The sucking behaviour and milk intake of 8-17 day old triplet lambs after 4 hours of separation from the ewe

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Abstract

In the first 3 days of life, triplet lambs, especially the smaller triplets, are less capable of surviving than twins and singles. However, whether differences in behaviour and survival of lambs within triplet litters persist after this period is unknown. This trial examined the differences in sucking behaviour and milk intake between the light, medium and heavy lambs within a triplet litter at 8-17 days of age, with the lamb rank based on birth weight. Of 28 triplet carrying ewes, only 10 complete litters survived to at least 8 days of age and could be used for this trial. After 4 hours of separation, the lambs were returned to the ewe and the behaviour of the lambs and ewe was observed for a period of 15 minutes. The abdominal girth and body weight of the lambs were measured before and after the sucking period. At 8-17 days of age, triplet lambs still differed in terms of weight (P=0.001) and abdominal girth (P=0.002); the triplet lambs born lightest were still lightest and had the smallest abdominal girth. However, there were no statistical relationships between lamb rank and sucking behaviour. Light, medium and heavy lambs did not differ in the number of sucking bouts, number of attempted bouts, total sucking duration, mean sucking bout duration, relative sucking duration, teat fidelity or number of butts. Nor did lamb rank affect the behaviour of the ewes (kicks or butts, walking away from lambs) or measures of milk intake such as relative weight gain or girth gain. However, there was a tendency for lamb rank to have an effect on one measure of milk intake (weight gain) (P=0.09).

The lambs that were lightest at birth tended to gain less weight during the observation period than heavier lambs, particularly those of medium birth weight. There was also a tendency for lamb rank to have an effect on relative girth gain (P=0.08). The lambs born lightest tended to gain more girth relative to their original girth than the heaviest lambs. The only measure of sucking behaviour that tended to differ with lamb rank was the frequency of teat switching (P=0.09); medium lambs tended to switch teats more often than the heaviest lambs. Overall, weight gain is likely to be the most accurate indicator of milk intake; based on this variable, lambs born lightest appear to have ingested less milk than their heavier siblings, despite the lack of differences in sucking behaviour. These lambs were still lighter and smaller at 8-17 days of age, suggesting that they had lower growth rates and potentially lower chances of survival. Further research should aim to determine whether ewes and lambs can adapt their sucking behaviour to cope with triplet litters, instead of twins or singles, or whether ewes should be selected for higher milk production. In addition, the effect of different management strategies (e.g. fostering or weaning of the lightest lamb or providing milk supplement) on triplet lambs' milk intake, growth and survival should be tested.

Key Words: triplets, lambs, sucking behaviour, milk intake

Introduction

Sheep reproductive performance

Improved lambing percentage is the biggest contributor to increased profits from sheep farming in New Zealand (Geenty, 1997). More lambs mean more income, particularly because the income from the sale of lambs has increased over the past decades (Morel *et al.,* 2008). To this aim, many sheep breeders have selected and bred ewes for increased fecundity over the last four decades (Everett-Hincks and Dodds, 2008). As a result, lambing percentage has increased from 98% in 1960 to 124% in 2006 (Morel *et al.,* 2008). This increase is due to a higher number of sheep pregnancies resulting in twins and triplets, instead of singles. However, ewes with triplets require considerably more attention for farmers to realize their production potential (Everett-Hincks and Dodds, 2008). The increased proportion of ewes having

triplets is of concern to farmers and to industry because neonatal lamb mortality is highest in triplets (Stafford *et al.,* 2007).

Lamb mortality

Overall, lamb mortality in the neonatal period ranges from 5-30%, but triplets are at an even higher risk of dying in the first 3 days of life than singles and twins (Dwyer and Lawrence, 2005; Everett-Hincks and Dodds, 2008). Production of more lambs followed by higher lamb mortality is unacceptable, both from an economic and animal welfare perspective (Nowak, 1996; Everett-Hincks *et al.,* 2005).

The events occurring in the first 3 days after birth are the most important for lamb survival (Dwyer, 2003). The ability of a lamb to survive this period is influenced by many factors, including genetics, physiology, the process of birth, the physical environment into which the lamb is born, on-farm management practices, and ewe nutrition during pregnancy (Stafford *et al.,* 2007; Everett-Hincks and Dodds, 2008). Large litter size is one of the most important risk factors for neonatal lamb mortality (Dwyer, 2003). Factors contributing to higher mortality of triplets include lower birth weight, lower body temperature, slower time to progress through neonatal behaviours, and lower growth rates (Dwyer and Morgan, 2006; Dwyer, 2008; Hudson and Trillmich, 2008).

Birth weight

Low lamb birth weight is the main risk factor for increased neonatal lamb mortality (Dwyer, 2008). Triplet lambs are born smaller and lighter than twin and single lambs (Stafford *et al*. 2007), and there is more variation in weight within triplet litters than between twins. This is because of increased competition for nutrients between foetuses in the same uterus horn (Everett-Hincks *et al.,* 2005). Within a litter, mortality is highest for the lightest lamb and lowest for the lamb born heaviest (Morel *et al.,* 2008). The optimum birth weight for lambs ranges between 3 and 5.5 kg (Nowak and Poindron, 2006). Lambs with very high birth weight die more often because of birth difficulties. Lambs with very low birth weights can die from starvation and exposure, because of lower energy reserves, weakness, immaturity and greater heat loss (Nowak and Poindron, 2006).

