Does ewe nutrition during pregnancy affect the neonatal behaviour of twin-born lambs?

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Abstract

In this experiment the effects of feeding treatments from mid pregnancy until lambing on the behaviour of twin-bearing ewes and their lambs were investigated. Fifty seven four-year-old Romney ewes were offered a medium (1164 ± 31.6 and 819 ± 16.0 kg DM/ha pre- and post-grazing pasture mass, respectively) or *ad libitum* (2181 ± 47.6 and 1431 ± 24.6 kg DM/ha pre- and post-grazing pasture mass, respectively) feeding treatment from day 76 of pregnancy until after lambing. At ear-tagging the time taken for the lambs to stand, make contact with, suck from and follow their dam was recorded. A maternal behaviour score based on the distance the ewe moved away from her lambs during tagging was also recorded. Survival analysis showed that lambs born to ewes offered the medium feeding treatment from mid pregnancy until lambing were quicker to stand, suck and follow compared with lambs born to ewes offered the *ad libitum* feeding treatment ($P<0.05$). It is unclear whether these behaviours indicate that lambs born to ewes in the medium treatment were more vigorous or that they had unmet needs that prompted the apparent increase in vigour. Ewe feeding treatments did not affect the maternal behaviour of the ewes.

Keywords: lamb behaviour; maternal behaviour; ewe BCS; ewe nutrition

Introduction

Appropriate ewe and lamb behaviours are important for the development of a strong ewe-lamb bond. A strong ewe-lamb bond is beneficial for ensuring lamb survival (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 & Alexander 1975). The behaviour of the newborn lamb, such as bleating and the progression to standing and sucking, is important to stimulate and reinforce maternal behaviours, such as grooming (Nowak 1990; Dwyer et al. 1999) and are essential in the ewe-lamb bonding process (O'Connor and Lawrence 1992).

Plane of nutrition of the ewe during pregnancy can affect maternal behaviours (Nowak, 1996; Everett-Hincks et al. 2005). For example, Dwyer et al. (2003) reported that ewes fed 65% of a pregnancy maintenance diet from 4 weeks of pregnancy until birth spent less time grooming their lambs in the first 30min after birth than ewe on the pregnancy maintenance. Lamb behaviours can also be affected by the ewes plane of nutrition during pregnancy (Muñoz et al. 2009). For example, Everett-Hincks et al. (2005) reported that the proportion of lambs that followed their dam after tagging increased as pasture allowance, from day 64 of pregnancy until birth, increased from 2cm (~800 kg DM/ha) to 8cm (~2000 kg DM/ha). With the exception of the study by Everett-Hincks et al. (2005), studies examining the impact of ewe nutrition on ewe and lamb behaviour have concentrated on the probable consequences of undernutrition. There is a lack of information on the possible advantages, or otherwise, of *ad libitum* feeding on ewe and lamb behaviour.

The process whereby environmental stimulus at a critical period of fetal development results in life-long effects of that individual is called "developmental programming" (Ross and Desai, 2005; Kenyon et al. 2014). In sheep, maternal nutrition can affect production traits such as the offspring's milk production and the grand-offspring's live weight to weaning (Kenyon et al. 2014). For an in-depth review see Kenyon et al. (2014). To date the potential role of developmental programming, resulting from differing feeding levels, on ewe and lamb behaviour has not been investigated.

Therefore, this experiment investigated the effects of ewe feeding from mid pregnancy until lambing on the behaviour of twin-bearing ewes and their lambs at tagging. Further, the dams of the ewes in this study had also been exposed to different maternal feeding levels when they were pregnant. A secondary aim was, therefore, to investigate the impact of different feeding levels during pregnancy on offspring and grand-offspring behaviour.

Materials and methods

The experiment was conducted in 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.

