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

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Abstract

Nutrition of ewes during pregnancy may affect the behaviour of ewes and their lambs in the first few days of life. In this experiment, triplet-bearing ewes were allocated 'medium' (pasture mass 800-1200 kg DM/ha) or *ad-libitum* nutrition (pasture mass 1500-2000 kg DM/ha) from day 93 until 114 of pregnancy. Both treatments included ewes of body condition score (BCS) ≤2.0, 2.5 or ≥3.0 as measured on day 92 of pregnancy. Behaviours of ewes and lambs were recorded in the paddock at tagging and a subset of lambs was subjected to a maternal-recognition test. Within the BCS≤2.0 ewe group, lambs born to ewes on the medium treatment emitted more high-pitched bleats and lower low-pitched bleats than lambs born to ewes on the *ad-libitum* treatment. In the paddock, there was no effect of ewe feeding treatment or BCS group on the median time taken for the lambs to stand, make contact with, suck from or follow their dam. In the maternal-recognition test, the median time taken for the lambs to reach the dam and the time the lambs spent with her was not affected by ewe BCS or feeding treatment. Overall the results indicate that feeding triplet-bearing ewes above pregnancy requirements has minimal effect on lamb behaviours. There is, however, some evidence to suggest that lambs born to ewes in the BCS≤2.0 group offered the medium treatments may have experienced a weaker ewe-lamb bond.

Introduction

Triplet-born lambs have lower survival rates than both single- and twin-born lambs (Hight & Jury 1970; 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 & Stafford 2004), with triplets becoming more common (Amer et al. 1999).

Nutrition during pregnancy has been shown to affect the behaviour of twin-bearing ewes and their lambs (Corner et al. 2010; Gronqvist et al. 2015; Gronqvist et al. 2016). There have, however, been few studies on triplets (Everett-Hincks et al. 2005a). The behaviour of the newborn lamb is vital for lamb survival as it motivates maternal behaviours (Nowak 1990; Dwyer et al. 1999) and helps develop the ewe-lamb bond (O'Connor & Lawrence 1992).

Body condition score (BCS) of the ewe during pregnancy affects lamb survival (Kleemann et al. 2006; Kenyon et al. 2014). Dwyer (2003) reported that ewes that lost body condition during pregnancy had lambs that were slower to stand and suck after birth. No studies have, however, examined the effect of ewe mid-pregnancy BCS on the behaviour of triplet-bearing ewes and their lambs. Therefore, this study investigates the effect of both ewe nutrition and BCS during mid-pregnancy on the behaviour of triplet-bearing ewes and their lambs at tagging under extensive pastoral conditions. The aim was to study whether *ad-libitum* compared with medium feeding improves the behaviour of triplet-bearing ewes and their lambs, and whether the response to *ad-libitum* feeding differs for ewes of different BCS.

Materials and methods

Background

This experiment included 88 primiparous triplet-bearing Romney ewes (two years of age) and their lambs (n=264). The data of ewe and lamb live weight to weaning have previously been published (Kenyon et al. 2011), however, ewe and lamb behaviour data has not. 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'* feeding treatment for 21 days (P93-P114). Pre- and post-grazing masses achieved were 1133±62 and 921±44 kg DM/ha, respectively for the medium treatment and 2012±71 and 1541±71 kg DM/ha, respectively for the *ad-libitum* treatment (Kenyon et al. 2011). Ewes of BCS ≤2.0, 2.5 and ≥3.0 (as measured on P92, scale of one to five, one=emaciated, five=obese; Jefferies, 1961) were randomly allocated, within BCS, to each feeding treatment. The ewe sub-groups contained the following number of ewes; *ad-libitum* BCS≤2.0 n=21, *ad-libitum* BCS2.5 n=13, *ad-libitum* BCS≥3.0 n=9, medium BCS≤2.0 n=14, medium BCS2.5 n=15 and medium BCS≥3.0 n=16.

From P114, the ewes were managed as a single mob and were offered *ad-libitum* feeding conditions until P141. At P142 ewes were set stocked in lambing paddocks. 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.

The 88 ewes in the current study were a subset of 144 ewes included in the study of Kenyon et al. (2011). Of the original 144 ewes, only complete sets of triplet-born lambs (all lambs alive at time of tagging) and their dams were included in the analysis. This was due to the potential variation in behaviour of a single- or twin-reared lamb, as opposed to a member of a complete triplet-set. Ewes requiring assistance during parturition were not included in this study.

Animal and behavioural measurements

Ewe BCS and live weights were recorded at P92, P113, and P142. During the lambing period ewes were inspected twice daily at 8 am and 4 pm. Lambs were tagged once they were mobile and their coat was dry. At tagging, the lambs were identified to their dam, ear-tagged and their sex, birth-rank and live weight were recorded. Immediately after tagging, the full sets of triplets were placed 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 recorded individual ewe and lamb behaviours, as follows, for five consecutive minutes.

