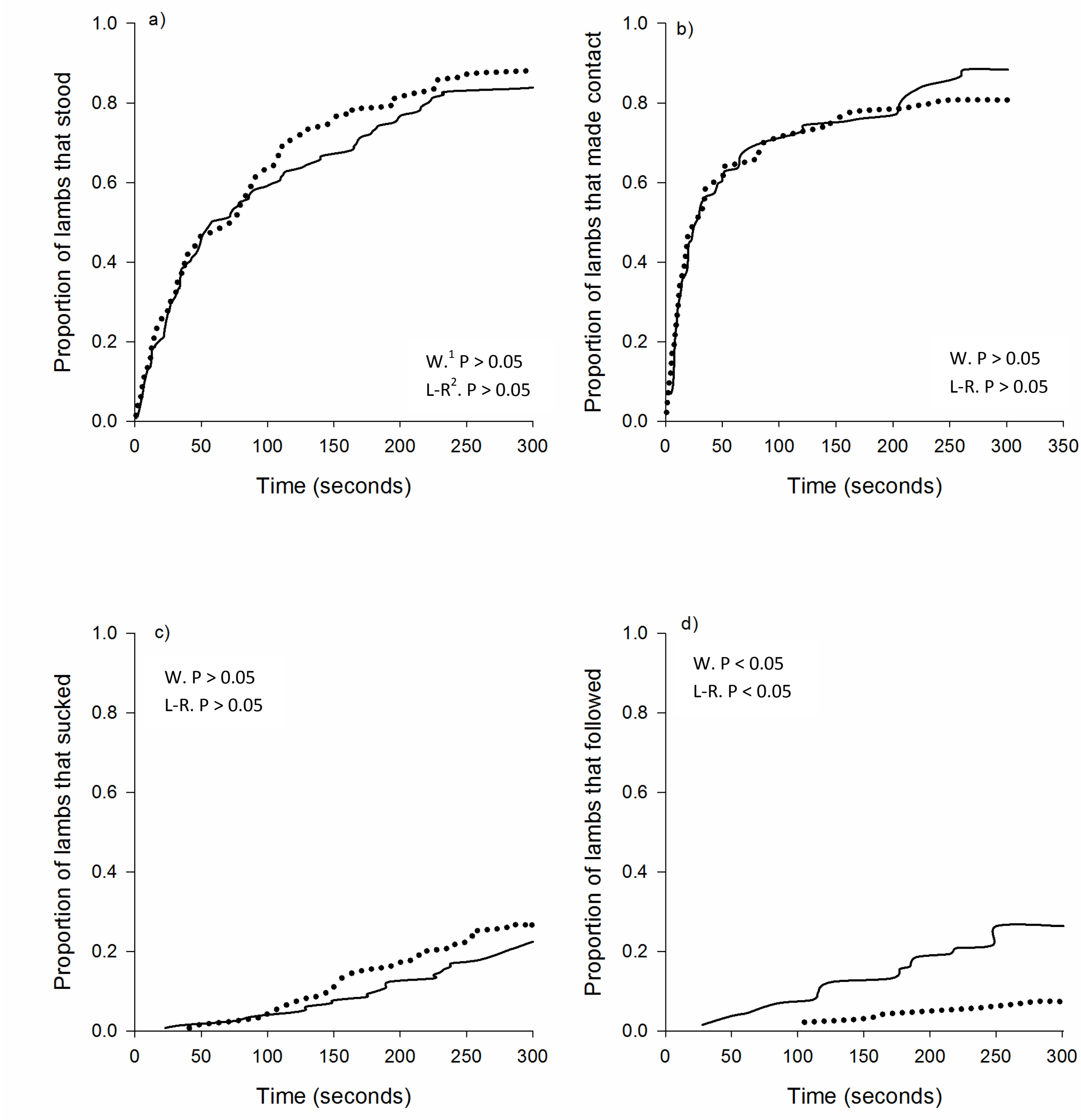
The survival analysis showed that the time for lambs to follow their dam during the observation period differed between lambs born to ewes in the two feeding treatments (Wilcoxon and log-rank $P < 0.05$, Fig. 3.2d). At approximately 245 seconds, 26% of lambs born to ewes in the *ad lib* treatment had followed compared with 7% of lambs born to ewes in the medium treatment. The median time to follow was not affected ($P > 0.05$, Table 3.5). Ewe feeding treatment did not affect the time taken for the lambs to stand, made contact or suck from their dam in the survival analysis nor the median time required to do so ($P > 0.05$, Fig. 3.2a, b, c, Table 3.5).

The survival analysis showed that the time for lambs to stand differed between lambs born to ewes in the BCS 2.0 and BCS 2.5 groups (Log-rank $P < 0.05$, Fig. 3.3a), but neither differed from those born to ewes in the BCS 3.0 group ($P > 0.05$). At the end of the five-minute observation period 93% of lambs born to ewes in the BCS 2.0 group had stood compared with 80% of lambs born to ewes on the BCS 2.5 group. The median time to stand was not affected by ewe feeding treatment or BCS ($P > 0.05$, Table 3.5).

The survival analysis showed that the time for lambs to follow their dam differed between lambs born to ewes in the BCS 3.0 and lambs born to ewes in either the BCS 2.0 or 2.5 groups (Wilcoxon and log-rank $P > 0.05$, Fig. 3.3d). At approximately 120 seconds, only 1% of lambs born to ewes in the BCS 3.0 group had followed their dam compared with 8.6% and 10.7% of lambs born to ewes in BCS 2.0 and 2.5 groups, which also differed from each other (Wilcoxon $P < 0.05$). The log-rank p-value show that BCS group

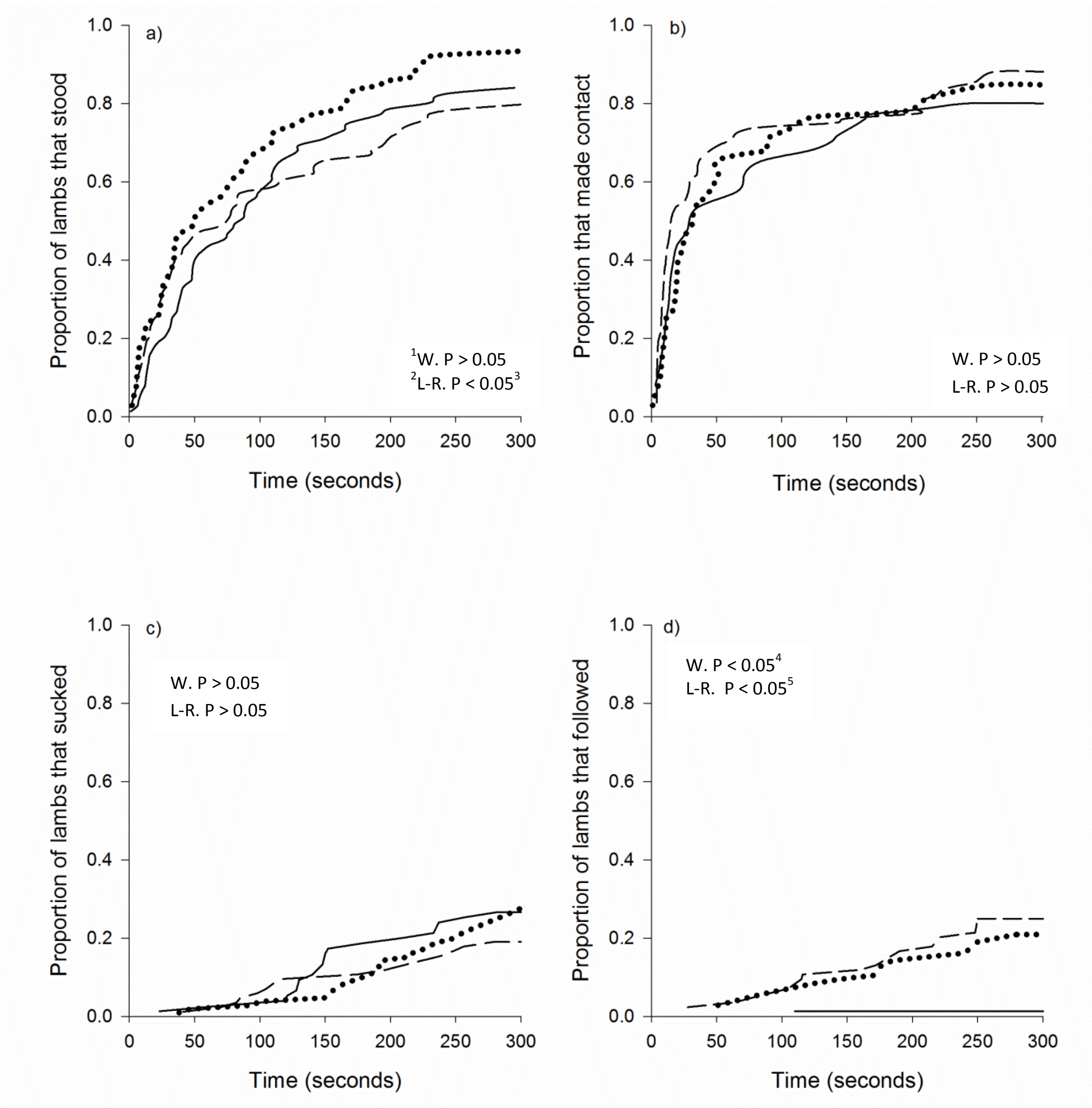
3.0 (1%) differ from BCS 2.0 and 2.5 at the end of the observation period (P < 0.05), however BCS 2.0 (21%) and 2.5 (25%) do not differ from each other (P > 0.05). In fact, only one lamb born to ewes in the BCS 3.0 group followed the ewe in this experiment. There were no effects of ewe feeding treatment or BCS on the median time to follow (P > 0.05, Table 3.5).

Figure 3.1. Experiment one; the effect of ewe feeding treatments (medium ..... and *ad lib* —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-Rank p-value.

Figure 3.2. Experiment one; the effect of ewe BCS group (BCS 2.0 ….., BCS 2.5 - - - and BCS 3.0—) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-Rank p-value. [3]The Log-Rank p-value showed a significant difference between BCS 2.0 and 2.5, but neither differed from BCS 3.0. [4]The Wilcoxon p-value showed a significant difference between BCS 2.0 and 3.0, and between BCS 2.5 and 3.0. [5]The Log-Rank p-value showed a significant difference between BCS 2.0 and 3.0, and between BCS 2.5 and 3.0.

Table 3.5. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) on the median time (seconds) required for the lambs to exhibit the various behaviours.

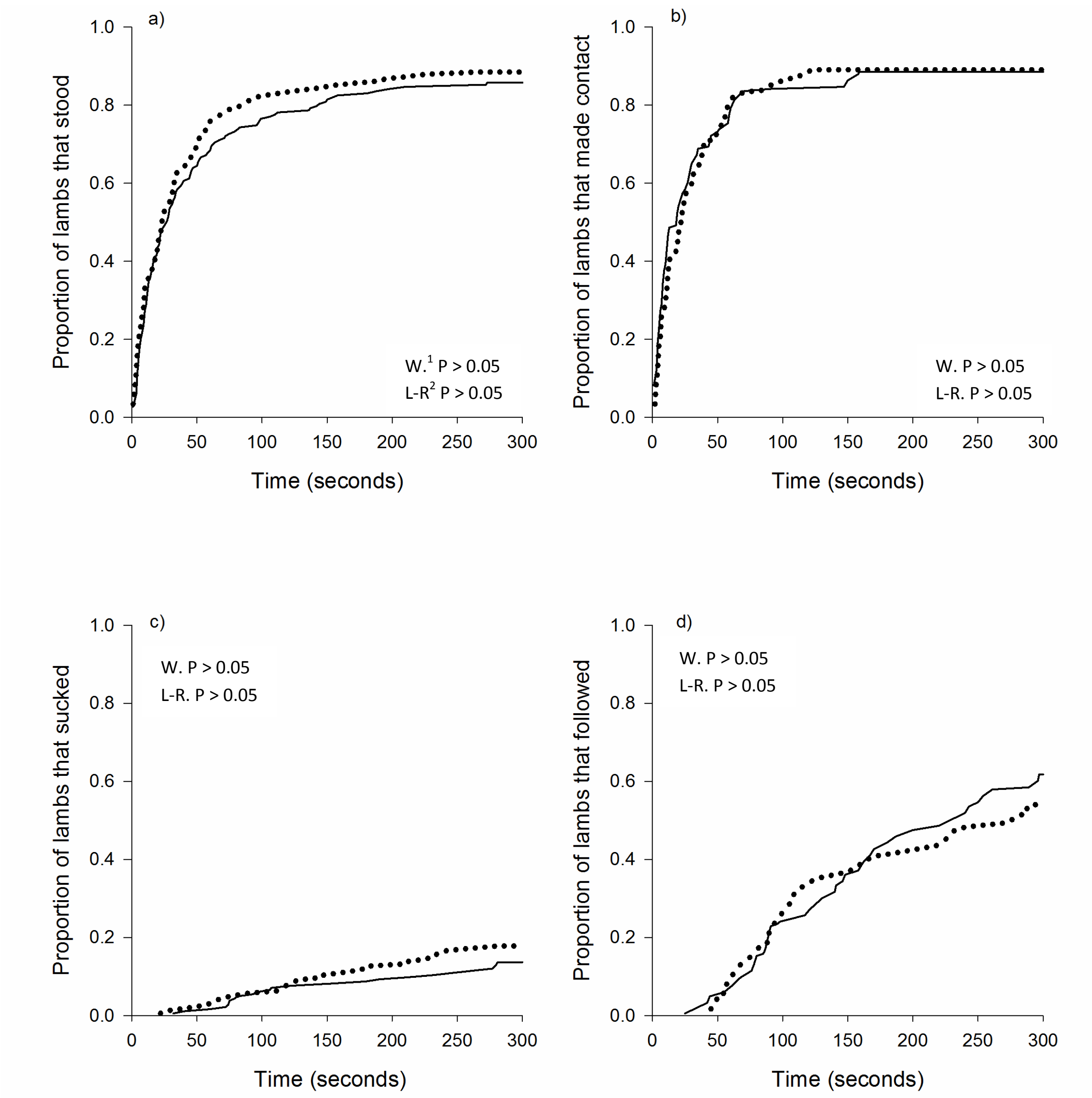
	n	Standing	Contact	Suckling	Follow
Experiment 1					
Feeding treatment					
medium	129	74.0	28.0	301.0	301.0
ad lib	135	60.0	29.0	301.0	301.0
BCS group					
BCS 2.0	105	50	32	301.0	301.0
BCS 2.5	84	77	17	301.0	301.0
BSC 3.0	75	87.0	35	301.0	301.0
Experiment 2					
Feeding treatment					
medium	183	23.5	22.5	301.0	280.0
ad lib	174	27.0	19.0	301.0	230.0
BCS group					
BCS 2.0	99	26.0	20.0	301.0	301.0
BCS 2.5	132	22.0	15.0	301.0	226.0
BSC 3.0	126	28.5	29.0	301.0	230.0
Experiment 3					
Feeding treatment					
medium	102	16.5	18.0	301.0	296.5
ad lib	110	26.0	26.0	301.0	255.0
BCS group					
BCS 2.0	74	24.0[b]	26.0	301.0	210.0[a]
BCS 2.5	78	16.0[a]	16.0	301.0	301.0[b]
BSC 3.0	60	23.5[ab]	12.0	301.0	231.5[a]

[abc] Different superscripts within experiments, columns and main effects indicate values that significantly differ ($P < 0.05$).

Experiment two –paddock behaviors

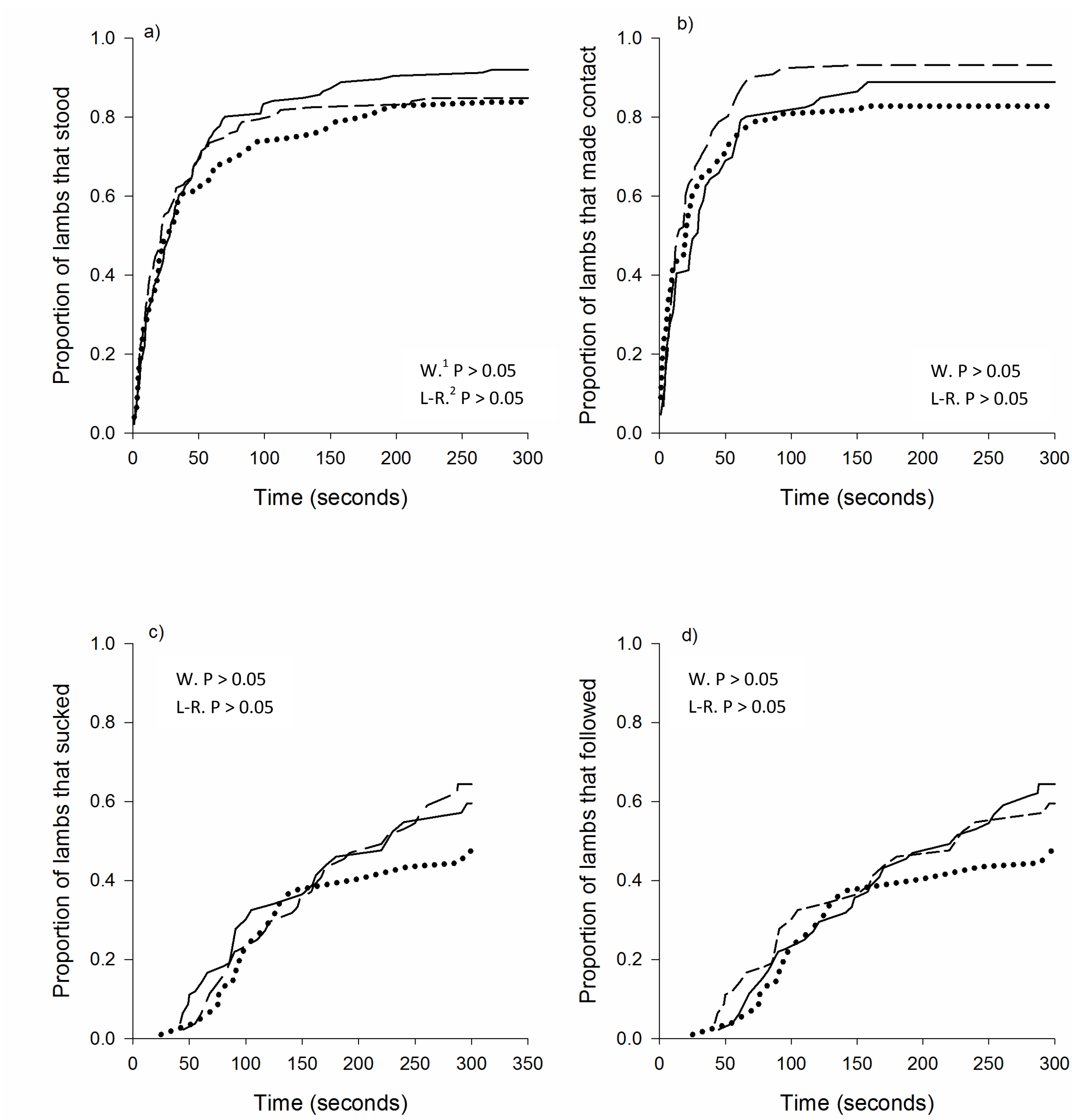
No lamb vigour behaviours (Fig. 3.4 and 3.5a-d, Table 3.5) were affected by ewe feeding treatment or BCS group (P > 0.05).

Figure 3.3. Experiment two; the effect of ewe feeding treatments (medium ….. and *ad lib* —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-Rank p-value.

Figure 3.4. Experiment two; the effect of ewe BCS group (BCS 2.0 ….., BCS 2.5 - - - and BCS 3.0—) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-Rank p-value.

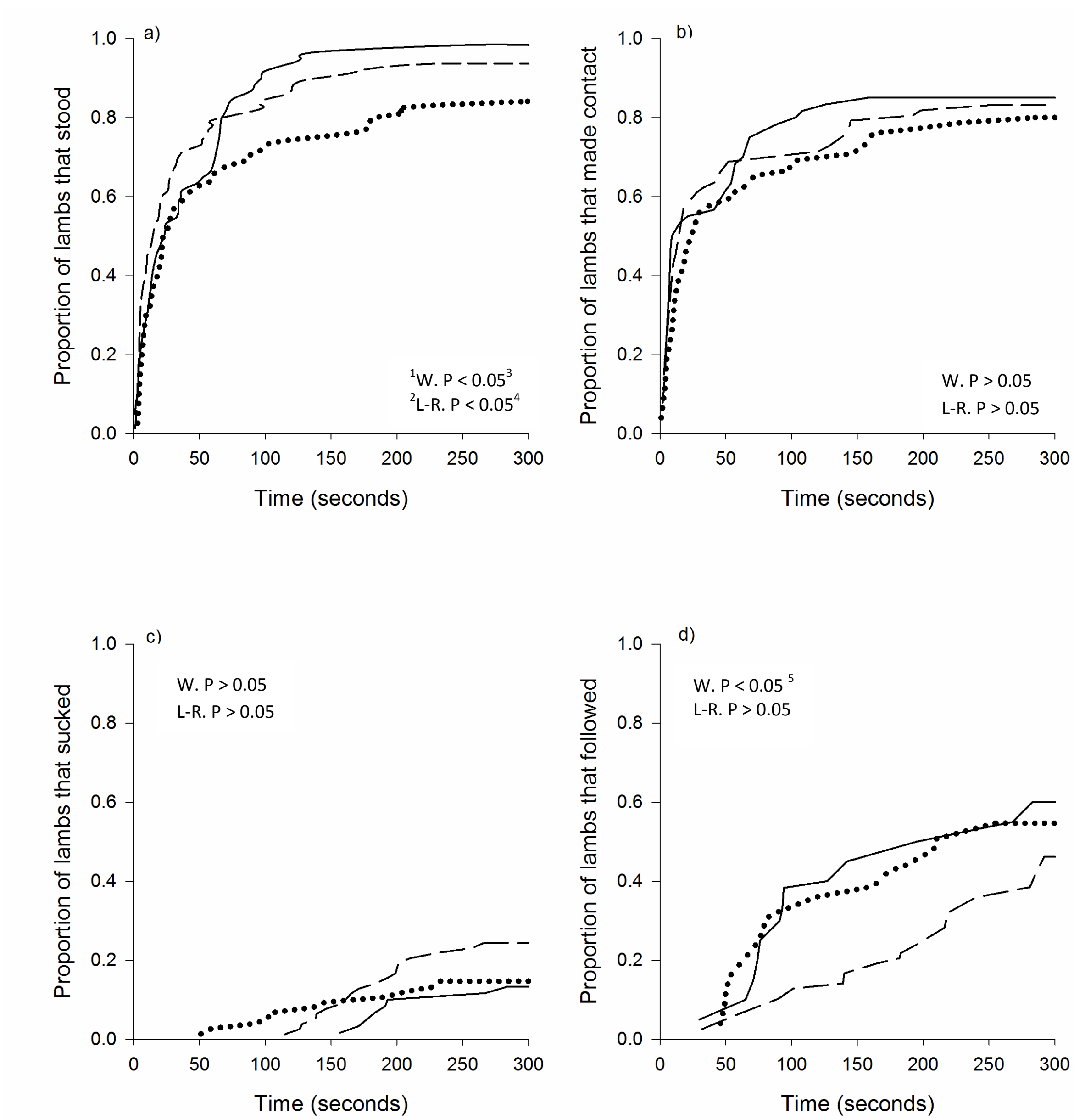
Experiment three –paddock behaviours

Survival analysis showed that the time for the lambs to stand differed between ewe BCS groups (Wilcoxon and log-rank P < 0.05, Fig. 3.6a and Table 3.5). Early in the observation period the Wilcoxon p-value showed a significant difference between BCS 2.0 and 2.5 (P < 0.05), but neither differed from BCS 3.0 (P > 0.05). Later in the observation period, the log-rank p-value showed a significant difference between BCS 2.0 and 2.5 (P < 0.05), and between BCS 3.0 and 2.0 (P < 0.05). At approximately 300 seconds, 84% of lambs in the BCS 2.0 group had stood compared with 94% and 100% of lambs in the BCS 2.5 and BCS 3.0 groups, respectively. Furthermore, the median time to stand was longer for lambs in the BCS 2.0 group than lamb in the BCS 2.5 (P < 0.05), but did not differ from the BCS 3.0 groups (P > 0.05).

Survival analysis showed that the time for the lambs to follow their dam differed between lambs in the BCS 2.5 group and those in either of the other BCS groups (Wilcoxon P < 0.05, Fig. 3.6d and Table 3.5). At approximately 140 seconds, 16% of lambs in the BCS 2.5 group had followed their dam compared with 45% and 36% for lambs in the BCS 2.0 and 3.0 groups, respectively. The median time to follow was also longer for lambs born to ewes in the BCS 2.5 compared with lambs in the BCS 2.0 and 3.0 groups (P < 0.05, Table 3.5).

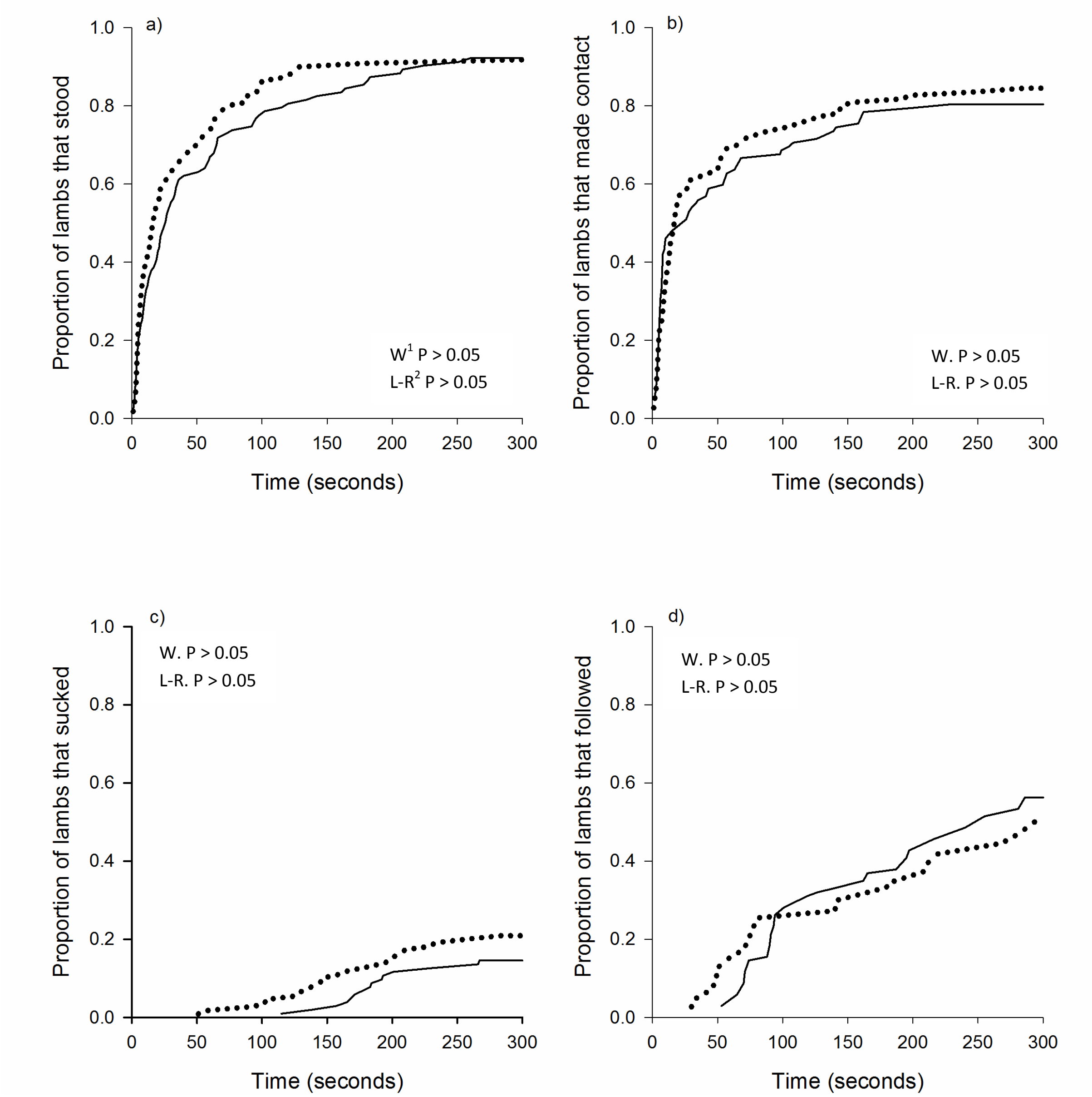
Ewe feeding treatment did not affect lamb behaviour (P < 0.05, Fig. 3.7a-d and Table 3.5).

Figure 3.5. Experiment three; the effect of ewe BCS group (BCS 2.0 …., BCS 2.5 - - - and BCS 3.0—) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-Rank p-value.[3]The Wilcoxon p-value showed a significant difference between BCS 2.0 and 2.5, but neither differed from BCS 3.0. [4]The Log-Rank p-value showed a significant difference between BCS 2.0 and 2.5, and between BCS 3.0 and 2.0. [5]The Wilcoxon p-value showed a significant difference between BCS 2.0 and 2.5, and between BCS 3.0 and 2.5.

Figure 3.6. Experiment three; the effect of ewe feeding treatments (medium ….. and *ad lib* —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-Rank p-value.

The effect of ewe feeding treatment and BCS on ewe behaviour

The MBS of the ewe was not affected by ewe BCS or feeding treatment in experiment one or two ($P > 0.05$, Table 3.6a&b). In experiment three, ewes from the BCS 2.0 group had a lower MBS compared with BCS 3.0 ewes ($P > 0.05$, Table 3.6c) but did not differ from ewes in the BCS 2.5 group ($P > 0.05$). Ewe feeding treatment had no effect on MBS in experiment three ($P > 0.05$). There were no significant interactions of ewe feeding treatment and BCS on ewe MBS in any of the three experiments ($P > 0.05$, Table 3.6a-c).

The percentage of ewes that emitted high- or low-pitched bleats was not affected by ewe feeding treatment, BCS group or their interaction in any of the three experiments ($P > 0.05$, Table 3.6a-c).

Experiment one

Of those ewes that bleated, there was a significant interaction of ewe feeding treatment and BCS on the number of high-pitched bleats, such that in the BCS 2.0 ewes in the medium feeding treatment emitted fewer high-pitched bleats than ewes in the *ad lib* treatment ($P < 0.05$, Table 3.6a). Within the BCS 3.0 group, ewes in the *ad lib* treatment emitted fewer high-pitched bleats than ewes the medium treatment ($P < 0.05$), while no difference was observed within the BCS 2.0 group ($P > 0.05$). The number of low-pitched bleats also varied among groups. Within BCS 2.5 and 3.0 groups, ewes in the medium treatment emitted more low-pitched bleats than ewes in the *ad lib* treatment ($P < 0.05$). There was no difference observed within the BCS 2.5 group ($P > 0.05$).

Table 3.6 a. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction on the percentage (95% CI) of ewes that emitted high- and low-pitched bleats and on the total number of bleats in the paddock.

	n	High-pitched bleat		Low-pitched bleat		MBS
		Percentage[1]	Number[2]	Percentage[1]	Number[2]	
Experiment 1						
Feeding treatment						
medium	45	93.7 (81.5-98.1)	16.6[a] (16.0-17.3)	87.8 (74.4-94.7)	38.6[b] (37.6-39.7)	2.9 (2.4-3.4)
ad lib	43	90.8 (76.4-96.8)	18.3[b] (17.5-19.1)	90.2 (76.4-96.3)	32.6[a] (31.6-33.6)	2.9 (2.4-3.5)
BCS group						
BCS 2.0	35	97.3 (82.9-99.6)	16.8[a] (16.1-17.7)	94.2 (79.5-98.5)	39.0[b] (37.8-40.2)	3.0 (2.5-3.7)
BCS 2.5	28	92.9 (75.5-98.2)	16.0[a] (15.2-16.9)	85.9 (67.7-94.6)	33.6[a] (32.4-34.9)	2.9 (2.2-3.7)
BSC 3.0	22	79.3 (58.6-91.2)	19.7[b] (18.7-20.7)	84.5 (64.5-94.2)	34.0[a] (32.7-35.3)	2.8 (2.2-3.50
Feeding x BCS[3]						
medium BCS 2.0	14	–	12.4[a] (11.4-13.5)	–	39.1[de] (37.3-41.1)	–
medium BCS2.5	15	–	16.8[bc] (15.6-18.0)	–	35.6[c] (33.9-37.4)	–
medium BCS 3.0	16	–	20.6[d] (19.3-21.9)	–	40.8[e] (39.0-72.7)	–
ad lib BCS 2.0	21	–	20.1[d] (19.0-21.2)	–	38.1[d] (36.6-39.7)	–
ad lib BCS 2.5	13	–	15.1[b] (13.9-16.3)	–	31.9[b] (30.2-33.7)	–
ad lib BCS 3.0	9	–	17.4[c] (15.9-19.1)	–	24.4[a] (22.6-26.4)	–

[1]The percentage of ewes that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- an low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscript within experiments, columns and effects indicate values that significantly differ (P < 0.05).

Ewe nutrition and BCS and triplet-born lamb behaviour

Table 3.6 b. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction on the percentage (95% CI) of ewes that emitted high- and low-pitched bleats and on the total number of bleats in the paddock.

	n	High-pitched bleat		Low-pitched bleat		MBS
		Percentage[1]	Number[2]	Percentage[1]	Number[2]	
Experiment 2						
Feeding treatment						
medium	58	86.2 (74.5-93.0)	9.1[b] (8.6-9.5)	91.6 (81.1-96.5)	14.9[a] (14.3-15.4)	2.7 (2.3-3.1)
ad lib	61	80.2 (68.2-88.5)	8.1[a] (7.7-8.5)	86.9 (75.8-93.4)	19.0[b] (18.4-19.7)	3.2 (2.7-3.7)
BCS group						
BCS 2.0	33	76.1 (58.9-87.7)	8.4[ab] (7.8-9.0)	85.2 (68.8-93.8)	18.2[b] (17.3-19.0)	2.9 (2.4-3.6)
BCS 2.5	44	86.5 (72.9-93.8)	8.3[a] (7.8-8.8)	88.8 (75.6-95.3)	17.5[b] (16.8-18.3)	3.1 (2.6-3.7)
BSC 3.0	42	86.2 (72.2-93.7)	9.1[b] (8.6-9.6)	93.2 (80.6-97.8)	14.9[a] (14.3-15.6)	2.8 (2.3-3.34)
Feeding x BCS[3]						
medium BCS 2.0	16	–	9.4[c] (8.5-10.3)	–	18.0[c] (16.8-19.2)	–
medium BCS2.5	23	–	8.7[bc] (8.0-9.4)	–	16.3[b] (15.4-17.3)	–
medium BCS 3.0	19	–	9.3[c] (8.6-10.1)	–	10.6[a] (9.8-11.5)	–
ad lib BCS 2.0	17	–	7.5[a] (6.8-8.3)	–	18.7[c] (17.6-20.0)	–
ad lib BCS 2.5	21	–	7.9[a] (7.2-8.6)	–	19.0[c] (17.9-20.1)	–
ad lib BCS 3.0	23	–	8.9[bc] (8.2-9.6)	–	19.0[c] (18.0-20.1)	–

[1]The percentage of ewes that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within experiments, columns and effects indicate values that significantly differ (P < 0.05).

Table 3.6 c. The effect of ewe feeding treatment (medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction on the percentage (95% CI) of ewes that emitted high- and low-pitched bleats and on the total number of bleats in the paddock.

	n	High-pitched bleat		Low-pitched bleat		MBS
		Percentage[1]	Number[2]	Percentage[1]	Number[2]	
Experiment 3						
Feeding treatment						
medium	37	74.0 (56.8-86.0)	5.4[a] (5.0-5.9)	91.0 (75.9-97.0)	24.7[b] (20.9-22.6)	2.6 (2.1-3.1)
ad lib	34	84.8 (68.4-93.5)	11.6[b] (11.0-12.3)	91.8 (76.3-97.5)	18.8[a] (18.0-19.7)	2.6 (2.1-3.2)
BCS group						
BCS 2.0	25	89.1 (70.3-96.6)	10.3[c] (9.6-11.1)	84.2 (64.2-94.0)	25.2[c] (24.1-26.4)	2.1[a] (1.6-2.8)
BCS 2.5	26	65.8 (45.8-81.4)	6.3[a] (5.8-6.9)	92.3 (74.0-98.1)	17.3[a] (16.4-18.2)	2.7[ab] (2.1-3.4)
BSC 3.0	20	80.0 (56.9-92.4)	7.6[b] (7.0-8.4)	95.0 (71.7-99.3)	19.0[b] (18.0-20.2)	3.1[b] (2.4-3.9)
Feeding x BCS[3]						
medium BCS 2.0	15	–	6.5[b] (5.7-7.3)	–	29.3[d] (27.7-30.9)	–
medium BCS2.5	13	–	6.4[b] (5.6-7.2)	–	12.4[a] (11.3-13.5)	–
medium BCS 3.0	9	–	3.2[a] (2.6-4.0)	–	25.8[c] (23.9-27.8)	–
ad lib BCS 2.0	10	–	15.4[d] (14.1-16.8)	–	20.5[b] (18.9-22.1)	–
ad lib BCS 2.5	13	–	7.2[b] (6.4-8.10	–	22.2[b] (20.8-23.8)	–
ad lib BCS 3.0	11	–	12.8[c] (11.7-14.1)	–	13.4[a] (12.2-14.7)	–

[1]The percentage of ewes that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within experiments, columns and effects indicate values that significantly differ (P < 0.05).

Ewe nutrition and BCS and triplet-born lamb behaviour

Experiment two

Of those ewes that bleated, there was a significant interaction of ewe feeding treatment and BCS on the number of high-pitched bleats such that, within the BCS 2.0 and 2.5 groups ewes in the medium treatment emitted more high-pitched bleats than the *ad lib* treatment (P < 0.05, Table 3.6b). There was no difference observed within the BCS 3.0 group (P > 0.05). There was also a significant interaction of ewe feeding treatment and BCS on the number of low-pitched bleats such that, within the BCS 2.5 and 3.0 groups ewes in the medium treatment emitted fewer high-pitched bleats than the *ad lib* treatment (P < 0.05, Table 3.6b), but no difference observed within the BCS 2.0 group (P > 0.05).