Body temperature

Triplet lambs often have lower body temperatures than twin lambs in the neonatal period (Dwyer and Morgan, 2006). The smaller triplet lambs lose more body heat to the environment because of their greater surface area to body mass ratio (Morel *et al.,* 2008). Furthermore, they have reduced body reserves and the thermogenic capacity of brown adipose tissue is probably poorer (Stafford *et al.,* 2007). This means that the lighter lambs from multiple litters have a reduced ability to maintain body temperature compared to heavier lambs (Dwyer, 2008). This can lead to death due to hypothermia in the first days after birth (Stafford *et al.,* 2007).

Neonatal behaviours

Newborn lambs need colostrum shortly after birth, and early interactions between the ewe and the lamb are important to achieve this (Nowak, 1996). Colostrum, provided by the ewe, is a source of energy, immunoglobulins and water for the lamb (Nowak, 1996). The neonatal behaviour sequence (Table 1) is very important to ensure early sucking.

Table 1. Definitions of the neonatal behaviours of lambs. Taken from Dwyer, 2003.

Behaviour	Definition
Shakes head	Lamb raises and shakes head
To knees	Lamb on chest, pushes up on knees, supporting part of body off the ground
Attempts to stand	Lamb on knees, supports part of its weight on at least one foot
Stands	Lamb supports itself on all four feet for at least 5 seconds
To udder	Lamb in parallel inverse position with head nudging ewe in udder region
Suck attempts	Lamb in parallel inverse position, head beneath ewe in udder region, prevented from sucking by ewe movement or leaves udder region within 5 seconds
Succesful suck	Lamb has teat in its mouth, in correct position, appears to be sucking for at least 5 seconds
Playing	Lamb running, jumping or frolicking, in a co-ordinated manner, with no apparent purpose

In precocial species, like the sheep, the speed with which the lamb stands and seeks the udder is related to lamb survival (Poindron, 2005; Dwyer and Morgan, 2006).

Lamb survival and lamb growth rate are better when lambs stand and suck quickly (Dwyer, 2003); these lambs gain the nutritional and immunological benefits of the early colostrum ingestion. Lambs that are slower to suck after birth have lower rectal temperatures than quick lambs, and this difference persists for at least 3 days after birth (Dwyer and Morgan, 2006). Thus, lambs that are behaviourally slow at birth are also less able to maintain their body temperature after birth, and are at greater risk of dying before weaning (Dwyer and Morgan, 2006). If the lamb has not sucked in the first 6 hours after birth, the sucking drive and the chances of ever sucking successfully are reduced (Nowak and Poindron, 2006).

Litter size appears to affect the expression of the neonatal behaviour sequence (Dwyer, 2003; Dwyer *et al.,* 2004; Dwyer and Morgan, 2006). Triplet lambs are slower than singles and twins to progress through the sequence of neonatal behaviours (Table 1) and are at greater risk of dying (Dwyer and Morgan, 2006). For example, triplet lambs are slower to stand and suck from their mothers (Dwyer and Morgan, 2006) and they ingest less colostrum than single- and twin-born lambs (Hess *et al.,* 1974; Stafford *et al.,* 2007).

Within triplet litters, birth weight appears to affect the time to progress through the neonatal behaviour sequence (Dwyer, 2008). Lighter lambs are less vigorous at birth and take longer to suck successfully than their heavier littermates, which leads to a higher risk of infection because of poorer immunological protection (Dwyer, 2008).

Growth rate

Triplet lambs are not only smaller at birth, they also grow more slowly than singles or twins (Hudson and Trillmich, 2008). The lower growth rate is because ewes do not seem to compensate fully with a higher milk yield for the increased demands of a triplet litter (Hudson and Trillmich, 2008). In other words, as the number of lambs increases, the ewe's milk production does not increase proportionally (Morris and Kenyon, 2004). For example, twin-bearing ewes provide half the amount of colostrum per kilogram of lamb compared with single-bearing ewes (Nowak, 1996). In addition, triplets have to compete for a milk resource that is suitable for rearing only two lambs (Hinch, 1989); ewes have only two teats (Everett-Hincks *et al.,* 2005).

Sucking behaviour and milk intake in older lambs

It is clear that triplet lambs, particularly triplets born smallest, are at higher risk of mortality in the critical neonatal period and triplets grow more slowly than singles and twin lambs (Hudson and Trillmich, 2008). Furthermore, even at older ages (1-9 weeks old), triplet lambs still have shorter sucking durations than twins and singles (Hinch, 1989). Similarly, 2 to 6 week old triplet lambs sucked for shorter durations and less frequently than single or twin lambs (Hess *et al.,* 1974). This means that an increased litter size results in a shorter duration of sucking and therefore less milk intake (Hinch, 1989).

Sucking behaviour and milk intake within triplet litters

Less is known about differences in sucking behaviour and milk intake *within* triplet litters after the critical neonatal period. It has been reported that at 2-6 weeks of age, there is a wide variation in milk intake in triplet lambs (Hess *et al.,* 1974; Stafford *et al.,* 2007). Within triplet litters, there can be a 24% difference in milk intake between the "best" and "worst" lamb at 1-9 weeks of age (Hinch, 1989). Competition for milk can become a problem for triplets (Everett-Hincks *et al.,* 2005) and can result in one lamb being consistently excluded from sucking (Hinch, 1989). However, whether the excluded lamb is always the lamb born lightest, or whether birth weight (lamb rank) predicts differences in sucking behaviour and milk intake within triplet litters at 1-2 weeks of age is unknown.