Four-year-old twin-bearing ewes were offered either medium or *ad libitum* feeding conditions, based on pasture masses, from 76 days after the start of the breeding season (day 76; P76) until birth (Table 1). The ewes were part of a large study that examined the effect of nutrition during pregnancy on the productivity of female offspring (Kenyon et al. 2014). The pre- and post-grazing pasture masses of the *ad libitum* feeding treatment were 2181 ± 47.6 and 1431 ± 24.6 kg DM/ha, respectively while they were 1164 ± 31.6 and 819 ± 16.0 kg DM/ha, respectively in the medium feeding treatment (Kenyon et al. 2014). Pasture was a white clover and ryegrass mix and metabolisable energy consisted of 12.7 ± 0.26 and 12.1 ± 0.26 MJ ME/kg DM,

Table 1 The number of ewes (n) in the grand-dam (P21-50 and P51-140) and ewe (P76-140) feeding treatment subgroups.

Grand-dam		Ewe	
P21-50	P51-140	P76-142	n
Low	Medium	Medium	6
Low	Medium	*Ad libitum*	5
Low	*Ad libitum*	Medium	2
Low	*Ad libitum*	*Ad libitum*	3
Medium	Medium	Medium	3
Medium	Medium	*Ad libitum*	5
Medium	*Ad libitum*	Medium	4
Medium	*Ad libitum*	*Ad libitum*	8
Ad libitum	Medium	Medium	8
Ad libitum	Medium	*Ad libitum*	3
Ad libitum	*Ad libitum*	Medium	3
Ad libitum	*Ad libitum*	*Ad libitum*	7

for *ad libitum* and medium respectively (P=0.09).

The 57 ewes in the present study were a random subset of ewes whose own dams (hereafter called 'grand-dams') had been offered either low, medium or *ad libitum* feeding treatments in early pregnancy (P21-P50), followed by either medium or *ad libitum* feeding treatments in mid to late pregnancy (P51-P140, Table 1, Kenyon et al. 2011). The grand-dams were part of a large study that examined the effect of nutrition during pregnancy on ewe and lamb performance. The full details of the feeding treatments of the grand-dams can be found in Kenyon et al. (2011). The effect of the early and late pregnancy feeding treatments of the grand-dams on lamb behaviours is briefly examined in this experiment.

The numbers of ewes presented above only include those who had a complete set of twin-born lambs (where both lambs were alive at tagging). Ewes with only a single lamb alive at tagging were not included in this study.

Animal and behavioural measurements

All ewes were weighed and had their body condition score (BCS) recorded at D76, and D142 (BCS, scale 1.0–5.0, including half units, Jefferies 1961) by a single experienced operator. During the lambing period, ewes were inspected twice daily. Lambs were ear tagged using FlexiTags by All Flex, New Zealand once their coat was dry and they were mobile. While the lambs were being tagged, 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 and makes contact with either the shepherd or lamb).

Immediately following tagging, the two lambs were placed together, lying on the ground while the observers moved approximately 10 metres away. The moment the lambs were released 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 two teams of three observers and the teams remained consistent for the majority of the study.

The lamb behaviours recorded during the 5-minute observation period 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 was 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 for at least five seconds) was noted. Only behaviours expressed by the lamb in the 5-minutes were recorded.

After the behavioural observations were conducted, the ewes and their lambs were moved to a new paddock and were offered *ad libitum* grazing until weaning (Kenyon et al. 2011; 2014).

Statistical Analysis

All statistical analyses were conducted using SAS v. 9.3 (SAS Institute Inc., Cary, 2011, NC, USA). Live weights of the ewes at day 76 and 142 of pregnancy were analysed using a mixed model allowing for repeated measures that included the fixed effects of ewe feeding treatment (*ad libitum* or medium) and included days to lambing as a covariate (based on actual lambing date).

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 (*ad libitum* or medium) 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. Due to low numbers of ewes in some BCS groups, ewes with a BCS above 3 on P76 were classified as ≥3, while ewes with a BCS above 4 on P142 were classified as ≥4.

The time taken for lambs to stand, make contact, suck and follow their dam during the observation period were not normally distributed and the distribution could not be normalised. Therefore, the time taken to exhibit these behaviours was analysed using survival analysis. Survival curves were generated using Kaplan-Meyer estimators. The effect of ewe feeding treatment and grand-dam feeding treatments were tested in separate models. Due to the non-parametric nature of the test covariates could not be added. Lambs that did not exhibit 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.