Experiment three

Of those ewes that bleated, there was a significant interaction of ewe feeding treatment and BCS on the number of high-pitched bleats such that, within the BCS 2.0 and the 3.0 group ewes in the *ad lib* treatment emitted the most high-pitched bleats (P < 0.05, Table 3.6c). No difference was observed between feeding treatments within the BCS 2.5 group (P > 0.05). The number of low-pitched bleats also varied among groups. Within the BCS 2.0 and the 3.0 group ewes in the *ad lib* treatment emitted fewer low-pitched bleats than the medium treatment (P < 0.05, Table 3.6c), while within the BCS 2.5 group ewes in the *ad lib* treatment emitted more low-pitched bleats than the medium treatment (P < 0.05).

The effect of ewe feeding treatment and BCS on lamb triangle pen test

The outcome of the preference test, as part of the lamb triangle pen test, was not affected by ewe feeding treatment, BCS group or age of lamb (P > 0.05). In total 48% and 52% of lambs made the 'correct choice', 22% and 20% made the 'incorrect choice' and 30% and 28% made 'no choice' for experiment one and three, respectively.

Experiment one

There was no effect of ewe feeding treatment and BCS on the percentage of lambs that emitted high- or low-pitched bleats (P > 0.05, Table 3.7). Of those that bleated, there was a significant interaction of ewe feeding treatment and BCS on the number of low-pitched bleats such that, within the BCS 2.5 and 3.0 groups lambs born to ewes in the *ad lib* feeding treatment emitted fewer low-pitched bleats than the medium treatment (P < 0.05). Within the BCS 2.0 group the opposite was observed (P < 0.05). Of lambs tested at 12 hours of age those in the medium treatment emitted more low-pitched bleats than the *ad lib* treatment (P < 0.05). No difference was observed within the lambs tested at 24 hours (P > 0.05). Of lambs in BCS group 2.0 and 2.5 there was no effect of age on the number of low-pitched bleats (P > 0.05). Within the BCS 3.0 group lambs tested at 24 hours emitted fewer low-pitched bleats (P < 0.05) than lambs tested at 12 hours.

Table 3.7. The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and age at testing (12h and 24h) and their interaction on the percentage (95% CI) of lambs that emitted low- and high-pitched bleats, and the total number of bleats in the triangle pen test.

	n	Low-pitched bleat		High-pitched bleat	
		Percentage[1]	Number [2]	Percentage[1]	Number [2]
Experiment 1					
Feeding treatment					
medium	81	55.3 (43.1-66.8)	4.8[b] (4.3-5.3)	95.7 (86.9-99.0)	29.8 (28.6-31.0)
ad lib	69	55.7 (44.6-66.3)	3.8[a] (3.3-4.3)	98.8 (91.9-99.8)	28.4 (27.1-29.7)
BCS group					
BCS 2.0	59	55.0 (42.4-67.1)	3.4[a] (3.0-4.0)	98.7 (90.3-99.8)	37.8[c] (36.2-39.4)
BCS 2.5	38	64.1 (48.1-77.5)	6.0[b] (5.5-7.0)	95.5 (82.1-99.0)	24.1[a] (22.6-26.0)
BSC 3.0	51	47.0 (33.7-60.8)	3.6[a] (3.0-4.2)	98.1 (87.0-99.7)	27.0[b] (25.6-28.5)
Age					
12h	106	57.4 (47.9-66.4)	4.7[b] (4.3-5.1)	96.3 (90.6-98.6)	31.5[b] (30.5-32.6)
24h	42	47.6 (33.2-62.5)	3.2[a] (2.7-3.8)	100	28.3[a] (26.7-30.0)
Feeding x BCS[3]					
medium BCS 2.0	27	–	2.4[a] (1.9-3.1)	–	40.3[d] (38.0-42.8)
medium BCS2.5	21	–	8.0[c] (6.9-9.3)	–	23.3[a] (21.3-25.4
medium BCS 3.0	33	–	4.6[b] (3.9-5.4)	–	27.3[b] (25.6-29.2)
ad lib BCS 2.0	33	–	4.2[b] (3.6-5.0)	–	35.5[c] (33.6-37.6)
ad lib BCS 2.5	18	–	4.3[b] (3.5-5.4)	–	25.2[ab] (23.0-27.6)
ad lib BCS 3.0	18	–	2.2[a] (1.6-3.2)	–	27.1[b] (25.6-29.2)

Feeding x age[3]					
medium 12h	57	–	5.3[c]	–	29.8[b]
			(4.7-5.9)		(28.4-31.3)
ad lib 12h	51	–	4.1[b]	–	33.4[c]
			(3.5-4.7)		(31.9-35.0)
medium 24h	24	–	3.5[ab]	–	32.5[bc]
			(2.8-4.3)		(30.3-34.9)
ad lib 24h	18	–	2.8[a]	–	22.7[a]
			(2.2-3.7)		(20.6-25.0)
BCS group x age[3]					
BCS 2.0 12h	42	–	3.7[bc]	–	39.5[d]
			(3.2-4.3)		(37.6-41.4)
BCS 2.5 12h	27	–	6.8[e]	–	26.8[b]
			(5.9-7.9)		(24.6-28.8)
BSC 3.0 12h	39	–	4.3[cd]	–	26.2[b]
			(3.7-5.0)		(24.6-27.8)
BCS 2.0 24h	18	–	2.8[ab]	–	33.5[c]
			(2.1-3.7)		(30.9-36.3)
BCS 2.5 24h	12	–	5.2[de]	–	18.2[a]
			(4.0-6.6)		(15.9-20.7)
BSC 3.0 24h	12	–	1.8[a]	–	30.7[c]
			(1.2-2.8)		(27.7-34.0)

[1]The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2] The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc]Different superscripts within experiments, columns and effects indicate values that significantly differ (P < 0.05).

The number of high-pitched bleats showed an interaction of ewe BCS and feeding treatment, such that within the BCS 2.0 group lambs born to ewes on the medium treatment emitted more high-pitched bleats than lambs born to ewes in the *ad lib* treatment (P > 0.05, Table 3.7). No differences were observed within the BCS 2.5 and 3.0 groups (P > 0.05). Within the *ad lib* feeding treatment lambs tested at 12 hours of age emitted more high-pitched bleats than lambs tested at 24 hours of age (P < 0.05). Within the BCS 2.0 and 2.5 groups lambs tested at 12 hours of age emitted more high-pitched bleats than at 24 hours of age ($P < 0.05$). The reverse effect was observed within the BCS 3.0 group ($P > 0.05$).

The percentage of lambs that sat during the triangle pen test was smaller for lambs born to ewes in the BCS 3.0 group compared with lambs born to ewes in the BCS 2.5 group ($P < 0.05$, Table 3.8a), but did not differ from the BCS 2.0 group ($P > 0.05$). Lambs in the BCS 3.0 group spent more time standing compared with in the other BCS groups ($P < 0.05$). Lambs born to ewes in the BCS 2.0 group spent more time walking ($P < 0.05$) compared with lambs born to ewes in the BCS 2.5 and BCS 3.0 group which did not differ ($P > 0.05$). Lambs tested at 24 hours of age spent more time walking than lambs tested at 12 hours ($P < 0.05$).

Table 3.8a. The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and. BCS 3.0), age (12h and. 24h) at testing on the time (seconds) spent by the lambs standing, the percentage of lambs (95% CI) that walked, sat and the time spent walking and sitting (for those exhibited the behaviours) in the triangle pen test.

	n	Time standing[1]	Walk		Sit	
			Percentage[2]	Time[3]	Percentage[2]	Time[3]
Experiment 1						
Feeding treatment						
medium	81	260	95.6 (87.1-98.6)	30	20.9 (12.5-32.8)	10
ad lib	67	260	92.9 (84.7-96.9)	40	9.7 (4.8-18.6)	75
BCS group						
BCS 2.0	59	250[a]	95.0 (85.6-98.4)	50.0[b]	13.6[ab] (7.0-24.9)	70
BCS 2.5	38	260[a]	95.0 (82.1-98.8)	30.0[a]	25.4[b] (14.0-41.5)	15
BSC 3.0	51	270[b]	92.8 (81.5-97.5)	30.0[a]	8.2[a] (3.1-20.4)	10
Age						
12h	106	260	94.4 (88.2-97.5)	30.0[a]	16.7 (10.8-24.9)	45
24h	42	260	92.9 (80.1-97.7)	50.0[b]	11.9 (5.0-25.6)	10

[1] All lambs were standing at the beginning of the triangle test. Standing is presented as the back-transformed median time in seconds. [2]The percentages of lambs that walked and sat are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [3]Time for those that expressed the behaviour is presented as the median time in seconds. [abc] Different superscripts within experiments, columns and main effects indicate values that significantly differ (P < 0.05).

Time with the alien ewe showed an interaction of ewe feeding treatment and BCS, such that within the medium treatment a greater percentage of lambs born to ewes in the BCS 2.0 group spent time with the alien ewe than lambs born to ewes in the BCS 3.0 group ($P < 0.05$, Table 3.9a), but neither differed from the BCS 2.5 group ($P > 0.05$). There were no differences within the *ad lib* treatment ($P > 0.05$). There was further an interaction of ewe BCS and age of lamb such that within the BCS 3.0 group a greater percentage of lambs tested at 12 hours spent time with the alien ewe than lambs tested at 24 hours ($P < 0.05$, Table 2.9b), there were no differences within the other two BCS groups ($P > 0.05$). No other behaviours exhibited in the triangle pen test were affected by ewe feeding treatment, BCS group, lamb age or their interactions ($P > 0.05$, Fig. 3.7, 3.8, Tables 3.8a, 3.9a, 3.10a).

Table 3.9a. The effect of ewe feeding treatment (medium and. *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) age (12h and 24h) at testing and their interaction on the percentage (95% CI) of lambs, and the median time (seconds) they spent with the dam, the alien ewe and in the contact zone (for those that exhibited the behaviour) in the triangle pen test.

	n	Spent time with dam		Spent time with alien ewe		Spent time in contact zone	
		Percentage[1]	Time[2]	Percentage[1]	Time[2]	Percentage[1]	Time[2]
Experiment 1							
Feeding treatment							
medium	81	79.7 (7.3-87.7)	190	65.8 (53.5-76.3)	100	93.5 (84.5-97.5)	270
ad lib	67	82.4 (7.4-89.3)	170	80.2 (69.9-87.6)	110	91.5 (82.9-96.0)	270
BCS group							
BCS 2.0	59	81.8 (70.0-90.0)	190	78.0 (65.6-86.8)	90	91.6 (81.4-96.5)	270
BCS 2.5	38	76.9 (61.2-87.5)	190	77.0 (61.2-87.8)	120	87.4 (72.9-94.7)	270
BSC 3.0	51	84.0 (71.0-91.8)	190	64.6 (50.2-76.8)	105	96.3 (86.0-99.1)	270
Age							
12h	106	79.6 (71.0-86.2)	190	74.1 (65.0-81.5)	110	90.7 (83.6-95.0)	270
24h	42	85.7 (71.7-93.4)	190	71.4 (56.1-83.0)	95	95.2 (82.9-98.8)	270
Feeding x BCS[3]							
medium BCS 2.0	27	–	–	92.8[b] (74.2-98.3)	–	–	–
medium BCS2.5	21	–	–	81.4[ab] (58.9-93.0)	–	–	–
medium BCS 3.0	33	–	–	55.1[a] (33.2-75.2)	–	–	–
ad lib BCS 2.0	33	–	–	67.0[a] (47.1-82.3)	–	–	–
ad lib BCS 2.5	18	–	–	77.2[ab] (46.8-92.9)	–	–	–
ad lib BCS 3.0	18	–	–	63.2[a] (37.9-82.8)	–	–	–

[1] The percentage of lambs that spent with dam, spent with alien and spent in contact zone is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]Time is presented as the back-transformed median time in seconds. [3]Non-significant interactions are not shown. [abc] Different superscripts within experiments, columns and effects indicate values that significantly differ ($P < 0.05$).

Table 3.9b. The effect of ewe feeding treatment (medium and. *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) age (12h and 24h) at testing and their interaction on the percentage (95% CI) of lambs, and the median time (seconds) they spent with the dam, the alien ewe and in the contact zone (for those that exhibited the behaviour) in the triangle pen test.

	n	Spent time with dam		Spent time with alien ewe		Spent time in contact zone	
		Percentage[1]	Time[2]	Percentage[1]	Time[2]	Percentage[1]	Time[2]
BCS group x age[3]							
BCS 2.0 12	42	–	–	80.9^{ab} (62.8-91.3)	–	–	–
BCS 2.5 12	27	–	–	74.8^{ab} (55.1-87.8)	–	–	–
BSC 3.0 12	39	–	–	74.8^{b} (57.7-86.6)	–	–	–
BCS 2.0 24	18	–	–	86.1^{b} (60.5-96.2)	–	–	–
BCS 2.5 24	12	–	–	83.2^{ab} (49.8-96.1)	–	–	–
BSC 3.0 24	12	–	–	41.5^{a} (18.2-69.4)	–	–	–
Experiment 3							
Feeding treatment							
medium	132	92.8 (86.5-96.3)	200	93.3 (87.5-96.5)	100	100	285
ad lib	133	96.9 (92.3-98.8)	180	89.6 (83.1-93.8)	100	100	280
BCS group							
BCS 2.0	87	97.9^{b} (91.9-99.5)	180	93.2 (85.6-96.9)	100	100	280
BCS 2.5	87	94.7^{ab} (87.6-97.9)	180	92.0 (84.0-96.1)	90	98.8(92.2-99.8)	285
BSC 3.0	90	90.4^{a} (82.3-95.1)	180	89.3 (81.0-94.2)	100	96.7 (90.2-98.9)	280
Age							
12h	168	91.7 (86.4-95.0)	190	90.5 (85.0-94.1)	90	98.2 (94.6-99.4)	280
24h	96	97.9 (92.0-99.5)	170	92.6 (85.3-96.5)	100	99.0 (92.9-99.9)	280

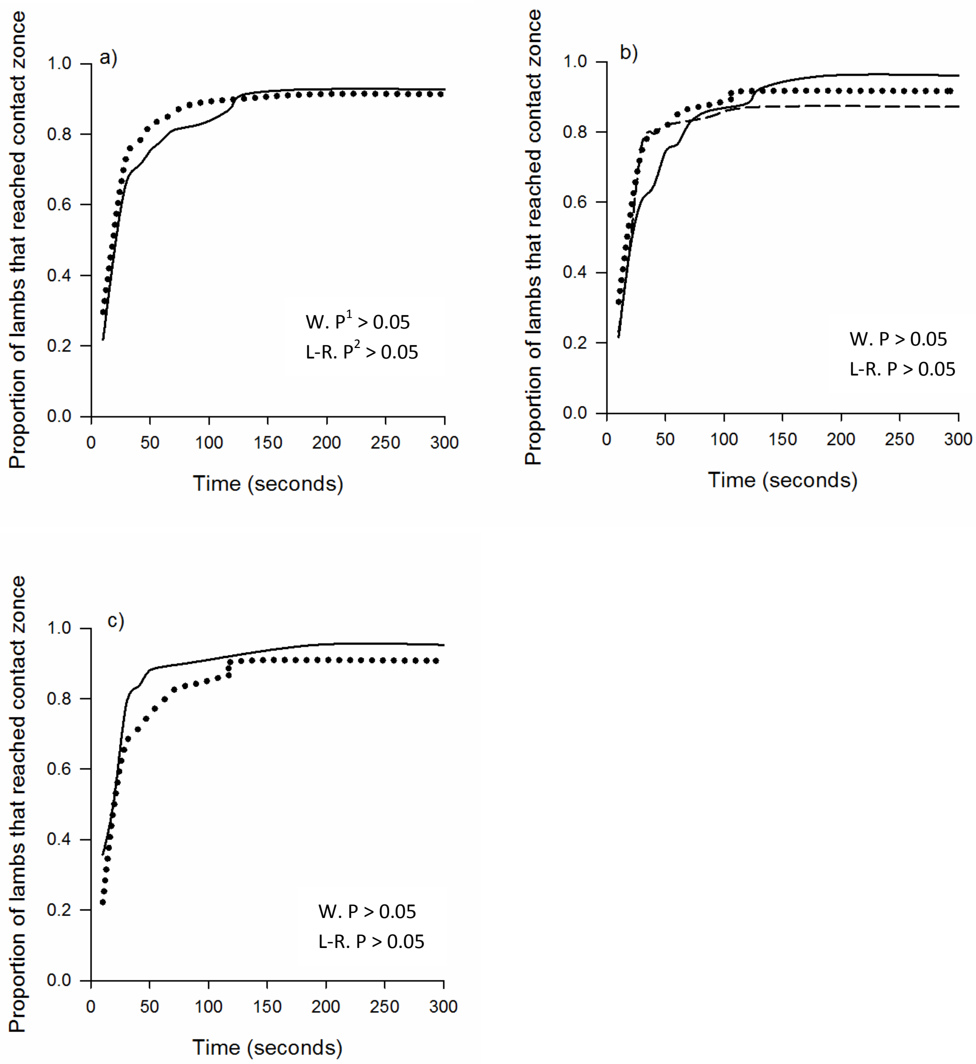
[1] The percentage of lambs that spent with dam, spent with alien and spent in contact zone is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]Time is presented as the back-transformed median time in seconds. [3]Non-significant interactions are not shown. [abc] Different superscripts within experiments, columns and effects indicate values that significantly differ (P < 0.0

Table 3.10a. The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and age (12h and 24h) at testing on the back-transformed percentage (95% CI) and median time (seconds) required for lambs to reach their dam and the contact zone (for those that exhibited the behaviour) in the triangle pen test.

	n	Reached dam		Reached contact zone	
		Percentage	Time	Percentage	Time
Experiment 1					
Feeding treatment					
medium	81	79.7 (68.3-87.7)	50	93.5 (84.5-97.5)	20
ad lib	67	82.4 (72.4-89.3)	60	91.5 (82.9-96.0)	30
BCS group					
BCS 2.0	59	81.8 (70.0-89.7)	40	91.6 (81.4-96.5)	20
BCS 2.5	38	76.8 (61.2-87.5)	60	87.4 (72.9-94.7)	30
BSC 3.0	51	84.0 (71.0-91.8)	50	96.3 (86.0-99.1)	30
Age					
12 h	106	79.6 (70.99-86.19)	60	90.7 (86.64-94.95)	20
24 h	42	85.7 (71.66-93.44)	40	95.2 (82.86-98.81)	20

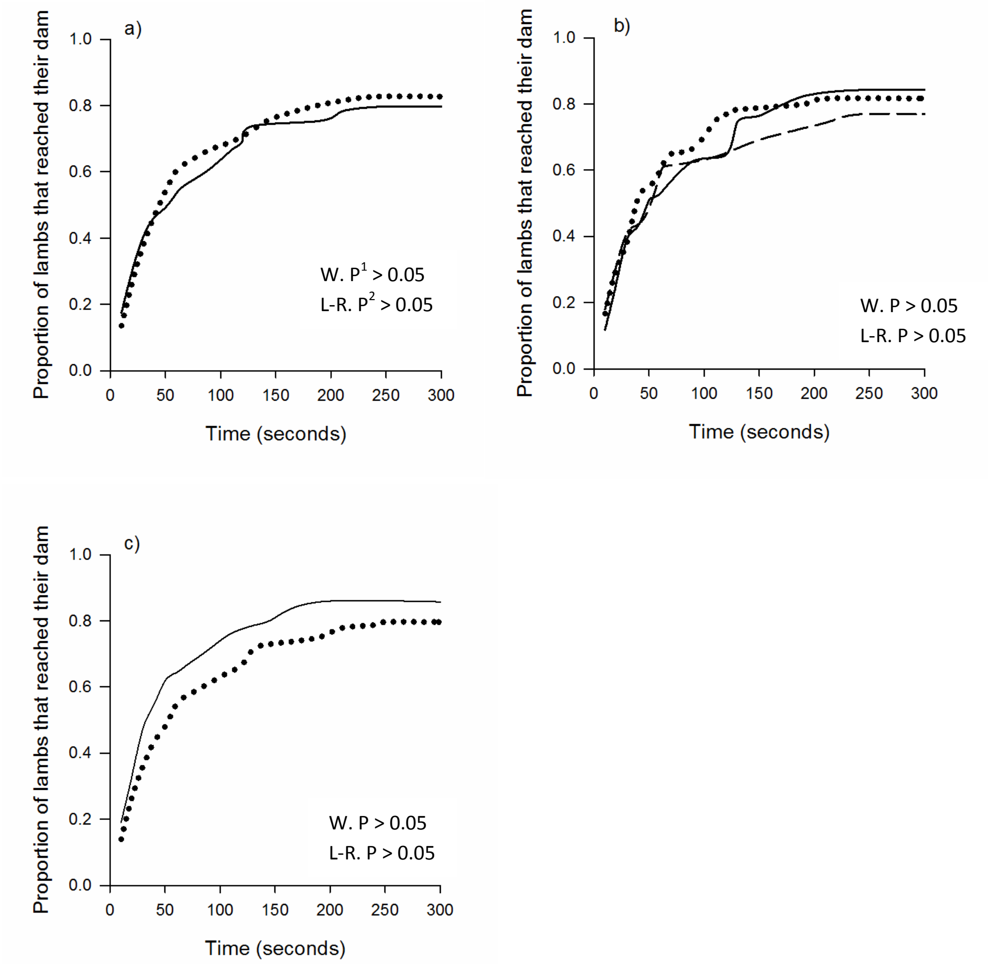
[abc] Different superscripts within experiments, columns and effects indicate values that significantly differ ($P < 0.05$).

Figure 3.7. Experiment one; the effect of ewe a) feeding treatments (medium ….. and *ad lib* —), b) body condition score (BCS 2.0 …., BCS 2.5 - - - and BCS 3.0 —) and c) age of lamb (12h ….. and 24h —) on the percentage of lambs that reached the contact zone in the triangle pen test. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

Figure 3.8. Experiment one; the effect of ewe a) feeding treatments (medium ….. and *ad lib* —), b) body condition score (BCS 2.0 ….., BCS 2.5 - - - and BCS 3.0 —) and c) age of lamb (12h ….. and 24h —) on the percentage of lambs that reached the contact zone in the triangle pen test. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

Experiment three

A greater percentage of lambs born to ewes in the medium feeding treatment emitted low-pitched bleats ($P < 0.05$, Table 3.11a). There were no other effects of ewe feeding treatment, BCS or age of lamb on the percentage of lambs that emitted low- and high-pitched bleats ($P > 0.05$).

Of those lambs that bleated, there was a significant interaction of ewe feeding treatment and BCS on the number of low-pitched bleats such that within the BCS 2.0 and 2.5 groups lambs in the medium treatment emitted more low-pitched bleats than lambs in the *ad lib* treatment ($P < 0.05$, Table 3.11a). There were no differences within the BCS 3.0 group ($P > 0.05$). The number of low-pitched bleats also showed an interaction of ewe feeding treatment and age of lamb on such that, within the lambs tested at 12 hours those born to ewes in the medium treatment emitted more low-pitched bleats than the *ad lib* treatment ($P < 0.05$). No difference was observed within the lambs tested at 24 hours ($P > 0.05$). There was a significant interaction of ewe BCS and age of lamb on the number of low-pitched bleats such that, within lambs tested at 12 hours those born to ewes in the BCS 2.0 group emitted more low-pitched bleats than the BCS 2.5 and 3.0 groups ($P < 0.05$). Within the lambs tested at 24 hours those born to ewes in the BCS 3.0 group emitted more low-pitched bleats than the 2.0 group which in turn emitted more bleats than the BCS 2.5 group ($P < 0.05$).

Table 3.11a. Experiment three; the effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and age at testing (12h and 24h) and their interaction on the percentage (95% CL) of lambs that emitted low- and high-pitched bleats, and the total number of bleats in the triangle pen test.

	n	Low-pitched bleat		High-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Feeding treatment					
medium	131	72.9[b]	6.7[b]	98.6	37.3[a]
		(64.7-79.8)	(5.1-5.9)	(94.2-99.7)	(36.3-38.4)
ad lib	132	55.6[a]	5.5[a]	97.8	42.8[b]
		(46.9-63.9)	(6.3-7.2)	(93.2-99.3)	(41.7-44.0)
BCS group					
BCS 2.0	87	65.7	7.1[b]	98.9	37.7[a]
		(55.0-75.0)	(6.5-7.7)	(92.4-99.8)	(36.4-39.0)
BCS 2.5	86	60.4	4.1[a]	97.7	39.9[b]
		(49.6-70.3)	(3.7-4.6)	(91.2-99.4)	(38.6-41.3)
BSC 3.0	90	67.8	7.7[b]	97.9	42.5[c]
		(57.3-76.7)	(7.1-8.3)	(91.7-99.5)	(41.1-43.8)
Age					
12h	168	61.9	3.8[a]	98.8	42.3[b]
		(54.34-98.93)	(3.5-4.1)	(95.37-99.7)	(41.3-43.3)
24h	96	68.4	10.8[b])	96.8	36.5[a]
		(58.43-76.96)	(10.1-11.4	(90.66-98.9)	(35.3-37.7)
Feeding x BCS[3]					
medium BCS 2.0	45	–	8.0[d]	–	35.3[b]
			(7.3-8.9)		(33.6-37.1)
medium BCS2.5	45	–	4.5[b]	–	32.4[a]
			(3.9-5.1)		(30.8-34.2)
medium BCS 3.0	42	–	8.4[d]	–	44.8[d]
			(7.5-9.3)		(42.8-46.8)
ad lib BCS 2.0	42	–	5.9[c]	–	39.5[c]
			(5.2-6.7)		(37.6-41.4)
ad lib BCS 2.5	42	–	3.8[a]	–	48.1[e]
			(3.3-4.5)		(46.0-50.3)
ad lib BCS 3.0	48	–	7.1[d]	–	41.0[c]
			(6.4-7.9)		(39.2-42.9)

[1]The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc]Different superscripts within experiments, columns and effects indicate values that significantly differ ($P < 0.05$).

Table 3.11b. Experiment three; the effect of ewe feeding treatment (medium and. *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and age at testing (12h and 24h) and their interaction on the percentage (95% CL) of lambs that emitted low- and high-pitched bleats, and the total number of bleats in the triangle pen test.

	n	Low-pitched bleat		High-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Feeding x age[3]					
medium 12h	79	–	4.4[b] (4.0-4.9)	–	41.4[b] (40.0-42.9)
ad lib 12h	89	–	3.3[a] (2.9-3.7)	–	42.6[b] (41.2-44.0)
medium 24h	63	–	10.7[c] (9.9-11.6)	–	31.2[a] (29.8-32.8)
ad lib 24h	42	–	10.9[c] (9.9-11.9)	–	43.0[b] (41.1-45.1)
BCS group x age[3]					
BCS 2.0 12h	58	–	4.3[c] (3.8-4.8)	–	41.6[b] (40.0-43.3)
BCS 2.5 12h	56	–	3.5[a] (3.1-4.0)	–	39.6[b] (38.0-41.3)
BSC 3.0 12h	54	–	3.6[ab] (3.1-4.1)	–	45.0[c] (43.2-46.8)
BCS 2.0 24h	29	–	12.5[e] (11.3-13.8)	–	28.7[a] (26.8-30.7)
BCS 2.5 24h	30	–	5.4[d] (4.7-6.3)	–	40.5[b] (38.3-42.8)
BSC 3.0 24h	36	–	13.8[f] (12.7-15.1)	–	39.4[b] (37.4-41.5)

[1]The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc]Different superscripts within experiments, columns and effects indicate values that significantly differ ($P < 0.05$).

Of those lambs that bleated, there was a significant interaction of ewe feeding treatment and BCS on the number of high-pitched bleats such that, within the BCS 3.0 group lambs born to ewes in the medium treatment emitted more high-pitched bleats than the *ad lib* treatment ($P < 0.05$, Table 3.11a). The opposite effect was observed within the BCS 2.0 and 2.5 groups ($P < 0.05$). There was also a significant interaction of ewe feeding treatment and age of lamb on the number of high-pitched bleats such that, within the medium feeding treatment lambs tested at 12 hours emitted more high-pitched bleats than lambs tested at 24 hours ($P < 0.05$, Table 3.11b). No effect was observed within the *ad lib* feeding treatment ($P > 0.05$). There was a significant interaction of ewe BCS and age of lamb on the number of high-pitched bleats such that, within the BCS 2.0 and 3.0 groups lambs tested at 12 hours emitted more high-pitched bleats than lambs tested at 24 hours ($P < 0.05$). No effect was observed within the BCS 2.5 group ($P > 0.05$).

Lambs tested at 24 hours of age spent more time walking than lambs tested at 12 hours, but less time standing ($P < 0.05$, Table 3.8b). A greater percentage of lambs born to ewes in the BCS 2.0 group spent time with their dam in the contact zone, compared with lambs in the BCS 3.0 group ($P < 0.05$, Table 3.9b).

Table 3.8b. The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and. BCS 3.0), age (12h and 24h) at testing on the time (seconds) spent by the lambs standing, the percentage of lambs (95% CI) that walked, sat and the time spent walking and sitting (for those exhibited the behaviours) in the triangle pen test.

	n	Time standing[1]	Walk		Sit	
			Percentage[2]	Time[3]	Percentage[2]	Time[3]
Experiment 3						
Feeding treatment						
medium	132	230	96.1 (90.7-98.4)	60	16.7 (11.2-24.0)	55
ad lib	133	230	98.1 (93.7-99.5)	50	17.5 (11.9-25.0)	80
BCS group						
BCS 2.0	87	230	95.7 (88.8-98.4)	60	17.3 (10.7-26.7)	50
BCS 2.5	87	220	98.9 (92.6-99.9)	60	16.3 (9.9-25.7)	70
BSC 3.0	90	240	95.7 (88.9-98.4)	50	17.7 (11.2-27.0)	70
Age						
12h	168	240.0[b]	96.4 (92.3-98.4)	50.0[a]	17.3 (12.3-23.7)	50
24h	96	220.0[a]	96.8 (90.7-99.0)	70.0[b]	16.8 (10.6-25.7)	70

[1] All lambs were standing at the beginning of the triangle test. Standing is presented as the back-transformed median time in seconds. [2]The percentages of lambs that walked and sat are presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [3]Time for those that expressed the behaviour is presented as the median time in seconds. [abc] Different superscripts within experiments, columns and main effects indicate values that significantly differ ($P < 0.05$).

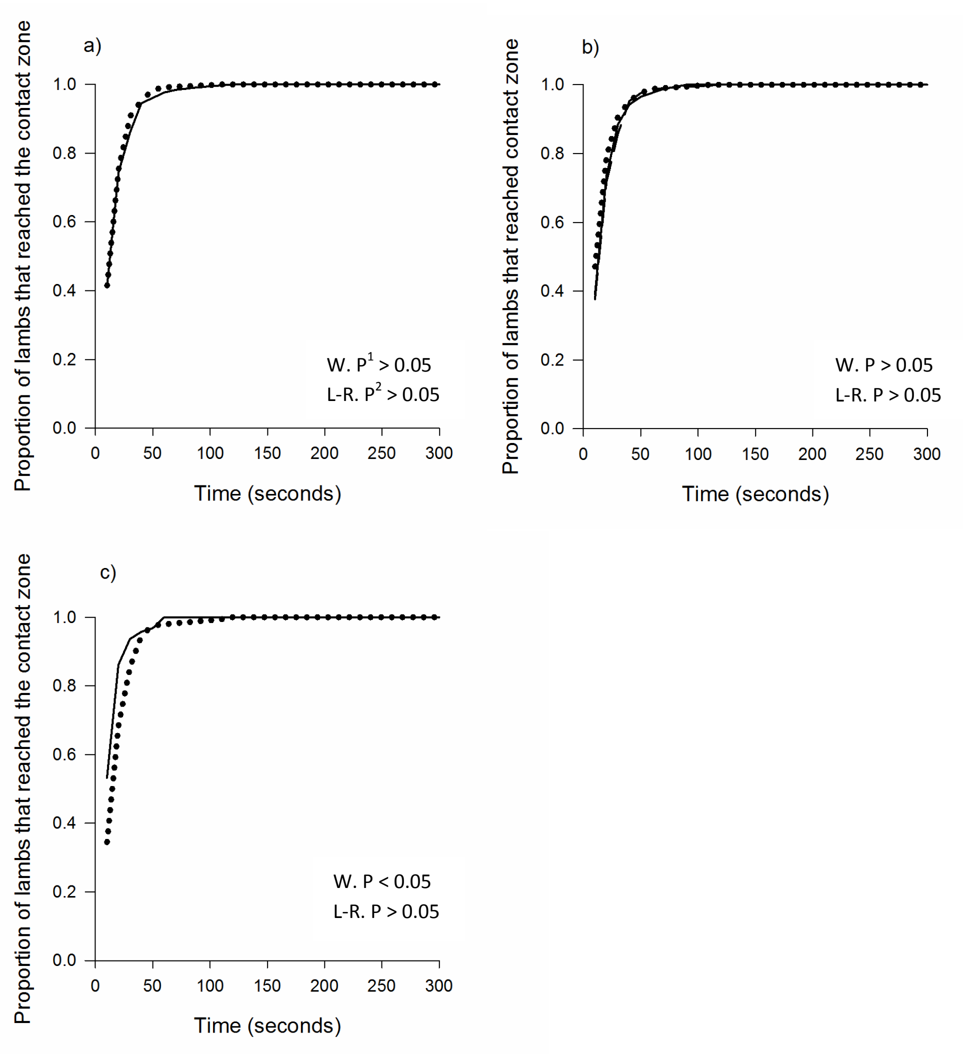
Table 3.10b. The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and age (12h and 24h) at testing on the back-transformed percentage (95% CI) and median time (seconds) required for lambs to reach their dam and the contact zone (for those that exhibited the behaviour) in the triangle pen test.

	n	Reached dam		Reached contact zone	
		Percentage	Time	Percentage	Time
Experiment 3					
Feeding treatment					
medium	132	92.8 (86.5-96.3)	20	100	20
ad lib	133	91.7 (86.4-98.8)	20	100	20
BCS group					
BCS 2.0	87	97.9 (91.9-99.5)	20	100	20
BCS 2.5	87	94.7 (87.6-97.9)	30	98.8 (92.2-99.8)	20
BSC 3.0	90	90.4 (82.3-95.1)	20	96.7 (90.2-98.9)	20
Age					
12 h	168	91.7 (86.42-95.00)	30	98.2 (94.61-99.42)	20^{b}
24 h	96	97.9 (91.97-99.47)	20	99.0 (92.91-99.85)	10^{a}

[abc] Different superscripts within experiments, columns and effects indicate values that significantly differ (P<0.05).

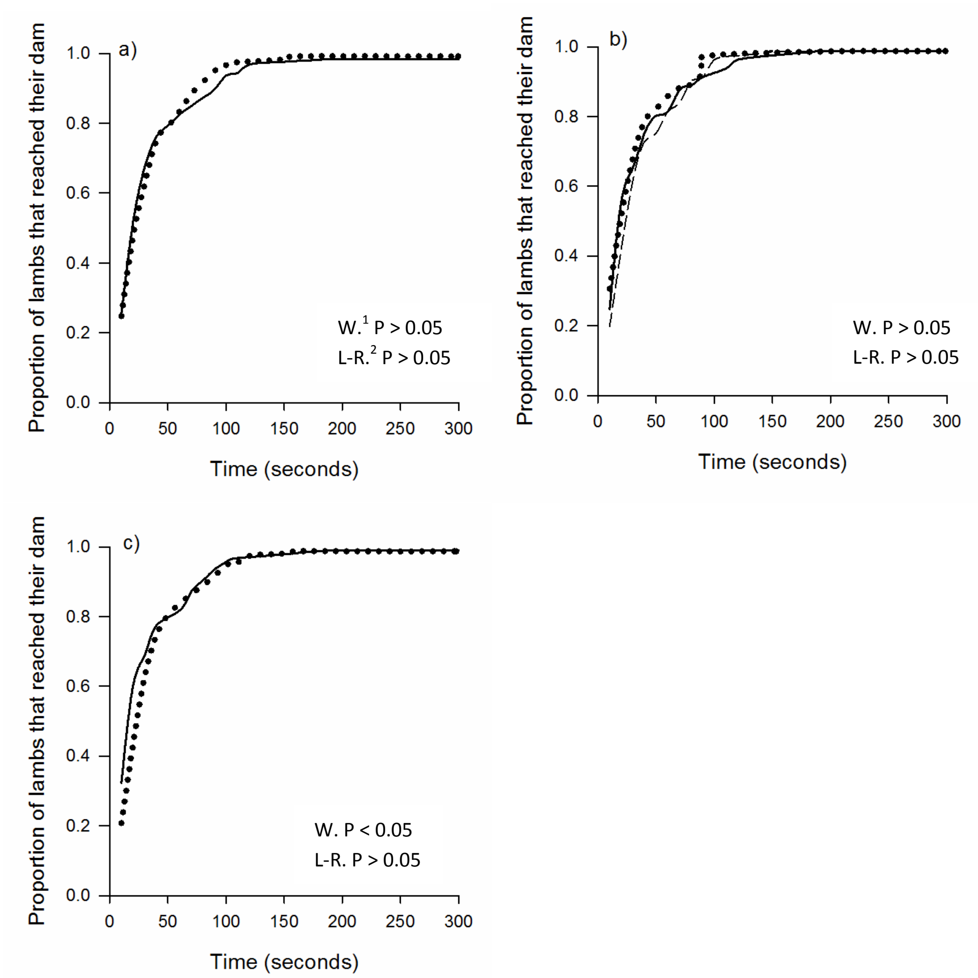
Survival analysis showed that the time taken for lambs to reached the contact zone and their dam differed early in the observation period between the ages of lamb at testing (Wilcoxon P < 0.05, Fig. 3.10c, 3.11c). At approximately 20 seconds 86% of lambs tested at 24 hours had reached the contact zone but this was 69% in lambs tested at 12 hours. The median time was shorter from lambs tested at 24h than for lambs tested at 12 hours (P < 0.05, Table 3.10b). At approximately 20 seconds 60% of lambs tested at 24 hours had reached their dam but only 44% of lambs tested at 12 hours (P < 0.05, Fig. 11c). There was no effect of ewe feeding treatment and BCS on the median time to reach the contact zone (P > 0.05, Table 3.10b).

Figure 3.10. Experiment three; the effect of ewe a) feeding treatments (medium ….. and *ad lib*—), b) body condition score (BCS 2.0 ….., BCS 2.5 - - - and BCS 3.0 —) and c) age of lamb (12h ….. and 24h —) on the percentage of lambs that reached the contact zone in the triangle pen test. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

Figure 3.11. Experiment three; the effect of ewe a) feeding treatments (medium ….. and *ad lib*—), b) body condition score (BCS 2.0 ….., BCS 2.5 - - - vs. BCS 3.0 —) and c) age of lamb (12h ….. and 24h —) on the percentage of lambs that reached their dam in the triangle pen test. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

Discussion

The aim of the current study was to investigate the effects of mid-pregnancy BCS and ewe nutrition during mid- to late-pregnancy on the behaviour of the triplet-bearing ewe and her lambs. The pre- and post-grazing masses in all three experiments indicated that the feeding treatments differed. Furthermore, ewes had been selected on the basis of three different ewe BCS in mid-pregnancy.