Measuring milk intake

There are a number of different ways of measuring the milk intake of ruminants (Dove, 1988). Most often, weighing of the lambs before and after sucking is used to give a direct estimate of milk intake. This method is easy, cheap and non-invasive. A disadvantage is that the normal milk intake can be underestimated if the handling and disturbance of the animals interferes with normal sucking behaviour of the lamb or with normal milk ejection of the ewe (Dove, 1988).

Another method of estimating milk intake is by measuring the total water turnover (Dove, 1988; Butte *et al.,* 1991). This can be done by injecting a dilution of isotopically labelled water (isotope-dilution method); the ewes receive an injection of TOH (tritiated water) and the lambs are injected with D_2O (deuterium oxide) (Dove,

1988). By taking a jugular blood sample, the turnover of D_2O in the lambs and the accumulation of TOH as a result of milk consumption are measured (Dove, 1988). In this way it is possible to track the movement of labelled water from the ewe to the lambs. The advantage is that milk intake is estimated in naturally sucking offspring; there is no disturbance during sucking (Dove, 1988). However, this method only gives an accurate estimate of the milk intake when milk is the only source of water intake (Dove, 1988). In addition, this method is costly and invasive.

Sucking behaviour as a predictor of milk intake

Measuring sucking behaviour as a predictor for milk intake has several advantages: it is easy, cheap and non-invasive. Some studies have reported a positive relationship between the sucking behaviour (e.g. sucking duration) and milk intake (based on weight gain). A significant correlation was found between the *total* duration of sucking bouts and milk intake (measured as weight gain) in lambs up to the age of 35 days (Cimen, 2007). There was also a significant correlation between the *median* duration of sucking bouts and the milk intake in lambs (Cimen, 2007). Therefore, milk intake appears to depend on both the frequency and the duration of sucking in lambs. Similarly, Hinch (1989) found a positive correlation between frequency of sucking bouts and milk intake. In addition, a decrease in the frequency and the duration of the sucking bouts coincided with a decline in milk intake (measured as the lamb's weight gain) (Cimen, 2007).

In contrast, other studies have found no consistent relationship between sucking behaviour and milk intake (Cameron, 1998). No significant linear relationship was found between measures of sucking behaviour and milk intake (using the isotope-dilution method) in horses (Cameron *et al.,* 1999). For example, female foals sucked for longer than male foals, but there was no difference in the milk intake. This means that measures of sucking behaviour may not adequately predict milk intake, at least in the domestic horse (Cameron *et al.,* 1999). If the accuracy of sucking behaviour as an estimate of milk intake is unknown, more direct indicators of milk intake, such as weight gain, should also be measured (Cimen, 2007).

Aim of this research

In the neonatal period, lambs within triplet litters differ in behaviour, milk intake and survival, such that lighter lambs are at higher risk of dying. The aim of this research project was to determine whether differences in the sucking behaviour and milk intake of lambs within triplet litters persist after the neonatal period. The sucking behaviour and milk intake of light, medium and heavy triplets were compared at 8-17 days of age, after they had been separated from the ewe for a period of 4 hours. The separation period was intended to increase hunger and therefore competition for milk between the lambs.

The hypotheses were:

1. Triplet lambs born lightest would still be the lightest lambs at 8-17 days old.
2. The lightest triplet lambs would have a lower milk intake than the other two (indicated by less weight gain and smaller abdominal girth gain).
3. The lightest triplet lambs would spend less time on the udder than the other two (number of sucking bouts, total sucking duration, mean sucking bout duration).
4. The lightest triplet lambs would attempt to suck more often and show more teat switching and butting than the other two lambs.

Materials and Methods

Animals

This study was conducted at the Massey University Keeble Farm, Palmerston North, New Zealand, with approval from the Massey University Animal Ethics Committee (Protocol Number 08/59).

There were 28 triplet-bearing ewes identified at the time of pregnancy scanning. These multiparous ewes were crossbreds: 50% Romney, 25% Texel and 25% Finnish Landrace. Romney rams were used for mating. At all times, the sheep had free access to water and pasture with sward height more than 4 cm, which has been shown to improve triplet litter survival by 4% compared to sward heights of 2 cm (Morris and Kenyon, 2004; Everett-Hincks *et al.,* 2005).

The group of 28 ewes was composed of 25 ewes that were mated for one cycle (17 days), and 3 ewes that got pregnant in the second mating cycle. The lambing period started at the beginning of September 2008; the first triplet was born on September 5th and the last triplet was born on September 29th. From the 28 ewes, only 25 gave birth to triplets; there were 3 sets of twins. Only 10 complete triplet litters survived to at least 8 days of age (Table 2). Other ewes lost at least one lamb; either it was born dead, or it died in the first days after birth. Therefore only 10 triplet litters were available for this trial.

Table 2. Lambs born to 28 ewes that were expected to have triplets

Lambs	Number of ewes
One lamb born dead	8
Two lambs born dead	3
One lamb died within 7 days	2
Two lambs died within 7 days	2
Only 2 lambs born (mis-scanned)	3
Three lambs alive at 8 days of age	**10**
Total	28

Treatment of ewes and lambs prior to start of this trial

The ewes carrying triplets were kept in a paddock with single- and twin-bearing ewes in the last few days of their pregnancy. The ewes were observed daily from dawn to dusk for signs of parturition, starting from day 130 of pregnancy. During this period, the ewes became acclimated to the observer, which minimised stress related to the presence of an observer during parturition.

Each ewe was moved into a pen (5m x 5m) as soon as the signs of lambing were observed. Neonatal behaviours were observed and video recorded for another PhD study. Ewes lambed without obstetric assistance as far as possible. Lambing assistance was provided if there was no appearance of parts of a lamb 60 minutes after the appearance of fluids, and/or if there was no other obvious progress made 120 minutes after parts of a lamb were seen at the vulva (Dwyer *et al.,* 1999). None of the 10 triplets used in this trial were assisted.