The median time to exhibit a listed behaviour was analysed by Kruskal-Wallis tests. The effect of ewe feeding treatment and grand-dam feeding treatments were tested in separate models. The analysis contained all lambs, including those that did not exhibit the behaviour during the observation period. The lamb was assigned a value of 301

for a behaviour variable if that behaviour was not exhibited. The association between the fixed effects and the behaviour variable was investigated using the Wilcoxon Test. If ewe feeding treatment or grand-dam feeding treatment was found to be significant, the Wilcoxon two-sample *post hoc* test was carried out with a Bonferroni adjustment.

Ewe MBS was analysed using a generalised model with a Poisson distribution containing the fixed effects of ewe nutrition treatments and grand-dam nutrition treatments and their two-way interactions.

Results

Ewe live weight and body condition score

The live weight of the ewes was similar for both feeding treatments at P76 (medium 69.6 ± 1.0 kg, *ad libitum* 70.7 ± 0.9 kg, P>0.05). On P142, ewes in the medium feeding treatment (84.2 ± 1.0 kg) were lighter than ewes in the *ad libitum* treatment (97.8 ± 0.9 kg, P<0.05).

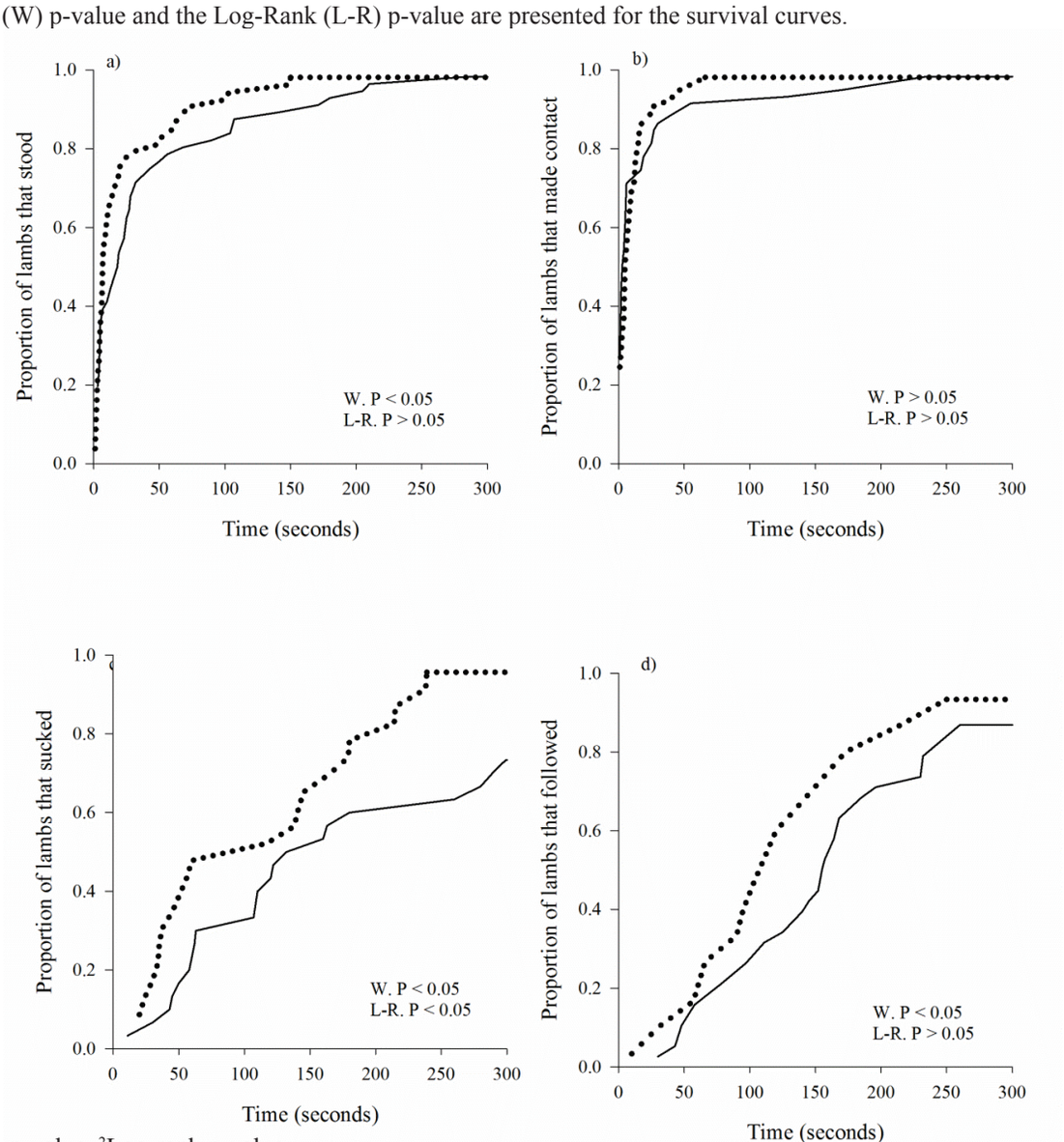
The BCS of the ewes were similar for both feeding treatments at P76 (medium 2.5 ± 0.08, *ad libitum* 2.5 ± 0.07, P>0.05). On P142, ewes in the medium feeding treatment had a lower BCS (2.4 ± 0.07) than ewes in the *ad libitum* feeding treatment (3.4 ± 0.07, P<0.05).