A ewe-lamb bond is an exclusive relationship and ewes will only offer maternal care to lambs they have bonded too (Nowak et al. 2011). Grooming and maintaining close contact with the lamb, low-pitched bleating, lamb recognition and co-operation with the lambs sucking attempts are all considered appropriate maternal bonding behaviours (Dwyer and Lawrence 2005). The behaviour of the newborn lamb is vital to motivate and strengthen maternal behaviors (Nowak 1990; Dwyer et al. 1999) and is necessary for the ewe-lamb bonding process (O'Connor and Lawrence 1992). Appropriate lamb bonding behaviours include ewe recognition and maintenance of close contact with the ewe, sucking and bleating (Nowak 1996; Dwyer and Lawrence 2005). Lamb bonding behaviours are critical as maternal behaviours decline rapidly if the responses from the newborn lamb are weak (Alexander et al. 1990; Dwyer et al. 1999).

In experiment three, ewes in the BCS 2.0 group displayed poorer MBS compared with ewes in the BCS 2.5 or 3.0 groups. This appears to be the first study to find a positive relationship between ewe BCS and MBS in triplet-bearing ewes managed under extensive pastoral conditions. Appropriate maternal behaviour is vital for lamb survival and it has been reported that lamb survival increases as MBS increases (O'Connor et al.

1985; Everett-Hincks et al. 2005b). However, in the larger study of Kenyon et al. (2013), of which these ewes were a subset, lambs born to ewes in the BCS 2.0 group did not display lower survival. In chapter two, ewe nutrition and BCS had no effect on MBS in twin-bearing ewes. It is, however, possible that the effects of mid-pregnancy BCS and mid- to late-pregnancy nutrition on ewe and lamb behaviour may be greater in triplet-bearing than in twin- and single-bearing ewes due to the greater nutritional demands during a triplet pregnancy (Nicol and Brookes 2007). However, if this was the case it may be expected that a similar effect would be present in all three experiment. There were no effects of ewe BCS on MBS in the other two experiments, however. Therefore, clear conclusions in regards to the relationship between BCS and MBS in triplet-bearing ewes cannot be drawn.

Ewe feeding treatments have, in earlier studies, been shown to affect MBS (Everett-Hincks et al. 2005a; Corner et al. 2010). In the present experiments this was not the case, supporting results reported in chapter two. Everett-Hincks et al. (2005a) and Corner et al. (2010) subjected ewes to lower feeding levels compared to the present experiments, and for longer periods. This may explain the lack of an effect on MBS. Furthermore, the ewes in the present experiments were not nutritionally restricted during pregnancy (Rattray et al., 1974). The results indicate that offering ewes feeding levels above pregnancy-maintenance requirement is probably not a useful management option to increase MBS.

Ewe and lamb high-pitched bleats are often emitted when the ewe and lamb are separated and may indicate a degree of need (Poindron et al. 1994; Dwyer et al. 1998).

Ewe and lamb low-pitched bleats help establish of the ewe-lamb bond and are also considered ‘care-giver’ bleats emitted exclusively between the ewe and her lamb (Shillito 1972; Nowak 1990; 1996; Dwyer et al. 1998). In all three experiments, within the BCS 2.0 group lambs born to ewes on the medium feeding treatment emitted more low-pitched bleats than lambs born to ewes on the *ad lib* treatment. The ewes within the BCS 2.0 group offered the medium feeding treatment did not, however, emit more low-pitched bleats than the ewes offered *ad lib*. Being of a lower BCS in mid-pregnancy and on lower feed intake during mid- to late-pregnancy, compared to ewes in BCS group 2.5 or 3.0 and in the *ad lib* treatment group, these ewes may have been more driven to graze after birth and thus have a weaker bond with their lambs. Perhaps the greater low-pitched bleating rates were an attempt by the lambs to strengthen and re-inforce that bond, or perhaps in response to bleats from the other littermates. The same effect was reported in experiment two (but not one) in chapter two. Everett-Hincks et al. (2005b) showed that triplet-bearing ewes on a restricted diet during pregnancy emitted more high-pitched bleats than well-fed ewes. The number of ewe and lamb high-pitched bleats did not appear to show a consistent pattern across the present experiments. These bleating rates results support those reported in chapter two. As indicated previously, the feeding levels used in the current experiments met or were above pregnancy requirements. This may explain the differences compared to the study by Everett-Hincks et al. (2005b). This again suggests that there is little advantage from offering ewes feeding levels above pregnancy needs.

The effects of ewe BCS and feeding treatment on the majority of lamb behaviours (time to stand, make contact with, suck from and follow the dam) were also inconsistent between experiments. In experiment one, lambs born to ewes offered *ad lib* feeding followed their dam more quickly than lambs born to ewes offered medium feeding. Alternatively, it may be that ewes in the *ad lib* treatment were quicker to gather and move their lambs away from the observers. The data collected does not, unfortunately, differentiate between the two. This supports the finding of Everett-Hincks et al. (2005b) who reported a positive relationship between triplet-bearing ewe feeding levels and the proportion of lambs that located the dams' udder and followed her when she moved away. However, the time taken for lambs to stand, contact, suck and follow was not affected by ewe feeding in the other two experiments. The effect of ewe feeding treatment on time to follow was also inconsistent in chapter two. Twin-born lambs born to ewes offered the medium treatment were quicker to follow in experiment one but slower in experiment two than *ad lib* lambs. Overall the results in this chapter suggest that feeding triplet-bearing ewes above pregnancy requirements has little or no effect on lamb behaviours.

No previous research appears to have investigated the effects of mid-pregnancy BCS in triplet-bearing ewe on the time taken for lambs to stand, contact, suck and follow the dam. The results reported in the current study were highly inconsistent between experiments and those results presented in the previous chapter. These inconsistencies may be due to the difference in length and timing of the feeding treatments. However, in all experiment they were implemented in mid- to late-pregnancy and as such any physiological effects due to them should be similar. The feeding treatments have been implemented too early in late-pregnancy to influence ewe and lamb behaviour as the majority of fetal growth occurs later in pregnancy (Rattray et al., 1974). The ewes in experiment one in this chapter were primiparous compared to multiparous in experiment two and three and all other chapters. Previous research have shown that primiparous ewes show more negative behaviours towards their lambs after birth and tend to have weaker attachments to their lambs (O'Connor et al. 1992; Dwyer et al. 1998; Dwyer and Lawrence 2000; Dwyer 2003; Otal et al. 2009). This difference in parity may have contributed to the inconsistent results between experiment one and two and three, and between chapters. Overall, there appears to be little advantage in feeding triplet-bearing ewes with BCS 2.0 to 3.0 800 kg DM/ha or above in mid- to late-pregnancy.

The effect of ewe mid-pregnancy BCS and feeding on lamb bleating behaviour in the triangle pen test differed from that recorded after tagging. In experiment two, within the BCS 2.0 ewe group lambs born to those ewes on the medium treatment emitted more high-pitched bleats and less low-pitched bleats than lambs born to ewes on the *ad lib* treatment. These results suggest that perhaps these lambs were more distressed by the separation and struggled to identify their dam. There is no other evidence however to support this. These results are in agreement with those reported in chapter two. The opposite effect was, however, observed in in this chapter in experiment three. Again, these inconsistencies may be due to the difference in length and timing of implementation of the feeding treatments or they may be artefacts of multiple comparisons.

The age of the lambs affected lamb behaviour in the lamb triangle pen test. Lambs tested at 24h of age spent more time walking (in experiment one and three) and were also faster at reaching the contact zones and their dam, supporting results from chapter two and earlier studies (Nowak et al., 1989). These results indicate that lambs are more mobile, have developed a stronger lamb-ewe bond and have a better ability to discriminate their dam at 24h of age.

Conclusion

While previous studies have indicated that poor maternal nutrition can negatively affect ewe and lamb behaviours there was little effect of nutrition in the present study. This may be explained by the fact that ewes were offered feeding levels that either met pregnancy-maintenance requirements or were above them, and the methodological inconsistencies between experiments. There is, however, some evidence to suggest that lambs born to ewes in the BCS 2.0 group offered the medium treatments experienced a weaker ewe-lamb bond. Further research is nevertheless required before a robust conclusion can be made.

Implications

The current chapter reported that there was little effect of ewe feeding treatment and BCS group on the behaviour of triplet-bearing ewes and their lambs. In the current experiments the feeding treatments ended on day 141 of pregnancy and the ewes were then offered *ad libitum* feeding conditions until lambing. It is possible that these feeding treatments may have been implemented too early in pregnancy to impact ewe and lamb behaviours. The following chapter will therefore examine the effects of ewe feeding treatments in very late-pregnancy and throughout the lambing period.

CHAPTER 4

The effect of ewe nutrition and body condition score during very late pregnancy and the perinatal period on the behaviour of twin-bearing ewes and their lambs

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Gronqvist, GV, Hickson, RE, Corner-Thomas, RA, Kenyon P. R., Stafford K. J., Morris S. T. (2015) The effect of ewe nutrition and body condition score during very late pregnancy and the perinatal period on the behaviour of twin-bearing ewes and their lambs. In 'New Zealand Society of Production Animals. Dunedin, New Zealand'. Volume 75 pp. 219-222.

Abstract

Ewe feeding levels during pregnancy have been shown to affect the behaviour and survival of lambs. This chapter describes the effect of maternal nutrition and body condition score (BCS) during very late-pregnancy and the lambing period on the behaviour of twin-bearing ewes and their lambs under extensive pastoral conditions. Ewes were allocated either a low (800 to 1000 kg DM/ha), medium (1200 to 1400 kg DM/ha) or *ad libitum* (‘*ad lib’* 1500 to 1700 kg DM/ha) feeding treatment from P141 until weaning. All feeding treatments included ewes with a BCS of 2.0, 2.5 or 3.0 as measured on day 98 of pregnancy. At tagging the behaviours of lambs and ewes were recorded in the paddock for five minutes after tagging. The behaviours recorded included the percentage of lambs that stood, made contact with, sucked from and followed their dam. For the lambs that performed each of these behaviours, the time to do so was noted. Both ewe and lamb high- and low-pitched bleats were counted during the five minute observation period and the maternal behaviour score (MBS) of the dam was determined. The time taken for lambs to make contact, suck and follow the dam did not differ between ewe mid-pregnancy BCS or treatments and the impacts on vocalisation were variable and of questionable practical application. However, the time taken for lambs to stand was significantly longer for lambs born to ewes managed on low pasture masses compared to lambs born to ewes managed on medium or *ad lib* pasture masses.

Introduction

Under pastoral grazing conditions, low levels of feeding in late-pregnancy and at lambing have been reported to have undesirable effects on ewe and lamb behaviour (Everett-Hincks et al. 2005). Nutritional restriction of the ewe during pregnancy has been shown to adversely affect maternal behaviour including reduced lamb grooming, suckling and mothering ability and the behaviour and/or survival of their lambs (Thomson and Thomson 1949; Putu et al. 1988; Corner et al. 2006; Corner et al. 2010; Kenyon et al. 2011). The results from chapter two and three, however, showed that feeding ewes *ad libitum* over pregnancy-maintenance requirements during mid- to late-pregnancy had little effect on the behaviour of twin- and triplet-bearing ewes and their lambs at tagging and during the triangle test. However, in these chapters the feeding treatments were completed between day 114 and 141 of pregnancy. At the end of these feeding periods ewes were then offered *ad libitum* feeding conditions until lambing. Therefore, it may not be surprising that these feeding treatments had little impact on ewe and lamb behaviours.

Few studies have investigated whether ewe body condition score (BCS) during pregnancy influences ewe and lamb perinatal behaviours. Chapter two and three also reported inconsistent effects of ewe mid-pregnancy BCS on ewe and lamb behaviour. It is also possible that the lack of consistent behavioural effects from ewe BCS was due to the timing of the feeding treatments. Therefore, the aim of this experiment was to investigate the effect of ewe mid-pregnancy BCS and feeding treatment in very late-pregnancy and throughout the lambing period on the behaviour of twin-bearing ewes and their lambs under extensive pastoral conditions.

Materials and methods

The experiment was conducted on Massey University's Keeble Farm, 5 km south of Palmerston North, New Zealand (40°S, 175°E), with the approval of the Massey University Animal Ethics Committee. The current experiment included 93 twin-bearing multiparous Romney ewes (aged 3 to 5 years) bred over a 17-day period and their lambs (n=186). These ewes were a subset of ewes (n=297) utilised by Corner-Thomas et al. (2015b) who reported the effects of feeding treatments and BCS (a scale of one to five including half units, one = emaciated, five = obese, Jeffries 1961, Kenyon et al. 2014) on twin-bearing ewe and lamb performance to weaning.

The aim of this analysis was to explore the behaviours of the ewe and her twin-born lambs. Only complete sets of twin-born lambs (where both lambs were alive at investigation) and their dams were included in the analysis due to the potential variation in behaviour of a single-reared lamb as opposed to a complete set.

Background

In the study by Corner-Thomas et al. (2015b) twin-bearing ewes were selected based on BCS (BCS 2.0 [n= 24, 64.5 ± 1.0kg], 2.5 [n=28, 69.5 ± 0.9kg] or 3.0 [n=40, 74.1 ± 1.1kg]) at 98 days after the start of breeding (P98). From P98 until P141, ewes were managed as one group with average pre- and post-grazing pasture masses of 1154 ± 19.5 and 823 ± 20.0 kg DM/ha, respectively (Corner-Thomas et al. 2015b). At P141, ewes from each BCS group were randomly allocated to either a 'low', 'medium' or *ad libitum* ('*ad lib*') feeding treatment until the weaning of their lambs 79 days after the mid-point of the lambing period (L79). The aim of the low feeding treatment was to offer pasture masses between 800 to 1000 kg DM/ha, the medium treatment 1200 to 1400 kg DM/ha and the *ad lib* treatment 1500 to 1700 kg DM/ha. The mean pasture masses during the feeding treatments for the low, medium and *ad lib* treatments were 902 ± 33, 1226 ± 33 and 1718 ± 34 kg DM/ha, respectively (Corner-Thomas et al. 2015b). Previous studies have demonstrated that ewe intake is not restricted above pasture masses of 1200 kg DM/ha (Morris et al. 1993; Morris and Kenyon 2004). Therefore the low feeding treatment was designed to restrict ewe intake in the feeding period. Lambing began at P146 and continued for 19 days. The median date of lambing (L0) occurred on the 17th of September.

The number of twin-rearing ewes included within each of the potential nine groups (three feeding treatments by three BCS groups) was as follows: low BCS 2.0 n=7, low BCS 2.5 n=10, low BCS 3.0 n=7, medium BCS 2.0 n=11, medium BCS 2.5 n=11, medium BCS 3.0 n=7, *ad lib* BCS 2.0 n=13, *ad lib* BCS 2.5 n=14 and *ad lib* BCS 3.0 n=13.

Animal and behavioural measurements

During the lambing period ewes were inspected twice daily at 8 am and 4 pm. Lambs were tagged once their coat was dry and the lamb was mobile (at approximately

3 to 18 hours of age). During tagging all lambs were identified to their dam, ear-tagged and had their weight, birth-rank and sex recorded. Immediately following tagging, behavioural observations were conducted as described in chapter two (page 50).

Statistical Analysis

Statistical analyses were conducted using SAS v. 9.3 (SAS Institute Inc., Cary, 2011, NC, USA). The statistical analyses are similar to those described in previous chapters; however, they are also described below.

The effect of ewe BCS and feeding treatment on lamb behaviours

The time required for lambs to stand, make contact with their dam, suck from their dam and follow her when she moved away during the paddock observations was tested for normality using the Kolmogorov-Smirnov test. All data were found not normally distributed and could not be normalised using standard methods. The time to exhibit each behaviour was analysed using both survival analysis and Kruskal-Wallis test. Survival curves were obtained using Kaplan-Meyer estimates. The effect of ewe BCS group and feeding treatment was tested in separate models. Lambs that did not perform a particular behaviour within the five minute observation period were censored at 301 seconds. For the behavioural parameters 'suck' and 'follow' the model was analysed with and without removing lambs that never gained their feet. This was done to ensure data was not skewed by those individuals who never stood in the five minutes and hence could not have sucked or followed. When analysing the effect of ewe BCS and feeding treatment on suck and follow, there were no differences in the results depending on whether the lambs that stood were included or not and hence only those results including all lambs are presented below.

The median time for a lamb to exhibit a behaviour was also calculated using the non-parametric Kruskal-Wallis test. The effect of ewe BCS or feeding treatment was tested in separate models and the analysis contained all lambs including those that did not show the behaviour during the observation period. If a particular behaviour was not exhibited the lamb was given a value of 301 for that variable. The association between feeding treatment or BCS and the behaviour variable were analysed using the Wilcoxon Test. If ewe BCS and feeding treatment was found to be significant, the Wilcoxon two-sample *post hoc* test was then carried out. Wilcoxon two-sample tests were used together with a Bonferroni adjustment to reduce the chance of Type 1 errors (Field et al. 2010). For the behavioural parameters 'suck' and 'follow' the model was analysed with and without removing lambs that did not gain their feet.

The effect of ewe BCS and feeding treatment on vocalisation

The percentage of lambs that emitted at least one high-pitched bleat and the percentage of lambs that emitted at least one low-pitched bleat was analysed using a generalised model based on a binomial distribution and a logit transformation. The number of bleats emitted was analysed using a generalised model based on a Poisson distribution.

The effect of ewe BCS and feeding treatment on ewe maternal behaviour score

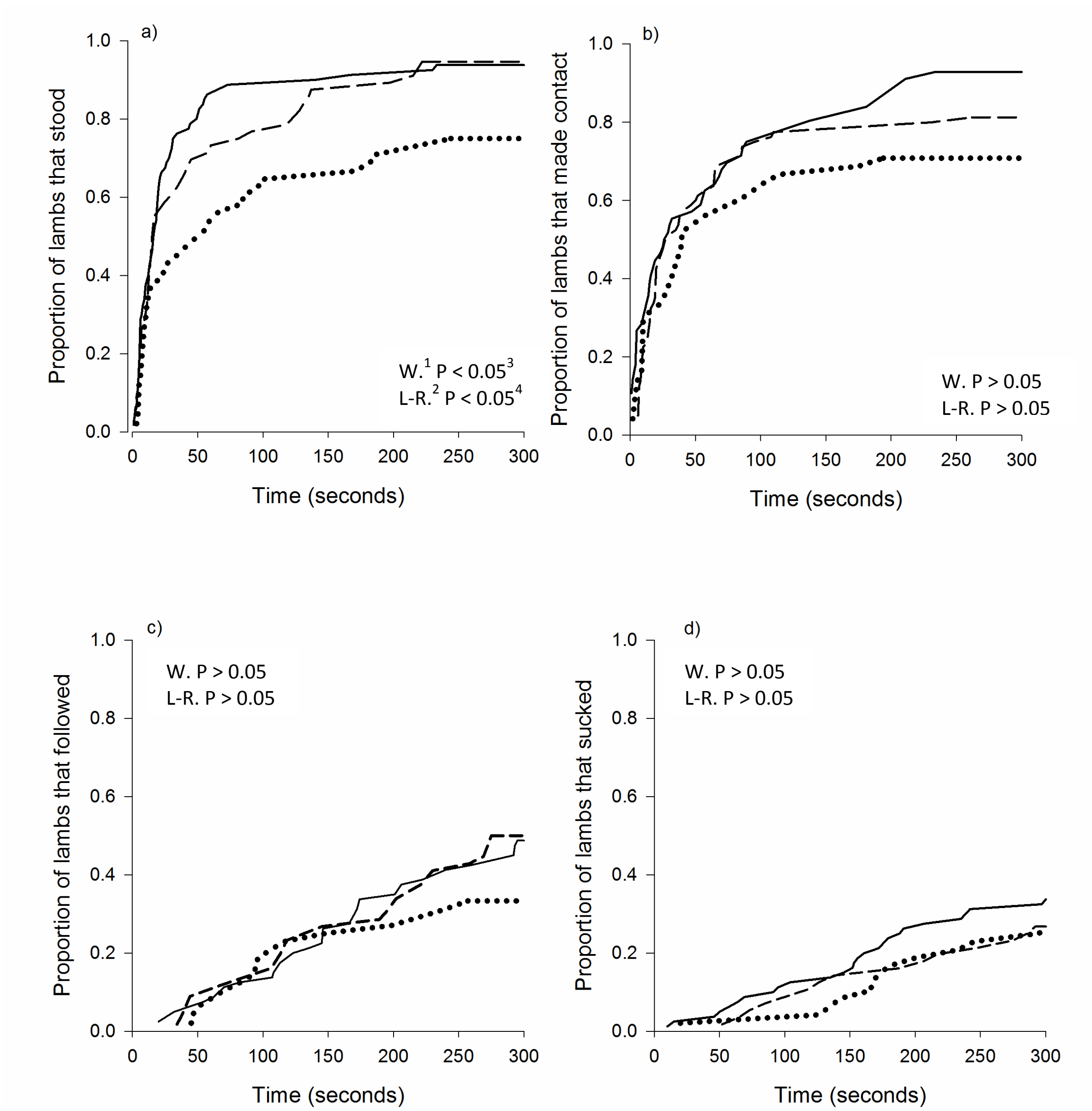
Ewe MBS was analysed using a generalised model based on a Poisson distribution containing the fixed effects of ewe BCS, feeding treatment and their interaction. The results are presented as the back-transformed means with 95% confidence interval in parenthesis.

Results

The effect of ewe BCS and feeding treatment on lamb behaviours

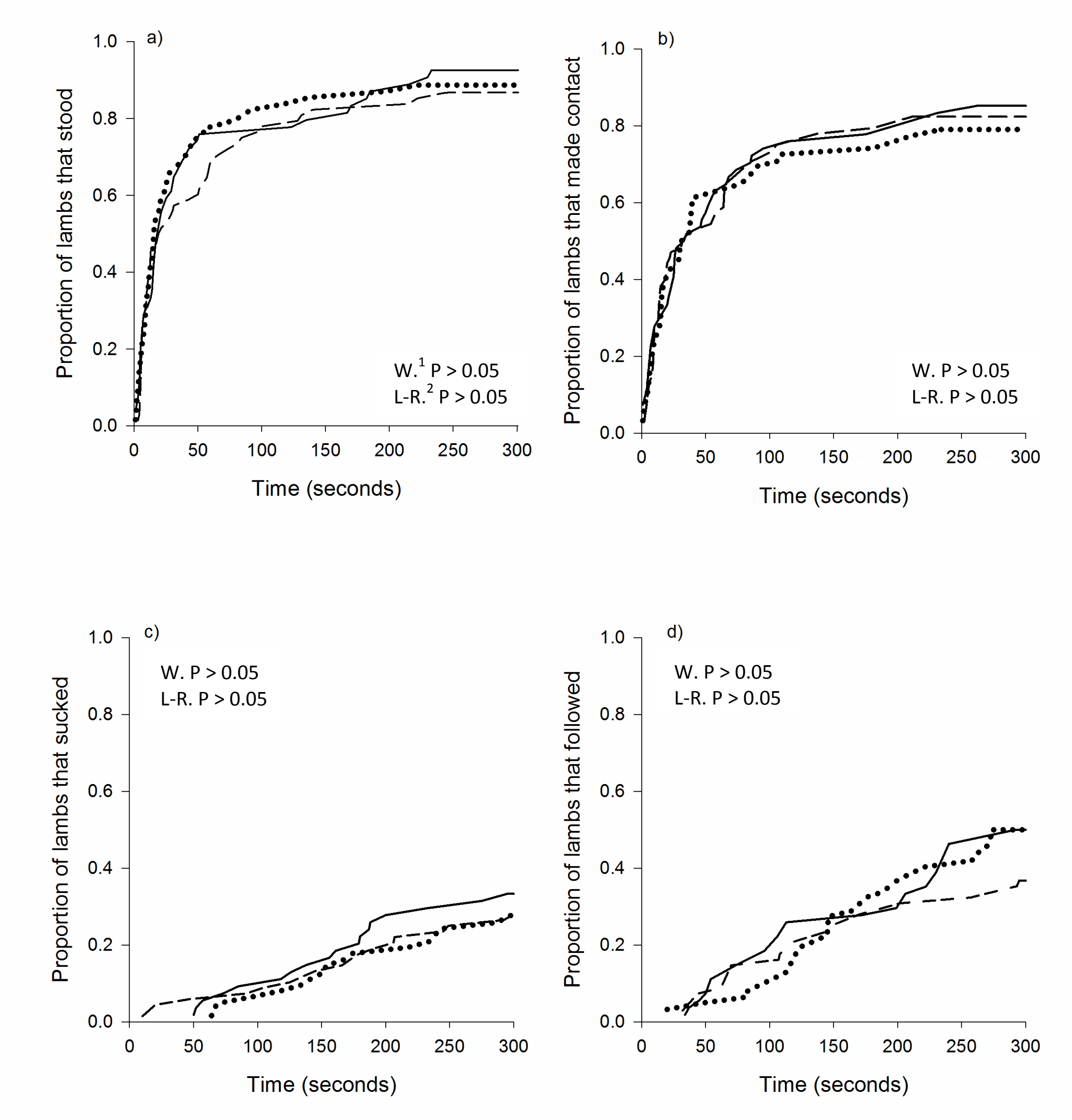
Survival analysis of the time taken for a lamb to stand showed that lambs born to ewes in the *ad lib* were quicker to stand than those in the low feeding treatment (Wilcoxon and log-rank $P < 0.05$, Figure 4.1a), but did not differ from those born to ewes in the medium treatment ($P > 0.05$). At approximately 60 seconds, 86% of lambs born to ewes in the *ad lib* treatment had stood compared with 54% of lambs born to ewes on the low treatment. The median time to stand was also less ($P < 0.05$, Table 4.1) for lambs born to ewes in the *ad lib* treatment compared to lambs in the low treatment. Lambs born to ewes in the medium treatment did not differ from either ($P > 0.05$).

Figure 4.1. The effect of ewe feeding treatment (low ....., medium - - - and *ad lib* —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1] The Wilcoxon p-value. [2] The Log-rank p-value. [3] The Wilcoxon p-value shows a significant difference between the high and low feeding treatments and the medium and low feeding treatments. [4] The Log-Rank p-value shows a significant difference between the high and low feeding treatments.

Figure 4.2. The effect of ewe BCS group (BCS 2.0 ….., BCS 2.5 - - - and BCS 3.0 —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]The Wilcoxon p-value. [2]The Log-rank p-value.

Table 4.1. The effect of ewe feeding treatment (low, medium and *ad lib*) and body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0 as on P98) on the median time (seconds) required for the lambs to exhibit the various behaviours.

	n	Stand	Contact	Suck	Follow
Feeding treatment					
low	48	53.5[b]	28.5	301.0	301.0
medium	56	16.5[ab]	40.0	301.0	301.0
ad lib	80	15.5[a]	27.5	301.0	296.5
BCS group					
BCS 2.0	62	16.0	34.0	301.0	288.0
BCS 2.5	68	20.0	33.5	301.0	301.0
BSC 3.0	54	18.5	34.0	301.0	296.5

[abc] Different superscripts within columns and main effects indicate values that significantly differ (P < 0.05)

Ewe feeding treatment did not affect the time taken for lambs to make contact, suck or follow their dam during the observation period nor the median time required to do so (P > 0.05, Figure 4.1b, c, d, Table 4.4 and 4.5). Ewe BCS did not affect any lamb behaviours measured (P > 0.05, Figure 4.2a, b, c, d, Table 4.1).

The effect of ewe BCS and feeding treatment on lamb vocalisation

There was no effect of ewe BCS or feeding treatment on the percentage of lambs that emitted high- or low-pitched bleats (P < 0.05, Table 4.2).

However, when the numbers of bleats emitted by those that bleated was analysed there was an interaction between ewe feeding treatment and BCS on the number of high-pitched bleats (Table 4.2). Within the BCS 2.0 group lambs born to ewes in the low feeding treatment emitted fewer high-pitched bleats than those in the medium, which in turn emitted fewer high-pitched bleats than those in the *ad lib* feeding treatment (P < 0.05). Within the BCS 2.5 group lambs born to ewes in the low and *ad lib* feeding treatments emitted fewer high-pitched bleats than those in the medium treatment (P < 0.05), but did not differ from each other (P > 0.05). Within the BCS 3.0 group lambs born to ewes in the low feeding treatment emitted fewer high-pitched bleats than those both in the medium or *ad lib* treatments (P < 0.05), furthermore lambs in the *ad lib* treatment emitted fewer high-pitched bleats than those in the medium treatment (P < 0.05).

There was also an interaction of ewe feeding treatments and BCS group on the number of low-pitched bleats emitted by the lamb (Table 4.2). In the BCS 2.0 group lambs born to ewes in the low feeding treatment emitted more low-pitched bleats than those in the medium and the *ad lib* feeding treatments (P < 0.05), which did not differ from each other (P > 0.05). Within the BCS 2.5 group lambs born to ewes in the *ad lib* feeding treatment emitted fewer low-pitched bleats than those in the low treatment (P < 0.05), which in turn emitted fewer low-pitched bleats than those in the medium treatment (P < 0.05). While, within the BCS 3.0 group lambs born to ewes in the low feeding treatment emitted more low-pitched bleats than those both in the medium or *ad lib* treatments (P < 0.05), which did not differ from each other (P > 0.05).

Table 4.2. The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction on the percentage of lambs that emitted high- and low-pitched bleats and the total number of bleats.

	n	High-pitched bleat		Low-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
Feeding treatment					
low	48	85.7 (72.6-93.1)	14.8[a] (13.7-15.9)	64.2 (49.8-76.5)	7.6[c] (6.9-8.5)
medium	56	90.3 (79.4-95.7)	22.4[b] (21.1-23.7)	80.6 (68.1-88.9)	6.3[b] (5.7-7.0)
ad lib	80	91.5 (82.9-95.9)	22.5[b] (21.4-23.5)	77.2 (66.7-85.1)	5.0[a] (4.5-5.5)
BCS group					
BCS 2.0	62	85.0 (73.5-92.0)	22.2[b] (21.1-23.5)	73.2 (60.6-83.0)	8.0[b] (7.3-8.8)
BCS 2.5	68	90.0 (80.3-95.2)	14.1[a] (13.2-15.0)	77.2 (65.8-85.7)	5.7[a] (5.2-6.3)
BSC 3.0	54	92.1 (80.7-97.0)	23.6[b] (22.3-25.0)	73.1 (59.1-83.6)	5.3[a] (4.6-5.9)
Feeding treatment x BCS group[3]					
low BCS 2.0	14	-	13.1[a] (11.4-15.2)	-	11.3[e] (9.7-13.2)
low BCS 2.5	20	-	12.4[a] (10.9-14.0)	-	5.7[bc] (4.7-6.8)
low BCS 3.0	14	-	19.1[b] (16.9-21.5)	-	6.9[cd] (5.7-8.5)
medium BCS 2.0	22	-	20.9[b] (19.1-22.9)	-	7.1[cd] (6.1-13.2)
medium BCS 2.5	22	-	19.3[b] (17.5-21.3)	-	7.9[d] (6.8-9.2)
medium BCS 3.0	28	-	30.1[d] (27.2-33.2)	-	3.9[a] (3.0-5.2)
ad lib BCS 2.0	26	-	31.5[d] (29.4-33.7)	-	6.6[cd] (5.7-7.6)
ad lib BCS 2.5	28	-	12.8[a] (11.5-14.1)	-	4.1[a] (3.4-4.9)
ad lib BCS 3.0	26	-	25.0[c] (23.2-27.1)	-	4.7[ab] (3.9-5.7)

[1]The percentage of lambs that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscripts within columns and effects indicate values that significantly differ ($P < 0.05$).

The effect of ewe BCS and feeding treatment on ewe behaviour and vocalisation

The maternal behaviour score of the ewe was not affected by either ewe BCS group, feeding treatment or their interaction ($P > 0.05$, Table 4.3).

The percentage of ewes that emitted high- and low-pitched bleats was not affected by ewe feeding treatment or BCS group ($P > 0.05$, Table 4.3). When analysing the numbers of bleats emitted, by those that bleated, there was an interaction between ewe feeding treatment and BCS on the number of high-pitched bleats. Within the BCS 2.0 group ewes in the medium feeding treatment emitted fewer high-pitched bleats than those in the low and the *ad lib* feeding treatments ($P < 0.05$, Table 4.3), which did not differ from each other ($P > 0.05$). Within the BCS 2.5 group ewes in the *ad lib* feeding treatment emitted fewer high-pitched bleats than those in the low and medium treatment ($P < 0.05$), which did not differ from each other ($P > 0.05$). While, within the BCS 3.0 group ewes in the *ad lib* feeding treatment emitted more high-pitched bleats than those in the low and medium treatments ($P < 0.05$), which did not differ from each other ($P > 0.05$).

There was also an interaction of ewe BCS and feeding treatment for the number of low-pitched bleats, such that within both the BCS 2.0 and 3.0 groups ewes in the low feeding treatment emitted fewer low-pitched bleats than those in the medium and the *ad lib* feeding treatments ($P < 0.05$, Table 4.3), which did not differ from each other ($P > 0.05$). However, within the BCS 2.5 group ewes in the low feeding treatment emitted more low-pitched bleats than those in the medium and *ad lib* treatment ($P < 0.05$).

Table 4.3. The effect of ewe feeding treatment (medium and *ad lib*), body condition score group (BCS 2.0, BCS 2.5 and BCS 3.0) and their interaction on the percentage of ewes that emitted high- and low-pitched bleats, maternal behaviour score (MBS) and on the total number of bleats.

	n	High-pitched bleat		Low-pitched bleat		MBS
		Percentage[1]	Number[2]	Percentage[1]	Number[2]	
Feeding treatment						
low	24	100	14.9[c] (13.9-16.1)	79.9 (59.3-91.6)	20.1[a] (18.9-21.4)	3.1 (2.4-3.9)
medium	28	100	11.0[a] (10.2-11.9)	96.8 (80.3-99.6)	25.3[c] (18.9-21.4)	3.1 (2.5-3.8)
ad lib	40	100	13.5[b] (12.7-14.3)	80.4 965.2-90.0)	22.5[b] (21.5-23.5)	2.8 (2.3-3.4)
BCS group						
BCS 2.0	31	100	15.8[c] (14.8-16.8)	86.8 (68.4-95.2)	21.7[a] (20.6-22.9)	2.9 (2.3-3.6)
BCS 2.5	35	89.7 (74.0-96.4)	12.6[b] (11.8-13.5)	86.6 (69.5-94.8)	21.7[a] (20.6-22.9)	2.8 (2.3-3.4)
BSC 3.0	27	90.4 (71.8-97.2)	11.1[a] (10.3-12.1)	92.2 (75.2-97.9)	24.2[b] (22.9-25.6)	3.3 (2.6-4.1)
Feeding x BCS group[3]						
low BCS 2.0	7	-	18.9[c] (16.7-21.3)	-	14.7[a] (12.8-16.9)	-
low BCS2.5	10	-	16.8[c] (15.1-18.7)	-	26.3[d] (18.8-22.7)	-
low BCS 3.0	7	-	8.6[a] (7.2-10.3)	-	16.4[a] (14.4-18.7)	-
medium BCS 2.0	11	-	9.4[a] (8.2-10.7)	-	26.4[d] (24.3-28.6)	-
medium BCS 2.5	11	-	14.9[bc] (13.3-16.6)	-	20.7[bc] (18.8-22.7)	-
medium BCS 3.0	14	-	9.7[a] (8.2-11.5)	-	29.3[d] (26.6-32.3)	-
ad lib BCS 2.0	13	-	19.2[c] (17.6-21.0)	-	22.5[c] (20.7-24.4)	-
ad lib BCS 2.5	14	-	8.4[a] (7.4-9.6)	-	19.4[b] (17.9-21.1)	-
ad lib BCS 3.0	13	-	13.6[b] (12.3-15.1)	-	25.8[d] (23.9-27.8)	-

[1]The percentage of ewes that bleated is presented as the back-transformed mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high-low-pitched bleats is presented as the mean value (with the 95% confidence limit in parenthesis). [3]Non-significant interactions are not shown. [abc] Different superscr within columns and effects indicate values that significantly differ (P < 0.05).

Discussion

The aim of the current experiment was to investigate the effect of ewe BCS at mid-pregnancy and feeding treatment in very late-pregnancy and throughout the lambing period on the behaviour of twin-bearing ewes and their lambs. The pre- and post-grazing masses indicate that nutritional conditions differed between the feeding treatments and met their aimed targets. In contrast to previous chapters this chapter included a feeding treatment where ewes were fed at a restricted level in very-late pregnancy and during the lambing period.

The studies of Moore et al. (1986) and Corner et al. (2008) which had similar feeding levels in mid- to late-pregnancy (but not during the lambing period) to the current experiment, reported that ewes on a lower plane of nutrition during mid- to late-pregnancy gave birth to weaker lambs. In the current study, 25% of lambs in the low treatment did not gain their feet within the five-minute observation period. These results support those of Everett-Hincks et al. (2005) who reported that lambs born to ewes grazing lower sward heights from mid-pregnancy until 24 hours after lambing were less likely to stand, locate their dam's udder and follow her. Failing to stand within five-minutes of a disturbance event may indicate low levels of energy and could affect lamb survival in adverse conditions.

The time taken for the lamb to make contact with the dam, suck and follow her was not affected by ewe feeding. Corner et al. (2010) with similar feeding regimes in mid- to late-pregnancy reported also no effect of feeding treatment on lamb behaviours.

Furthermore, chapters two and three reported minimal effect of ewe feeding treatment on lamb behaviours. It is possible that the feeding treatments may have been too short in duration to induce physiological changes and subsequently affect behaviour of ewes and their lambs, as they only started at day 141 of pregnancy. Corner-Thomas et al. (2015b), with the larger cohort of ewes which the present study were a subset of, reported that there was no effect of ewe feeding treatment on lamb birth weight and no effect of ewe BCS group or feeding treatment on lamb serum glucose or GGT concentrations at approximately 24 hours of age or lamb survival. It may thus not be surprising that very few behavioural traits were affected by BCS group and feeding treatment in the current study.

A ewe-lamb bond is described as an exclusive relationship where dams will only provide maternal care, such as grooming and suckling, to lambs with which they have bonded (Nowak et al. 2011). The MBS assesses the strength of the bond by measuring the distance the ewe moves away from her lambs while they are being handled by a shepherd (O'Connor et al. 1985). Dwyer et al. (2003) reported that restricted feeding, causing ewes to lose body condition reduced ewes' MBS. However, neither ewe mid-pregnancy BCS and late-pregnancy feeding treatment affected MBS in the current study, supporting previous research of Everett-Hincks et al. (2005) Corner et al. (2010) and in chapters two and three. This may be due to the fact that the ewes did not lose body condition during pregnancy as in the study by Dwyer et al. (2003). Therefore, combined this indicates that in terms of MBS, there is little advantage of having ewes in BCS 3.0 compared to 2.0 when ewes are fed at a level of the present study.