The time each lamb was born was recorded and the lambs were identified at birth or shortly after using spray of different colours. Two hours after the birth of the last lamb, the video recording was stopped, and the lambs were ear-tagged, weighed, measured and blood samples were taken for another study. The lambs born at night were ear-tagged, weighed, measured and blood sampled the next morning.

At 6-24 hours of age, the lambs completed a ewe selection test to determine their ability to recognize and return to their own mother within the test period of 5 minutes. After the test the ewe and lambs were reunited with the flock. This test was also part of the PhD research.

Experimental procedure

The sucking behaviour test occurred when the triplets were 8-17 days old. All litters were tested on 6 days over a period of about two weeks (Table 3). On the first 4 days, 2 ewes with their triplets were tested. On those days, one ewe started the test 15 minutes after the other ewe so that the author could do all observations herself. On the last 2 days, only one ewe with her triplets was tested per day (Table 3).

Table 3. The testing days and ages of the triplet lambs in the sucking behaviour test

Ewe	Date of testing	Testing day	Age of lambs (days)
1	22/09/2008	1	17
2	22/09/2008	1	12
3	23/09/2008	2	13
4	23/09/2008	2	12
5	24/09/2008	3	12
6	24/09/2008	3	11
7	26/09/2008	4	10
8	26/09/2008	4	8
9	28/09/2008	5	8
10	6/10/2008	6	8

On the day of testing, at 9 am, the ewe with her lambs was brought from the paddock into a lambing pen (Fig. 1). Each lamb was weighed and the abdominal girth was measured. Lambs were weighed by placing them in a bucket and subtracting the

weight of the bucket. The abdominal girth behind the last rib was determined using a tape measure. The behaviour of the ewe and the lambs was observed for 2 hours before being weighed and measured again. The results of these observations are reported elsewhere.

At 11 am, the lambs were placed in a corner of the pen, behind a gate (Fig. 1). They could still see, smell and hear the mother, but could not suck. The lambs were thus separated from the ewe for a period of 4 hours. This time was chosen to stimulate hunger so that competition between siblings could be observed after they were reunited with the ewe (Lévy *et al*., 1991; Cimen, 2007).

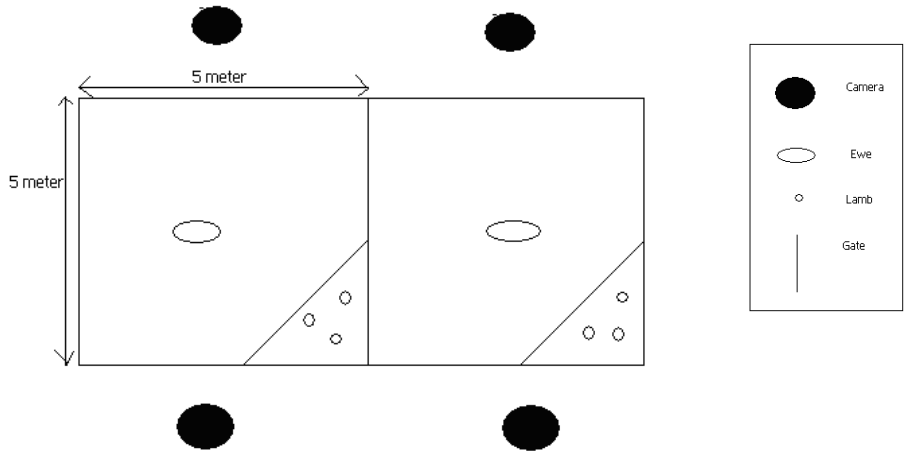

Figure 1. Observation pens for the sucking behaviour test, including the location of the lambs and ewe, during the 4 hours of separation.

Data collection

At 3 pm, the lambs were weighed and the abdominal girth was measured again. These measurements were used in the analysis presented here. Two cameras, positioned on opposite sides of the pen, were turned on (Fig. 1). The cameras were at a height of 3 metres and both video-taped the whole pen, but from opposite sides. This position allowed all maternal and lamb behaviours to be observed from the videotape. The 3 lambs were returned to the ewe simultaneously, and the behaviour of the lambs and the ewe was observed for a period of 15 minutes (Table 4).

At 3.15 pm the lambs were weighed and the abdominal girth was measured for the last time. The differences between the measurements before and after the observation period were used to calculate the weight gain and girth gain.

As well as scoring behaviour from videotapes, some live observations of behaviour were also made. If the three lambs were fighting for the udder at the same time, the two that succeeded were recorded at the time to aid scoring from the video. In addition, the frequency of urination and defecation were recorded directly. All the other durations and frequencies were recorded using the videos.

The term 'sucking' refers to the behaviour of the lamb that leads to milk release from the mother's teat (Nowak *et al.*, 1996).

Data analysis

For all the behaviours measured (Table 4), the results of the light, medium and heavy lambs, based on birth weights, were compared. Statistical comparisons of the variables were made between the three triplet lambs to determine whether lamb rank affected sucking behaviour and milk intake at 8-17 days of age. Variables were analysed using a mixed model with lamb rank (light, medium, heavy) as the fixed effect and ewe as a random effect, using SAS Version 9 (SAS Institute Inc., Cary, NC, USA, 2002). When there was a significant effect of lamb rank, post hoc tests were used to determine differences between ranks. The contribution of ewe identity to variation in lamb behaviour was calculated by dividing the variance due to the ewe by the total variance. Differences are reported as significant at $P<0.05$ and as tendencies at $P<0.10$.