Lamb behaviour

The survival analysis showed that the time for lambs to stand differed between lambs born to ewes in the medium and *ad libitum* feeding treatment (Wilcoxon P<0.05, Fig. 1a). At approximately 10 seconds, 61% of lambs born to ewes in the medium feeding treatment had stood compared with 41% of lambs born to ewes in the *ad libitum* feeding treatment.

After 43 seconds 90% of all lambs had made contact their dam. The time for the ewe and lamb to make contact after tagging did not differ between feeding treatments (P>0.05, Fig. 1b).

Figure 1 The effect of ewe feeding treatments (medium ….. and *ad libitum* ──) 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 The effect of grand-dam and ewe feeding treatments (grand-dam early pregnancy; low, medium and *ad libitum,* grand-dam mid to late pregnancy; medium and *ad libitum* and ewe; medium and *ad libitum*) 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
Grand-dam, P21-P50					
low	32	5.5	6.0	162.0	140.0
medium	40	20.0	4.0	121.0	168.5
ad libitum	42	11.0	5.0	90.0	120.0
Grand-dam, P51-P140					
medium	60	18.0	6.0	147[b]	152.0
ad libitum	54	7.0	5.0	114.0[a]	138.0
Ewe, Feeding treatments					
medium	52	7.0	5.0	132.0	120.0
ad libitum	62	19.0	3.5	117.0	156.0

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

Lambs born to ewes in the medium feeding treatment were quicker to suck than lambs born to ewes in the *ad libitum* feeding treatment (Wilcoxon and log-rank $P<0.05$, Fig. 1c). At approximately 50 seconds, 36% of lambs born to ewes in the medium feeding treatment had sucked compared with 17% of lambs born to ewes in the *ad libitum* feeding treatment.

Lambs born to ewes in the medium feeding treatment were quicker to follow their dam than lambs born to ewes in the *ad libitum* feeding treatment (Wilcoxon $P<0.05$, Fig. 1d). At approximately 120 seconds, 60% of lambs born to ewes in the medium feeding treatment had followed compared with 35% of lambs born to ewes in the *ad libitum* feeding treatment.

The median times for the lambs to stand contact, suck or follow were not affected by ewe feeding treatment ($P<0.05$, Table 2).

The grand-dam early pregnancy feeding treatment did not affect any lamb or ewe behaviours ($P>0.05$, data not shown).

The median time to suck was less ($P<0.05$) for lambs whose grand-dams were offered the *ad libitum* (114 seconds) feeding treatment compared with lambs whose grand-dams were offered the medium (147 seconds) feeding treatment in mid to late pregnancy. No other ewe or lamb behaviours were affected by the grand-dam feeding treatments in mid to late pregnancy ($P>0.05$, data not shown).

Discussion

In pastoral farming systems, ewes are frequently fed "pregnancy maintenance levels" – that is, to gain conceptus weight whilst maintaining their own body weight. By feeding pregnancy maintenance levels, pasture can be saved for lambing and lactation. It has been reported that ewes and lambs benefit from increased feeding levels during lactation (West et al. 2009), but there is limited evidence as to the advantage of increased feeding throughout pregnancy. The two aims of this study were to examine the effects of ewe feeding treatments (medium and *ad libitum*) and grand-dam nutrition (low, medium and *ad libitum* during P21-50 and medium and *ad libitum* during P51-142) on the behaviour of ewes and their twin-born lambs after birth.