Low-pitched bleats are emitted almost exclusively in the first 24 hours between the ewe and her neonates to provide reassurance (Shillito 1972; Dwyer et al. 1998) and maintain the ewe-lamb bond (Nowak 1990). Whereas high-pitched are usually emitted by the ewe and lamb when they are separated from each other (Poindron et al. 1994; Dwyer et al. 1998). The percentage of ewes or lambs that emitted high- or low-pitched bleats in the current study was not affected by ewe BCS or feeding treatment.

When investigating the interaction of ewe BCS and feeding treatment on the number of ewe and lamb low-pitched bleats and lamb high-pitched bleats only the extremes of the BCS range showed an effect. Overall, within the BCS 2.0 and 3.0 groups lambs born to ewes in the low treatment emitted fewer high-pitched bleats and within all BCS groups these lambs emitted more low-pitched bleats. The opposite effect, however, was seen in the number of ewe low-pitched bleats. These results do not support those in chapter two. This may be a result of the variation in treatment length and timing (P112-136 and P128-141 for experiment one and two, respectively in chapter two). Furthermore, chapter two did not investigate the effect of sub-maintenance feeding. These bleating results, when combined with the effects of treatment on standing suggest that perhaps the lambs on the low treatments were too weak to high-pitch bleat. If this was the case, however, it may be expected that theses lambs would also be too weak to emit low-pitched bleats, but this was not the case. Why the ewes did not appear to be returning the same levels of low-pitched bleats are unknown, perhaps the sub-maintenance nutrition prompted her to focus on grazing. However, without additional information of the ewes behavior during the observation period it is not possible to draw a conclusion as to the meaning of the bleating behavior. It is probable that the effects on bleating in the present experiment are not biologically significant but merely an artifact of multiple comparisons.

Conclusion

In conclusion, the time taken for lambs to make contact, suck and follow the dam did not differ between treatments and the impacts on vocalisation were variable and of questionable practical application. However, the time taken for lambs to stand was significantly longer for lambs born to ewes managed on pasture masses of 800 to 1000 kg DM/ha during very late-pregnancy compared to lambs born to ewes managed on pasture masses above 1200 kg DM/ha. Failure to stand could have significant implications for lamb survival in extensive sheep production systems.

Implications

Ewe mid-pregnancy BCS group and feeding treatment in very late-pregnancy and throughout lambing had little impact on other behaviours of twin-bearing ewes and their lambs. Previous studies, however, have reported an effect of ewe nutrition on ewe and lamb behaviour when longer feeding treatments prior to and during lambing are imposed on the ewes (Everett-Hincks et al. 2005; Corner et al. 2008). The next chapter will therefore investigate the effects of ewe feeding during an extended period of pregnancy on ewe and lamb behaviour. There is increasing evidence to indicate fetal programming effects in sheep (van der Linden et al. 2009; Paten et al. 2013; Kenyon and

Blair 2014b). However, to date there has been no studies which have investigated if the nutritional conditions a ewe fetus was exposed to in pregnancy affect its behaviours when it becomes a dam or the behaviour of its lambs. This will be examined in the following chapter.

CHAPTER 5

Does ewe nutrition from mid-pregnancy until lambing affect the behaviour of twin-born lambs?

Abstract

In this chapter the effects of grand-dam (G0) and dam (G1) feeding treatments during early- and late-pregnancy on the behaviour of twin-bearing ewes and their lambs (G2) under extensive pastoral conditions are described. Fifty eight ewes (G1) whose dams (G0) had been offered a low, medium or high feeding treatment between days 21 and 50 (P21-P50) of pregnancy followed by either medium or high feeding for days 51 to 140 (P51-P140) of pregnancy were used in the present study. Subsequently, these G1 ewes at 4 year of age were offered either a medium or *ad lib* feeding treatment from day 76 (P76) of pregnancy until term. At tagging (3 to 18 hours after birth) the behaviours of ewes (G1) and lambs (G2) was recorded in the paddock. Survival analysis showed that lambs (G2) born to ewes (G1) offered the medium feeding treatment from mid-pregnancy until term were quicker to stand, suck and follow compared with lambs born to ewes (G1) offered the *ad lib* feeding treatment ($P < 0.05$). Lambs born to ewes on the medium treatment emitted more low-pitched bleats but fewer high-pitched bleats than lambs born to ewes on the *ad lib* treatment ($P < 0.05$). Ewes on the medium treatment also emitted fewer high-pitched bleats and fewer low-pitched bleats than ewes on the ad lib treatment ($P < 0.05$). The results suggest that lambs born to ewes on the medium feeding treatment had a greater drive to remain close to their dam. The majority of ewe (G1) and lamb (G2) behaviours remained unaffected by the feeding treatments of the grand-dams (G0). Therefore, under the conditions of the present study there was little evidence of fetal programming effects on ewe and lamb behaviour.

Introduction

Nutrition of the ewe during pregnancy can affect maternal (Thomson and Thomson 1949; Putu et al. 1988; Nowak 1996; Corner et al. 2006) and lamb behaviours (Everett-Hincks et al. 2005; Muñoz et al. 2009). Results from chapters two, three and four investigating the effects of ewe feeding treatments during mid- to late-pregnancy (medium and *ad lib*) and throughout lambing (low, medium and *ad lib*) reported few consistent effects on twin- and triplet-bearing ewe and lamb behaviour at 3 to 18 hours after birth. The differences between previous experiments is likely explained by a lack or prolonged underfeeding in mid- to late-pregnancy in these chapters.

The body condition score (BCS) of the ewe during pregnancy has been shown to affect ewe and lamb behaviour (Dwyer 2003). Results from chapters two, three and four indicated that ewe BCS (BCS 2.0, 2.5 and 3.0) as measured between day 72 and 98 of pregnancy also failed to consistently effects on twin- and triplet-bearing ewe and lamb behaviour.

There was some evidence of a mid-pregnancy BCS and mid- to late-pregnancy feeding treatment effect on twin- and triplet-lambs in previous chapters. However, these effects were not, repeatable across chapters. The length of the feeding treatments in the previous chapters varied, with some commencing as late as five days prior to lambing (chapter 4) and others ending as early as P114 (chapter 3, experiment 1). It is possible that the relatively short time frame of the treatments may have limited any effects on ewe and lamb behaviour. It would therefore be of interest to investigate the effects of maternal feeding treatments implemented for a longer duration of ewe pregnancy on ewe and lamb behaviour.

The process whereby stress or environmental stimulus at a critical period of fetal development results in life-long effects of that individual is called “fetal programming” (Ross and Desai, 2005). Fetal programming enables animals to develop adaptive responses to challenges faced in that particular environment if encountered later in life (Bateson et al., 2004; Rickard and Lummaa, 2007). The in-utero environment can affect future productivity and reproductive performance of sheep (Paten et al. 2013; Kenyon and Blair 2014b). To date, it appears that very little work has investigated the potential fetal effects of nutrition on ewe and lamb behaviour. Therefore, in the current experiment the potential fetal programming effects were examined by comparing behaviour of ewes and their lambs, for ewes that were themselves exposed to different maternal feeding when they were in-utero, i.e. as a fetus.

This study, therefore, investigated the effects of ewe feeding treatments from mid-pregnancy until lambing on the behaviour of twin-bearing ewes and their lambs at tagging.

Materials and methods

The experiment was conducted on Massey University's Keeble Farm, 5 km south of Palmerston North, New Zealand (40°S, 175°E) with approval of the Massey University Animal Ethics Committee. This experiment included ewes (G0) that were fed different feeding treatments during both early- and mid- to late-pregnancy while pregnant with the G1 ewes (Kenyon et al. 2011). The G1 ewes were subsequently fed different feeding treatments during mid- pregnancy and until term while pregnant with the G2 lambs whose behaviours are measured in the current experiment.

Background

Four-year-old twin-bearing ewes (G1, n=57) used in the present study and were a subset of 114 ewes whose dams (G0) had been offered either low, medium or *ad lib* (*ad libitum*) feeding levels in early pregnancy between days 21 and 50 (P21-P50), followed by either medium or *ad lib* feeding in mid to late pregnancy (P51-P140, Table 5.1 and 5.2). The ewes were part of a large study examining the effect of pregnancy nutrition on the productivity of the female offspring, i.e. a fetal programming effect (Kenyon et al. 2011). During the period of P21 to P50 mean live weight change of the G0 ewes in the low, medium and *ad lib* feeding treatment were -0.15 ± 0.02, -0.02 ± 0.02 and +0.15 ± 0.02 kg/d, respectively. During P51 to P140 the mean G0 ewe live weight change were 0.119 ± 0.01 and 0.260 ± 0.01 kg/d in the medium and *ad lib* feeding treatments, respectively (Kenyon et al. 2011).

The 57 ewes (G1) in the present study were offered either medium or *ad libitum* (*ad lib*) feeding conditions from P76 until term. The pre- and post-grazing masses of the *ad lib* feeding treatment (2181 ± 47.6 and 1431 ± 24.6 kg DM/ha, respectively) were greater ($P < 0.05$) than the medium feeding treatment (1164 ± 31.6 and 819 ± 16.0 kg DM/ha, respectively). Within 24 hours of lambing the ewes (G1) and their lambs (G2) were managed under *ad lib* grazing conditions until weaning. The average grazing mass during lactation was 1858 ± 80.8 kg DM/ha (Kenyon et al. 2014a).

Only complete sets of twin-born lambs (G2) (where both lambs were alive at tagging) and their dams (G1) were included in the analysis due to the potential variation in behaviour of a single-reared lamb as opposed to a twin-born and twin-reared lamb.

Table 5.1. Pre-and post-grazing grazing masses of the grand-dams (G0) (Kenyon et al. 2011).

Parameter	Herbage mass (kg DM/ha)			
	P21-50		P51-140	
	Pre-grazing	Post-grazing	Pre-grazing	Post-grazing
low	996a ± 89.3	814a ± 54.2	-	-
medium	1479b ± 107.7	1112b ± 59.4	1450a ± 83.9	1011a ± 32.8
ad lib	2331c ± 82.0	1649c ± 54.2	1828b ± 76.0	1301b ± 37.8

Table 5.2. The number (n) of ewes in each combination of grand-dam (G0) feeding treatments during early pregnancy (P21-50; low [L], medium [M] and *ad lib* [A]) and mid- to late-pregnancy (P51-140; medium [M] and *ad lib* [A]) and ewe (G1) feeding treatments (P76-term; medium and *ad lib*) and their lambs (G20).

G0		G1	G2
P21-50	P51-140	P76-lambing	
L (n=16)	M (n=11)	Medium (n=6)	(n=12)
		Ad lib (n=5)	(n=10)
	A (n=5)	Medium (n=2)	(n=4)
		Ad lib (n=3)	(n=6)
M (n=20)	M (n=8)	Medium (n=3)	(n=6)
		Ad lib (n=5)	(n=10)
	A (n=12)	Medium (n=4)	(n=8)
		Ad lib (n=8)	(n=16)
A (n=21)	M (n=11)	Medium (n=8)	(n=16)
		Ad lib (n=3)	(n=6)
	A (n=10)	Medium (n=3)	(n=6)
		Ad lib (n=7)	(n=14)

Animal and behavioural measurements

The live weights of the ewes (G1) were recorded at P76 and P142. During the lambing period ewes were inspected twice daily at 8 am and 4 pm. Lambs (G2) were tagged once their coat was dry and they were mobile (3 to 18 hours of age). During tagging all lambs were identified to their dam, ear-tagged and had their weight, birth-rank and sex recorded. Behavioural observations were conducted as per chapter two (page 50).

Statistical Analysis

All statistical analyses were conducted using SAS v. 9.3 (SAS Institute Inc., Cary, 2011, NC, USA). The statistical analyses were conducted in a similar manner to that in previous chapters, with the addition of the G0 ewe feeding treatments and ewe BCS not included in the models. The tested used are described below.

The effect of feeding treatments on ewe live weight

Live weights of the ewes (G1) at day 70 and 140 of pregnancy were analysed using a mixed model allowing for repeated measures that included the fixed effects of ewe (G1) feeding treatment (*ad lib* or medium) and included days to lambing as a covariate (based on actual lambing date).

The effect of grand-dam (G0) and ewe (G1) feeding treatments on vocalisation

The proportions of ewes (G1) and lambs (G2) that emitted at least one high-pitched bleat and the proportion of ewes (G1) and lambs (G2) that emitted at least one low-pitched bleat were analysed using a generalised model based on a binomial distribution and a logit transformation. The number of bleats emitted was analysed using a generalised model based a Poisson distribution. The models included the fixed effects of grand-dam (G0) and ewe (G1) feeding treatment and the two-way interaction of G0 early with mid to late pregnancy, G0 early pregnancy with G1 treatment and G0 mid to late pregnancy with G1 treatments. High- and low-pitched bleats were analysed separately.

The effect of grand-dam (G0) and ewe (G1) feeding treatments on the time taken by lambs to exhibit behaviours

The time required for the lambs (G2) to stand, make contact with their dam, suck from their dam (G1) and follow her when she moved away during the paddock observations was tested for normality using the Kolmogorov-Smirnov test. All data was not normally distributed and could not be normalised with transformations and was subsequently analysed using survival analysis. Survival curves were obtained using Kaplan-Meyer estimates. Lambs that did not perform a particular behaviour were treated as censored data at 301 seconds. The effect of grand-dam (G0), early-pregnancy followed by mid- to late-pregnancy, and ewe (G1) feeding treatments were tested in separate models. Both the Wilcoxon and the Log-rank p-value are presented. Interactions could not be tested for due to limitations of the analytical techniques.

The association between the fixed effects and the behaviour variable was investigated using the Wilcoxon Test. If a fixed effect was found to be significant the Wilcoxon two-sample *post hoc* test was carried out with a Bonferroni adjustment.

The effect of grand-dam (G0) and ewe (G1) feeding treatments on the ewe (G1) maternal behaviour score

Ewe MBS was analysed using a generalised model based on a Poisson distribution containing the fixed effects of grand-dam (G0) and ewe (G1) feeding treatments and their two-way interaction.

Results

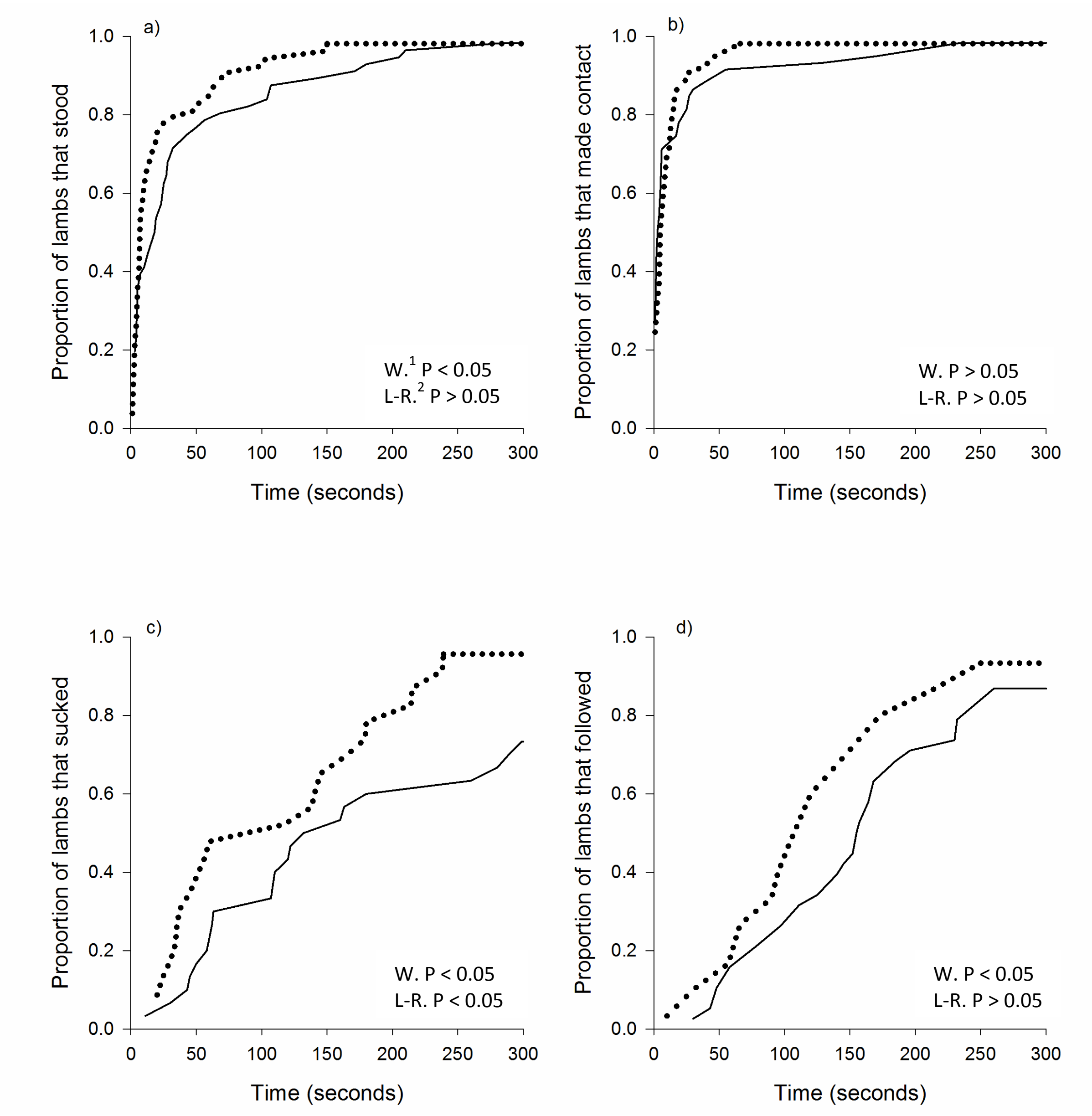
The effect of ewe (G1) feeding treatments on ewe (G1) live weight

No difference was observed between ewes (G1) in the medium (69.6 kg ±1.0) and the *ad lib* treatment (70.7 kg ± 0.9) on P70 ($P > 0.05$). On P140, ewes (G1) in the medium feeding treatment (84.2 kg ± 1.0) were lighter than ewes in the *ad lib* treatment (97.8 kg ± 0.9, $P < 0.05$).

The effect of grand-dam (G0) and ewe (G1) feeding treatments on lamb (G2) behaviour

The survival analysis showed that the time for lambs (G2) to stand differed between lambs born to ewes (G1) in the medium and *ad lib* feeding treatment (Wicoxon $P < 0.05$, Fig 5.1a). At approximately 10 seconds, 61% of lambs born to ewes (G1) in the medium treatment had stood compared with 41% of lambs born to ewes (G1) in the *ad lib* treatment. However, the median time for standing was not affected (P > 0.05, Table 5.3). Grand-dam (G0) feeding treatments did not affect the time taken for lambs to stand (P > 0.05, Fig 5.2a, 5.3a, Table 5.3).

Figure 5.1. The effect of G1 feeding treatments (medium ….. and *ad lib* —) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

Lambs born to ewes (G1) in the medium treatment were quicker to suck than lambs born to ewes (G1) in the *ad lib* feeding treatment (Wilcoxon and log-rank P < 0.05, Figure 5.1c). At approximately 50 seconds, 36% of lambs born to ewes (G1) in the medium feeding treatment had sucked compared with 17% of lambs born to ewes (G1) in the *ad lib* feeding treatment. The median time to suck was not affected (P > 0.05, Table 5.3).

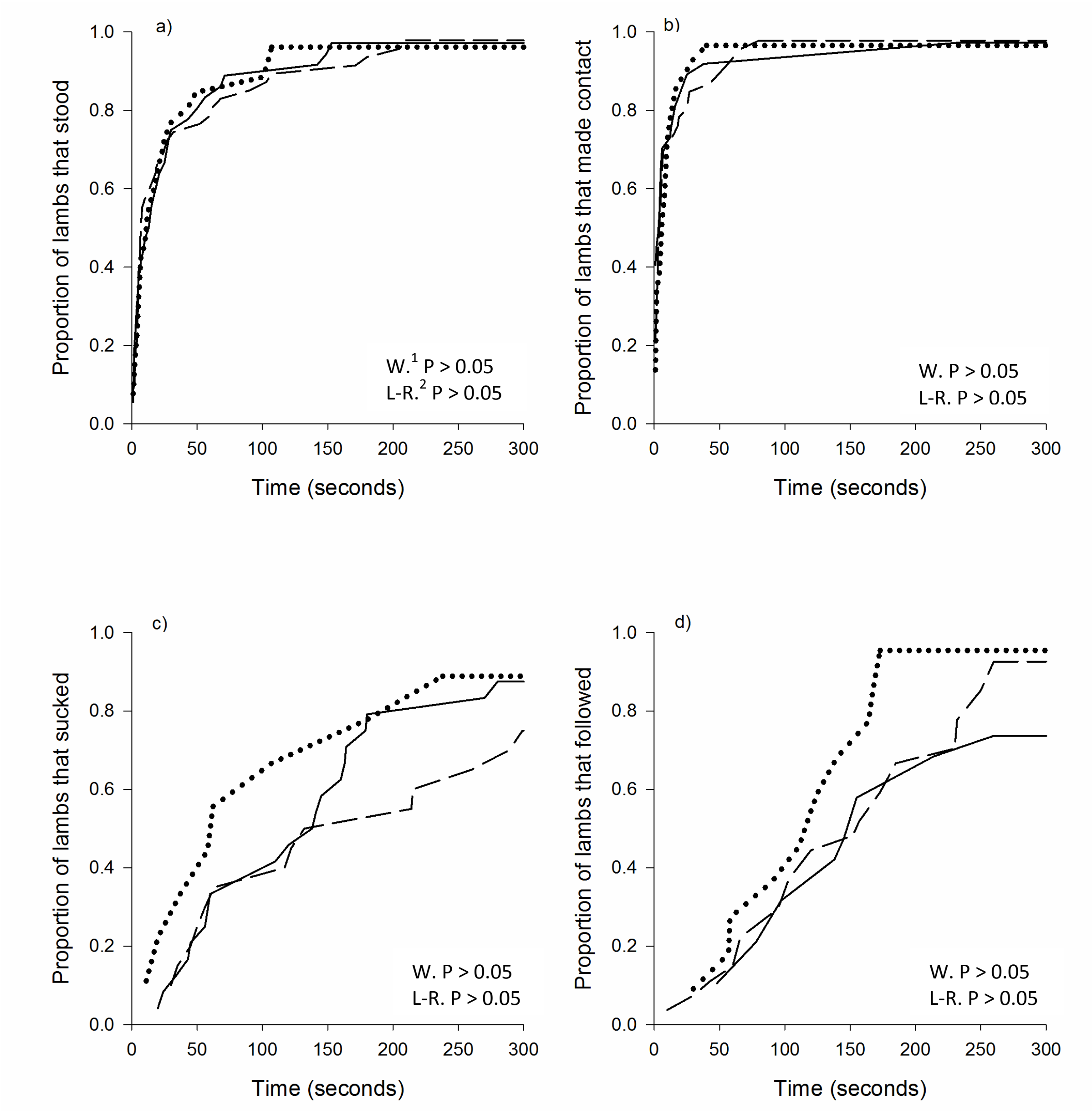
Lambs born to ewes (G1) in the medium treatment were quicker to follow their dam than lambs born to ewes (G1) in the *ad lib* feeding treatment (Wilcoxon P < 0.05, Figure 5.1d). At approximately 120 seconds, 60% of lambs born to ewes (G1) in the medium feeding treatment had followed compared with 35% of lambs born to ewes (G1) in the *ad lib* feeding treatment. The median time for following was not affected (P > 0.05, Table 5.3). There was no effect of grand-dam feeding on the time taken for lambs to follow their dam (P > 0.05, Fig 5.2d, 5.3d, Table 5.3).

Table 5.3. The effect of G0 and G1 feeding treatments (G0 early-pregnancy; low, medium and *ad lib,* G0 mid- to late-pregnancy; medium and *ad lib* and G1; medium and *ad lib*) on the median time (seconds) required for the lambs to stand, contact, suck and follow their dam (for those that exhibited the behaviours).

	n	Stand	Contact	Suck	Follow
G0, P21-P50					
low	32	5.5	6.0	162.0	140.0
medium	40	20.0	4.0	121.0	168.5
ad lib	42	11.0	5.0	90.0	120.0
G0, P50-P140					
medium	60	18.0	6.0	147[b]	152.0
ad lib	54	7.0	5.0	114.0[a]	138.0
G1, Feeding treatments					
medium	52	7.0	5.0	132.0	120.0
ad lib	62	19.0	3.5	117.0	156.0

[abc] Different superscripts within columns and main effects indicate values that significantly differ (P < 0.05)

Figure 5.2. The effect of G0 early-pregnancy (P21-50) feeding treatments (low ….., medium - - - and *ad lib*—) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.

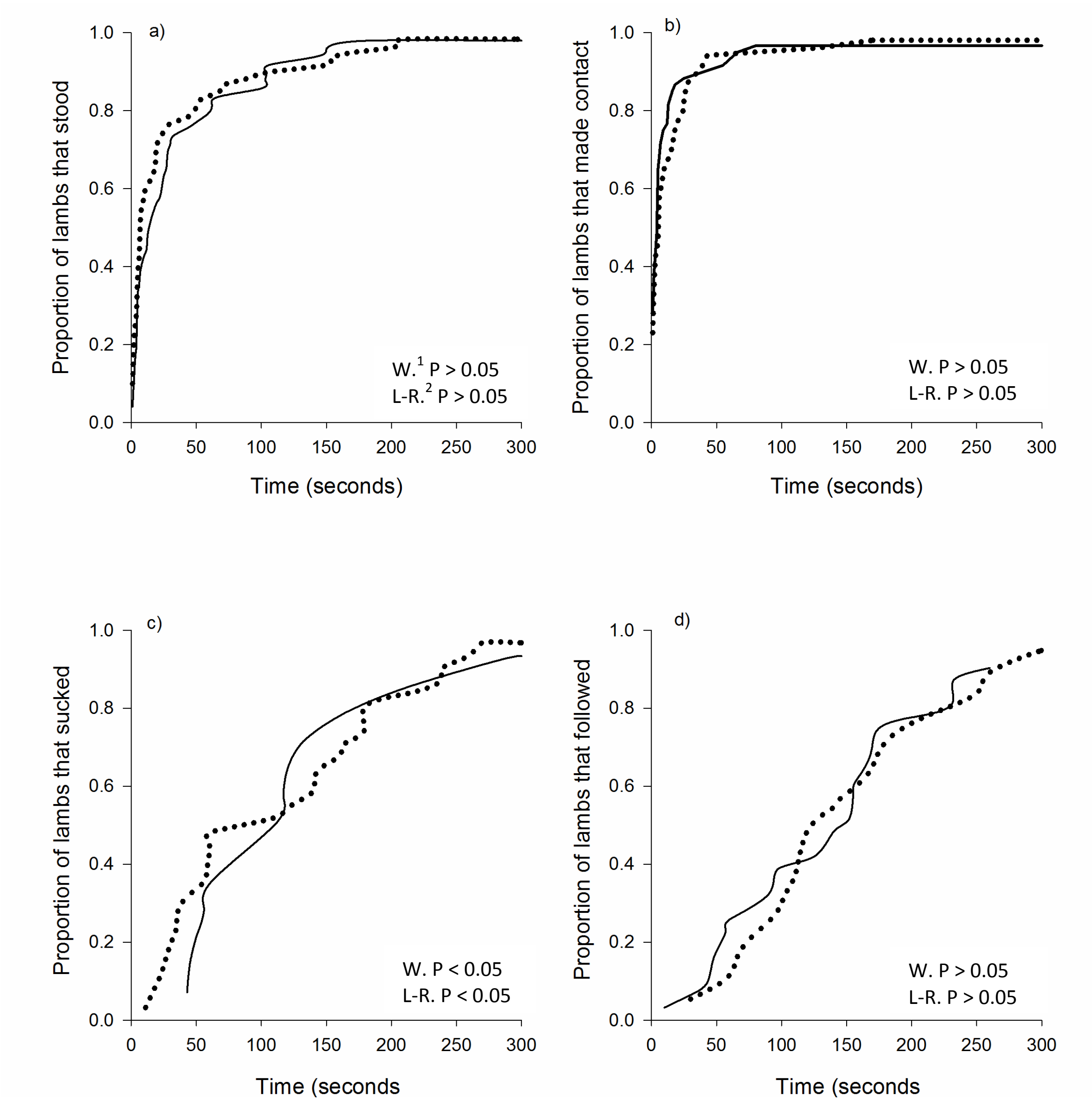


[1]Wilcoxon p-value. [2]Log-rank p-value.

The survival analysis showed that the time for lambs to suck differed between those whose grand-dams (G0) were offered the *ad lib* and the medium feeding treatment in mid to late pregnancy (Wilcoxon and log-rank $P < 0.05$, Figure 5.3c). At approximately 60 seconds, 48% of lambs whose grand-dams (G0) were offered the *ad lib* feeding treatment had sucked from their dam (G1) compared with 25% of lambs whose grand-dams (G0) were on the medium feeding treatment. The median time to suck was also less ($P < 0.05$, Table 5.3) for lambs whose grand-dams (G0) were on the *ad lib* feeding treatment compared with lambs whose grand-dams (G0) were on the medium feeding treatment in mid to late pregnancy. Early-pregnancy feeding of grand-dams (G0) had no effect on the time taken for lambs to suck ($P > 0.05$, Fig 5.2c, Table 5.3)

Grand-dam (G0) and ewe (G1) feeding treatments had no effect on the time taken for the lambs to make contact with their dam or the median time to do so ($P > 0.05$, Figure 5.1b, 5.2b, 5.3b, Table 5.3).

Figure 5.3. The effect of G0 mid- to late-pregnancy (P51-140) feeding treatments (medium ….and *ad lib*—) on the percentage of the lambs that (a) stood, (b) made contact with dam, (c) sucked and (d) followed the dam in the 300 seconds after tagging at tagging. Both the Wilcoxon (W) p-value and the Log-Rank (L-R) p-value are presented for the survival curves.



[1]Wilcoxon p-value. [2]Log-rank p-value.

The effect of grand-dam (G0) and ewe (G1) feeding treatment on lamb (G2) vocalisation

The percentage of lambs that emitted low-pitched bleats did not differ between grand-dam (G0) or ewe (G1) feeding treatments (P > 0.05, Table 5.4a).

More low-pitched bleats were emitted by lambs whose grand-dams (G0) were in the low or medium feeding treatment in early-pregnancy than lambs whose grand-dams (G0) were in the *ad lib* treatment (P < 0.05, Table 5.4a). Lambs whose grand-dams (G0) were in the *ad lib* feeding treatment in mid- to late-pregnancy emitted more low-pitched bleats than lambs whose grand-dams (G0) were in the medium treatment (P < 0.05). Lambs born to ewes (G1) in the medium feeding treatment emitted more low-pitched bleats than lambs born to ewes (G1) in the *ad lib* feeding treatment (P < 0.05).

The percentage of lambs that emitted high-pitched bleats showed an interaction between G0 early-pregnancy feeding treatment and G0 mid- to late-pregnancy feeding treatment (Table 5.4b). Grand-dam (G0) feeding treatment in early-pregnancy had no effect on the percentage of lambs that emitted high-pitched bleats if the grand-dams (G0) were offered medium in mid- to late-pregnancy (P > 0.05, Table 5.4b). However, if the grand-dams (G0) were offered *ad lib* in mid- to late-pregnancy, lambs whose grand-dams (G0) were in the *ad lib* feeding treatment in early-pregnancy emitted fewer high-pitched bleats than those whose grand-dams (G0) were in the low or medium treatment in early-pregnancy (P < 0.05).

The percentage of lambs that emitted high-pitched bleats showed an interaction between G0 early-pregnancy feeding treatment and G1 feeding treatment (Table 5.4b). A lesser percentage of lambs born to ewes (G1) in the *ad lib* treatment emitted high-pitched bleats if their grand-dams (G0) were offered the *ad lib* feeding treatment in early-pregnancy compared with the medium or low (P < 0.05, Table 5.4b) treatment. In contrast, the percentage of lambs born to ewes (G1) in the low treatment that high-pitched bleated was not affected by their grand-dams early-pregnancy feeding treatment (P > 0.05).

The percentage of lambs that emitted high-pitched bleats showed an interaction between G0 mid- to late-pregnancy feeding treatment and G1 feeding treatment (Table 5.4b). A smaller percentage of lambs born to ewes (G1) in the medium treatment emitted high-pitched bleats if their grand-dams (G0) were offered the medium feeding treatment in mid- to late-pregnancy, compared with the *ad lib* (P < 0.05) treatment. The percentage of lambs in the *ad lib* treatment that emitted high-pitched bleated was not affected by the feeding treatment of their grand-dams in mid-to late-pregnancy feeding (P > 0.05).

Less high-pitched bleats were emitted by lambs whose grand-dams (G0) were offered medium in mid- to late-pregnancy than lambs whose grand-dams were offered *ad lib* (P < 0.05, Table 5.4a). Lambs whose grand-dams were offered the low feeding treatment in early-pregnancy emitted more high-pitched bleats than lambs whose grand-dams (G0) were offered the medium treatment, who in turn emitted more high-pitched bleats than those whose grand-dams (G0) were offered the *ad lib* (P < 0.05) treatment. Fewer high-pitched bleats were emitted by lambs born to ewes (G1) in the medium treatment than lambs born to ewes (G1) in the *ad lib* treatment ($P < 0.05$).

Table 5.4a. The effect of G0 and G1 feeding treatments (G0 early-pregnancy P21-50; low, medium and *ad lib*, G0 mid- to late-pregnancy P51-140; medium and *ad lib* and G1; medium and *ad lib*) on the percentage (95% CI) of lamb that emitted high- and low-pitched and on the number of bleats.

	n	High-pitched bleat		Low-pitched bleat	
		Percentage[1]	Number[2]	Percentage[1]	Number[2]
G0, early-pregnancy feeding treatment					
low	32	86.4[ab]	12.4[c]	76.9	6.0[b]
		(65.4-95.5)	(11.2-13.7)	(59.3-88.4)	(5.2-6.9)
medium	40	88.3[b]	9.4[b]	78.9	5.4[b]
		(68.7-96.3)	(8.5-10.5)	(62.5-89.4)	(4.7-6.3)
ad lib	42	50.4[a]	7.0[a]	71.0	4.2[a]
		(30.8-69.9)	(6.2-7.8)	(54.8-83.1)	(3.7-4.9)
G0, mid- to late-pregnancy feeding treatment					
medium	60	78.5	6.8[a]	73.9	3.5[a]
		(61.2-89.4)	(6.1-7.5)	(60.5-84.0)	(3.1-4.1)
ad lib	54	78.5	12.8[b]	77.5	7.5[b]
		(60.8-89.6)	(11.9-13.9)	(63.8-87.0)	(6.8-8.4)
G1, Feeding treatments					
medium	52	86.0	8.4[a]	82.3	7.4[b]
		(71.2-93.9)	(9.5-11.2)	(69.1-90.6)	(6.7-8.2)
ad lib	62	68.4	10.3[b]	67.8	3.6[a]
		(51.2-81.7)	(9.5-11.2)	(54.6-78.7)	(3.2-4.1)

[1]The percentage of lambs that bleated are presented as the mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats are presented as the mean value (with the 95% confidence limit in parenthesis). [abc]different superscripts within columns and main effects indicate values that significantly differ ($P < 0.05$).

Table 5.4b. The effect of the two-way interactions of G0 and G1 feeding treatments (G0 early-pregnancy P21-50; low, medium and. *ad lib*, G0 mid- late-pregnancy P51-140; medium and *ad lib* and G1; medium and *ad lib*) on the percentage (95% CI) of lambs that emitted high- and low-pitched and on the number of bleats.

	n	High-pitched bleat
		Percentage[1]
G0, early-pregnancy feeding treatment x		
G0, mid- to late-pregnancy feeding treatment[3]		
low *ad lib*	10	89.9[b] (49.8-98.8)
low medium	21	82.0[b] (59.3-93.4)
medium *ad lib*	24	75.3[b] (53.2-89.1)
medium medium	16	94.9[b] (68.6-99.4)
ad lib ad lib	21	64.3[ab] (40.3-82.8)
ad lib medium	21	36.4[a] (12.9-68.8)
G0, early-pregnancy feeding treatment x		
G1 feeding treatment[3]		
low *ad lib*	16	86.4[b] (57.4-96.8)
low medium	16	86.3[b] (53.2-97.2)
medium *ad lib*	26	78.1[b] (57.5-90.4)
medium medium	14	94.1[b] (64.6-99.3)
ad lib ad lib	18	31.1[a] (10.5-63.5)
ad lib medium	24	69.6[b] (46.0-86.0)
G0, mid- to late-pregnancy feeding treatment x		
G1 feeding treatment		
medium *ad lib*	26	83.1[ab] (60.7-94.0)
medium medium	36	48.9[a] (24.9-73.3)
ad lib ad lib	34	73.1[ab] (46.3-89.5)
ad lib medium	18	93.9[b] (75.9-98.4)

[1]The percentage of lambs that bleated are presented as the mean percentage (with the 95% confidence limit in parenthesis). [3] Non-significant interactions are not shown. [abc] different superscripts within columns indicate values that significantly differ ($P < 0.05$).

The effect of grand-dam (G0) and ewe (G1) feeding treatments on ewe (G1) behaviours

The percentage of ewes (G1) that emitted high- or low-pitched bleats and their MBS were not affected by grand-dam (G0) or ewe (G1) feeding treatments or their two-way interaction ($P > 0.05$, Table 5.5).

More high-pitched bleats were emitted by ewes (G1) whose dams (G0) were offered the medium feeding treatment in early-pregnancy than by ewes (G1) whose dams (G0) were offered the low or *ad lib* treatment in early-pregnancy ($P < 0.05$, Table 5.5). Ewes (G1) whose dams (G0) were offered the *ad lib* feeding treatment in late-pregnancy emitted more high-pitched bleats than those whose dams were offered the medium treatment ($P < 0.05$). More high-pitched bleats were also emitted by ewes (G1) offered the *ad lib* feeding treatment than by ewes (G1) offered the medium feeding treatment ($P < 0.05$).

More low-pitched bleats were emitted by ewes (G1) whose dams (G0) were offered the low feeding treatment in early-pregnancy than ewes whose dams (G0) were offered the medium or *ad lib* treatment in early-pregnancy ($P < 0.05$, Table 5.5). Ewes (G1) whose dams (G0) were offered the medium feeding treatment in late-pregnancy emitted more low-pitched bleats than those whose dams (G0) were offered the *ad lib* treatment ($P < 0.05$). More low-pitched bleats were also emitted by ewes (G1) that were offered the *ad lib* feeding treatment than by ewes offered the medium treatment ($P < 0.05$).