Table 4. Behaviours observed during the 15 min observation period of triplet lambs and ewes, after 4 hours of separation

Variable	Definition	Unit	Reference
Milk intake			
Weight gain	The gain in lamb's body weight during the observation period, due to milk intake	grams	Dove, 1988; Cimen, 2007
Relative weight gain	Weight gain as a percentage of the original weight measured before drinking (after 4 hours of fasting)	%	-
Abdominal girth gain	The gain in lamb's abdominal girth, measured just behind the last rib of the lamb	cm	-
Relative girth gain	Girth gain as a percentage of the original abdominal girth measured before drinking (after 4 hours of fasting)	%	-
Sucking behaviour			
Sucking bout	Period during which the lamb had the ewe's teat in its mouth for 5 seconds or longer. If sucking stopped and started again within 5 seconds, only 1 bout was scored. A new bout was scored if the interval was longer than 5 seconds	frequency	Cimen, 2007
Attempted bout	The lamb had the ewe's teat in its mouth for less than 5 seconds. Sucking was interrupted because of ewe movement, other lambs or the lamb leaving the udder	frequency	Dwyer *et al*., 2004
Total sucking duration	The sum of all sucking bout durations in the observation period	seconds	Hess *et al*., 1974
Mean sucking bout duration	Total sucking duration divided by the number of sucking bouts in the observation period	seconds	-
Relative sucking duration	Individual lamb's sucking duration as a percentage of the total sucking duration of the 3 lambs	%	-
Teat switching	The lamb stopped sucking one teat and went to the other teat to continue sucking	frequency	de Passille and Rushen, 2006
Teat fidelity	Percentage of the individual lamb's total sucking period spent on the most used teat	%	de Passille and Rushen, 2006
Non- sucking behaviour			
Butt	The lamb hit the udder with a rapid, upwards movement of the head, which usually caused the udder to lift	frequency	de Passille and Rushen, 2006
Urination	The lamb urinated	frequency	-
Defecation	The lamb defecated	frequency	-
Ewe behaviour			
Ewe kicks	The ewe lifted a foot towards a lamb	frequency	de Passille and Rushen, 2006
Ewe walks	The ewe stepped backwards, sideways or forwards to prevent the lamb from sucking	frequency	Dwyer *et al*., 2004
Ewe butts	The ewe pushed or knocked a lamb down or away with a rapid downwards, forwards or sideways movement of the head	frequency	Dwyer *et al*., 2004

Results

Physical characteristics

Birth weight

It was confirmed that birth weight did, in fact, differ statistically between the different lamb ranks (Table 5). The light lambs weighed less at birth than the medium lambs, which weighed less than the heavy lambs.

Table 5. Means ± pooled SE of the birth weight, the weight on the day of testing and the abdominal girth on the day of testing of the light, medium and heavy lambs within the triplet litters (lamb rank based on birth weight)

Measurement	Light lambs	Medium lambs	Heavy lambs	F value	P value	Effect of ewe (%)
Birth weight (g)	3506 ± 142 [a]	3958 ± 142 [b]	4471 ± 142 [c]	43.3	<0.0001	73
Weight (g)	5216 ± 336.9[a]	6274 ± 336.9[b]	6798 ± 336.9[b]	10.1	0.001	43
Girth (cm)	41.1 ± 1.0[a]	44.0 ± 1.0[b]	45.9 ± 1.0[b]	8.9	0.002	40

[a,b,c] Within rows, lamb ranks with different superscripts are significantly different (P<0.05)

Weight on testing day

There was a significant relationship between the lamb rank and the weights of the lambs at 8-17 days of age (Table 5). The lamb that was lightest at birth was still lighter than the other two at the time of the sucking behaviour test (light vs. medium: t = -2.95, P = 0.009; light vs. heaviest t = -4.41, P = 0.0003). However, there was no difference between the medium and heaviest lambs at this age (t = -1.46, P = 0.16).

Abdominal girth

There was a significant relationship between lamb rank and the abdominal girth at the time of the sucking behaviour test (Table 5). The lambs that were born lightest had smaller abdominal girths than the medium lambs (t = -2.56, P = 0.02) and than the heaviest lambs (t = -4.19, P = 0.0005) at 8-17 days of age. There was no significant difference in abdominal girth between the medium and heaviest lambs at the time of testing (t = -1.63, P = 0.12).

The means and pooled standard errors of the data recorded of the lambs and ewes during the 15 min observation period are presented in Table 6.

Table 6. Means ± pooled SE of the behaviours and indicators of milk intake of the triplet lambs and ewes during the 15 min observation period after 4 hours of separation, with the lamb rank based on birth weight