The difference in ewe live weight at P142 indicates that the aim of the ewe feeding treatments was fulfilled. Lambs born to ewes offered the medium feeding treatment (i.e. pregnancy maintenance) from mid pregnancy until lambing were quicker to stand, suck and follow their dam compared with lambs born to ewes in the *ad libitum* feeding treatment. The results of 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 sward heights of 2 cm (~700 kg dry matter (DM)/ha) had a lower median time to stand and a greater percentage of lambs moved towards their dam than those managed on sward heights of 4 cm (~1300 kg DM/ha) in mid and late pregnancy. On the other hand, Gronqvist et al. (2015) reported that, of the lambs born to ewes offered 800 to 1000 kg DM/ha in very late pregnancy (from day 141), 25% failed to stand in the 300 seconds following tagging compared to only 6% of lambs in the *ad libitum* fed group. However, the time taken for lambs to contact, suck and follow in that experiment was not affected by ewe feeding treatments (Gronqvist et al. 2015). Given that only one lamb behaviour trait was affected in the study by Gronqvist et al. (2015) this result may have been a spurious effect and not of biological significance. Alternatively, the current results may differ from previous research due to the increased length of the feeding treatments in this study (P76 until birth compared with P141 until birth in Gronqvist et al. 2015), which may have caused a greater treatment effect.

The results of the feeding treatments in the present study might indicate that either lambs born to ewes on the medium feeding treatment were more 'vigorous' or that these lambs had unmet 'needs' that prompted the increased activity. '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. Kenyon et al. (2014) reported that the lambs born to ewes offered *ad libitum* (of which the present lambs were a subset) were lighter at birth than those born to ewes offered medium. Perhaps greater birth weight of the lambs in the medium feeding treatment compared to the *ad libitum* treatments might explain why they were quicker to stand, suck and follow their dam.

Nowak (1996) reported that maternal nutrition during pregnancy can influence maternal behaviours Previous

studies have reported that restricted feeding of ewes from the 4th week of pregnancy (65% of pregnancy maintenance, Dwyer et al. [2003]) or the 10th week of pregnancy (to achieve 5% decrease in ewe live weight, Thomson and Thomson [1949]) 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 on ewe MBS. This may be due to the ewes not being nutritionally restricted. The ewes in the medium feeding treatment gained almost 15 kg in late pregnancy, suggesting that they were, indeed, not nutritionally restricted. The conceptus mass for twin-bearing ewes fed *ad libitum* has been reported to be approximately 11 kg (Rattray et al. 1974). The ewes in the medium treatment in the present study, therefore, appear to have gained live weight in addition to expected conceptus weight. Previous studies have tended to compare maintenance with sub-maintenance nutrition. However, studies by Everett-Hincks et al. (2005) and Corner et al. (2010), which utilised similar feeding treatments but implemented during different stages of pregnancy, reported similar results. Combined the results indicate that there is no advantage, in terms of MBS, in offering twin-bearing ewes feeding levels above pregnancy requirements.

Lambs whose grand-dams 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 developmental programming effect on lamb behaviour. No other recorded ewe or lamb behaviour traits were affected, however, and it is recognised that the number of ewes in this study was small so this may be a spurious finding. Nevertheless, further research is warranted and will help in drawing a clear conclusion regarding developmental programming effects on ewe and lamb behaviour. It would be particularly interesting to investigate the developmental programming effect of maternal undernutrition in late pregnancy when the majority of fetal growth occurs.

Conclusion

Lambs born to ewes offered medium feeding levels, aimed at meeting 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 fed *ad libitum*. However, it is unclear why lambs born to ewes in the medium treatment are compelled to perform these behaviours sooner after tagging than lambs born to ewe in the *ad libitum* treatment. The maternal behaviour of the ewes was unaffected by feeding treatment. There may be a developmental programming effect of grand-dam nutrition on lamb behaviours, further research in this area is thus warranted.

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