Table 5.5. The effect of G0 and G1 feeding treatments (G0 early-pregnancy P21-50; low, medium and *ad lib*, G0 mid- to late-pregnancy P51-140; medium and *ad lib* and G1; medium and *ad lib*) on the percentage (95% CI) of ewes that emitted high- and low-pitched bleats, maternal behaviour score (MBS) and on the number of bleats.

	n	Ewe high-pitched bleat		Ewe low-pitched bleat		MBS
		Percentage[1]	Number[2]	Percentage[1]	Number[2]	
G0, P21-P50						
low	16	63.5 (37.3-83.5)	5.8[a] (5.0-6.7)	100	36.1[b] (34.1-38.3)	3.4 (2.6-4.4)
medium	20	84.0 (60.7-94.7)	8.1[b] (7.2-9.0)	100	22.5[a] (21.0-24.1)	3.6 (3.0-4.4)
ad lib	21	78.5 (54.4-91.8)	6.0[a] (5.3-6.8)	100	24.3[a] (22.9-25.9)	3.5 (2.8-4.3)
G0, P50-P140						
medium	30	78.2 (58.4-90.1)	5.7[a](5.1-6.4)	100	30.7[b] (29.3-32.2)	3.6 (2.8-4.5)
ad lib	27	74.4 (52.7-88.3)	7.5[b] (6.8-8.3)	100	23.8[a] (22.5-25.2)	3.6 (2.9-4.6)
G1, Feeding treatments						
medium	26	85.6 (65.6-94.9)	6.1[a] (5.4-6.8)	100	24.8[a] (23.5-26.2)	3.3 (2.7-4.1
ad lib	31	63.5 (44.7-78.9)	7.1[b] (6.4-7.8)	100	29.5[b] (28.2-30.9)	3.7 (3.1-4.5)

[1]The percentage of ewes that bleated are presented as the mean percentage (with the 95% confidence limit in parenthesis). [2]The number of high- and low-pitched bleats are presented as the mean value (with the 95% confidence limit in parenthesis). [abc] different superscripts within columns and main effects indicate values that significantly differ (P < 0.05).

Discussion

The aim of this experiment was to study the effects of grand-dam (G0) and ewe (G1) feeding treatments on the behaviour of ewes (G1) and their twin-born lambs (G2) at tagging, 3 to 18 hours after birth.

Lambs (G2) born to ewes (G1) offered the medium feeding treatment from mid-pregnancy until term were quicker to stand, suck and follow their dam compared with lambs born to ewes in the *ad libitum* feeding treatment. The results in the current study are not in agreement with the results in previous chapters. Chapter four reports that, of lambs born to ewes offered 800 to 1000 kg DM/ha in very late-pregnancy, a quarter failed to stand in the 300 seconds following tagging. These lambs may have been weaker than lambs born to ewes offered feeding levels above 1200 kg DM/ha, however there were no physiological evidence to support this theory. The time taken for lambs to contact, suck and follow was not affected by ewe feeding levels in chapter four. Furthermore, the ewes had only been subjected to the feeding treatments between P141 and time of tagging (3-18 hours after birth). The effect of feeding on time to stand in chapter two may not be biological significance due to the short duration (total days) of the feeding treatments and the lack of physiological evidence that the ewes or the lambs differed between feeding treatments. In the present study the feeding treatments were of longer duration than those in previous research by the group.

The cause of the behavioural differences between feeding treatments is unclear. The results might indicate that lambs born to ewes on the medium feeding treatment were more active ('vigorous') or that these lambs had unmet needs that prompted the seeming increase in behaviour ('needy'). Unfortunately, from the results it is not possible to conclude whether the behaviour differences were due to 'vigour' or 'need'. Perhaps exhibiting 'needy' behaviours were advantageous for lambs born to ewes fed medium as these ewes may have been hungry and more compelled to graze than interact and bond with their lambs. Kenyon et al. (2014a), with the larger cohort of ewes which the present study were a subset of, reported that the lambs born to ewes offered *ad libitum* were lighter at birth than those born to ewes offered medium. Perhaps the difference in lamb birthweight could explain the differences observed in lamb behaviour. Unfortunately, the statistical analyses used in this study did not allow birthweight to be added as a covariate when investigating the effect of feeding on behaviour. Kenyon et al. (2014a) further reported that there was no effect of ewe feeding treatments on lamb survival and consequently there does not appear to be any obvious advantage, in terms of production, in feeding ewes *ad libitum* over feeding levels designed to meet pregnancy maintenance requirements from mid-pregnancy until lambing.

The results in the current study are partly supported by those of Corner et al. (2010), who conducted similar experiments with ewes managed under comparable feeding regimes. Corner et al. (2010) reported that lambs born to ewes managed on lower feeding levels (sward height of 2 cm in mid and/or late pregnancy, equivalent to 800-900 kg DM/ha [Morris and Kenyon 2004]) exhibited a lower median time to stand and a greater percentage of lambs moving towards their dam than those managed on sward heights of 4 cm in mid- and late-pregnancy. Time to ewe-lamb contact was not affected by ewe feeding treatments in the current study. However, majority of ewe-lamb contact was noted to be instigated by the ewe approaching the lamb and 'contact' may thus be a better indicator of the motivation of the ewe than the lamb.

Low-pitched bleats are involved in developing and maintaining the ewe-lamb bond (Nowak 1990). Lambs in the medium feeding treatment emitted more low-pitched bleats than lambs in the *ad lib* feeding treatment. This may have been a trade-off due to the greater high-pitched bleating rates of ewes in the *ad lib* treatment. Similar bleating results have been presented in previous research (Dwyer et al. 2003; Corner et al. 2010) and in all previous chapters. If the lambs born to ewes on the medium treatment were indeed more 'needy' they may have emitted more low-pitched bleats in an attempt to strengthen the ewe-lamb bond. Higher low-pitched bleating rates were not reciprocated by the medium ewes, however, as they bleated less than *ad lib* ewes. This further suggests that the medium feeding treatment may have had a negative effect on ewe maternal behaviours.

High-pitched 'distress' bleats are most commonly emitted by the ewe and the lambs during separation and may be a sign of 'need' for their dam (Poindron et al. 1994; Dwyer et al. 1998). If lambs (G2) in the medium treatment were more 'needy' it may be expected that they would also emit more high-pitched bleats than those in the *ad lib* treatment. However, the opposite effect was observed. These results support those reported in chapter four, where lambs in the *ad lib* treatment emitted more high-pitched bleats than those in the low treatment. A large number of high-pitched bleats may be in response to separation (Corner et al. 2010), or it may simply mean that they are generally more vocal. The greater high-pitched bleating rates may be a reflection of the increased amount of time the lambs (G2) born to ewes (G1) in the *ad lib* feeding treatment took to follow their dam (G1), compared to lambs born to ewes (G1) in the medium treatment. From the data collected it was, unfortunately, not possible to differentiate whether a lamb emitted a 'distress bleat' or bleated in response to its dam. However, as ewes on the *ad lib* treatment also emitted more high-pitched bleats than ewes on the medium treatment, it is plausible that lamb high-pitched bleats were in response to ewe high-pitched bleats.

Nowak (1996) reported that improved maternal nutrition during pregnancy may result in improved maternal behaviours. Likewise, Dwyer et al. (2003) and Thomson and Thomson (1949) reported that restricted feeding of ewes during pregnancy resulted in the ewes being more likely to exhibit poor maternal behaviour and have lower MBS compared with well-fed ewes. However, in the present study no effect of ewe feeding treatment was found for ewe MBS. This may be due to the ewes not being nutritionally restricted (Rattray et al. 1974); rather the comparison was between maintenance and above-maintenance feeding levels, whereas most previous studies have compared maintenance with sub-maintenance nutrition. Further, it is possible that the nutrition affected maternal behaviours as the results indicate, but not directly the MBS which is only one way of measuring the strength of the ewe-lamb bond. These results support those by Everett-Hincks et al. (2005b) and Corner et al. (2010), who studied the effects of similar feeding levels on maternal behaviour as in the current study. Results, therefore, indicate that there is no advantage, in terms of MBS, in offering twin-bearing ewes feeding levels above pregnancy maintenance requirements. However, *ad libitum* feeding may have a positive effect on the ewe-lamb bond and further research is warranted.

Lambs (G2) whose grand-dams (G0) were offered *ad libitum* rather than medium feeding during late-pregnancy were quicker to suck from their dam. This is the first study to report a fetal programming effect on lamb behaviour. This difference could potentially be due to a difference in milk yield. Using the same ewes and the current study, Paten et al. (2013) reported that the ewes born to dams fed *ad libitum* during late-pregnancy tended to have greater accumulated milk yields (P = 0.10) compared with ewes born to dams fed medium. Standing, contact and following were not affected by G0 feeding in the current study. Further the effects on ewe and lamb bleating were highly inconsistent and their biological significance questionable.

Conclusion

Lambs born to ewes offered feeding levels to meet pregnancy-maintenance-requirements from P76 until term were quicker to stand, suck and follow their dam when she moved away, than lambs born to ewes in the *ad lib* treatment. These results, combined with the effects reported on ewe and lamb bleating behaviour, suggest that these lambs may have been more ‘needy’. It is further possible that the medium feeding treatment may have had a negative effect on the strength of the ewe-lamb bond. Under conditions of the present study there was little evidence of fetal programming effects on ewe and lamb behaviour.

CHAPTER 6

Are ewe and lamb behaviours at the time of tagging and during a triangle pen test predictors of lamb survival?

Abstract

This chapter reports on an investigation into the relationships between maternal and lamb behaviour and twin- and triplet-born lamb survival from tagging until weaning. It combines data from chapters two, three, four and five. In each of these studies the lambs were ear-tagged, identified to their dam and had their weight, sex and birth-rank recorded within 3 to 18 hours after birth. Lamb behaviours were recorded immediately after tagging in the paddock. These behaviours included time to stand, make contact with dam, suck from dam and follow dam if she moved away. High- and low-pitched bleats from both ewes and lambs were also recorded, as was a maternal behaviour score (MBS) of the dam. In chapters two and three, a triangle pen test was also conducted. Data was analysed using logistic regression to examine the effect of ewe and lamb behaviour on lamb survival with twin- and triplet-born lambs being analysed separately. The majority of ewe and lamb paddock and triangle pen test behaviours were not related to the survival of twin- or triple-born lambs and therefore may be of little use as indicators of survival. In the paddock, for each additional second required for twin-born lambs to follow their dam, lambs were more likely to survive ($P < 0.05$). The opposite relationship was, however, seen in triplet lambs, such that for each additional second required for triplet-born lambs to follow their dam lambs were less likely to survive ($P < 0.05$). During the triangle test, for every additional second required for twin-born lambs to reach the contact zone they were less likely to survive ($P < 0.05$). Similarly, for every additional second required for triplet-born lambs to reach their dam they were less likely to survive ($P < 0.05$). The difference between twin- and triplet-born lambs may be due to the difference in milk competition within twin- and triplet litters.

Yet in both litter types these behaviours may give an indication of the strength of the ewe-lamb bond.

Introduction

The behaviours of the ewe and the lamb during the first few days after birth affect lamb survival (Alexander et al. 1986; Nowak et al. 2000; Dwyer and Lawrence 2005). In order to survive, the neonatal lamb must exhibit key behaviours to ensure maternal acceptance and facilitate milk consumption (Nowak et al. 2000). These behaviours include standing soon after birth, sucking soon after standing, following the dam closely and returning to her if separated (Alexander et al. 1986; Nowak et al. 2000; Dwyer 2003; 2008). The time required for the lamb to progress through this series of behaviours immediately after birth has been used to assess the likelihood of lamb survival (Alexander 1988; Dwyer 2003; Dwyer et al. 2003). Cloete (1993) reported that lambs that died before weaning were slower to stand and tended to be slower at progressing from standing to sucking than lambs that survived until weaning. These results were similar to those of Dwyer et al. (2001) who reported that lambs that started moving more quickly after birth were more likely to survive to eight weeks of age. Further, survival has been reported to decrease by almost 1% for every minute increase in the time taken by a lamb to attempt to stand, stand or attempt to find the udder immediately after birth (Owens et al. 1985).

Lamb survival can also be affected by ewe behaviour (Murphy et al. 1994; Everett-Hincks et al. 2005; Plush et al. 2011; Dwyer 2014). O'Connor (1985) reported a positive relationship between maternal behaviour score (MBS) and lamb survival. A more recent study conducted under New Zealand farming conditions also reported that an increase in MBS resulted in greater lamb survival rates (Everett-Hincks et al. 2005).

In most of the studies that have reported that ewe and lamb behaviour affected lamb survival the behavioural measures were taken immediately post birth a (O'Connor et al. 1985; Owens et al. 1985; Alexander 1988; Cloete 1993; Dwyer et al. 2001; Dwyer 2003; Dwyer et al. 2003; Dwyer and Buenger 2012). However, the nature of extensive pastoral farming conditions in New Zealand makes observations immediately post birth impractical. In addition, disturbance during this period can have negative consequences for ewe-lamb bonding, which may lead to the ewe rejecting the lamb(s) (Fisher and Mellor 2002). Moreover, of lambs that die, up to 94% have been reported to do so after 12 hours of age (Hatcher et al. 2009; Hinch and Brien 2014), thus knowledge of how the behaviour post the immediate lambing period affects the survival is also of interest. It is likely also that under extensive farming systems the farmers wait a few hours before they interact with the ewe and her lambs.

If a behaviour trait that was displayed a few hours post-birth could be identified as a predictor of lamb survival it could be used to recognise lambs that need intervention to improve survival rate. It could also be used as a research tool to evaluate the effects of various ewe interventions in pregnancy and their potential impact on lamb survival. There are many potential lamb behaviour traits that have been observed under extensive conditions (Owens et al. 1985; Dwyer 2003). These can, however, be time consuming to measure and often more than one test has been utilized. Further, few studies to date have actually included enough numbers of lambs to be able to determine if these behaviours recorded relate to lamb survival.

Therefore, the aim of this chapter was to determine whether the behaviours of ewes and lambs at tagging (3 to 18 hours after birth) and during a triangle pen test (12 or 24 hours after birth) were associated with survival of twin- and triplet-born lambs.

Materials and methods

In this chapter the data from chapters two, three, four and five were combined. Therefore, only a brief description of the individual experiments is given here (Table 6.1). The analysis included data from 423 twin-bearing ewes and their 846 lambs and 278 triplet-bearing ewes and their 834 lambs. The aim of this analysis was to explore the behaviours of the twin- and triplet-bearing ewe and her twin- and triplet-born lambs. Only complete sets of twin- and triplet-born lambs (where the full litter was alive when behaviours were observed) and their dams were included in the analysis due to the potential variation in behaviour of a complete litter as opposed to a 'partial-litter'.

Animal and behavioural measurements

During the lambing period ewes were inspected twice daily at 8 am and 4 pm. Lambs were tagged once their coat was dry and the lamb was mobile (at approximately 3 to 18 hours of age). During tagging lambs were ear-tagged, identified to their dam and had their weight, sex and birth-rank recorded. Immediately after tagging, behavioural observations were conducted on the ewe and her lambs as described in chapter two (page 50).

A triangle pen test based on that described by Nowak et al. (1989) was conducted on 597 lambs (twins n=190 and triplets n=407, Table 6.2) at either approximately 12 or 24 hours after birth (twins 12h n=130, 24h n=60; triplets 12h n=273, 24h n=134) as described in chapter two (page 51). Lamb live weight at weaning was used as an indicator of survival (no record was a proxy of lamb pre-weaning mortality).

Statistical analysis

All statistical analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC, 2011, USA). Twin- and triplet-born lambs were analysed separately. Ewe BCS and feeding treatments were not included in the present analysis. In the analysis 'year' and 'study' are synonymous since only one triplet and/or twin study was conducted each year. Therefore only 'year' was included as a fixed effect. All models were run with and without lamb birth weight and lamb birth weight as a linear and quadratic effect. The linear but not the quadratic effect of birth weight was significant, so only those models that included birth weight have been detailed here.

General

The mortality rates for twin- and triplet-litters were calculated based on whether live weight of the lamb had been recorded at weaning. The impact of year of study and sex of lamb on lamb survival was analysed using a logistic regression. The model contained the fixed effects of year and sex of lamb with lamb birth weight included as a covariate.

All lambs were assigned a ‘vigour score’ depending on how many different behaviours (stand, contact, suck and follow) they exhibited in the paddock. The scores were: 1= lamb did not exhibit any of the four behaviours, 2= lamb exhibited one behaviour, 3= lamb exhibited two behaviours, 4= lamb exhibited three behaviours and 5= lamb exhibited all four behaviours. The effect of the vigour scores on survival was analysed using a logistic regression. The model included the fixed effects of year and sex of lamb. Lamb birth weight and vigour score were added as covariates.

The relationship between ewe and lamb behaviour and lamb survival

The association between behaviour and survival was analysed using a logistic regression model that included the fixed effects of year and sex of lamb, with lamb birth weight included as a covariate. Each behaviour was added to the model individually. The behaviour trait was added as a fixed effect when investigating the relationship between whether or not a lamb or ewe exhibited a behaviour and survival. The behaviour trait was added as a covariate when investigating the relationship between time taken to exhibit (or time spent exhibiting) a behaviour and survival, and when analysing the relationship between the number of bleats and MBS and survival.

The age of the lamb at triangle pen testing (at approximately 12 or 24 hours) was also included as a fixed effect when testing for all triangle pen test behaviours. The interaction between the behaviour variable and age of lamb was included in the analysis for the triangle test behaviours, however, none of the interactions were significant (P > 0.05) and hence removed.

The relationship between the outcome of the preference test, i.e. whether the lambs made a 'correct choice', an 'incorrect choice' or made 'no choice' (correct choice =1, incorrect choice =2 and no choice = 3) and survival was also analysed using a using a logistic regression models. The model contained the fixed effects of year, sex and age of lamb and lamb birth weight as a covariate.

Further investigation of those behaviours related to survival

Behaviours that had an significant association with survival (P < 0.05) were subjected to further analysis. These behaviours were 'time to follow the ewe' for both twin- and triplet-born lambs and number of low-pitched bleats for triplet-born lambs in the paddock. In the triangle pen test these behaviours were 'time to reach the contact zone' and number of high-pitched bleats for twin-born lambs and 'time to reach the dam' for triplet-born lambs.

The relationship between whether the behaviour (follow, reach contact zone, reach dam) was exhibited in under 30 seconds, 1 minute, 2 minutes, 3 minutes or 4 minutes and survival was further examined using a logistic regression. For each behaviour the model contained the fixed effects of the behaviour variable, year and sex of lamb with lamb birth weight included as a covariate.

Within both twin- and triplet-litters the relationship between survival and the time from ewe-lamb contact until the lamb followed the dam was also analysed using a logistic regression. The models included the fixed effects of the behaviour variable, year and sex of lamb with lamb birth weight included as a covariate.

Are different ewe and lamb behaviours correlated?

The behavioural data collected made it possible to analyse the existence of behavioural correlations, however, these results are outside the scope of this thesis and only presented in appendix 1 and 2. The statistical methods used in analysing the correlations are also presented in appendix 1 and 2.

Results

General

Lamb mortalities from tagging to weaning across all studies were 12% and 25% for twin- and triplet-born lambs, respectively (Table 6.1 and 6.2). Birth weight and the sex of the lamb influenced survival of both twin- and triplet-born lambs (Fig. 6.1). For every additional 100 g of birth weight twin- and triplet-born lambs were 1.468 (1.102-1.956, $P < 0.05$) and 1.840 (1.497-2.286, $P < 0.05$) times more likely to survive, respectively. Twin- and triplet-born female lambs were 1.552 (1.002-2.404, $P < 0.05$) and 1.599 (1.152-2.225, $P < 0.05$) times more likely to survive as their male counterparts. The vigour score (the number of behaviours exhibited) of twin- and triplet-born lambs had no effect on survival ($P < 0.05$, data not shown).

Table 6.1. The number (n) of twin- and triplet-born lambs utilised in the paddock test each study and their mortality rate (%).

Year of study	Birth rank	n	Mortality rate
2010[1]	Twins	308	17.50%
2011[1]	Twins	240	7.10%
2012[3]	Twins	186	9.80%
2013[4]	Twins	112	9.70%
2009[2]	Triplets	264	26.10%
2010[2]	Triplets	357	24.90%
2011[2]	Triplets	213	24.00%

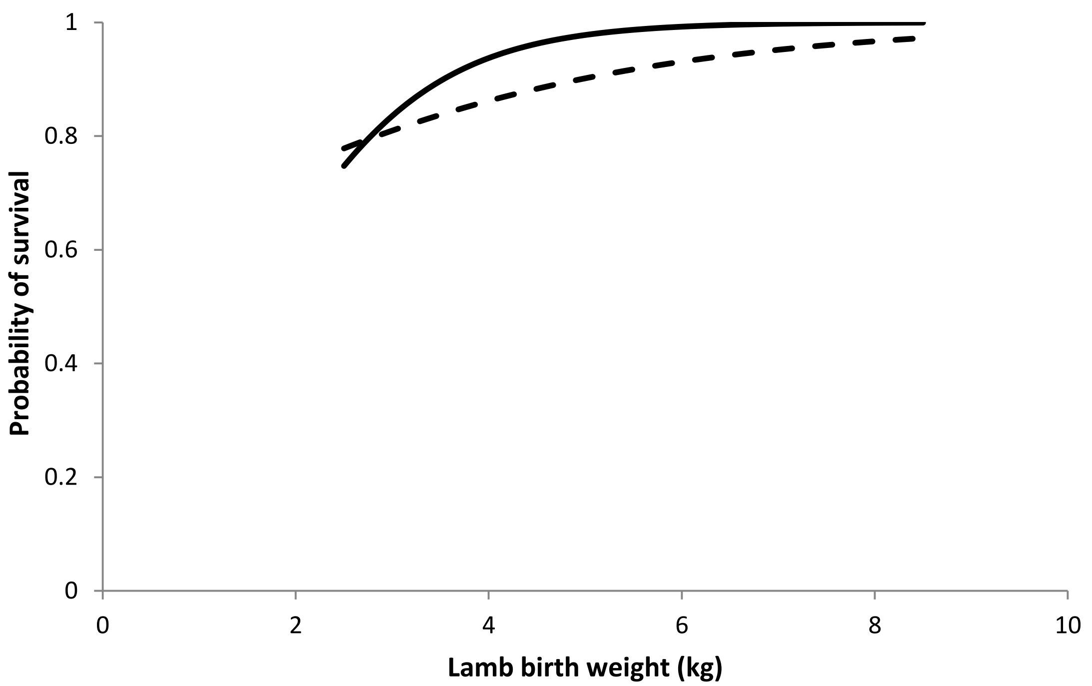
[1] Chapter two, [2] chapter three, [3] chapter four, [4] chapter five.

Table 6.2. The number (n) of twin- and triplet-born lambs utilised in the triangle test in each study and their mortality rate (%).

Year of study	Birth rank	n	Mortality rate
2010[1]	Twins	190	12.9%
2009[2]	Triplets	150	20.0%
2011[2]	Triplets	260	20.2%

[1] Chapter two, [2] chapter three.

Figure 6.1. The effect of lamb birth weight (kg) on the probability of survival for twin-(- -) and triplet-born (—) lambs (presented as a back transformed log-function).



The relationship between ewe and lamb behaviour at tagging and survival

Lamb behaviours and vocalisation

Survival of twin- and triplet-born lambs was not associated with whether or not the lamb stood, made contact with, sucked from or followed their ewe in the five-minute observation period ($P > 0.05$, Table 6.3).

When the time to exhibit a behaviour was considered, twin-born lambs were 1.004 (1.000-1.008) times more likely to survive for every additional second taken to follow their dam ($P < 0.05$, Table 6.4). In addition, if a twin-born lamb had not followed its dam by the end of three minutes it was 2.405 (1.170-4.943) times more likely to survive ($P < 0.05$) than if it has followed her already. There were no relationships between whether a twin-born lamb followed its dam at 30 sec, 1 min, 2 min or 4 min and the probability of survival ($P > 0.05$, data not shown). There was also no relationship between survival and the time from ewe-lamb contact until the twin-born lamb followed ($P > 0.05$, data not shown).

Among triplet-born lambs the opposite relationship was seen. For each additional second triplet-born lambs took to follow their dam slightly decreased odds of survival (0.996 [0.993-0.999], $P < 0.05$). If a triplet-born lamb had not followed its dam by the end of four minutes it was 0.405 (0.226-0.725) times less likely to survive ($P < 0.05$) than if it had followed its dam. There were, however, no relationships between whether a triplet-born lamb followed its dam at 30 seconds, 1 minute, 2 minute or 3 minute and its probability of survival ($P > 0.05$, data not shown). Among triplet-born lambs, for each additional second between ewe-lamb contact and the point at which the triplet-born lamb followed its dam the probability of survival decreased by 0.996 ([0.993-0.999], $P < 0.05$).

There were no relationships between the time taken for lambs to stand, make contact with their dam and suck from their dam and the odds of survival in either twin- or triplet-born lambs ($P > 0.05$).

Table 6.3. The effect of whether or not a lamb exhibited a particular behaviour in the paddock on the probability of survival.

	n[1]	Odds ratio	P
Twin-born lambs	846		
Stand	780	1.720 (0.872-3.391)	0.118
Contact	776	0.773 (0.302-1.776)	0.491
Suck	239	1.220 (0.737-2.018)	0.440
Follow	512	1.098 (0.705-1.711)	0.678
High-pitch bleat	726	1.016 (0.556-1.854)	0.960
Low-pitch bleat	694	1.460 (0.872-2.443)	0.150
Triplet-born lambs	834		
Stand	731	1.265 (0.787-2.035)	0.332
Contact	711	0.852 (0.532-1.366)	0.506
Suck	159	1.447 (0.942-2.224)	0.092
Follow	361	1.002 (0.707-1.421)	0.991
High-pitch bleat	708	0.916 (0.577-1.454)	0.710
Low-pitch bleat	495	1.026 (0.739-1.423)	0.880

[1]The total number of lambs followed by the number of lambs that exhibited the various behaviour traits. The effect of whether or not a lamb exhibited a particular behaviour on the probability of survival is presented as the point estimate (with the 95% confidence limit in parenthesis) followed by the p-value.

Table 6.4. The effect of the time required to exhibit a particular behaviour in the paddock on the probability of survival, for those performed the behaviour.

	n[1]	Median time	Odds ratio	P
Twin-born lambs	846			
Stand	780	20	1.001 (0.997-1.005)	0.603
Contact	776	14	1.002 (0.997-1.006)	0.482
Suck	239	139	1.003 (0.997-1.010)	0.282
Follow	512	120	1.004 (1.000-1.008)	0.031
Triplet-born lambs	834			
Stand	731	25	0.999 (0.996-1.002)	0.507
Contact	711	16	1.000 (0.997-1.003)	0.962
Suck	159	165	0.998 (0.995-1.007)	0.674
Follow	361	120	0.996 (0.993-0.999)	0.007

[1]The total number of lambs followed by the number of lambs that exhibited the various behaviour traits. The effect of the time required to exhibit a particular behaviour on the probability of survival is presented as the point estimate (with the 95% confidence limit in parenthesis) followed by the p-value.

Lamb vocalisation

There was no relationship between survival of twin- and triplet-born lambs and whether or not the lamb emitted high- or low-pitched bleats, ($P > 0.05$, Table 6.3).

For every additional low-pitched bleat emitted by triplet-born lambs they were 1.059 (1.007-1.112, $P < 0.05$, Table 6.5) times more likely to survive. The number of high-pitched bleats emitted by triplet-born lambs was not associated with survival ($P > 0.05$).

Table 6.5. The effect of the number of high- and low-pitched bleats emitted by the ewes and their lambs in the paddock on the probability of survival, for those that bleated.

	n[1]	Median number	Odds ratio	P
Twin-born lambs				
High-pitch bleat	726	10	0.996 (0.983-1.009)	0.557
Low-pitch bleat	694	5	0.992 (0.969-1.015)	0.485
Triplet-born lambs				
High-pitch bleat	708	6	0.994 (0.986-1.003)	0.169
Low-pitch bleat	495	15	1.059 (1.007-1.112)	0.024
Twin-bearing ewes				
High-pitch bleat	363	13	0.998 (0.980-1.017)	0.865
Low-pitch bleat	376	3	0.999 (0.988-1.010)	0.862
Triplet-bearing ewes				
High-pitch bleat	233	8	1.006 (0.993-1.020)	0.354
Low-pitch bleat	247	20	1.002 (0.994-1.010)	0.597

[1]The number of ewes and lambs that exhibited the behaviour trait. The effect of the number of bleats on the probability of survival is presented as the point estimate (with the 95% confidence limit in parenthesis) followed by the p-value.

Table 6.6. The effect of whether or not a ewe emitted high- or low-pitched bleats in the paddock on the probability of survival.

Bleating	n[1]		Odds ratio	P
Twin-bearing ewes	423			
High-pitch bleat		363	1.109 (0.605-2.033)	0.737
Low-pitch bleat		376	0.890 (1.100-1.954)	0.750
Triplet-bearing ewes	278			
High-pitch bleat		233	1.337 (0.872-2.051)	0.184
Low-pitch bleat		247	1.156 (0.698-1.914)	0.574

[1]The total number of ewes followed by the number of ewes that exhibited the various behaviour traits The effect of ewe bleating on the probability of survival is presented as the point estimate (with the 95% confidence limit in parenthesis) followed by the p-value.

Ewe behaviour and vocalisation

There were no relationships between whether or not the dam bleated ($P > 0.05$, Table 6.6) or the number of ewe high- or low-pitched bleats and twin- and triplet-born lamb survival ($P > 0.05$, Table 6.5). Furthermore, ewe MBS was not associated with twin- or triplet-born lamb survival (1.022 [0.844-1.237] and 1.029 [0.888-1.192], respectively, $P > 0.05$).

The relationship between lamb behaviour during the triangle pen test and survival

Whether or not a twin- or triplet-born lamb emitted high- or low-pitched bleats or exhibited any of the recorded triangle pen test behaviours in the five-minute observation period had no relationship with survival ($P > 0.05$, Table 6.7). The outcome of the triangle pen test (correct 52%, incorrect 20% or no choice 29% for twin- and triplet-born lambs combined) were not associated with lamb survival for twin- (0.896 (0.517-1.553) or triplet-born lambs (1.023 [0.728-1.438], $P > 0.05$).

In twin-litters, there were no associations between the amount of time that the lamb spent walking, standing, sitting, in the contact zone, with an alien ewe or with their dam and survival ($P > 0.05$, Table 6.8). The time required for the twin-born lamb to reach their dam was not associated with survival either ($P > 0.05$). For each additional second the twin-born lamb took to reach the contact zone it was 0.989 (0.979-1.000) times less likely to survive ($P < 0.05$).

Furthermore, there were no associations between whether a twin-born lamb reached the contact zone at 30 sec, 1 min, 2 min, 3 min or 4 min and survival ($P > 0.05$, data not shown).

In triplet-litters, the amount of time the lamb spent walking, standing, sitting, in the contact zone, with the alien ewe or with their dam was not associated with survival ($P > 0.05$, Table 6.8). For every additional second the triplet-born lamb took to reach its dam it was 0.994 (0.989-0.999) times less likely to survive ($P < 0.05$). There were no relationships between whether a triplet-born lamb reached its dam at 30 sec, 1 min, 2 min, 3 min or 4 min and its probability of survival ($P > 0.05$, data not shown).

For each additional high-pitched bleat emitted in the triangle pen test twin-born lambs were 0.987 (0.964-0.992) times less likely to survive ($P < 0.05$, Table 6.9), but this effect was not seen within triplet-litters. The number of low-pitched bleats was not associated with survival of either twin- or triplet-born lambs ($P > 0.05$).

Table 6.7. The effect of whether or not a lamb exhibited a particular behaviour in the triangle pen test on the probability of survival.

	n[1]	Odds ratio	P
Twin-born lambs	190		
Walked	182	1.002 (0.117-8.590)	0.9986
Stood	188	6.177 (0.360-105.971)	0.2092
Sat	66	2.263 (0.801-6.391)	0.1232
Reached dam	159	1.377 (0.469-4.045)	0.5606
Reached contact zone	178	2.280 (0.567-9.173)	0.2458
Spent time with dam	159	1.455 (0.493-4.293)	0.4972
Spent time with alien	144	1.053 (0.388-2.856)	0.9192
Spent time in contact zone	179	2.609 (0.637-10.696)	0.1827
High-pitch	186	3.636 (0.308-42.930)	0.3054
Low-pitch	133	0.527 (0.182-1.522)	0.2362
Triplet-born lambs	410		
Walked	389	1.661 (0.552-4.992)	0.3665
Stood	404	1.645 (0.144-18.784)	0.6887
Sat	65	0.602 (0.293-1.235)	0.1662
Reached dam	363	0.8325 (0.241-2.047)	0.4620
Reached contact zone	391	1.044 (0.276-3.955)	0.9489
Spent time with dam	363	1.352 (0.785-2.328)	0.6932
Spent time with alien	346	0.841 (0.376-1.883)	0.6738
Spent time in contact zone	391	1.044 (0.276-3.955)	0.9489
High-pitch	398	6.003 (0.960-37.531)	0.0553
Low-pitch	250	1.158 (0.668-2.009)	0.6013

[1]The total number of lambs followed by the number of lambs that exhibited the various behaviour traits. The effect of behaviour on the probability of survival is presented as the point estimate (with the 95% confidence limit in parenthesis) followed by the p-value.

Table 6.8. The effect of the time required to exhibit a particular behaviour in the triangle pen test on the probability of survival, for those performed the behaviour.

	n[1]	Median time	Odds ratio	P
Twin-born lambs				
Walked	182	50	1.001 (0.990-1.011)	0.905
Stood	188	240	0.996 (0.987-1.005)	0.358
Sat	66	30	0.993 (0.983-1.004)	0.224
Reached dam	159	30	0.999 (0.991-1.008)	0.838
Reached contact zone	178	20	0.989 (0.979-1.000)	0.047
Spent time with dam	159	170	1.002 (0.997-1.006)	0.431
Spent time with alien	144	70	1.001 (0.996-1.006)	0.718
Spent time in contact zone	179	280	1.003 (0.999-1.008)	0.157
Triplet-born lambs				
Walked	389	50	1.001 (0.995-1.007)	0.748
Stood	404	240	1.002 (0.997-1.007)	0.348
Sat	65	60	0.996 (0.990-1.002)	0.173
Reached dam	363	30	0.994 (0.989-0.999)	0.029
Reached contact zone	391	20	0.991 (0.981-1.002)	0.098
Spent time with dam	363	170	1.001 (0.998-1.004)	0.449
Spent time with alien	346	80	1.001 (0.997-1.004)	0.742
Spent time in contact zone	391	280	1.003 (0.999-1.006)	0.151

[1] The number of lambs that exhibited the behaviour trait. The effect of lamb behaviour on the probability of survival is presented as the point estimate (with the 95% confidence limit in parenthesis) followed by the p-value.

Table 6.9. The effect of the number of high- and low-pitched bleats emitted by the lambs in the triangle pen test on the probability of survival, for those that bleated.

	n[1]	Median number	Odds ratio	P
Twin-born lambs				
High-pitch bleat	186	27	0.978 (0.964-0.992)	0.002
Low-pitch bleat	133	4	1.050 (0.974-1.133)	0.204
Triplet-born lambs				
High-pitch bleat	398	30	0.998 (0.998-1.008)	0.685
Low-pitch bleat	250	5	1.013 (0.978-1.050)	0.465

[1] The number of lambs that exhibited the behaviour trait. The effect of lamb bleating on the probability of survival is presented as the point estimate (with the 95% confidence limit in parenthesis) followed by the p-value.

Discussion

In this chapter, using the behaviours of twin- and triplet-bearing ewes and their lambs as predictors of lamb survival to weaning were examined. Lambs were observed at tagging in the paddock at 3 to 18 hours of age and/or in a triangle pen test at approximately 12 or 24 hours of age.

The mortality rates reported are typical of those previously reported for twin- and triplet-born lambs in New Zealand (Kerslake et al. 2005) as are the effects of sex (Lopez-Villalobos and Garrick, 1999) and birth weight (e.g. Holst et al. 2002) of the lambs on survival. Twin-born lambs had a greater mortality rate in 2010 than 2011, 2012 and 2013 however the reason for this is unknown. It is possible that this was due to adverse weather conditions; this data was not captured on farm, however, according to the National Institute of Water and Atmospheric Research, 2010 was a particularly wet spring (NIWA, 2016).

Paddock behaviours

The majority of lamb behaviours observed at tagging had no effect on survival. This suggests that using behaviours such as standing, ewe-lamb contact, suck and ewe bleating, at 3 to 18 hours after birth, as indicators of survival in twin- and triplet-born lambs is not suitable. However, triplet-born lambs that bleated more in a low-pitch had better odds of survival, possibly suggesting a stronger ewe-lamb bond. Low-pitched bleats are emitted almost exclusively between the ewe and her neonates and help maintain the ewe-lamb bond (Nowak 1990). It is, therefore, not surprising that a triplet-born lamb with a stronger maternal bond would display greater survival. If this is the case, however, it may also be expected that triplet-born lambs that emitted at least one low-pitched bleat would have better odds of survival than lambs that did not emit any low-pitched bleats. However, this connection was not present possibly due to the number of lambs being too low for a binomial effect to be detected.

The quicker the triplet-born lamb followed its dams, the greater its probability of survival. Twin-bearing ewes produce around 40% more milk than single-bearing ewes but triplet-bearing ewes only produce 0% to 10% more milk than twin-bearing ewes (Treacher 1978; 1983; Snowder and Glimp 1991). As such there is a significant competition for milk within triplet-litters. It could thus be hypothesised that the quicker the triplet-born lamb was to follow its dam and emit low-pitched bleats which would help the dam recognise her lambs (Nowak 1990) the more likely it was to suck from her and hence increase its survival. Triplet-born lambs that sucked from their dam after tagging also tended (P = 0.09) to have better odds of survival than those that did not suck, further supporting this theory.

Interestingly, the opposite effect was observed within twin-born lambs. The quicker twin-born lambs followed their dam, the lower their probability of survival. Milk competition is not generally an issue within twin-litters (Treacher 1978; 1983; Snowder and Glimp 1991) and as such being quick to follow the dam when she moves away may not affect survival in twins in the same way as it appear to do in triplets. Perhaps, twin-born lambs that are quick to follow their dams are those that have not received adequate maternal care and nutrition and therefore, at the ages tested, are more motivated to stay close to their dams to receive nutrition. However, if these were true explanations, whether or not the twin- and triplet-born lamb followed at all during the observation period, should have been associated with survival but it was not. Again, it is possible that an association between following as a behaviour and survival could not be found when analysing due to numbers with this binomial trait.