Variable	Light lambs	Medium lambs	Heavy lambs	F value	P value	Effect of lamb rank	Effect of ewe[2] (%)
Milk intake							
Weight gain (g)	116.0 ± 33.4	224.0 ± 33.4	196.0 ± 33.4	2.8	0.09	tendency	0
Relative weight gain (%)	2.3 ± 0.6	3.6 ± 0.6	3.0 ± 0.6	1.2	0.33	NS[1]	0
Abdominal girth gain (cm)	2.8 ± 0.5	2.0 ± 0.5	1.2 ± 0.5	2.5	0.11	NS	9
Relative girth gain (%)	6.8 ± 1.2	4.6 ± 1.2	2.8 ± 1.2	2.9	0.08	tendency	9
Sucking behaviour							
Sucking bouts	2.6 ± 0.6	3.0 ± 0.6	2.5 ± 0.6	0.3	0.73	NS	48
Attempted bouts	5.6 ± 1.4	5.5 ± 1.4	4.3 ± 1.4	0.4	0.68	NS	31
Total sucking duration (s)	146.5 ± 25.0	151.8 ± 25.0	125.5 ± 25.0	0.4	0.67	NS	26
Mean sucking bout duration (s)	80.4 ± 20.7	79.3 ± 20.7	52.7 ± 20.7	0.6	0.57	NS	0
Relative sucking duration (%)	33.5 ± 5.0	36.0 ± 5.0	30.5 ± 5.0	0.3	0.74	NS	0
Frequency of teat switching	0.4 ± 0.2	0.8 ± 0.2	0.0 ± 0.2	2.7	0.09	tendency	0
Teat fidelity (%)	91.0 ± 4.3	86.6 ± 4.5	100.0 ± 4.5	2.3	0.13	NS	0
Non-sucking behaviour							
Number of butts	22.6 ± 3.8	23.0 ± 3.8	20.0 ± 3.8	0.2	0.79	NS	21
Urination frequency	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	1.5	0.25	NS	20
Defecation frequency	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	1.0	0.39	NS	8
Ewe behaviour							
Ewe kicks	0.8 ± 0.4	1.1 ± 0.4	0.5 ± 0.4	0.7	0.52	NS	29
Ewe walks	6.0 ± 1.5	5.2 ± 1.5	4.0 ± 1.5	1.1	0.37	NS	56
Ewe butts	0.6 ± 0.4	0.1 ± 0.4	0.1 ± 0.4	1.0	0.39	NS	34

[1] Non Significant

[2] The effect of the ewe on the variables was determined by dividing the variance due to the ewe identity by the total variance

Milk intake

Weight gain

There was a tendency (P = 0.09) for lamb rank to affect weight gain, and therefore milk intake (Fig. 2). The lambs that were lightest at birth tended to gain less weight in the observation period than heavier lambs, particularly those of medium birth weight.

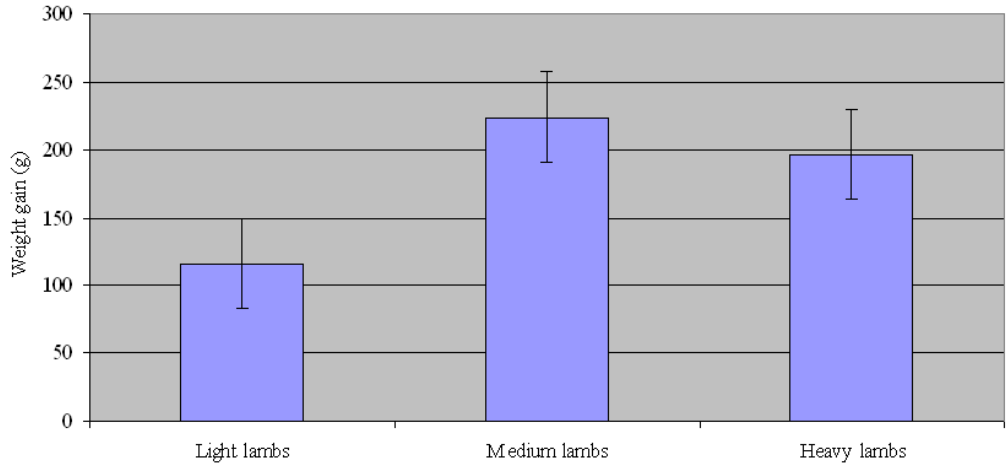

Figure 2. Mean ± SE weight gain due to milk intake in light, medium and heavy triplet lambs in the 15 min observation period.

Relative weight gain

When the weight gain was calculated as a percentage of the original weight, there was no difference between the lamb ranks. In other words, lambs of all ranks gained a similar percentage of their original body weight (Table 6).

Abdominal girth gain

Lamb rank did not have a significant effect on abdominal girth gain (Table 6).

Relative girth gain

There was a tendency (P = 0.08) for lamb rank to affect the relative girth gain (Fig. 3). Lambs that were lightest at birth tended to gain more abdominal girth, relative to their original girth, than lambs that were heaviest at birth.

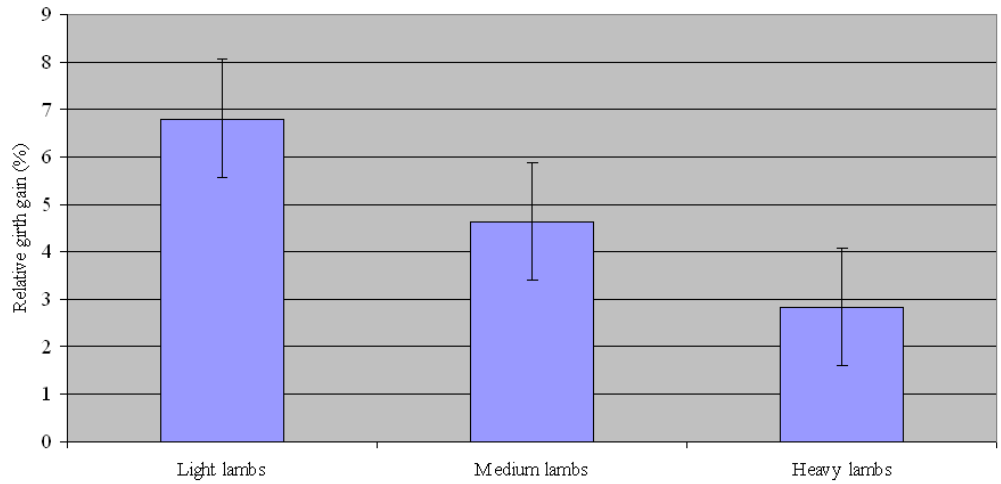

Figure 3. Mean ± SE girth gain relative to the original girth measured after 4 hours of separation of the light, medium and heavy triplet lambs in the 15 min observation period.

Sucking behaviour

There was no effect of lamb rank on most of the sucking behaviours measured in the 15 min observation period. The number of sucking bouts, number of attempted bouts, total sucking duration, mean sucking bout duration, relative sucking duration and teat fidelity were similar between the 3 lamb ranks (Table 6). Ewe identity had a moderate to large effect on the number of sucking bouts, attempted bouts and total sucking duration of the lambs (Table 6).

Frequency of teat switching

There was a tendency (P = 0.09) for lamb rank to have an effect on the frequency of teat switching (Fig. 4). The lambs with medium weights at birth tended to switch teats more often than the heaviest lambs.

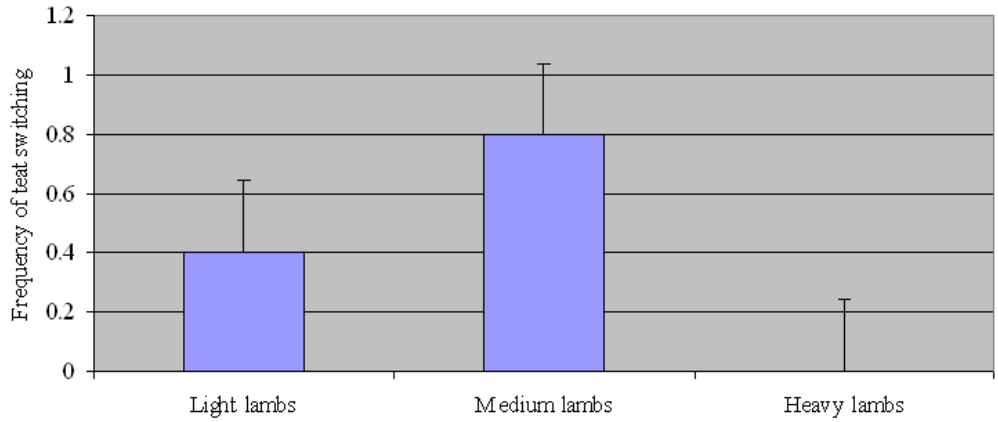

Figure 4. Mean ± SE frequency of teat switching of the light, medium and heavy triplet lambs in the 15 min observation period.

Non- sucking behaviour

Number of butts

There was no significant relationship between lamb rank and the number of butts (Table 6).

Urination and defecation frequency

Most lambs did not urinate or defecate within the observation period. However, some lambs did both. There were 6 lambs that urinated once, 3 lambs that defecated once and 2 lambs that both urinated and defecated once. However, there were no significant differences in the urination or defecation frequency between the light, medium and heavy triplet lambs (Table 6).

Ewe behaviour

There was no effect of lamb rank on the number of times the ewes kicked the lambs, walked away or butted the lambs (Table 6).

Discussion

There is much evidence that lambs born lighter are at higher risk of dying in the first week of life than heavier lambs (Morel *et al.,* 2008). Triplet lambs tend to be lighter than twin and single lambs (Stafford *et al*. 2007). Within triplet litters, mortality in the neonatal period is highest for the lightest lamb and lowest for the lamb born heaviest (Morel *et al.,* 2008). Lighter lambs are less vigorous at birth and take longer to suck successfully than their heavier littermates, which leads to a higher risk of infection because of poorer immunological protection (Dwyer, 2008). Based on these findings, we wanted to determine whether differences in sucking behaviour and milk intake between triplet lambs persist after the first week of life, and whether birth weight can predict which lamb is excluded from sucking and therefore less likely to prosper. We hypothesized that at 8-17 days of age, the triplet lambs born lightest would be less competitive and receive less milk than their heavier siblings after a period of separation from the ewe.

Milk intake

The results of this study provide some evidence that the hypotheses were correct. Triplet lambs born lightest tended to gain less weight than their heavier siblings after 15 minutes with the ewe. They were also still significantly lighter than the other 2 triplets at 8-17 days of age, suggesting that their milk intake and therefore growth rate was consistently lower.

In addition to weight gain, the abdominal girth was measured as an indication of milk intake. This was never done before, but was expected to provide an additional measure of milk intake. However, the results using this measure appeared to contradict those obtained by measuring weight gain. The lightest lambs at birth tended to gain *more* girth relative to their original girth than the heavier lambs. Therefore, the abdominal girth did not seem to be that accurate. Probably the location measured did not reflect the filling of the stomach accurately.

Sucking behaviour

Sucking behaviours did not differ according to lamb rank. Contrary to the hypotheses, the lightest lambs did not appear to be less competitive; they spent as much time on

the udder as the heavier lambs. Nor did light lambs show more signs of milk shortage; they did not have more unsuccessful sucking attempts, nor did they show more butting or teat switching than the heavier lambs. In fact, lambs born of medium weight tended to switch teats more often than heavy lambs, but at similar frequencies to light lambs. This suggests that the heavy lambs got on teat and stayed there until they were satiated whereas medium lambs had to switch teats to get enough milk.