In the present study lamb behaviours were observed at 3 to 18 hours of age, and thus it may not be surprising that the results differ from research examining the ewe and lamb behaviours exhibited immediately after birth (Owens et al. 1985; Cloete 1993; Dwyer 2003; Miller et al. 2010). It is important to note that by allowing for a minimum of three hours after birth before recording behaviour, in the present study, those lambs that died prior to tagging, and their siblings, were therefore excluded from this study. Further, there are environmental differences, i.e. between confined indoor structures (Owens et al. 1985; Dwyer 2003) compared to the extensive pastoral outdoor environment.

In contrast to previous studies (Lambe et al. 2001; Everett-Hincks et al. 2005; Plush et al. 2011) ewe MBS was not associated with the survival of twin- or triplet-born lambs. Lambe et al. (2001) and Plush et al. (2011) both reported a positive relationship between ewe MBS and lamb survival. Furthermore, Everett-Hincks et al. (2005) reported that litter survival increased for dams that had a MBS of three compared with dams with an MBS of one or two. Why similar effects were not seen in the current study is unknown. One difference between the studies is the breed of the ewes, with previously mentioned studies using Scottish Blackface (Lambe et al. 2011), Merinos (Plush et al. 2011) and Coopworths (Everett-Hincks et al., 2005) while the present study used Romney crosses. All previous studies also utilised a mixture of single-, twin- and triplet-born lambs while the current study analysed twin- and triplet-lambs separately. Perhaps the relationship between MBS and survival is more complex for triplet-born lambs than single- and twin-born lambs. Perhaps the advantages of a dam with a great MBS are overshadowed by the difficulties, in regards to survival, associated with lower birth weights (Dwyer et al., 2003) and increased milk competition within litters (Snowder and Glimp 1991).

Triangle pen test

Nowak and Lindsay (1992), with fewer lambs, reported that single- and twin-born lambs that survived past seven days of life had spent a larger proportion of the total time with their dam when tested at 12 hours of age. This effect was not seen in the present study for twin- or triplet-born lambs. In the same study by Nowak and Lindsay (1992), when twin-born lambs were tested at 72 hours of age no relationship between the time spent with their dam and survival was reported. It is possible that this age progression effect is observed earlier in Romney lambs compared to Merinos as utilised by Nowak and Lindsay (1992). Further, Nowak and Lindsay (1992) measured lamb survival until only seven days of age, while the present study measured survival only at weaning.

In the present study, the quicker triplet-born lambs reached their dam, the better their odds of survival. While within twin-born lambs, simply reaching the contact zone increased their odds of survival, but there was no benefit of reaching the dam. These results may, as previously discussed on page 221, reflect the difference in milk competition within twin- versus triplet-litters. Triplet-born lambs may be more motivated to quickly get close to their dam in order to improve their chance of drinking and subsequent survival, while the same urgency may not be prevalent in twin-born lambs where milk competition is less.

In the triangle pen test, the more high-pitched bleats emitted by twin-born lambs, the lower their survival. High-pitched bleats are considered a distress bleat and are frequently emitted when the lamb and ewe are separated (Poindron et al. 1994; Weary and Fraser 1995; Dwyer et al. 1998) such as during the triangle test. It is thus possible that twin-born lambs that died before weaning had poorer recognition skills and a weaker bond with their dam, as evident by more high-pitched bleats. Twin-born lambs high-pitched bleats were also negatively correlated with ewe MBS (appendix 2, table 3). These results further suggest that during a triangle pen test at 12-24 hours after birth twin-born lamb high-pitched bleats are an indication of ewe-lamb bond strength. Greater high-pitched bleating rates in lambs that died may be a reflection of the fact that they took longer to enter the contact zone than those that survived, and may have spent this time calling out or responding to calls from their dam. As the behaviour and vocalisation of the dam during the triangle pen test was not recorded it is not possible to determine whether the high-pitched bleats were a response or merely a distress bleat.

These results could indicate that using these measures at 12 to 24 hours of age could be used as proxy measure of lamb survival. Although, if this was the case it might be expected that whether or not the lamb exhibited these behaviours would also have been associated with survival, this was not the case, which again may be due to low numbers of lambs. As such, the importance of the time taken to reach the contact zone and the dam are somewhat questionable and further research is warranted before any conclusions can be drawn.

Conclusion

It is clear that majority of the behaviours observed both during tagging and during a triangle pen test had no relationship with survival of both twin- and triplet-born Romney lambs. This suggests that, when measured in the paddock at 3 to 18 hours after birth and at approximately 12 or 24 hours after birth in a triangle pen test they are not suitable behaviours as proxy indicators for lamb survival. The time taken for twin- and triplet-born lambs to follow their dam after tagging appears to be associated with survival. Likewise, the time taken for a triplet-born lamb to reach the contact zone and for the twin-born lambs to reach their dam in the triangle pen test is associated with lamb survival. The difference observed between twin- and triplet-born lambs may be a result of milk competition within litters. Yet in both twins and triplets these behaviours may give an indication of the strength of the ewe-lamb bond which is known to affect survival. However, the practical application of these behaviours to be used as a tool to predict survival of lambs is limited.

CHAPTER 7

Concluding discussion

Outline

The overall aim of this thesis was to investigate the effects of mid-pregnancy BCS and feeding during mid- to late-pregnancy on the behaviour of twin- and triplet-bearing ewes and their lambs in the first 24 hours after birth. In addition the relationship between ewe and lamb behaviours and lamb survival was investigated. Behavioural data was collected over a five-year period and only full sets (the whole litter alive at time of measuring) of twins and triplets were included in the studies.

General Introduction

Lamb deaths are of concern to the New Zealand sheep industry from both an economic and an animal welfare perspective (Mellor and Stafford 2004). The national average lambing percentage in New Zealand has increased from approximately 100% in 1990 to 128% in 2013 (Anon. 2013). This increase has been associated with a greater percentage of twin- and triplet-litters (Amer et al. 1999) which have greater mortality rates than single lambs (Hight and Jury 1970; Dalton et al. 1980; Hinch et al. 1983; Kenyon et al. 2002; Kerslake et al. 2005), even when birth weight is accounted for (Oldham et al. 2011). A better understanding of the factors involved in twin- and triplet-born lamb mortality may help develop strategies to improve ewe flock productivity.

Ewe and lamb behaviours have been shown to affect lamb survival (Alexander et al. 1959; Murphy et al. 1994; Lambe et al. 2001; Plush et al. 2011). Previous studies have shown that ewe BCS and nutrition during pregnancy can impact both maternal and lamb behaviours (Putu et al. 1988; Dwyer 2003; Everett-Hincks et al. 2005; Kleemann et al. 2006; Corner et al. 2010; Kenyon et al. 2011; Kenyon et al. 2014). To date, it appears that few studies have investigated the potential effects of above-pregnancy-maintenance level nutrition during mid- to late-pregnancy on the behaviour of twin- and triplet-bearing ewes and their lambs managed under extensive pastoral conditions (Everett-Hincks et al 2005). Information about the effects of ewe BCS during mid-pregnancy on ewe and lamb behaviour is also lacking. In addition, most previous studies of ewe and lamb behaviour have been conducted immediately after birth (Dwyer 2003). The nature of extensive pastoral farming conditions in New Zealand make such observations problematic and disturbance during this period can have negative consequences for ewe-lamb bonding, which could result in ewes rejecting their lambs (Fisher and Mellor 2002). Therefore in this thesis ewe and lamb behaviour was observed between 3 and 24 hours after birth. Further, many behavioural studies have not had the statistical power to examine the relationship between behaviour and survival. The data collected over the seven experiments and subsequent high number of lambs included in the final analysis allowed this.

Summary of chapters

Chapter two

The aim of this study was to investigate the effect of ewe mid-pregnancy BCS (BCS 2.0, 2.5 and 3.0 as measured on P89) and two different feeding treatments (medium <1200 kg DM/ha and *ad lib* >1200 kg DM/ha) during mid- to late-pregnancy (P112 to

P136 and P128 to P141, in experiment one and two, respectively) on the behaviour of twin-bearing ewes and their lambs in two separate experiments. Behavioural observations were conducted in the paddock following tagging between 3 and 18 hours of age and in a triangle pen test at approximately 12 or 24 hours of age. Overall, the effects of mid-pregnancy BCS and feeding treatment on ewe and lamb behaviours were either minor or inconsistent. Results showed, however, that in experiments one lambs born to ewes in the medium feeding treatment were quicker to make contact with their dam than lambs born to ewes in the *ad lib* treatment, while the opposite was true in experiment two. In experiment two, within the BCS 2.0 and 3.0 group, less ewes in the medium treatment emitted low-pitched bleats than the *ad lib* treatment. Yet as similar effect was not seen in experiment one. Overall, feeding ewes of BCS 2.0 to 3.0 *ad libitum* during mid- to late-pregnancy appears to provide very little advantage in ewe and lamb behaviours compared with feeding ewes medium feeding levels.

Chapter three

Three separate experiments were conducted during consecutive years to investigate the effect of ewe mid-pregnancy BCS (BCS 2.0, 2.5 and 3.0 as measured on P92, P72 and P72 respectively for the three different experiments) and feeding treatments ('medium' <1200 kg DM/ha versus '*ad lib*' >1200 kg DM/ha) during mid- to late-pregnancy (P93 to P114, P115 to P136 and P128 to P141) on the behaviour of triplet-bearing ewes and their lambs. Behaviours were recorded in the paddock and in a triangle pen test as in chapter 2. Across the experiments there were few consistent effects of ewe BCS and feeding treatment on ewe and lamb behaviours. It was concluded that from a behavioural perspective there is little advantage offering triplet-bearing ewes *ad lib* feeding compared with medium. Furthermore, results show that there is little advantage to the behaviour of the triplet-bearing ewe and her lambs under pastoral feeding conditions from having a ewe at a BCS 3.0 compared with 2.0 in mid-pregnancy.

Chapter four

The aim of this experiment was to investigate the effect of ewe mid-pregnancy BCS (BCS 2.0, 2.5 and 3.0 as measured on P98) and three ewe feeding treatments ('low' 800-1000 kg DM/ha, 'medium' 1200-1400 kg DM/ha and '*ad lib'* 1500-1700 kg DM/ha) from day 141 of pregnancy and throughout the lambing period on the behaviour of twin-bearing ewes and their lambs under extensive pastoral conditions. Behaviours of the ewe and lambs were recorded in the paddock following tagging between 3 and 18 hours of age. Lambs born to ewes in the low feeding treatment took longer to stand compared with lambs born to ewes in the medium and *ad lib* treatment. No other ewe or lamb behaviours were affected by mid-pregnancy BCS or feeding treatments and vocalisation results were inconsistent.

Chapter five

The aim of this chapter was to examine the effects of grand-dam (G0) and ewe (G1) feeding treatments during pregnancy on the behaviour of ewes (G1) and their lambs (G1) at tagging. The G0 ewes were offered either 'low', 'medium' or '*ad lib'* (*ad libitum*) feeding in early-pregnancy (P21-P50), followed by either 'medium' or '*ad lib'* feeding in late-pregnancy (P51-P140; Kenyon et al. 2011b). The G1 ewes were fed either 'medium' (800-1100 kg DM/ha) or '*ad lib'* (1400-2100 kg DM/ha) feeding conditions from P76 until term. Lambs born to G1 ewes offered the medium feeding treatment from P76 until term were quicker to stand, suck and follow their dam when she moved away, than lambs born to G1 ewes in the *ad lib* treatment. Ewes offered the medium feeding treatment emitted fewer high-pitched bleats and more low-pitched bleats than ewes in the *ad lib* treatment. The results contradict those reported in chapters two and three, maybe because the feeding treatments were implemented for a longer period of time and ended at term, compared with earlier chapters. The feeding treatments of the grand-dams (G0) did not affect the majority of ewe (G1) and lamb (G2) behaviours, and overall no consistent developmental programming effect was found.

Chapter six

The aim of this chapter was to determine whether the behaviour of the ewes and lambs at tagging and during a triangle pen test were related to twin- and triplet-born lamb survival to weaning. In this chapter the data from chapters two, three, four and five were combined. The analysis included data from 423 twin-bearing ewes and their 846 lambs, as well as 278 triplet-bearing ewes and their 834 lambs. Triplet-born lambs that reached their dam more quickly and twin-born lambs that reached the contact zone more quickly during the triangle pen test had a slightly improved chance of survival. The time taken for twin- and triplet-born lambs to follow their dam after tagging may also be linked to survival. However, this effect was not consistent between rearing rank; survival was greater for faster triplet-born lambs but for slower twin-born lambs. No other behaviours were related to survival.

Discussion

The effects of ewe mid-pregnancy BCS and feeding treatment on lamb behaviours (stand, contact, suck and follow) were minor and inconsistent in all experiments. These inconsistencies may be due to the timing and length of the feeding treatments which varied across studies. In chapters 2-4 the treatments varied from approximately 20 days to only a few days and the effects on lamb behaviours were minimal. However, when the duration of the feeding treatments were extended in chapter 5 the results on behaviour became greater and more consistent. All ewes gained liveweight in accordance to expected conceptus mass (Rattray et al. 1974) and it is, thus, perhaps not surprising that few behavioural differences were identified. Previous studies have, however, shown that even when no liveweight differences existed the difference between feeding ewes pregnancy maintenance requirements and above pregnancy maintenance still resulted in changes in progesterone, insulin, cortisol and triiodothyronine concentrations (Vonnahme et al., 2013). This study has been discussed in more detail on page 32 in chapter 1.

Overall the results show that, under the conditions of these studies, mid-pregnancy BCS and ewe feeding in mid- to late-pregnancy cannot be used as a means to influence ewe and lamb behaviour.

Whether the lamb stood, sucked or made ewe-lamb contact, and the time taken to do, so was not associated with lamb survival in chapter six. The majority of studies that have reported on associations between lamb behaviour and survival have been conducted immediately after birth (Owens et al. 1985; Cloete 1993; Dwyer 2003; Miller et al. 2010). Why similar associations between lamb behaviour exhibited at 3 to 18 hours after birth and survival is unknown. The main causes for lamb mortality in New Zealand are dystocia and starvation/exposure or a combination of the two (Hinch et al., 1986; Kerslake et al., 2005). Lambs suffering dystocia often die within the first 24 hours after birth (Hinch et al. 1986). When observations are done at birth the behavioural changes recorded may perhaps mainly identify those lambs that suffer from dystocia and are likely to die within 24 hours. As such it is possible that lambs suffering from dystocia had already died by the time the behavioural observations were conducted in the present studies, and were hence excluded. The main cause of death in the current studies was not identified, but twin- and triplet-born lambs have lower birth weights than singletons (Holst et al. 2002) and hence often die from starvation/exposure (Dalton et al. 1980; Kerslake et al. 2005), which deaths largely occurring within 3 to 7 days after birth (Hinch et al. 1986). Perhaps the threat of death by starvation/exposure can not be readily identified through behavioural observations at 3 to 18 hours after birth.

Chapter six reported that the time taken for lambs to follow their dam after tagging and reaching her or her contact zone in the triangle pen test was associated with survival. This suggests that at 3 to 24 hours after birth, behaviours that seem to indicate the strength of the ewe-lamb bond may be used as indicators of survival. The results showed that the faster twin-born lambs followed their dam after tagging the lower their chance of survival. This supports the notion in chapter five that lambs born to ewes on the low feeding treatment displayed a greater 'need' by following their dam more quickly than lambs on the *ad lib* treatment. The quicker triplet-born lambs were to follow their dam, however, the better their chances of survival. This may be driven by the increased milk competition within triplet-litters, compared to twin-litters. And the ability to follow the dam quickly may indicate a strong ewe-lamb bond and more opportunities to suck. Further, being quick at following could reduce the risk of dying from starvation/exposure which is a major threat for triplet-born lambs due to their lighter birthweight and greater milk competition compared with twins. In both litter types these behaviours may give an indication of the strength of the ewe-lamb bond.

It is further possible that the time taken for an individual lamb to exhibit a behaviour is not a good indicator of survival. Instead the behaviour of a lamb in relation to its litter mates might give a better indication of its survival chances. The behaviour of one lamb may have influenced the behaviour of another hence creating non-independence of individual lambs within a litter. This could not unfortunately be accounted for during analysis due to the non-normal distribution of the behaviour data. It was, nevertheless, anecdotally noted during behaviour observations that the behaviour of a lamb appeared to be independent to that of its littermates.

The effects of ewe BCS and feeding treatments on ewe and lamb vocalisation were inconsistent. Even within chapters, where lambs of the same birth-rank born to ewes that had been exposed to feeding treatments of similar lengths were compared, the vocalisation results of both ewes and lambs varied greatly. The number of ewe and lamb high- and low-pitched bleats, and to a lesser extent, the percentage of ewes and lambs that bleated, differed among ewe BCS and feeding treatments in chapters two to five. Chapter six reported that the more low-pitched bleats emitted by triplet-born lambs the better their probability of survival. When applying that knowledge to chapter three where triplet-born lambs emitted more low-pitched bleats if they were born to medium ewes compared to *ad lib* ewes (within the BCS 2.0 group), it appears that this combination of ewe treatments does not result in weaker lambs or a weaker ewe-lamb bond.

The behavioural data collected made it possible to analyse the existence of behavioural correlations (Appendix 1 and 2). The results of these correlations (Appendix 1) support previous research which reported that high-pitched bleats indicate a lower MBS and ewe and lamb separation. On the contrary, low-pitched bleats are positively correlated with MBS (Appendix 1) and aid in creating and maintaining a strong ewe and lamb bond (Kiley 1972; Shillito 1972; Dwyer et al. 1998; Everett-Hincks et al. 2007). Few studies have, however, investigated the effects of ewe BCS and feeding during pregnancy on ewe and lamb vocalisation. The results presented in chapters two to five are highly inconsistent and based on these results no clear conclusion can be made. It is possible that the way in which the observations were recorded did not adequately reflect vocalisation behaviour. High-pitched bleats may have different meanings depending on the situation the in which they are emitted, however, this data was not recorded. Furthermore, bleats were categorised as either high- or low-pitched bleats depending on mouth movement. These measurement may have been too crude and perhaps bleat acoustic range or latency to bleat (Morton et al. 2015) need to be investigated to better understand ewe and lamb vocalisation.

It is possible that the manner in which the number of bleats were analysed did not adequately display the effects of ewe BCS and feeding. With low numbers of ewes in each treatment group, and especially when interactions of BCS and feeding were investigates, spurious results due to type 1 errors are more likely. Post hoc comparisons were not done to correct for this. Furthermore, the mean and the median number of bleats differed in some instances suggesting that the effects seen may have been driven by one or two individuals with very high bleating rates. However, individuals with high bleating rates were not excluded as bleating frequencies have been reported to vary greatly between individuals (Nowak 1990). Moreover, no clear cut off point could be identified as to which individuals could potentially be excluded.

Results by Kenyon et al. (2012a; 2012b; 2013) and Corner-Thomas et al. (2015), using the same sheep as used in chapters two and three, reported no advantage, in terms of production, in feeding twin- and triplet-bearing ewes *ad libitum* grazing in pregnancy compared with levels designed to meet, but not exceed pregnancy-maintenance requirements. Therefore, in addition to no overall production advantage, the results from this thesis show that there are no behavioural advantages in farmers feeding twin- and triplet-bearing ewes above-pregnancy-maintenance levels.

Overall summary

If twin- and triplet-bearing ewes had a mid-pregnancy BCS between 2.0 and 3.0 there was little advantage for farmers in offering *ad libitum* grazing from mid- to late-pregnancy to manipulate ewe and lamb behaviour (chapter 2, 3 and 5).

Feeding levels below pregnancy-maintenance requirements in very late-pregnancy and throughout the lambing period had a negative effect on the time taken for twin-born lambs to stand after tagging (chapter 4).

The majority of the behaviours in the paddock and in the triangle pen test had no relationship with lamb survival in both twin- and triplet-litters. The time taken for twin- and triplet-born lambs to follow their dam after tagging and the time taken for twin-born lambs to reach the contact zone and triplet-born lambs to reach their dam in the triangle pen test were associated with survival. However, the practical application of these behaviours to be used as a tool to predict survival of lambs is limited.

Limitations of the study

Chapters two and three utilised pre-existing data, while chapters four and five consisted of data collected specifically for this thesis. Therefore, a potential limitation of this thesis is that the data was collected over a five-year period by a number of individuals with various experiences in animal behaviour observation and recording. All observations were conducted by teams of two or three people following a clearly defined protocol using pre-printed recording sheets. In an attempt to reduce inconsistency each experiment included observers who had previously measured ewe and lamb behaviour and training was given to all observers. Within experiments, all observers rotated between treatments groups.

The majority of studies have investigated lamb behaviour immediately after birth (e.g. Cloete et al. 1993; Dwyer 2003; Banchero et al. 2010). However, extensive pastoral farming conditions in New Zealand make such observations impractical. Therefore, in the present study behaviours were observed between 3 and 18 hours after birth. The age at which lambs were observed in this study makes it difficult to compare the results presented in this thesis to previous research. Observing ewe and lamb behaviours both at birth and at 3 to 18 hours and comparing the two would have been advantageous, but unfortunately it was not possible under the conditions in which these ewes lambed.

Chapters two and three showed that lambs subjected to the triangle pen test at 12 hours of age behaved differently to lambs tested at 24 hours of age. Lambs tested at 24 hours were more mobile and better at discriminating their dams than lambs tested at 12 hours. As such, it is likely that a similar age effect may have been observed among the behaviours recorded in the paddock at tagging. Ideally the time of birth would have been recorded to allow for adjustment. Alternatively, behavioural observations of lambs could be conducted in such a way that all lambs were of a similar age at time of observation. However, the mean age of lambs tested in each treatment group is likely similar and as such it is unlikely that the age of lamb would have affected the results of these treatments.

The number of ewes in each ewe BCS group and feeding treatment was another limitation of this thesis, particularly when investigating the effects of two-way interactions between ewe BCS and feeding treatment. Further, analyses that examined the association between whether or not a behaviour trait was exhibited and survival would require many more lambs per group to have sufficient power to examine the binomial outcome. For example, to detect a difference between 83% and 89% of twin-born lambs that stood after tagging with 80% power and 5% level of significance, 248 lambs would be needed in the group that did not stand.

In this thesis MBS was affected only by ewe BCS in chapter 3 (experiment 3) and not at all by ewe feeding treatment. Only data from complete sets of either twin- or triplet-litters at time of tagging were included in the analyses of these experiments. By only including complete litters at the time of tagging (3 to 18 hours after birth), the study may have excluded ewes with very poor maternal behaviour and/or very weak lambs. The decision to only include complete litters was made due to the potential behavioural variation of ewes and lambs between complete and incomplete litters (e.g. a triplet-litter with all lambs alive versus a triplet-litter with only one lamb alive). The majority of previous research does not clarify whether incomplete litters were included in the studies.

Lambs that did not stand (and were given a nominal value of 301 seconds) in the five minute observation period were not removed when analysing the time taken to suck and follow. By including these lambs it is possible that the effects of ewe BCS and nutrition on suck and follow may have been obscured. However, in chapter four lambs that did not stand were excluded from analyses on suck and follow. When analysed this way, there was no change in the results. This indicates that including lambs that did not stand did not obscure the results.

Not investigating the effect of below-pregnancy-maintenance feeding levels on ewe and lamb behaviour in all experiments may be another limitation. However, a number of previous studies have already established that very low feeding levels can negatively affect behaviour (e.g. Dwyer et al. 2003), while no research has investigated the effects of *ad lib* feeding on behaviour. Moreover, reducing feed intake in pregnant ewes below pregnancy-maintenance levels would likely cause a drop in production (Monteath 1971; Mellor and Murray 1985) and therefore feeding ewes at such levels, even if it improved behaviour, would be impractical for New Zealand farmers.

Another possible limitation of this thesis is the small range of ewe BCS investigated. However, BCS 2.0 to 3.0 are most commonly observed within New Zealand sheep flocks and keeping ewes below BCS 2.0 has already been shown to negatively affect pregnancy and lambing (Kenyon et al. 2014) and is unlikely to be targeted by New Zealand farmers. Furthermore, the current experiments utilised ewes with a natural variation in their BCS range, meaning that the ewes were not manipulated in order to achieve BCS of 2.0, 2.5 and 3.0. It is possible that the effects of ewe BCS on behaviour may have been different had the BCS been induced. Further, ewe BCS may be more relevant at other times (Dwyer 2003), but this was not investigated here.

Practical implications of the results

Ewe BCS and feeding treatments

From a behavioural perspective there appears to be no overall advantage for farmers to feed ewes, managed under extensive pastoral conditions, *ad libitum* instead of feeding levels aimed to meet pregnancy maintenance requirements in mid- to late-pregnancy (chapters two to five). Furthermore, literature reports little advantage, in terms of production outcome (lamb live weight, total litter weight at weaning and lamb survival), in feeding twin- and triplet-bearing ewes *ad libitum* nutrition (Kenyon et al. 2012a; 2012b; 2013; Corner-Thomas et al. 2015). Ewe and lamb behavioural changes are more likely to appear under nutritional restriction (Dwyer et al. 2003).

Chapters two to five showed that a BCS of between 2.0 and 3.0, as measured between P72 and P98, had a minimal effect on ewe and lamb behaviour. Literature on the effect of twin- and triplet-bearing ewe mid-pregnancy BCS on production traits agree (Kenyon et al. 2012a; 2012b; 2013; Corner-Thomas et al. 2015). The results suggest that, over this range, lifting ewe BCS from 2.0 to 3.0 before mid-pregnancy does not improve behaviour.

Survival

It is clear that the association between lamb behaviour and survival differs between twin- and triplet-born lambs. The time taken for twin- and triplet-born lambs to follow their dam after tagging and the time taken for twin-born lambs to reach the contact zone and triplet-born lambs to reach their dam in the triangle pen test were associated with survival. Nevertheless, the practical application of these behaviours to be used as a tool to predict survival of lambs is limited.

Future research that could be undertaken

Ewe and lamb behaviour

It is possible that within litter variation and the behaviour of a lamb in relation to that of its litter mates is more important that the behaviour of an individual lamb. Behavioural variation within litters, such as the time taken for a lamb to stand, suck or follow in relation to its litter mates could therefore be further explored. Time to follow was found to be linked to survival in both twin- and triplet-born lambs. Additional research exploring the time taken for a lamb to follow its dam in relation to the time of its litter-mates could provide further evidence as to the link between following and survival. Further research could also investigate the time between the ewe leaving her lambs (after contact has initially been made) and the lambs following. This may provide a more accurate picture of the behavioural interactions of the ewe and her lambs than the time from the start of the observation period until the lamb follows the ewe.

Additional research into the behaviour of the ewe in response to her lambs during the five-minute observation period would also be advantageous. Poindron et al. (1980) reported that the behaviour of the lamb is influenced by the behaviour of the ewe, and Everett-Hincks et al. (2005) suggested that ewe and lamb behaviour should be studied conjointly. The response of the ewe to lamb behaviours such as sucking attempts could provide additional information of the strength of the ewe-lamb bond.

Further research may benefit from investigating ewe and lamb behaviours conjointly over a longer period of time, for example several 5-minute observation periods over the first three days after birth, without the 'catch-and-release' part. This may provide a broader picture of the ewe-lamb bond and relationship. Especially sucking behaviour may be benefit from this method because better dams may not allow their lambs to suckle in the immediate aftermath of tagging but rather contact them and move them away from the observers.

Chapters two and three reported behavioural differences between lambs tested at 12 and 24 hours of age in the triangle pen test. Lambs tested at 24 hours were more mobile and displayed an increased ability to discriminate their dam than younger lambs. Future research may wish to investigate the association between lamb behaviour in the triangle at various ages (such as 6 hours, 12 hours and 24 hours) and survival in order to identify an optimal age window when survival could be predicted.

Future research may also benefit from recording the behaviour of the ewe during the triangle pen test. This could be especially useful when trying to interpret the bleating behaviour of the lamb and whether it was bleating in response to the dam.

Additional research may want to trial other forms of ewe-lamb discrimination or temperament tests. These tests may be used to further investigate the effects of ewe feeding levels during pregnancy on the test outcome or how the outcome effects survival. A number of these tests have previously been used by various authors to predict lamb survival and this has been discussed in more detail on page 11 and 12.

Overall, however, the results and methodologies from previous studies have been highly inconsistent.

Vocalisation

Ewe BCS and feeding treatments influenced the number of ewe and lamb high- and low-pitched bleats, and to a lesser extent, the percentage of ewes and lambs that bleated in chapters two to five. The results presented in chapters two to five are highly inconsistent and based on these results no clear conclusion can be made about the meaning of the vocalisation. Is possible that the way in which the observations were recorded did not adequately reflect vocalisation behaviour. A large number of high-pitched bleats, for example, may have different meanings depending on the situation the in which they are emitted. Recording the distance between the ewe and her lambs at the time of vocalisation, or the relationship of vocalisation to other behaviours expressed (i.e. walking, sitting etc.) could provide additional information regarding ewe and lamb communications. Observations of whether or not a lamb bleat was reciprocated by the ewe could also provide valuable information as to the ewe-lamb relationship. Lastly, in the current study high- and low-pitched bleats were distinguished by both mouth movement (open mouth for high-pitched bleats and closed mouth for low-pitched bleats) and sound (high-pitched versus low-pitched). Future studies may wish to record the audio of the bleats for more thorough investigation between pitch-type. Further research in vocalisation is warranted, especially since both high- and low-pitched vocalisation was shown to be associated with lamb survival in chapter six.

Conclusion

The results presented indicate that for ewes with BCS in the range of 2.0 to 3.0 at mid-pregnancy, offering unrestricted grazing in mid- to late-pregnancy had little effect on ewe and lamb behaviour. Therefore, it appears that there is no advantage, in ewe and lamb behaviour, for New Zealand farmers to feed their ewes *ad libitum* when the ewes have a BCS of 2.0 to 3.0 in mid-pregnancy.

The majority of the behaviours observed in the paddock and in the triangle pen test were not associated with survival in either twin- or triplet-litters. These behaviours are, hence, not suitable to predict the survival of lambs. There is evidence, however, to suggest that a twin-born lamb that appears to have a greater need for its dam by quickly following her when she moves away may have a decreased chance of survival, while the opposite appears to be the case for triplet-born lambs. Further, the time taken for twin-born lambs to reach the contact zone and for triplet-born lambs to reach the dam during the triangle pen test was linked to survival. However, the practical application of these behaviours as a tool to predict lamb survival is limited.

Appendix 1

The aim of this study was to determine if the behaviours recorded after tagging are correlated. This analysis included data from 425 twin-bearing ewes, 850 twin-born lambs, 277 triplet-bearing ewes and 831 triplet-born lambs.

Twin- and triplet-born lambs were analysed separately. Lambs that did not stand, contact, suck or follow within the five minute observation period were censored at 301 seconds. Blank cells in the tables indicate insufficient data to conduct the analysis.

The effect of the probability of a behaviour being exhibited on the probability of another behaviour being exhibited was calculated using a logistic regression procedure. All behaviours were tested as both the dependant and the independent variable. For example the effect of the probability of standing was tested on all other behaviour variables (ewe-lamb contact, suck from and follow dam, emitted high- and low-pitched bleats) and the effect of the probability of all other behaviour variables were tested on the probability of standing. Year of study, sex of the lamb and the behaviour variable were included as fixed effects and lamb birth weight as a covariate. The effect of the time taken to exhibit a behaviour and the number of bleats emitted on the probability of another behaviour being exhibited was also analysed using the logistic procedure using the same fixed effects and covariate.

The time taken for lambs to exhibit each vigour behaviour, ewe MBS and the number of high- and low-pitched bleats emitted by the ewe and the lamb were analysed for correlations using a Spearman's Correlation analysis.

The effect of the probability of the behaviour being exhibited on the time taken to exhibit other behaviours or on the number of bleats emitted was analysed using the non-parametric Kruskal-Wallis test. The probability of the behaviour being exhibited was used as the fixed effect with the time taken to exhibit other behaviours (or number of bleats) included as the dependant variable.

Table 1. The effect, within twin-born lambs, of the probability of a behaviour being exhibited in the in the triangle pen test on of the probability of a behaviour being exhibited in the in the paddock. The independent variable is displayed horizontally at the top, with the dependent variable displayed vertically on the left side.

	Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat
Stand		13.9 (7.5-25.6) <.0001			6.4 (3.7-11.1) <.0001	9.4 (5.5-16.4) <.0001	2.0 (1.1-3.8) 0.0263	6.7 (3.7-12.2) <.0001
Contact	13.7 (7.5-25.0) <.0001				5.4 (3.0-9.6) <.0001	3.7 (2.2-6.3) <.0001	5.0 (2.7-9.1) <.0001	22.7 (11.8-43.5) <.0001
Suck				1.2 (0.9-1.6) 0.3123	1.6 (1.0-2.5) 0.0603	1.9 91.2-3.0) 0.0052	1.0 (0.6-1.5) 0.8683	1.7 (1.0-2.8) 0.0578
Follow			1.2 (0.9-1.6) 0.3045		2.6 (1.7-3.9) <.0001	2.3 (1.6-3.4) <.0001	1.8 (1.2-2.8) 0.0031	4.0 (2.5-6.5) <.0001
Lamb high bleat	6.4 (3.7-11.1) <.0001	5.5 (3.0-9.7) <.0001	1.6 (1.0-2.6) 0.0565	2.6 (1.7-3.9) <.0001		2.7 (1.7-4.3) <.0001	1.8 (1.1-3.1) 0.0168	2.2 (1.3-3.8) 0.0033
Lamb low bleat	9.4 (5.5-16.4) <.0001	3.7 (2.2-6.3) <.0001	1.9 (1.2-3.0) 0.0049	2.3 (1.63-3.4) <.0001	2.8 (1.7-4.3) <.0001		1.0 (0.6-1.7) 0.8835	3.2 (1.9-5.2) <.0001
Ewe high bleat	2.1 (1.1-3.9) 0.0214	5.1 (2.8-9.3) <.0001	1.0 (0.6-1.5) 0.8631	1.9 (1.2-2.8) 0.0002	1.9 (1.1-3.1) <.0001	1.0 (0.6-1.7) 0.9394		2.8 (1.7-4.8) <.0001
Ewe low bleat	6.7 (3.7-12.2) <.0001	22.7 (11.8-43.5) <.0001	1.7 (1.0-2.8) 0.0548	4.0 (2.5-6.5) <.0001	2.2 (1.3-3.8) 0.0034	3.2 (1.9-5.3) <.0001	2.8 (1.7-4.9) <.0001	

Table 2. The effect within triplet-born lambs, of the probability of one behaviour being exhibited on of the probability of another behaviour being exhibited. The independent variable is displayed horizontally at the top, with the dependent variable displayed vertically on the left side

	Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat
Stand		14.9 (9.0-25.0) <.0001			10.6 (6.5-17.2) <.0001	8.3 (4.9-14.3) <.0001	1.3 (0.8-2.4) 0.2984	8.6 (5.2-14.5) <.0001
Contact	14.7 (8.8-24.4) <.0001				4.9 (3.1-7.7) <.0001	3.2 (2.1-4.8) <.0001	1.9 (1.2-3.1) 0.0083	47.6 (26.3-90.9) <.0001
Suck				0.6 (0.4-0.9) 0.024	1.3 (0.8-2.2) 0.32	1.7 (1.1-2.4) 0.0075	0.8 (0.5-1.2) 0.2571	23.8 (3.3-166.7) 0.0017
Follow			0.6 (0.4-0.9) 0.0239		2.3 (1.4-3.6) 0.0004	1.5 (1.1-2.1) 0.0083	1.7 (1.2-2.6) 0.0083	6.7 (3.5-12.8) <.0001
Lamb high bleat	10.6 (6.5-17.2) <.0001	4.9 (3.2-7.7) <.0001	1.3 (0.8-2.2) 0.3232	2.3 (1.4-3.6) 0.0004		2.2 (1.5-3.3) <.0001	1.0 (0.6-1.7) 0.9052	3.8 (2.3-6.3) <.0001
Lamb low bleat	8.3 (4.9-14.3) <.0001	3.2 (2.1-4.8) <.0001	1.7 (1.1-2.4) 0.0076	1.5 (1.1-2.1) 0.0084	2.2 (1.5-3.2) <.0001		1.3 (0.9-1.9) 0.2509	3.8 (2.4-6.2) <.0001
Ewe high bleat	1.3 (0.8-2.4) 0.3017	1.9 (1.2-3.1) 0.0083	0.8 (0.5-1.2) 0.2604	1.7 (1.1-2.6) 0.0088	1.0 (0.6-1.7) 0.9161	1.3 (0.9-1.9) 0.0956		4.4 (2.7-7.2) <.0001
Ewe low bleat	8.6 (5.2-14.30) <.0001	47.6 (26.3-90.9) <.0001	23.8 (3.3-166.7) 0.0017	6.7 (3.5-12.8) <.0001	3.8 (2.3-6.3) <.0001	3.8 (2.4-6.2) <.0001	4.3 (2.6-7.1) <.0001	

Table 3. The effect, within twin-born lambs, of the time taken to exhibit a particular behaviour, the number of bleats emitted and ewe MBS on the time taken to exhibit another behaviour, the number of bleats emitted and ewe MBS.

		Time to				Number of				Scale
		Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat	MBS
Time to	Stand		0.2997 <.0001			-0.0637 0.0714	-0.1728 <.0001	0.1082 0.002	-0.0939 0.0067	-0.1097 0.0016
	Contact	830				0.23696 <.0001	-0.2002 <.0001	0.3817 <.0001	-0.1674 <.0001	-0.5348 <.0001
	Suck				0.0058 0.8746	0.1129 0.002	-0.1792 <.0001	0.1055 0.0036	0.0023 0.9488	-0.1656 <.0001
	Follow			756		-0.0116 0.7504	-0.0796 0.0258	0.0647 0.0723	-0.0053 0.8828	0.0875 0.0143
Number of	Lamb high bleat	802	758	746	805		-0.1203 0.0006	0.2478 <.0001	0.1684 <.0001	-0.1904 <.0001
	Lamb low bleat	828	784	772	831	808		0.0157 0.6543	0.07 0.0432	0.1131 0.0011
	Ewe high bleat	816	772	760	819	799	820		-0.0773 0.0265	-0.3887 <.0001
	Ewe low bleat	832	788	776	835	810	836	824		0.1666 <.0001
Scale	MBS	827	783	773	830	805	831	819	835	

Table 4. The effect, within triplet-born lambs, of the time taken to exhibit a particular behaviour, the number of bleats emitted and ewe MBS on the time taken to exhibit another behaviour, the number of bleats emitted and ewe MBS.