It was difficult to time the sucking behaviours of the lambs, which makes it a less reliable indicator of milk intake. Sometimes all 3 lambs of a triplet waggled their tails at the same time and made real sucking movements, but one of them was faking. Because of the fact that both direct observations and video recording were done, most mysteries could be solved. However, it was clear to see that behaviour of the lambs could be exactly the same when they were really drinking milk, and when they were just sucking some wool near the udder. It seemed that non-nutritive sucking was rewarding, just like some lambs accepted sucking an artificial teat each time it was presented to them in the study of Goursaud and Nowak (1999).

The lightest triplet lambs

Given the difficulties outlined above, it is likely that weight gain is the most reliable indicator of milk intake measured in this study. Based on this measure, the lightest triplet lambs had a lower milk intake than their heavier siblings at 8-17 days of age. They were also still lighter at this age than the two heavier lambs, which had equalized in terms of weight, size and milk intake. These results are consistent with the notion put forward by Hinch that one lamb out of a triplet litter is handicapped in its milk intake compared to the other two (Hinch, 1989). In our study, the lightest lamb appears to be the runt in triplet litters. The two lambs born medium and heavy appear to equalize by a week or 2 old, but the one born lightest appears to do poorly. This lightest lamb gets less milk, and grows less quickly. This suggests that farmers should either try to rescue these light lambs at an early stage, for example by weaning or fostering them. Or there should be a selection for ewes with a higher milk production, to prevent one lamb getting not enough milk.

Sucking behaviour as a predictor of milk intake

The current study differed from previous studies comparing triplet lamb sucking behaviour and milk intake, because we did not find a relationship between milk intake and sucking behaviour, whereas they did (Hinch, 1989; Cameron, 1998; Cimen, 2007). However, there are difficulties in using sucking behaviour to measure milk intake: longer sucking durations may result in a greater milk intake or indicate a greater difficulty in obtaining the milk (de Passille and Rushen, 2006). Likewise, increased sucking frequency may reflect a high milk intake or a lower milk intake per sucking bout (Dwyer, 2003). Therefore, caution should be taken in inferring milk intake from measures of sucking behaviour in lambs.

Competition for milk

The sucking behaviour in the current study was observed after 4 hours of separation from the ewe, which meant that there was a strong competition for all lambs to get milk. Probably the undisturbed sucking behaviour in the triplets would have been different. On the other hand, by separating all triplets for 4 hours, it was for sure that all lambs really wanted to get a drink, and there was no difference that maybe one lamb just had a drink before the observation period. Furthermore, there is evidence that the ewe often decides whether she lets the lambs drink or not. At pasture, ewes often call the lambs; if one arrives at her side before the other, she will not allow the first lamb to suck until the second is present (Ewbank, 1964). As soon as 2 lambs are present, the ewe will stand still and the lambs can suck. So it is realistic to have a period of a few hours without drinking and then have competition for milk during the sucking period. The current study suggests that there is natural competition indeed and the lightest lamb does not get the same amount of milk as the heavier two, who have equalized in weight and size at 8-17 days of age.

Limitations of this study

One limitation of this study was the small sample size. Of the 28 triplet bearing ewes, only 10 complete litters survived to at least 8 days of age and could be used for this trial. A larger group of animals would have reduced the variation within lamb ranks making it more likely that any real differences between ranks would have been found. As a consequence of the small sample sizes, ewe identity had a moderate to large effect on some behaviours of the lambs, like the number of sucking bouts, attempted

bouts and total sucking duration. However, ewes did not have an effect on other behaviours. For example fighting for a position at the teat (measured by teat switching and teat fidelity) was largely left to the lambs (Hudson and Trillmich, 2008).

Another limitation of this study was the wide range in the age of the triplet litters on the day of testing. If the ewes had been synchronised, the variations between the triplets would have been smaller. In that case, the age of the lambs would have been almost the same for all of them, instead of varying between 8 and 17 days like in this trial. Now, the date of testing and the age of the lambs were strongly related. Younger litters were tested later. This could have affected the test results, but it was not possible to do the sucking behaviour test at exactly the same age for all the triplet lambs.

The last limitations of this study were the different weather conditions on the 6 days of observation, and the fact that by the age of 8-17 days, only the strong triplet litters were left. The weakest lambs had already died.

However, the results are still relevant to farmers because if lambs survive until 8-17 days of age, the lightest ones at birth still tend to ingest less milk than the heavier lambs, are therefore still the weaker lambs within a set of triplets and will have a lower weight at weaning causing less profit.

Conclusions

There were no differences in the sucking behaviour of the 1-2 week old triplet lambs in a competitive situation. However, the lightest lambs tended to gain less weight than their heavier siblings during the observation period. Given the limitations of using behaviour to indicate milk intake, these results suggest that the lightest lambs had a lower milk intake than the other two siblings and that the lamb born lightest is likely to be the one excluded from sucking most often. In other words, triplet lambs born lightest are runt. This explanation is consistent with the fact that triplet lambs born lightest were still lighter than their siblings at 1-2 weeks of age, while the other two lambs had equalized in weight and size. The lightest lambs appeared to have a lower growth rate and therefore lower chance of survival.

This research should be repeated with a greater number of triplets to clarify the differences between light, medium and heavy lambs within a set of triplets. In addition, further research should be done to determine whether ewes and lambs can adapt their sucking behaviour to cope with triplet litters, instead of twins or singles, or whether ewes should be selected for higher milk production. In addition, the effect of different management strategies (e.g. fostering or weaning of the lightest triplet or providing milk supplement) on triplet lambs' milk intake, growth and survival should be tested.

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