		Time to				Number of				Scale
		Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat	MBS
Time to	Stand		0.2997 <.0001			-0.0616 0.1143	0.021 0.6462	0.2971 <.0001	0.1505 <.0001	-0.0734 0.0543
	Contact	680				0.19 <.0001	0.007 0.8817	0.4908 <.0001	-0.0919 0.0157	0.1773 0.0012
	Suck				-0.0262 0.5251	0.0447 0.6015	-0.0027 0.9781	0.1984 0.022	-0.0654 0.414	-0.143 0.0819
	Follow			153		0.0311 0.5751	-0.1169 0.075	0.2269 <.0001	0.3237 <.0001	0.1773 0.0012
Number of	Lamb high bleat	658	327	139	635		-0.038 0.4253	0.1732 <.0001	-0.021 0.5928	-0.2076 <.0001
	Lamb low bleat	479	233	111	453	443		0.069 0.1533	0.1515 0.001	0.0121 0.7922
	Ewe high bleat	623	311	133	613	598	429		-0.0116 0.7679	-0.3389 <.0001
	Ewe low bleat	681	349	158	690	649	470	649		0.2389 <.0001
Scale	MBS	688	333	149	668	666	478	660	705	

Table 5. The effect, within twin-born lambs, of the time taken to exhibit a behaviour and the number of bleats emitted (displayed vertically on the left side) on the probability of another behaviour being exhibited (displayed horizontally at the top).

		Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat
Time to	Stand		-0.0080 <0.0001			-0.0061 <.0001	-0.0063 <.0001	-0.0009 0.4121	-0.0071 <.0001
	Contact	-0.00711 <0.0001				-0.0036 0.0006	-0.0040 <.0001	-0.0029 0.0072	-0.0119 <.0001
	Suck				-0.0002 0.8318	0.0005 0.7248	-0.0025 0.0677	0.0024 0.0624	-0.0036 <.0001
	Follow			0.0012 0.1800		-0.0039 0.0013	-0.0020 0.0444	-0.0013 0.2542	0.0072 <.0001
Number of	Lamb high bleat	0.0648 <.0001	0.0036 0.6320	0.0053 0.0186	0.0072 0.1105		-0.0179 0.0003	0.0030 0.5912	0.0141 0.0862
	Lamb low bleat	0.2632 <.0001	0.0718 0.0027	0.0064 0.3569	0.0053 0.4392	0.0083 0.3507		0.0317 0.0014	0.0210 0.0461
	Ewe high bleat	-0.0122 0.1731	-0.0343 <.0001	-0.0236 0.0018	-0.0066 0.2583	0.00001 0.9984	-0.0081 0.2465		-0.0284 <.0001
	Ewe low bleat	0.0028 0.0028	0.0636 <.0001	0.0108 0.0052	0.0056 0.1414	0.0065 0.2619	0.0162 0.0026	0.0236 <.0001	

Table 6. The effect, within triplet-born lambs, of the time taken to exhibit a behaviour and the number of bleats emitted (displayed vertically on the left side) on the probability of another behaviour being exhibited (displayed horizontally at the top).

		Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat
Time to	Stand		-0.0033 0.0843			-0.0030 0.1088	0.0018 0.1934	0.0043 0.0547	0.0029 0.1877
	Contact	0.0319 0.0030				0.0031 0.2262	-0.0024 0.1185	0.0646 <.0001	-0.0012 0.8470
	Suck				0.0021 0.4412	-0.0073 0.0515	0.0001 0.9543	0.0010 0.7590	
	Follow			0.0015 0.4278		0.0029 0.2460	0.0037 0.0157	0.0023 0.2464	
Number of	Lamb high bleat	0.0710 0.2637	-0.0166 0.0042	-0.0313 <.0001	-0.0034 0.4518		-0.0050 0.2386	0.0110 0.0191	-0.0664 <.0001
	Lamb low bleat	0.0548 0.0005	-0.0034 0.8975	-0.0193 0.3488	0.0043 0.8034	-0.0220 0.3042		-0.0084 0.1845	-0.0145 0.0195
	Ewe high bleat	-0.0303 <.0001	-0.0716 0001	-0.0399 <.0001	-0.0303 <.0001	-0.0100 0.1646	-0.0161 0.0048		-0.0286 0.5136
	Ewe low bleat	0.0097 0.1364	0.0261 0.0052	0.0059 0.1161	-0.0034 0.3717	0.0046 0.3807	0.0094 0.0104	-0.0696 0.0002	

Table 7 a & b. The effect, within (a) twin- and (b) triplet-born lambs, of the probability of a behaviour being exhibited (displayed horizontally at the top) on the time taken to exhibit another behaviour and the number of bleats emitted (displayed vertically on the left).

	(a)	Stand		Contact		Suck		Follow		Lamb high bleat		Lamb low bleat		Ewe high bleat		Ewe low bleat	
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Time to	Stand			15[a]	33[b]					21[a]	37[b]	21[a]	41[b]	22	25	21[a]	52[b]
	Contact	21[a]	73[b]							12[a]	16[b]	14.5[a]	25[b]	9[a]	17[b]	14	17
	Suck							301	301	301	301	301	301	301	301	301	301
	Follow					212	201			190[a]	301[b]	186[a]	301[b]	200	233	189.5[a]	301[b]
Number of	Lamb high bleat	9[b]	3[a]	9	6.5	7	9	7[a]	9[b]			11	8	10	3	9[b]	4[b]
	Lamb low bleat	5[b]	1[a]	4[b]	1[a]	5[b]	4[a]	5[b]	3[a]	4	3			4	3	4	3
	Ewe high bleat	5	4	5	5	5	5	5	5	6[b]	4[a]	5	6			5	7
	Ewe low bleat	13[b]	6[a]	14[b]	0.5[a]	12	12	14[b]	10[a]	13[b]	9[a]	13[b]	9[a]	13[b]	9[a]		

	(b)	Stand		Contact		Suck		Follow		Lamb high bleat		Lamb low bleat		Ewe high bleat		Ewe low bleat	
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Time to	Stand			25	35					24[a]	32[b]	28[b]	23[a]	26.5[b]	16[a]	24	31
	Contact	18	14							18	14	16	18	20[b]	5[a]	18	11
	Suck							147	174	158[a]	195[b]	168	165	173	160	165.5	32
	Follow					129	117			121	90	3	2	119	140	120	118.5
Number of	Lamb high bleat	14[b]	6[a]	12	22	8[a]	15[b]	15[b]	11[a]			12	13	13	11	13	17
	Lamb low bleat	3[b]	2[a]	3	3	3	3	3	4	3[a]	5[b]			3[a]	5[b]	3	3
	Ewe high bleat	8[a]	15[b]	7[a]	29[b]	5[a]	9[b]	7[a]	10[b]	8	10	7[a]	10[b]			8[a]	30[b]
	Ewe low bleat	20	19	21[b]	12.5[a]	26	19	14[a]	26[b]	21	18	25[b]	15.5[a]	21	17		

Appendix 2

The aim of the current study was to determine whether the behaviours of ewes and lambs at tagging were related to the behaviours observed during the five minute triangle pen test.

The analysis included data from 425 twin-bearing ewes and their 850 twin-born lambs, in addition to 277 triplet-bearing ewes and their 831 triplet-born lambs.

The analyses of twin- and triplet-born lambs were conducted separately. Lambs that did not perform a particular behaviour within the five minute observation period were censored at 301 seconds. Blank cells in the tables indicate insufficient data to conduct the analysis.

The probability of one behaviour being expressed on the expression of another behaviour was calculated using the logistic regression procedure. Year of study, the behaviour of interest and sex of the lamb were included in the model as fixed effects and lamb birth weight as a covariate. All behaviours were tested as both the dependant and the independent variable. The relationship between the time taken to exhibit a behaviour, or the number of bleats emitted, and the probability of a behaviour being exhibited was also analysed using the logistic procedure using the same fixed effects and covariate.

The correlations of time taken for lambs to exhibit each behaviour, the time spent exhibiting the behaviour, ewe MBS and the number of high- and low-pitched bleats emitted by the ewe and the lamb were analysed for correlations using a Spearman's Correlation analysis.

The probability of a behaviour being exhibited on the time taken to exhibit other behaviours, the time spent exhibiting behaviours or the number of bleats emitted was analysed using the non-parametric Kruskal-Wallis test. The probability of the behaviour being exhibited was used as the fixed effect with the time taken to exhibit behaviours (or the number of bleats) included as the dependant variable. The probability of a lamb spending time in the contact zone and spending time with the dam in the triangle test were excluded from analysis because these behaviours were conditional to the probability of the lamb reaching the contact zone and reaching the dam. They hence produced the same results.

Table 1. The odds of a twin-born lamb exhibiting a paddock behaviour for those lambs that expressed a triangle pen test behaviour compared with those that did not express that behaviour. The results are presented as odds ratio with the 95% confidence interval given in parenthesis and p-value given below.

			Paddock behaviours							
Triangle pen test behaviours			Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat
	Time spent	Walking	2.3 (0.3-21.3) 0.4596		0.6 (0.1-2.7) 0.5030	0.3 (0.03-2.2) 0.2132	2.9 (0.5-16.9) 0.2262	2.2 (0.4-11.8) 0.3724	0.6 (0.1-5.5) 0.6729	
		Sitting	0.9 (0.3-2.8) 0.8254	1.6 (0.4-6.2) 0.5178	1.2 (0.6-2.5) 0.5327	0.8 (0.4-1.6) 0.5489	0.8 (0.3-1.9) 0.5898	1.4 (0.6-3.2) 0.4900	1.2 (0.6-2.7) 0.5997	1.4 (0.5-4.1) 0.5596
		With dam	0.4 (0.1-3.2) 0.3924	0.5 (0.1-4.0) 5120	0.6 (0.3-1.4) 0.2279	1.0 (0.5-2.4) 0.9140	1.6 (0.6-4.5) 0.3741	0.5 (0.1-1.7) 0.2598	2.3 (0.9-5.8) 0.0769	0.7 (0.1-3.1) 0.6117
		With alien ewe	1.4 (0.4-5.0) 0.5610	1.1 (0.5-2.3) 0.7725	1.0 (0.4-2.1) 0.9217	1.3 (0.3-5.0) 0.7524	0.9 (0.3-2.5) 0.8552	0.4 (0.1-1.2) 0.1145	1.4 (0.6-3.2) 0.4724	0.4 (0.1-1.8) 0.2355
		In contact zone	1.4 (0.2-12.2) 0.7482	1.8 (0.2-15.9) 0.5955	0.9 (0.2-3.6) 0.8975	1.2 (0.3-4.1) 0.8146	1.6 (0.3-8.1) 0.5928		2.8 (0.7-11.1) 0.1377	1.0 (0.1-8.8) 0.9685
	Time to	Reach dam	0.4 (0.05-3.1) 0.3762	0.5 (0.1-3.9) 0.4958	0.5 (0.2-1.2) 0.1338	1.0 (0.4-2.2) 0.9650	1.6 (0.6-4.4) 0.3973	0.5 (0.1-1.7) 0.2423	2.2 (0.9-5.4) 0.1006	0.6 (0.1-3.0) 0.5807
		Reach contact zone	1.3 (0.2-11.1) 0.8060	1.7 (0.2-14.5) 0.6417	0.7 (0.2-2.4) 0.5491	1.0 (0.3-3.5) 0.9990	1.5 (0.3-7.5) 0.6415		2.4 (0.6-9.0) 0.2017	1.0 (0.1-8.0) 0.9669
	Number of	Low bleats	1.1 (0.3-3.7) 0.9199	0.2 (0.03-1.8) 0.1639	1.2 (0.6-2.5) 0.6605	0.6 (0.3-1.2) 0.1223	0.3 (0.1-1.0) 0.0471	1.4 (0.6-3.2) 0.4789	0.6 (0.2-1.5) 0.2705	0.7 (0.2-2.4) 0.6233
		High bleats						2.5 (0.2-29.4) 0.4769		

Table 2. The odds of a twin-born lamb exhibiting a triangle pen test behaviour for those lambs that expressed a paddock behaviour compared with those that did not express that behaviour. The results are presented as odds ratio with the 95% confidence interval given in parenthesis and p-value given below.

			Triangle pen test behaviours								
			Walking	Sitting	With dam	With alien ewe	Time in contact zone	To reach dam	To reach contact zone	Low bleats	High bleats
Paddock behaviours	Time to	Stand	2.1 (0.2-19.2) 0.5288	0.9 (0.3-2.8) 0.8252	0.4 (0.03-3.2) 0.3873	1.4 (0.4-5.0) 0.4083	1.4 (0.2-11.9) 0.7622	0.4 (0.02-3.1) 0.3722	1.3 (0.1-10.8) 0.8261	1.1 (0.3-3.7) 0.9369	
		Contact		1.6 (0.4-6.3) 0.5203	0.5 (0.1-4.0) 0.4973	1.2 (0.3-4.9) 0.7710	1.7 (0.2-15.2) 0.6141	0.5 (0.1-3.9) 0.4837	1.6 (0.2-13.9) 0.6720	0.2 (0.02-1.8) 0.1584	
		Suck	0.6 (0.1-2.6) 0.4818	1.2 (0.6-2.5) 0.5291	0.6 (0.3-1.4) 0.2314	1.0 (0.4-2.1) 0.9216	0.9 (0.2-3.6) 0.9014	0.5 (0.2-1.2) 0.1358	0.7 (0.2-2.4) 0.5498	1.2 (0.6-2.5) 0.6580	
		Follow	0.2 (0.03-2.1) 0.1925	0.8 (0.4-1.6) 0.5502	1.0 (0.5-2.4) 0.9171	1.1 (0.5-2.3) 0.7772	1.2 (0.3-4.1) 0.8199	1.0 (0.4-2.2) 0.7353	1.0 (0.3-3.4) 0.9867	0.6 (0.3-1.2) 0.1209	
	Number of	Lamb high bleat	2.6 (0.5-14.7) 0.2807	0.8 (0.3-1.9) 0.5827	1.6 (0.6-4.5) 0.3929	0.9 (0.3-2.5) 0.8530	1.5 (0.3-7.6) 0.6334	1.5 (0.5-4.4) 0.4126	1.4 (0.3-7.1) 0.6855	0.3 (0.1-1.0) 0.0473	
		Lamb low bleat	1.9 (0.4-10.4) 0.4514	1.4 (0.6-3.2) 0.4928	0.5 (0.1-1.7) 0.2489	0.4 (1.2-0.1) 0.1136		0.5 (0.1-1.7) 0.2349		1.4 (0.6-3.2) 0.4794	2.5 (0.2-28.6) 0.4751
		Ewe high bleat	0.6 (0.1-5.2) 0.6355	1.2 (0.6-2.7) 0.5915	2.3 (0.9-5.9) 0.0730	1.3 (3.1-0.6) 0.4950	2.8 (0.7-10.8) 0.1268	2.2 (0.9-5.4) 0.0983	2.4 (0.6-8.7) 0.1927	0.6 (0.2-1.5) 0.2542	
		Ewe low bleat		1.4 (0.5-4.1) 0.56.5	0.7 (0.1-3.1) 0.6023	0.4 (0.1-1.8) 0.2341	1.0 (0.1-8.7) 0.9703	0.6 (0.1-3.0) 0.5743	0.9 (0.1-7.8) 0.9541	0.7 (0.2-2.5) 0.6294	

Table 3. The odds of a triplet-born lamb exhibiting a paddock behaviour for those lambs that expressed a triangle pen test behaviour compared with those that did not express that behaviour. The results are presented as odds ratio with the 95% confidence interval given in parenthesis and p-value given below.

			Paddock behaviours							
			Stand	Contact	Suck	Follow	Lamb High-bleat	Lamb Low-bleat	Ewe High-bleat	Ewe Low-bleat
Triangle pen test behaviours	Time spent	Walking	1.3 (0.3-5.8) 0.7658	0.8 (0.2-3.0) 0.7510	1.7 (0.4-7.5) 0.5146	1.7 (0.5-5.4) 0.3944	1.5 (0.5-4.9) 0.4857	1.7 (0.6-4.8) 0.2847	1.7 (0.5-5.7) 0.3537	0.4 (0.1-3.4) 0.4326
		Sitting	0.5 (0.2-1.3) 0.1415	1.0 (0.5-2.2) 0.9961	1.1 (0.5-2.5) 0.7520	0.7 (0.3-1.5) 0.3651	0.8 (0.3-1.7) 0.5403	1.0 (0.5-2.0) 0.9824	1.0 (0.4-2.3) 0.9583	0.7 (0.3-1.7) 0.3947
		With dam	0.8 (0.3-2.4) 0.6864	0.5 (0.2-1.3) 0.1407	0.9 (0.4-2.0) 0.8454	2.7 (0.6-12.7) 0.2022	1.8 (0.8-3.8) 0.1286	0.8 (0.4-1.6) 0.6133	0.7 (0.3-1.8) 0.4799	0.6 (0.2-1.8) 0.3797
		With alien ewe	0.7 (0.1-5.6) 0.7290	1.2 (0.3-4.4) 0.8231	1.4 (0.3-6.7) 0.6496	2.2 (1.0-4.6) 0.0396	2.1 (0.6-7.2) 0.2399	1.1 (0.4-3.4) 0.8432	0.9 (0.2-4.3) 0.8982	2.3 (0.6-9.2) 0.2221
		In contact zone	0.8 (0.2-2.7) 0.6727	1.8 (0.8-4.0) 0.1307	1.6 (0.6-4.3) 0.3734	2.2 (0.9-5.2) 0.0776	1.1 (0.4-2.7) 0.8338	1.6 (0.8-3.2) 0.1907	0.6 (0.2-1.8) 0.3560	1.9 (0.8-5.0) 0.1585
	Time to	Reach dam	0.7 (0.1-5.6) 0.7290	1.2 (0.3-4.4) 0.8231	1.4 (0.3-6.7) 0.6496	2.7 (0.6-12.7) 0.2022	2.1 (0.6-7.2) 0.2399	1.1 (0.4-3.4) 0.8432	0.9 (0.2-4.3) 0.8109	2.3 (0.6-9.2) 0.2221
		Reach contact zone	0.8 (0.2-2.7) 0.6727	1.8 (0.8-4.0) 0.1307	1.6 (0.6-4.3) 0.3734	2.2 (0.9-5.2) 0.0776	1.1 (0.4-2.7) 0.8338	1.6 (0.8-3.2) 0.1907	0.6 (0.2-1.8) 0.3560	1.9 (0.8-5.0) 0.1585
	Number of	Low- bleats	1.1 (0.5-2.3) 0.8409	1.4 (0.8-2.5) 0.1925	0.8 (0.5-1.5) 0.5598	1.2 (0.7-2.0) 0.4301	0.6 (0.3-1.1) 0.1203	1.2 (0.7-1.9) 0.5048	0.5 (0.3-1.0) 0.0570	0.9 (0.5-1.9) 0.8411
		High-bleats			0.3 (0.1-2.1) 0.2431	0.9 (0.1-5.3) 0.8711	1.1 (0.1-10.0) 0.9392	0.4 (0.01-4.0) 0.4649		

Table 4. The odds of a triplet-born lamb exhibiting a paddock behaviour for those lambs that expressed a triangle pen test behaviour compared with those that did not express that behaviour. The results are presented as odds ratio with the 95% confidence interval given in parenthesis and p-value given below.

			Triangle pen test behaviours								
			Walking	Sitting	With dam	With alien ewe	Time in contact zone	To reach dam	To reach contact zone	Low bleats	High bleats
Paddock behaviours	Time to	Stand	1.3 (0.3-5.8) 0.7679	0.5 (0.2-1.3) 0.1422	0.7 (0.1-5.8) 0.7545	0.8 (0.3-2.4) 0.6966	0.7 (0.2-2.7) 0.6548	0.7 (0.1-5.8) 0.7545	0.7 (0.2-2.7) 0.6548	1.1 (0.5-2.3) 0.8422	
		contact	0.8 (0.2-3.0) 0.7511	1.0 (0.5-2.2) 0.9974	1.2 (0.3-4.4) 0.8284	0.5 (0.2-1.2) 0.1343	1.8 (0.8-4.0) 0.1295	1.2 (0.3-4.4) 0.8284	1.8 (0.8-4.0) 0.1295	1.4 (0.8-2.5) 0.1927	
		Suck	1.7 (0.4-7.5) 0.5149	10.0 (0.2-1.3) 0.7512	1.5 (0.3-6.9) 0.6378	0.9 (0.4-2.0) 0.8452	1.6 (0.6-4.3) 0.3748	1.5 (0.3-6.9) 0.6378	1.6 (0.6-4.3) 0.3748	0.8 (0.5-1.5) 0.5594	0.3 (0.1-2.1) 0.2456
		Follow	1.6 (0.5-5.2) 0.4193	0.7 (0.3-1.5) 0.3633	2.7 (0.6-12.5) 0.2088	2.2 (1.0-4.6) 0.0434	2.2 (0.9-5.3) 0.0716	2.7 (0.6-12.5) 0.2088	2.2 (0.9-5.3) 0.0716	1.2 (0.7-2.0) 0.4340	0.8 (0.1-5.2) 0.8565
	Number of	Lamb high bleat	1.5 (0.5-4.9) 0.4886	0.8 (0.3-1.7) 0.5458	2.0 (0.6-6.9) 0.2839	1.8 (0.8-3.7) 0.1373	1.1 (0.4-2.6) 0.8912	2.0 (0.6-6.9) 0.2839	1.1 (0.4-2.6) 0.8912	0.6 (1.1-0.3) 0.1197	1.1 (0.1-9.9) 0.9492
		Lamb low bleat	1.7 (0.6-4.8) 0.2838	1.0 (0.5-2.0) 0.7099	1.1 (0.4-3.5) 0.8175	0.8 (0.4-1.6) 0.6132	1.6 (0.8-3.3) 0.1809	1.1 (0.4-3.5) 0.8175	1.6 (0.8-3.3) 0.1809	1.2 (0.7-1.9) 0.5036	0.4 (0.03-4.0) 0.4592
		Ewe high bleat	1.7 (0.5-5.7) 0.3549	1.0 (0.4-2.3) 0.9573	0.9 (0.2-4.5) 0.9209	0.7 (0.3-1.8) 0.4917	0.6 (0.2-1.8) 0.3624	0.9 (0.2-4.5) 0.9209	0.6 (0.2-1.8) 0.3624	0.5 (0.3-1.0) 0.0570	
		Ewe low bleat	0.4 (0.1-3.4) 0.5806	0.7 (0.3-1.7) 0.3968	2.4 (0.6-9.3) 0.2203	0.6 (0.2-1.8) 0.3717	1.9 (0.8-4.9) 0.1644	2.4 (0.6-9.3) 0.2203	1.9 (0.8-4.9) 0.1644	0.9 (0.5-1.9) 0.8411	

Table 5. Among twin-born lambs, the correlations between MBS, the time taken to exhibit a behaviour and the number of bleats emitted in the paddock and the time taken to express a behaviour in the triangle pen test. The results are presented as the correlation coefficient with the p-value given below.

		Triangle pen test behaviours								
		Time spent					Time to		Number of	
		Walk	Sit	With dam	With alien ewe	In contact zone	Reach dam	Reach contact zone	Low bleats	High bleats
Paddock behaviours	Stand	-0.04565 0.5361	0.00978 0.8946	0.05277 0.4744	-0.00855 0.9078	0.09619 0.1915	-0.06344 0.3897	-0.05461 0.4591	0.06504 0.3778	0.00287 0.9691
	Follow	-0.03081 0.6763	-0.03843 0.6026	0.08376 0.2557	-0.07356 0.3183	0.02615 0.7231	-0.07738 0.2938	-0.00018 0.9981	0.01818 0.8054	0.01012 0.8912
	Suck	-0.20104 0.0059	0.02616 0.723	0.07104 0.3352	-0.05276 0.4745	0.01002 0.892	-0.0396 0.5915	0.05027 0.4956	-0.05497 0.4561	-0.0583 0.4305
	Contact	0.06028 0.4138	-0.02685 0.716	0.00237 0.9743	0.08386 0.2551	0.06316 0.3918	-0.02494 0.7354	-0.13507 0.066	0.11041 0.1336	-0.22227 0.0024
	Lamb high-bleat	0.09816 0.2029	-0.03026 0.6953	0.08733 0.2575	-0.04979 0.519	0.15051 0.0501	0.02485 0.7477	-0.07939 0.3034	-0.10004 0.1943	0.22113 0.0039
	Lamb low-bleat	0.01093 0.8829	0.10209 0.1679	-0.01217 0.8697	0.02733 0.7127	0.09036 0.2225	-0.0111 0.8811	-0.07576 0.3067	0.16887 0.0219	0.14084 0.0572
	Ewe high-bleat	-0.03302 0.6635	-0.06749 0.3735	0.05732 0.4499	0.00126 0.9867	0.04326 0.5687	0.01072 0.8877	-0.04057 0.5929	-0.02355 0.7564	-0.04102 0.5899
	Ewe low-bleat	0.02372 0.748	0.04391 0.5517	0.14701 0.0452	-0.2020 0.0057	0.01892 0.7977	-0.06579 0.3723	0.01049 0.887	0.04998 0.4981	0.01795 0.8084
	MBS	-0.09447 0.1996	-0.1394 0.0577	-0.01908 0.796	-0.13686 0.0625	-0.06229 0.3983	0.12058 0.1011	0.17896 0.0145	-0.21812 0.0028	0.14647 0.0467

Table 6. Among triplet-born lambs, the correlations between MBS, the time taken to exhibit a behaviour and the number of bleats emitted in the paddock on the time taken to express a behaviour in the triangle pen test. The results are presented as the correlation coefficient with the p-value given below.

	Triangle pen test behaviours								
	Time spent					Time to		Number of	
Paddock behaviours	Walk	Sit	With dam	With alien ewe	In contact zone	Reach dam	Reach contact zone	Low bleats	High bleats
Stand	-0.16373 0.006	0.02737 0.6484	-0.06775 0.2585	-0.01044 0.8619	-0.09673 0.1063	0.10895 0.0831	0.10765 0.0780	0.09499 0.2207	-0.09283 0.1246
Follow	-0.1513 0.1163	0.03863 0.69	0.03219 0.7397	-0.01921 0.8429	0.04198 0.6647	0.09861 0.3193	0.13112 0.1762	-0.0859 0.4828	0.1503 0.1223
Suck	0.03958 0.7640	-0.01864 0.8876	0.0267 0.8395	-0.14902 0.2558	-0.09593 0.4659	0.03296 0.8094	0.2072 0.1186	0.10052 0.5716	0.17498 0.1889
Contact	-0.10561 0.0991	0.05776 0.3680	-0.12813 0.0451	0.06334 0.3235	-0.09371 0.1436	0.11712 0.0796	0.11261 0.0843	-0.05604 0.4943	-0.10202 0.1150
Lamb high bleat	0.06997 0.2647	0.00374 0.9525	-0.06221 0.3215	-0.01088 0.8625	-0.10263 0.1014	0.0146 0.8246	0.08868 0.1638	0.01764 0.8315	0.24863 <.0001
Lamb low bleat	0.01438 0.8411	-0.07266 0.3103	-0.05692 0.4269	-0.0254 0.7232	-0.03897 0.5866	0.06653 0.3709	0.01516 0.8351	0.26686 0.0031	0.18003 0.0122
Ewe high bleat	-0.06177 0.3202	0.0575 0.3548	-0.17102 0.0056	0.05411 0.384	-0.16681 0.0069	0.06515 0.3211	0.0336 0.597	-0.0293 0.7219	-0.0254 0.6859
Ewe low bleat	-0.17642 0.0035	0.07832 0.1978	0.07411 0.2231	-0.17094 0.0047	-0.07979 0.1896	0.03801 0.5505	0.07834 0.2054	0.13996 0.0757	-0.07907 0.1978
MBS	-0.07413 0.2035	-0.06957 0.2327	0.14491 0.0126	-0.1138 0.0505	0.03798 0.5151	-0.08893 0.1450	-0.01617 0.7857	-0.04109 0.5840	0.01035 0.8604

Table 7. Among twin-born lambs, the relationship between MBS, the time taken to express a behaviour and the number of bleats emitted in the paddock on the probability of a behaviour being expressed in the triangle pen test. The results are presented as the regression coefficient with the p-value given below.

			Triangle pen behaviour								
Paddock behaviours			Walk	Sit	With dam	With alien ewe	In contact zone	Reach dam	Reach contact zone	Low bleats	High bleats
	Time to	Stand	0.00007 0.9856	0.000475 0.7830	0.00281 0.5064	0.00004 0.9856	0.000878 0.7049	0.00105 0.7719	0.00151 0.5343	0.00216 0.2784	-0.00605 0.2336
		Contact	0.012 0.2751	-0.00089 0.6591	0.00134 0.7662	0.00163 0.4988	0.000293 0.9091	0.00202 0.6611	0.00006 0.9804	0.00571 0.0521	0.00647 0.1850
		Suck	0.00331 0.4086	0.00068 0.7287	0.000828 0.8295	0.000454 0.8353	0.00110 0.6515	0.00168 0.6355	0.000703 0.7799	0.3986 0.5278	
		Follow	0.00411 0.2421	0.000322 0.8301	0.00253 0.3966	-0.00038 0.8177	0.00171 0.3683	0.00188 0.5113	0.00197 0.3063	0.00103 0.5162	0.000258 0.9626
	Number of	Lamb high bleat	-0.0528 0.0049	0.00571 0.5810	0.00973 0.6938	0.00345 0.7685	0.00539 0.7128	0.0155 0.5435	0.0131 0.6374	-0.0146 0.1684	-0.0299 0.2635
		Lamb low bleat	0.2556 0.1404	-0.0349 0.2207	-0.00070 0.7801	0.0132 0.6441	0.0131 0.6319	-0.0130 0.7385	0.00309 0.8315	0.0876 0.0277	0.0399 0.5023
		Ewe high bleat	0.0534 0.3958	0.0067 0.6148	0.0147 0.6626	0.0204 0.2445	0.00183 0.9193	0.0200 0.5632	0.00395 0.8258	0.0114 0.4615	0.0351 0.3171
		Ewe low bleat	0.0165 0.2320	0.0101 0.2546	-0.0125 0.3262	-0.0146 0.0709	0.00076 0.9386	-0.0160 0.1749	0.00222 0.8326	0.00656 0.4457	0.0338 0.5285
		MBS	-0.3121 0.1722	0.2736 0.0761	-0.4592 0.0094	-0.5631 0.0896	-0.3907 0.0509	-0.6422 0.0504	-0.3539 0.0786	-0.3887 0.0194	2.0102 0.0598

Table 8. Among twin-born lambs, the relationship between the time taken to express a behaviour, time spent expressing a behaviour and the number of bleats emitted in the triangle pen test on the probability of a behaviour being expressed in the paddock. The results are presented as the regression coefficient with the p-value given below.

			Paddock behaviours							
Triangle pen test behaviours			Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat
	Time spent	Walking	-0.0016 0.7340	0.0025 0.6138	0.0032 0.2230	0.0068 0.0390	0.0017 0.6982	0.0043 0.1597	0.0008 0.0333	0.0056 0.3431
		Sitting	0.0013 0.5834	0.0004 0.8701	0.0009 0.5087	0.0016 0.2192	0.00015 0.9365	-0.0011 0.5256	-0.0013 0.4303	-0.0004 0.8715
		With dam	-0.0066 0.0708	0.0011 0.7297	-0.0023 0.1726	0.0006 0.7061	0.0002 0.9408	0.0006 0.7917	0.0031 0.1109	0.0017 0.4986
		With alien ewe	0.0093 0.0593	0.0034 0.4023	0.0026 0.1753	0.0022 0.2396	0.0013 0.6060	-0.0007 0.7797	0.0022 0.3009	0.0009 0.7654
		In contact zone	0.0006 0.8827	0.0021 0.5807	0.0005 0.8168	0.0018 0.3838	0.0020 0.4729	0.0022 0.4938	0.0022 0.3621	0.0017 0.5806
	Time to	Reach dam	0.0091 0.0952	0.0031 0.3964	0.0022 0.1498	0.0006 0.7046	0.00003 0.9886	0.0023 0.2848	-0.0029 0.0862	0.0007 0.7720
		Reach contact zone	0.0026 0.6080	0.0004 0.9172		-0.0019 0.3353	0.0011 0.6837	0.0048 0.2399	0.0021 0.3600	0.0005 0.9023
	Number of	Low bleats	0.0024 0.9451	0.0149 0.4111	-0.0362 0.1612	0.0234 0.1784	-0.0449 0.0304	0.0273 0.3725	0.0025 0.9038	0.0393 0.3639
		High bleats	0.0106 0.4093	0.0282 0.1241	0.0028 0.6553	0.0087 0.1685	0.0254 0.0292	0.0062 0.4621	0.0145 0.0932	0.0067 0.4433

Table 9. Among triplet-born lambs, the relationship between MBS, the time taken to express a behaviour and the number of bleats emitted in the paddock on the probability of a behaviour being expressed in the triangle pen test. The results are presented as the regression coefficient with the p-value given below.

			Triangle pen behaviour								
Paddock behaviours			Walk	Sit	With dam	With alien ewe	In contact zone	Reach dam	Reach contact zone	Low bleats	High bleats
	Time to	Stand	-0.00311 0.3977	0.00427 0.0808	0.00097 0.7188	0.00156 0.6928	0.00264 0.2654	0.00097 0.7188	0.00156 0.6928	0.00113 0.5602	0.00817 0.4534
		Contact	-0.00363 0.3642	0.00106 0.7101	-0.00752 0.0055	0.00353 0.3697	0.00079 0.7587	-0.00752 0.0055	0.00353 0.3697	0.00073 0.7311	0.0336 0.3512
		Suck	0.00983 0.4746	0.00137 0.8187	0.0108 0.1740		0.00254 0.6407	0.0108 0.1740		0.00471 0.2673	0.0291 0.1373
		Follow	-0.00752 0.2434	0.00232 0.5412	0.00547 0.2801		0.00654 0.1976	0.00547 0.2801		-0.00679 0.0090	
	Number of	Lamb high bleats	0.00889 0.6196	0.00249 0.7879	0.00145 0.8892	0.00900 0.5681	0.00473 0.6390	0.00145 0.8892	0.00900 0.5681	0.00205 0.7538	0.1746 0.1561
		Lamb low bleats	0.0314 0.4566	0.00847 0.8060	0.0129 0.6888	0.0505 0.1545	0.0312 0.2041	0.0129 0.6888	0.0505 0.1545	0.0516 0.0594	0.0766 0.1743
		Ewe high bleats	-0.0130 0.4357	0.0038 0.7225	-0.0180 0.0822	0.00976 0.5229	0.00467 0.6583	-0.0180 0.0822	0.00976 0.5229	0.00738 0.3463	0.4012 0.1270
		Ewe low bleats	-0.0164 0.0626	0.00361 0.5895	0.00546 0.4307	0.0134 0.1785	-0.0147 0.0108	0.00546 0.4307	-0.0134 0.1785	0.00407 0.3996	0.0546 0.1454
		MBS	-0.3121 0.1722	-0.2165 0.1600	-0.1104 0.4830	-0.0975 0.4848	-0.0590 0.8091	-0.1104 0.4830	0.0590 0.8091	-0.0244 0.8116	-0.2289 0.5629

Table 10. Among twin-born lambs, the relationship between the time taken to express a behaviour, time spent expressing a behaviour and the number of bleats emitted in the triangle pen test on the probability of a behaviour being expressed in the paddock. The results are presented as the regression coefficient with the p-value given below.

			Paddock behaviours							
Triangle pen test behaviours			Stand	Contact	Suck	Follow	Lamb high bleats	Lamb low bleats	Ewe high bleats	Ewe low bleats
	Time spent	Walking	0.0008 0.8394	0.0025 0.4061	0.0017 0.5782	0.0021 0.4257	0.0034 0.2720	0.0032 0.2170	0.0032 0.2170	0.0028 0.3996
		Sitting	0.0010 0.8273	0.0011 0.7809	0.0017 0.6836	0.0034 0.3786	0.0027 0.4188	0.00005 0.9869	0.0008 0.8502	0.0001 0.9877
		With dam	0.0019 0.3541	0.0044 0.0054	0.0009 0.5726	0.0018 0.1918	0.0007 0.6888	0.0026 0.0499	0.0004 0.8178	0.0055 0.0052
		With alien ewe	0.0005 0.8161	-0.0035 0.0308	0.0005 0.7794		0.0015 0.4208	-0.0030 0.0315	0.0002 0.9486	0.0030 0.1228
		In contact zone	0.0024 0.3429	0.0021 0.2912	0.0009 0.6841	0.0037 0.0786	0.0030 0.1254	0.0001 0.9389	0.0006 0.8189	0.0040 0.0616
	Time to	Reach dam	-0.0067 0.0405	-0.0072 0.0074	0.0015 0.6171	-0.0058 0.0445	0.0015 0.6195	0.0037 0.1311	0.0005 0.8807	-0.0099 0.0010
		Reach contact zone	0.0066 0.3082	-0.0014 0.8065	0.0075 0.1479	-0.0138 0.0357	0.0041 0.4748	0.0002 0.9751	0.0061 0.2740	0.0056 0.3696
	Number of	Low bleats	0.0048 0.8337	0.0142 0.4494	0.0123 0.4241	0.0144 0.2781	-0.0271 0.0570	0.0557 0.0087	0.0012 0.9423	0.0024 0.8980
		High bleats	0.0091 0.1557	0.0059 0.2247	0.0018 0.7429	0.0085 0.0509	0.0034 0.5525	0.0047 0.2935	-0.0144 0.0059	0.0061 0.3030

Table 11 a & b. Within (a) twin- and (b) triplet-born lambs, the relationship between the probability of a behaviour being expressed in the triangle pen test on the median time taken to express a behaviour and the median number of bleats emitted in the paddock. Results presented as the median value and ^{ab} signifies a significant effect within a behaviour.

			Paddock behaviours															
Triangle pen test behaviours		(a)	Stand		Contact		Suck		Follow		Lamb high bleat		Lamb low bleat		Ewe high bleat		Lamb low bleat	
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
	Time spent	Walking	50	60	40	60	40^{a}	60^{b}	50	40	40	50	50	50	40	60	50	50
		Sitting	301	301	301	301	301	301	301	301					301	301	301	301
		With dam	170^{a}	250^{b}	180	140	180	180	170	190	160	170	170^{b}	140^{a}	180	140	180	140
		With alien	70	30	70	80	90	60	70	50	80	80	70^{a}	100^{b}	60	80	60	80
		In contact zone	280	290	280	270	280	280	280	270	270	270	270	270	280	270	280	270
	Time to	Reach dam	40^{b}	10^{a}	40^{b}	20^{a}	50	40	40	40	30	35	30	30	30	50	40	40
		Reach contact zone	20^{b}	10^{a}	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Number of	Low bleat	2	3	2^{a}	8^{b}	3	2	2	2	4^{a}	8^{b}	4^{a}	7^{b}	2	3	2	1
		High bleat	27	26	27	20	24	32	26	26	31	3127	32	29	27	20	27	26

			Paddock behaviours															
Triangle pen test behaviours		(b)	Stand		Contact		Suck		Follow		Lamb high bleat		Lamb low bleat		Ewe high bleat		Lamb low bleat	
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
	Time spent	Walking	50	40	50	45	50	50	40	40	40	50	50	50	40	60	50	40
		Sitting																
		With dam	160	130	170^{b}	130^{a}	170	160	180	160	160	170	170^{b}	140^{a}	170	160	170^{b}	110^{a}
		With alien	80	80	70^{a}	115	80	80	80	75	80	80	70^{a}	100^{b}	80	95	80	110
		In contact zone	270^{b}	260^{a}	270	270	270	270	280^{b}	270^{a}	270	270	270	270	270	270	270	270
	Time to	Reach dam	30	40	30	35	30	30	30	30	30	35	30	30	30	30	30^{a}	50^{b}
		Reach contact zone	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Number of	Low bleat	5	5	5	5	5	5.5	5	5	4^{a}	8^{b}	7^{b}	4^{a}	5	8	5	8
		High bleat	29	38	28.5	37	25	32	35^{b}	27^{a}	31	27	32	29	27.5^{a}	39^{b}	30	33

Table 12 a & b. The effect, within (a) twin- and (b) triplet-born lambs, of the probability of a behaviour being exhibited in the paddock on the time taken to exhibit a particular behaviour and the number of bleats emitted in the triangle pen test.

			Triangle pen test behaviours													
		(a)	Walk		Sit		With dam		With alien		In contact zone		Low bleat		High bleat	
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Paddock behaviours	Time to	Stand	23	30	22	24	24	10	23.5	20	23	8	19	12	22a	141[b]
		Contact	11	12.5	11	12	12	11	12	9	11	11	12	10	11[a]	123[b]
		Suck	301	301	301	301	301	301	301	301	301	301	301	301	301	301
		Follow	200	134	271	190	208	154	186	218	200	137	204	186	200	198
	Number of	Lamb high bleat	7[a]	20[b]	9	7	7	10	7.5	9	8	10	7	10	8	24
		Lamb low bleat	3	1.5	2	3	2	3	3	2	2	3	3	2	2	4
		Ewe high bleat	5	5	6	4	5	5	5	4	5	3	5	6	5	15
		Ewe low bleat	8.5	12	8	10	9	7.5	7a	17b	9	17	10	6	9	5

			Triangle pen test behaviours													
		(a)	Walk		Sit		With dam		With alien		In contact zone		Low bleat		High bleat	
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Paddock behaviours	Time to	Stand	30	46	25.5	30.5	29	36	29	35	30	37	27.5	32	30	25
		Contact	14	8	22.5	14	13^{a}	31^{b}	13.5	20	14	10	12.5	15	14	10
		Suck	171	213	165	183	177	156	180	157	174	176	187	153	180	112
		Follow	119	208	164	127	119	185	127	108	127	147	93^{a}	182^{b}	119	158
	Number of	Lamb high bleat	12	16	10.5	12	11	18	12	12	11.5	22	10	13	12^{b}	2^{a}
		Lamb low bleat	4	3	4	5	4	3	4	6	4	7.5	5	3.5	4	5.5
		Ewe high bleat	8	10.5	8.5	7	7.5^{a}	13^{b}	8	8	8	13	7.5	8	8^{b}	2^{a}
		Ewe low bleat	28	26	28	26	26^{a}	34^{b}	25^{a}	36^{b}	26^{a}	41^{b}	26.5	27.5	27	7

Appendix 3

Twin- and triplet-born lambs were analysed separately. Lambs that did not stand, contact, suck or follow after tagging or exhibit any of the behaviours in the triangle pen test within the five minute observation period were censored at 301 seconds. Blank cells in the tables indicate insufficient data to conduct the analysis. The results of conditional behaviours, i.e. if one behaviour was depended of that of another behaviour, e.g. standing and following, are not presented.

The probability of one behaviour being expressed on the expression of another behaviour was analysed using the Logistic Regression procedure. Year of study, the behaviour of interest and sex of the lamb were included in the model as fixed effects and lamb birth weight as a covariate. All behaviours were tested as both the dependant and the independent variable. The relationship between the time taken to exhibit a behaviour, or the number of bleats emitted, and the probability of a behaviour being exhibited was also analysed using the logistic procedure using the same fixed effects and covariate.

The correlations of time taken for lambs to exhibit each behaviour, the time spent exhibiting the behaviour, ewe MBS and the number of high- and low-pitched bleats emitted by the ewe and the lamb were analysed for correlations using a Spearman's Correlation analysis.

The probability of a behaviour being exhibited on the time taken to exhibit other behaviours, the time spent exhibiting behaviours or the number of bleats emitted was analysed using the non-parametric Kruskal-Wallis test. The probability of the behaviour being exhibited was used as the fixed effect with the time taken to exhibit behaviours (or the number of bleats) included as the dependant variable. The probability of a lamb spending time in the contact zone and spending time with the dam in the triangle test were excluded from analysis because these behaviours were conditional to the probability of the lamb reaching the contact zone and reaching the dam. They hence produced the same results.

Table 1. The odds of a twin-born lamb exhibiting a paddock behaviour for those lambs that expressed a triangle pen test behaviour compared with those that did not express that behaviour. The results are presented as odds ratio with the 95% confidence interval given in parenthesis and p-value given below.

			Paddock behaviours							
Triangle pen test behaviours			Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat
	Time spent	Walking	2.3 (0.3-21.3) 0.4596		0.6 (0.1-2.7) 0.5030	0.3 (0.03-2.2) 0.2132	2.9 (0.5-16.9) 0.2262	2.2 (0.4-11.8) 0.3724	0.6 (0.1-5.5) 0.6729	
		Sitting	0.9 (0.3-2.8) 0.8254	1.6 (0.4-6.2) 0.5178	1.2 (0.6-2.5) 0.5327	0.8 (0.4-1.6) 0.5489	0.8 (0.3-1.9) 0.5898	1.4 (0.6-3.2) 0.4900	1.2 (0.6-2.7) 0.5997	1.4 (0.5-4.1) 0.5596
		With dam	0.4 (0.1-3.2) 0.3924	0.5 (0.1-4.0) 5120	0.6 (0.3-1.4) 0.2279	1.0 (0.5-2.4) 0.9140	1.6 (0.6-4.5) 0.3741	0.5 (0.1-1.7) 0.2598	2.3 (0.9-5.8) 0.0769	0.7 (0.1-3.1) 0.6117
		With alien ewe	1.4 (0.4-5.0) 0.5610	1.1 (0.5-2.3) 0.7725	1.0 (0.4-2.1) 0.9217	1.3 (0.3-5.0) 0.7524	0.9 (0.3-2.5) 0.8552	0.4 (0.1-1.2) 0.1145	1.4 (0.6-3.2) 0.4724	0.4 (0.1-1.8) 0.2355
		In contact zone	1.4 (0.2-12.2) 0.7482	1.8 (0.2-15.9) 0.5955	0.9 (0.2-3.6) 0.8975	1.2 (0.3-4.1) 0.8146	1.6 (0.3-8.1) 0.5928		2.8 (0.7-11.1) 0.1377	1.0 (0.1-8.8) 0.9685
	Time to	Reach dam	0.4 (0.05-3.1) 0.3762	0.5 (0.1-3.9) 0.4958	0.5 (0.2-1.2) 0.1338	1.0 (0.4-2.2) 0.9650	1.6 (0.6-4.4) 0.3973	0.5 (0.1-1.7) 0.2423	2.2 (0.9-5.4) 0.1006	0.6 (0.1-3.0) 0.5807
		Reach contact zone	1.3 (0.2-11.1) 0.8060	1.7 (0.2-14.5) 0.6417	0.7 (0.2-2.4) 0.5491	1.0 (0.3-3.5) 0.9990	1.5 (0.3-7.5) 0.6415		2.4 (0.6-9.0) 0.2017	1.0 (0.1-8.0) 0.9669
	Number of	Low bleats	1.1 (0.3-3.7) 0.9199	0.2 (0.03-1.8) 0.1639	1.2 (0.6-2.5) 0.6605	0.6 (0.3-1.2) 0.1223	0.3 (0.1-1.0) 0.0471	1.4 (0.6-3.2) 0.4789	0.6 (0.2-1.5) 0.2705	0.7 (0.2-2.4) 0.6233
		High bleats						2.5 (0.2-29.4) 0.4769		

Table 2. The odds of a twin-born lamb exhibiting a triangle pen test behaviour for those lambs that expressed a paddock behaviour compared with those that did not express that behaviour. The results are presented as odds ratio with the 95% confidence interval given in parenthesis and p-value given below.

			Triangle pen test behaviours								
			Walking	Sitting	With dam	With alien ewe	Time in contact zone	To reach dam	To reach contact zone	Low bleats	High bleats
Paddock behaviours	Time to	Stand	2.1 (0.2-19.2) 0.5288	0.9 (0.3-2.8) 0.8252	0.4 (0.03-3.2) 0.3873	1.4 (0.4-5.0) 0.4083	1.4 (0.2-11.9) 0.7622	0.4 (0.02-3.1) 0.3722	1.3 (0.1-10.8) 0.8261	1.1 (0.3-3.7) 0.9369	
		Contact		1.6 (0.4-6.3) 0.5203	0.5 (0.1-4.0) 0.4973	1.2 (0.3-4.9) 0.7710	1.7 (0.2-15.2) 0.6141	0.5 (0.1-3.9) 0.4837	1.6 (0.2-13.9) 0.6720	0.2 (0.02-1.8) 0.1584	
		Suck	0.6 (0.1-2.6) 0.4818	1.2 (0.6-2.5) 0.5291	0.6 (0.3-1.4) 0.2314	1.0 (0.4-2.1) 0.9216	0.9 (0.2-3.6) 0.9014	0.5 (0.2-1.2) 0.1358	0.7 (0.2-2.4) 0.5498	1.2 (0.6-2.5) 0.6580	
		Follow	0.2 (0.03-2.1) 0.1925	0.8 (0.4-1.6) 0.5502	1.0 (0.5-2.4) 0.9171	1.1 (0.5-2.3) 0.7772	1.2 (0.3-4.1) 0.8199	1.0 (0.4-2.2) 0.7353	1.0 (0.3-3.4) 0.9867	0.6 (0.3-1.2) 0.1209	
	Number of	Lamb high bleat	2.6 (0.5-14.7) 0.2807	0.8 (0.3-1.9) 0.5827	1.6 (0.6-4.5) 0.3929	0.9 (0.3-2.5) 0.8530	1.5 (0.3-7.6) 0.6334	1.5 (0.5-4.4) 0.4126	1.4 (0.3-7.1) 0.6855	0.3 (0.1-1.0) 0.0473	
		Lamb low bleat	1.9 (0.4-10.4) 0.4514	1.4 (0.6-3.2) 0.4928	0.5 (0.1-1.7) 0.2489	0.4 (1.2-0.1) 0.1136		0.5 (0.1-1.7) 0.2349		1.4 (0.6-3.2) 0.4794	2.5 (0.2-28.6) 0.4751
		Ewe high bleat	0.6 (0.1-5.2) 0.6355	1.2 (0.6-2.7) 0.5915	2.3 (0.9-5.9) 0.0730	1.3 (3.1-0.6) 0.4950	2.8 (0.7-10.8) 0.1268	2.2 (0.9-5.4) 0.0983	2.4 (0.6-8.7) 0.1927	0.6 (0.2-1.5) 0.2542	
		Ewe low bleat		1.4 (0.5-4.1) 0.56.5	0.7 (0.1-3.1) 0.6023	0.4 (0.1-1.8) 0.2341	1.0 (0.1-8.7) 0.9703	0.6 (0.1-3.0) 0.5743	0.9 (0.1-7.8) 0.9541	0.7 (0.2-2.5) 0.6294	

Table 3. The odds of a triplet-born lamb exhibiting a paddock behaviour for those lambs that expressed a triangle pen test behaviour compared with those that did not express that behaviour. The results are presented as odds ratio with the 95% confidence interval given in parenthesis and p-value given below.

			Paddock behaviours							
			Stand	Contact	Suck	Follow	Lamb High-bleat	Lamb Low-bleat	Ewe High-bleat	Ewe Low-bleat
Triangle pen test behaviours	Time spent	Walking	1.3 (0.3-5.8) 0.7658	0.8 (0.2-3.0) 0.7510	1.7 (0.4-7.5) 0.5146	1.7 (0.5-5.4) 0.3944	1.5 (0.5-4.9) 0.4857	1.7 (0.6-4.8) 0.2847	1.7 (0.5-5.7) 0.3537	0.4 (0.1-3.4) 0.4326
		Sitting	0.5 (0.2-1.3) 0.1415	1.0 (0.5-2.2) 0.9961	1.1 (0.5-2.5) 0.7520	0.7 (0.3-1.5) 0.3651	0.8 (0.3-1.7) 0.5403	1.0 (0.5-2.0) 0.9824	1.0 (0.4-2.3) 0.9583	0.7 (0.3-1.7) 0.3947
		With dam	0.8 (0.3-2.4) 0.6864	0.5 (0.2-1.3) 0.1407	0.9 (0.4-2.0) 0.8454	2.7 (0.6-12.7) 0.2022	1.8 (0.8-3.8) 0.1286	0.8 (0.4-1.6) 0.6133	0.7 (0.3-1.8) 0.4799	0.6 (0.2-1.8) 0.3797
		With alien ewe	0.7 (0.1-5.6) 0.7290	1.2 (0.3-4.4) 0.8231	1.4 (0.3-6.7) 0.6496	2.2 (1.0-4.6) 0.0396	2.1 (0.6-7.2) 0.2399	1.1 (0.4-3.4) 0.8432	0.9 (0.2-4.3) 0.8982	2.3 (0.6-9.2) 0.2221
		In contact zone	0.8 (0.2-2.7) 0.6727	1.8 (0.8-4.0) 0.1307	1.6 (0.6-4.3) 0.3734	2.2 (0.9-5.2) 0.0776	1.1 (0.4-2.7) 0.8338	1.6 (0.8-3.2) 0.1907	0.6 (0.2-1.8) 0.3560	1.9 (0.8-5.0) 0.1585
	Time to	Reach dam	0.7 (0.1-5.6) 0.7290	1.2 (0.3-4.4) 0.8231	1.4 (0.3-6.7) 0.6496	2.7 (0.6-12.7) 0.2022	2.1 (0.6-7.2) 0.2399	1.1 (0.4-3.4) 0.8432	0.9 (0.2-4.3) 0.8109	2.3 (0.6-9.2) 0.2221
		Reach contact zone	0.8 (0.2-2.7) 0.6727	1.8 (0.8-4.0) 0.1307	1.6 (0.6-4.3) 0.3734	2.2 (0.9-5.2) 0.0776	1.1 (0.4-2.7) 0.8338	1.6 (0.8-3.2) 0.1907	0.6 (0.2-1.8) 0.3560	1.9 (0.8-5.0) 0.1585
	Number of	Low- bleats	1.1 (0.5-2.3) 0.8409	1.4 (0.8-2.5) 0.1925	0.8 (0.5-1.5) 0.5598	1.2 (0.7-2.0) 0.4301	0.6 (0.3-1.1) 0.1203	1.2 (0.7-1.9) 0.5048	0.5 (0.3-1.0) 0.0570	0.9 (0.5-1.9) 0.8411
		High-bleats			0.3 (0.1-2.1) 0.2431	0.9 (0.1-5.3) 0.8711	1.1 (0.1-10.0) 0.9392	0.4 (0.01-4.0) 0.4649		

Table 4. The odds of a triplet-born lamb exhibiting a paddock behaviour for those lambs that expressed a triangle pen test behaviour compared with those that did not express that behaviour. The results are presented as odds ratio with the 95% confidence interval given in parenthesis and p-value given below.

			Triangle pen test behaviours								
			Walking	Sitting	With dam	With alien ewe	Time in contact zone	To reach dam	To reach contact zone	Low bleats	High bleats
Paddock behaviours	Time to	Stand	1.3 (0.3-5.8) 0.7679	0.5 (0.2-1.3) 0.1422	0.7 (0.1-5.8) 0.7545	0.8 (0.3-2.4) 0.6966	0.7 (0.2-2.7) 0.6548	0.7 (0.1-5.8) 0.7545	0.7 (0.2-2.7) 0.6548	1.1 (0.5-2.3) 0.8422	
		contact	0.8 (0.2-3.0) 0.7511	1.0 (0.5-2.2) 0.9974	1.2 (0.3-4.4) 0.8284	0.5 (0.2-1.2) 0.1343	1.8 (0.8-4.0) 0.1295	1.2 (0.3-4.4) 0.8284	1.8 (0.8-4.0) 0.1295	1.4 (0.8-2.5) 0.1927	
		Suck	1.7 (0.4-7.5) 0.5149	10.0 (0.2-1.3) 0.7512	1.5 (0.3-6.9) 0.6378	0.9 (0.4-2.0) 0.8452	1.6 (0.6-4.3) 0.3748	1.5 (0.3-6.9) 0.6378	1.6 (0.6-4.3) 0.3748	0.8 (0.5-1.5) 0.5594	0.3 (0.1-2.1) 0.2456
		Follow	1.6 (0.5-5.2) 0.4193	0.7 (0.3-1.5) 0.3633	2.7 (0.6-12.5) 0.2088	2.2 (1.0-4.6) 0.0434	2.2 (0.9-5.3) 0.0716	2.7 (0.6-12.5) 0.2088	2.2 (0.9-5.3) 0.0716	1.2 (0.7-2.0) 0.4340	0.8 (0.1-5.2) 0.8565
	Number of	Lamb high bleat	1.5 (0.5-4.9) 0.4886	0.8 (0.3-1.7) 0.5458	2.0 (0.6-6.9) 0.2839	1.8 (0.8-3.7) 0.1373	1.1 (0.4-2.6) 0.8912	2.0 (0.6-6.9) 0.2839	1.1 (0.4-2.6) 0.8912	0.6 (1.1-0.3) 0.1197	1.1 (0.1-9.9) 0.9492
		Lamb low bleat	1.7 (0.6-4.8) 0.2838	1.0 (0.5-2.0) 0.7099	1.1 (0.4-3.5) 0.8175	0.8 (0.4-1.6) 0.6132	1.6 (0.8-3.3) 0.1809	1.1 (0.4-3.5) 0.8175	1.6 (0.8-3.3) 0.1809	1.2 (0.7-1.9) 0.5036	0.4 (0.03-4.0) 0.4592
		Ewe high bleat	1.7 (0.5-5.7) 0.3549	1.0 (0.4-2.3) 0.9573	0.9 (0.2-4.5) 0.9209	0.7 (0.3-1.8) 0.4917	0.6 (0.2-1.8) 0.3624	0.9 (0.2-4.5) 0.9209	0.6 (0.2-1.8) 0.3624	0.5 (0.3-1.0) 0.0570	
		Ewe low bleat	0.4 (0.1-3.4) 0.5806	0.7 (0.3-1.7) 0.3968	2.4 (0.6-9.3) 0.2203	0.6 (0.2-1.8) 0.3717	1.9 (0.8-4.9) 0.1644	2.4 (0.6-9.3) 0.2203	1.9 (0.8-4.9) 0.1644	0.9 (0.5-1.9) 0.8411	

Table 5. Among twin-born lambs, the correlations between MBS, the time taken to exhibit a behaviour and the number of bleats emitted in the paddock and the time taken to express a behaviour in the triangle pen test. The results are presented as the correlation coefficient with the p-value given below.

		Triangle pen test behaviours								
		Time spent					Time to		Number of	
		Walk	Sit	With dam	With alien ewe	In contact zone	Reach dam	Reach contact zone	Low bleats	High bleats
Paddock behaviours	Stand	-0.04565 0.5361	0.00978 0.8946	0.05277 0.4744	-0.00855 0.9078	0.09619 0.1915	-0.06344 0.3897	-0.05461 0.4591	0.06504 0.3778	0.00287 0.9691
	Follow	-0.03081 0.6763	-0.03843 0.6026	0.08376 0.2557	-0.07356 0.3183	0.02615 0.7231	-0.07738 0.2938	-0.00018 0.9981	0.01818 0.8054	0.01012 0.8912
	Suck	-0.20104 0.0059	0.02616 0.723	0.07104 0.3352	-0.05276 0.4745	0.01002 0.892	-0.0396 0.5915	0.05027 0.4956	-0.05497 0.4561	-0.0583 0.4305
	Contact	0.06028 0.4138	-0.02685 0.716	0.00237 0.9743	0.08386 0.2551	0.06316 0.3918	-0.02494 0.7354	-0.13507 0.066	0.11041 0.1336	-0.22227 0.0024
	Lamb high-bleat	0.09816 0.2029	-0.03026 0.6953	0.08733 0.2575	-0.04979 0.519	0.15051 0.0501	0.02485 0.7477	-0.07939 0.3034	-0.10004 0.1943	0.22113 0.0039
	Lamb low-bleat	0.01093 0.8829	0.10209 0.1679	-0.01217 0.8697	0.02733 0.7127	0.09036 0.2225	-0.0111 0.8811	-0.07576 0.3067	0.16887 0.0219	0.14084 0.0572
	Ewe high-bleat	-0.03302 0.6635	-0.06749 0.3735	0.05732 0.4499	0.00126 0.9867	0.04326 0.5687	0.01072 0.8877	-0.04057 0.5929	-0.02355 0.7564	-0.04102 0.5899
	Ewe low-bleat	0.02372 0.748	0.04391 0.5517	0.14701 0.0452	-0.2020 0.0057	0.01892 0.7977	-0.06579 0.3723	0.01049 0.887	0.04998 0.4981	0.01795 0.8084
	MBS	-0.09447 0.1996	-0.1394 0.0577	-0.01908 0.796	-0.13686 0.0625	-0.06229 0.3983	0.12058 0.1011	0.17896 0.0145	-0.21812 0.0028	0.14647 0.0467

Table 6. Among triplet-born lambs, the correlations between MBS, the time taken to exhibit a behaviour and the number of bleats emitted in the paddock on the time taken to express a behaviour in the triangle pen test. The results are presented as the correlation coefficient with the p-value given below.

		Triangle pen test behaviours								
		Time spent					Time to		Number of	
		Walk	Sit	With dam	With alien ewe	In contact zone	Reach dam	Reach contact zone	Low bleats	High bleats
Paddock behaviours	Stand	-0.16373 0.006	0.02737 0.6484	-0.06775 0.2585	-0.01044 0.8619	-0.09673 0.1063	0.10895 0.0831	0.10765 0.0780	0.09499 0.2207	-0.09283 0.1246
	Follow	-0.1513 0.1163	0.03863 0.69	0.03219 0.7397	-0.01921 0.8429	0.04198 0.6647	0.09861 0.3193	0.13112 0.1762	-0.0859 0.4828	0.1503 0.1223
	Suck	0.03958 0.7640	-0.01864 0.8876	0.0267 0.8395	-0.14902 0.2558	-0.09593 0.4659	0.03296 0.8094	0.2072 0.1186	0.10052 0.5716	0.17498 0.1889
	Contact	-0.10561 0.0991	0.05776 0.3680	-0.12813 0.0451	0.06334 0.3235	-0.09371 0.1436	0.11712 0.0796	0.11261 0.0843	-0.05604 0.4943	-0.10202 0.1150
	Lamb high bleat	0.06997 0.2647	0.00374 0.9525	-0.06221 0.3215	-0.01088 0.8625	-0.10263 0.1014	0.0146 0.8246	0.08868 0.1638	0.01764 0.8315	0.24863 <.0001
	Lamb low bleat	0.01438 0.8411	-0.07266 0.3103	-0.05692 0.4269	-0.0254 0.7232	-0.03897 0.5866	0.06653 0.3709	0.01516 0.8351	0.26686 0.0031	0.18003 0.0122
	Ewe high bleat	-0.06177 0.3202	0.0575 0.3548	-0.17102 0.0056	0.05411 0.384	-0.16681 0.0069	0.06515 0.3211	0.0336 0.597	-0.0293 0.7219	-0.0254 0.6859
	Ewe low bleat	-0.17642 0.0035	0.07832 0.1978	0.07411 0.2231	-0.17094 0.0047	-0.07979 0.1896	0.03801 0.5505	0.07834 0.2054	0.13996 0.0757	-0.07907 0.1978
	MBS	-0.07413 0.2035	-0.06957 0.2327	0.14491 0.0126	-0.1138 0.0505	0.03798 0.5151	-0.08893 0.1450	-0.01617 0.7857	-0.04109 0.5840	0.01035 0.8604

Table 7. Among twin-born lambs, the relationship between MBS, the time taken to express a behaviour and the number of bleats emitted in the paddock on the probability of a behaviour being expressed in the triangle pen test. The results are presented as the regression coefficient with the p-value given below.

			Triangle pen behaviour								
Paddock behaviours			Walk	Sit	With dam	With alien ewe	In contact zone	Reach dam	Reach contact zone	Low bleats	High bleats
	Time to	Stand	0.00007 0.9856	0.000475 0.7830	0.00281 0.5064	0.00004 0.9856	0.000878 0.7049	0.00105 0.7719	0.00151 0.5343	0.00216 0.2784	-0.00605 0.2336
		Contact	0.012 0.2751	-0.00089 0.6591	0.00134 0.7662	0.00163 0.4988	0.000293 0.9091	0.00202 0.6611	0.00006 0.9804	0.00571 0.0521	0.00647 0.1850
		Suck	0.00331 0.4086	0.00068 0.7287	0.000828 0.8295	0.000454 0.8353	0.00110 0.6515	0.00168 0.6355	0.000703 0.7799	0.3986 0.5278	
		Follow	0.00411 0.2421	0.000322 0.8301	0.00253 0.3966	-0.00038 0.8177	0.00171 0.3683	0.00188 0.5113	0.00197 0.3063	0.00103 0.5162	0.000258 0.9626
	Number of	Lamb high bleat	-0.0528 0.0049	0.00571 0.5810	0.00973 0.6938	0.00345 0.7685	0.00539 0.7128	0.0155 0.5435	0.0131 0.6374	-0.0146 0.1684	-0.0299 0.2635
		Lamb low bleat	0.2556 0.1404	-0.0349 0.2207	-0.00070 0.7801	0.0132 0.6441	0.0131 0.6319	-0.0130 0.7385	0.00309 0.8315	0.0876 0.0277	0.0399 0.5023
		Ewe high bleat	0.0534 0.3958	0.0067 0.6148	0.0147 0.6626	0.0204 0.2445	0.00183 0.9193	0.0200 0.5632	0.00395 0.8258	0.0114 0.4615	0.0351 0.3171
		Ewe low bleat	0.0165 0.2320	0.0101 0.2546	-0.0125 0.3262	-0.0146 0.0709	0.00076 0.9386	-0.0160 0.1749	0.00222 0.8326	0.00656 0.4457	0.0338 0.5285
		MBS	-0.3121 0.1722	0.2736 0.0761	-0.4592 0.0094	-0.5631 0.0896	-0.3907 0.0509	-0.6422 0.0504	-0.3539 0.0786	-0.3887 0.0194	2.0102 0.0598

Table 8. Among twin-born lambs, the relationship between the time taken to express a behaviour, time spent expressing a behaviour and the number of bleats emitted in the triangle pen test on the probability of a behaviour being expressed in the paddock. The results are presented as the regression coefficient with the p-value given below.

			Paddock behaviours							
Triangle pen test behaviours			Stand	Contact	Suck	Follow	Lamb high bleat	Lamb low bleat	Ewe high bleat	Ewe low bleat
	Time spent	Walking	-0.0016 0.7340	0.0025 0.6138	0.0032 0.2230	0.0068 0.0390	0.0017 0.6982	0.0043 0.1597	0.0008 0.0333	0.0056 0.3431
		Sitting	0.0013 0.5834	0.0004 0.8701	0.0009 0.5087	0.0016 0.2192	0.00015 0.9365	-0.0011 0.5256	-0.0013 0.4303	-0.0004 0.8715
		With dam	-0.0066 0.0708	0.0011 0.7297	-0.0023 0.1726	0.0006 0.7061	0.0002 0.9408	0.0006 0.7917	0.0031 0.1109	0.0017 0.4986
		With alien ewe	0.0093 0.0593	0.0034 0.4023	0.0026 0.1753	0.0022 0.2396	0.0013 0.6060	-0.0007 0.7797	0.0022 0.3009	0.0009 0.7654
		In contact zone	0.0006 0.8827	0.0021 0.5807	0.0005 0.8168	0.0018 0.3838	0.0020 0.4729	0.0022 0.4938	0.0022 0.3621	0.0017 0.5806
	Time to	Reach dam	0.0091 0.0952	0.0031 0.3964	0.0022 0.1498	0.0006 0.7046	0.00003 0.9886	0.0023 0.2848	-0.0029 0.0862	0.0007 0.7720
		Reach contact zone	0.0026 0.6080	0.0004 0.9172		-0.0019 0.3353	0.0011 0.6837	0.0048 0.2399	0.0021 0.3600	0.0005 0.9023
	Number of	Low bleats	0.0024 0.9451	0.0149 0.4111	-0.0362 0.1612	0.0234 0.1784	-0.0449 0.0304	0.0273 0.3725	0.0025 0.9038	0.0393 0.3639
		High bleats	0.0106 0.4093	0.0282 0.1241	0.0028 0.6553	0.0087 0.1685	0.0254 0.0292	0.0062 0.4621	0.0145 0.0932	0.0067 0.4433

Table 9. Among triplet-born lambs, the relationship between MBS, the time taken to express a behaviour and the number of bleats emitted in the paddock on the probability of a behaviour being expressed in the triangle pen test. The results are presented as the regression coefficient with the p-value given below.

			Triangle pen behaviour								
Paddock behaviours			Walk	Sit	With dam	With alien ewe	In contact zone	Reach dam	Reach contact zone	Low bleats	High bleats
	Time to	Stand	-0.00311 0.3977	0.00427 0.0808	0.00097 0.7188	0.00156 0.6928	0.00264 0.2654	0.00097 0.7188	0.00156 0.6928	0.00113 0.5602	0.00817 0.4534
		Contact	-0.00363 0.3642	0.00106 0.7101	-0.00752 0.0055	0.00353 0.3697	0.00079 0.7587	-0.00752 0.0055	0.00353 0.3697	0.00073 0.7311	0.0336 0.3512
		Suck	0.00983 0.4746	0.00137 0.8187	0.0108 0.1740		0.00254 0.6407	0.0108 0.1740		0.00471 0.2673	0.0291 0.1373
		Follow	-0.00752 0.2434	0.00232 0.5412	0.00547 0.2801		0.00654 0.1976	0.00547 0.2801		-0.00679 0.0090	
	Number of	Lamb high bleats	0.00889 0.6196	0.00249 0.7879	0.00145 0.8892	0.00900 0.5681	0.00473 0.6390	0.00145 0.8892	0.00900 0.5681	0.00205 0.7538	0.1746 0.1561
		Lamb low bleats	0.0314 0.4566	0.00847 0.8060	0.0129 0.6888	0.0505 0.1545	0.0312 0.2041	0.0129 0.6888	0.0505 0.1545	0.0516 0.0594	0.0766 0.1743
		Ewe high bleats	-0.0130 0.4357	0.0038 0.7225	-0.0180 0.0822	0.00976 0.5229	0.00467 0.6583	-0.0180 0.0822	0.00976 0.5229	0.00738 0.3463	0.4012 0.1270
		Ewe low bleats	-0.0164 0.0626	0.00361 0.5895	0.00546 0.4307	0.0134 0.1785	-0.0147 0.0108	0.00546 0.4307	-0.0134 0.1785	0.00407 0.3996	0.0546 0.1454
		MBS	-0.3121 0.1722	-0.2165 0.1600	-0.1104 0.4830	-0.0975 0.4848	-0.0590 0.8091	-0.1104 0.4830	0.0590 0.8091	-0.0244 0.8116	-0.2289 0.5629

Table 10. Among twin-born lambs, the relationship between the time taken to express a behaviour, time spent expressing a behaviour and the number of bleats emitted in the triangle pen test on the probability of a behaviour being expressed in the paddock. The results are presented as the regression coefficient with the p-value given below.

			Paddock behaviours							
Triangle pen test behaviours			Stand	Contact	Suck	Follow	Lamb high bleats	Lamb low bleats	Ewe high bleats	Ewe low bleats
	Time spent	Walking	0.0008 0.8394	0.0025 0.4061	0.0017 0.5782	0.0021 0.4257	0.0034 0.2720	0.0032 0.2170	0.0032 0.2170	0.0028 0.3996
		Sitting	0.0010 0.8273	0.0011 0.7809	0.0017 0.6836	0.0034 0.3786	0.0027 0.4188	0.00005 0.9869	0.0008 0.8502	0.0001 0.9877
		With dam	0.0019 0.3541	0.0044 0.0054	0.0009 0.5726	0.0018 0.1918	0.0007 0.6888	0.0026 0.0499	0.0004 0.8178	0.0055 0.0052
		With alien ewe	0.0005 0.8161	-0.0035 0.0308	0.0005 0.7794		0.0015 0.4208	-0.0030 0.0315	0.0002 0.9486	0.0030 0.1228
		In contact zone	0.0024 0.3429	0.0021 0.2912	0.0009 0.6841	0.0037 0.0786	0.0030 0.1254	0.0001 0.9389	0.0006 0.8189	0.0040 0.0616
	Time to	Reach dam	-0.0067 0.0405	-0.0072 0.0074	0.0015 0.6171	-0.0058 0.0445	0.0015 0.6195	0.0037 0.1311	0.0005 0.8807	-0.0099 0.0010
		Reach contact zone	0.0066 0.3082	-0.0014 0.8065	0.0075 0.1479	-0.0138 0.0357	0.0041 0.4748	0.0002 0.9751	0.0061 0.2740	0.0056 0.3696
	Number of	Low bleats	0.0048 0.8337	0.0142 0.4494	0.0123 0.4241	0.0144 0.2781	-0.0271 0.0570	0.0557 0.0087	0.0012 0.9423	0.0024 0.8980
		High bleats	0.0091 0.1557	0.0059 0.2247	0.0018 0.7429	0.0085 0.0509	0.0034 0.5525	0.0047 0.2935	-0.0144 0.0059	0.0061 0.3030

Table 11 a & b. Within (a) twin- and (b) triplet-born lambs, the relationship between the probability of a behaviour being expressed in the triangle pen test on the median time taken to express a behaviour and the median number of bleats emitted in the paddock. Results presented as the median value and [ab] signifies a significant effect within a behaviour.

			Paddock behaviours															
Triangle pen test behaviours		(a)	Stand		Contact		Suck		Follow		Lamb high bleat		Lamb low bleat		Ewe high bleat		Lamb low bleat	
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
	Time spent	Walking	50	60	40	60	40[a]	60[b]	50	40	40	50	50	50	40	60	50	50
		Sitting	301	301	301	301	301	301	301	301					301	301	301	301
		With dam	170[a]	250[b]	180	140	180	180	170	190	160	170	170[b]	140[a]	180	140	180	140
		With alien	70	30	70	80	90	60	70	50	80	80	70[a]	100[b]	60	80	60	80
		In contact zone	280	290	280	270	280	280	280	270	270	270	270	270	280	270	280	270
	Time to	Reach dam	40[b]	10[a]	40[b]	20[a]	50	40	40	40	30	35	30	30	30	50	40	40
		Reach contact zone	20[b]	10[a]	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Number of	Low bleat	2	3	2[a]	8[b]	3	2	2	2	4[a]	8[b]	4[a]	7[b]	2	3	2	1
		High bleat	27	26	27	20	24	32	26	26	31	3127	32	29	27	20	27	26

			Paddock behaviours															
Triangle pen test behaviours		(b)	Stand		Contact		Suck		Follow		Lamb high bleat		Lamb low bleat		Ewe high bleat		Lamb low bleat	
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
	Time spent	Walking	50	40	50	45	50	50	40	40	40	50	50	50	40	60	50	40
		Sitting																
		With dam	160	130	170[b]	130[a]	170	160	180	160	160	170	170[b]	140[a]	170	160	170[b]	110[a]
		With alien	80	80	70[a]	115	80	80	80	75	80	80	70[a]	100[b]	80	95	80	110
		In contact zone	270[b]	260[a]	270	270	270	270	280[b]	270[a]	270	270	270	270	270	270	270	270
	Time to	Reach dam	30	40	30	35	30	30	30	30	30	35	30	30	30	30	30[a]	50[b]
		Reach contact zone	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Number of	Low bleat	5	5	5	5	5	5.5	5	5	4[a]	8[b]	7[b]	4[a]	5	8	5	8
		High bleat	29	38	28.5	37	25	32	35[b]	27[a]	31	27	32	29	27.5[a]	39[b]	30	33

Table 12 a & b. The effect, within (a) twin- and (b) triplet-born lambs, of the probability of a behaviour being exhibited in the paddock on the time taken to exhibit a particular behaviour and the number of bleats emitted in the triangle pen test.

			Triangle pen test behaviours													
		(a)	Walk		Sit		With dam		With alien		In contact zone		Low bleat		High bleat	
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Paddock behaviours	Time to	Stand	23	30	22	24	24	10	23.5	20	23	8	19	12	22a	141[b]
		Contact	11	12.5	11	12	12	11	12	9	11	11	12	10	11[a]	123[b]
		Suck	301	301	301	301	301	301	301	301	301	301	301	301	301	301
		Follow	200	134	271	190	208	154	186	218	200	137	204	186	200	198
	Number of	Lamb high bleat	7[a]	20[b]	9	7	7	10	7.5	9	8	10	7	10	8	24
		Lamb low bleat	3	1.5	2	3	2	3	3	2	2	3	3	2	2	4
		Ewe high bleat	5	5	6	4	5	5	5	4	5	3	5	6	5	15
		Ewe low bleat	8.5	12	8	10	9	7.5	7a	17b	9	17	10	6	9	5

			Triangle pen test behaviours													
		(a)	Walk		Sit		With dam		With alien		In contact zone		Low bleat		High bleat	
			Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Paddock behaviours	Time to	Stand	30	46	25.5	30.5	29	36	29	35	30	37	27.5	32	30	25
		Contact	14	8	22.5	14	13[a]	31[b]	13.5	20	14	10	12.5	15	14	10
		Suck	171	213	165	183	177	156	180	157	174	176	187	153	180	112
		Follow	119	208	164	127	119	185	127	108	127	147	93[a]	182[b]	119	158
	Number of	Lamb high bleat	12	16	10.5	12	11	18	12	12	11.5	22	10	13	12[b]	2[a]
		Lamb low bleat	4	3	4	5	4	3	4	6	4	7.5	5	3.5	4	5.5
		Ewe high bleat	8	10.5	8.5	7	7.5[a]	13[b]	8	8	8	13	7.5	8	8[b]	2[a]
		Ewe low bleat	28	26	28	26	26[a]	34[b]	25[a]	36[b]	26[a]	41[b]	26.5	27.5	27	7

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