The number of low-pitched bleats, involving little mouth movement, and high-pitched bleats, involving full mouth movement, emitted by the lamb were recorded (Everett-Hincks et al. 2007). The time taken until the lamb stood and fully supported itself on all legs for a minimum of five seconds. The time taken for the ewe and lamb to come within 0.5 metres of each other and to follow the ewe were noted. The time taken to follow was recorded from 'time zero' until the ewe and lamb moved a minimum of five meters from their initial point of contact. The time taken for the lamb to effectively suck from its dam was recorded from when the lamb held the teat in its mouth and appeared to be suckling for a minimum of five seconds (Everett-Hincks et al. 2005a). If a lamb did not exhibit a specific behaviour in the five-minute period, a value for that behaviour was not recorded. Maternal behaviour score (MBS), as defined 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 retreated from her litter while they 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). Ewe high- and low-pitched bleats were recorded (Corner et al. 2010).

Observers were trained prior to all measurements. Observers worked in teams of three, with at least one experienced recorded present in each group, to ensure measures were accurately recorded following a prescribed protocol. Teams were utilised as it would not have been possible for a single person to collect all of the data in these triplet ewe and lamb units.

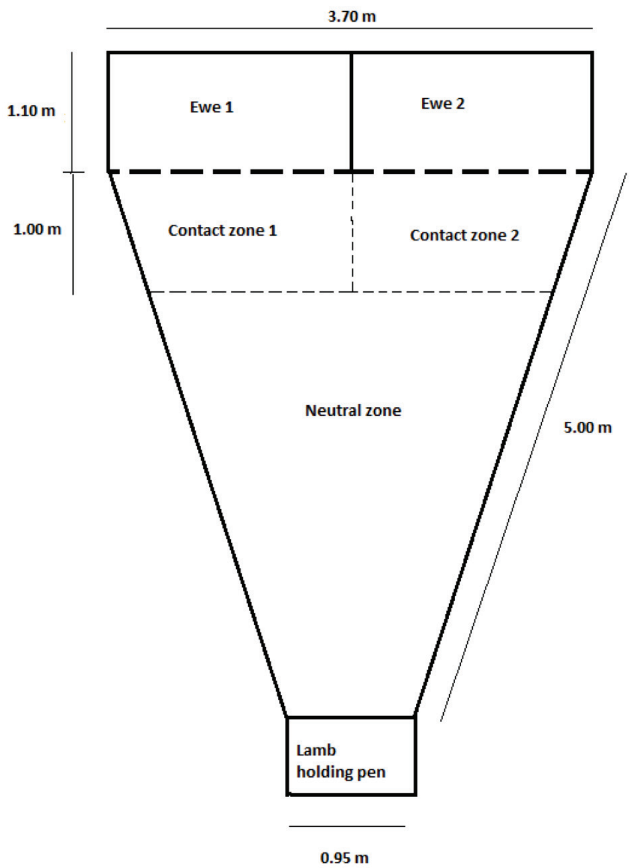
Maternal recognition test

A randomly chosen subset of lambs based on treatments (n=150, i.e. 50 triplet sets) were subjected to a maternal-recognition test (Nowak et al. 1987). The testing arena was of a triangular shape, fenced by one-metre-high solid walls (3.7 x 6.1 m.). Adjacent to the vertex of the triangle pen was a lamb-holding pen. At the opposite end there were two ewe pens (1.85 x 1.1 m) side by side, separated from the testing arena by wire-mesh gates. The arena itself was divided into three zones separated by 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 (Figure 1).

Testing occurred between 1 and 3 pm daily. As a result, lambs were tested at approximately 12 or 24 hours of age depending on when they were born and handled. 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 released into the neutral zone, the lamb could see both ewes. All other lambs, including the sibling of the lamb being tested and the lambs 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 & Alexander 1975; Nowak et al. 1987).

Each maternal-recognition test was conducted for five minutes and the location and activity of the lamb were recorded at 10-second intervals. This allowed the total time that the lamb spent in the contact zone adjacent to its dam, or in either contact zone, and the time spent sitting (supporting itself on less than four legs) and walking to be calculated. High- and low-pitched bleats were counted for the duration of the test. Trained observers watched and recorded from outside the pen.

Figure 1 Diagram of the maternal-recognition testing pen showing dimensions of the area, layout and zones. Based on that by Nowak et al. (1987)



Statistical Analysis

All statistical analyses were conducted using SAS v. 9.3 (SAS Institute Inc., Cary, 2011, NC, USA).

Live weight and BCS of the ewes were analysed using a mixed model allowing for repeated measures that included the fixed effects of feeding treatment, BCS group and their two-way interaction. 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 percentage of ewes or lambs that bleated in the paddock or maternal recognition test, or performed the various behaviours assessed during the maternal-recognition test was analysed using a generalised model based on a binomial distribution and a logit transformation. Number of bleats emitted and MBS were analysed using a generalised model based on a Poisson distribution. These models included the fixed effects of feeding treatment and BCS group in all cases, and included age of lamb (12 vs 24 h) and all two-way interactions for bleating behaviour in the maternal-recognition test.

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 not normally distributed and could not be normalised using transformations. The median time to exhibit a behaviour in the paddock or the maternal recognition test was analysed using the non-parametric Kruskal-Wallis test. The effects of feeding treatment or BCS group were tested in separate models and, for the paddock behaviours, the analysis contained all animals including those that did not show the behaviour during the observation period. In the maternal-recognition test, when analysing the median time the lamb spent walking, sitting, with the dam and in the contact zone, only lambs that exhibited those behaviours were included. 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.

Results

Ewe weight and BCS

At P93, ewes in the BCS≤2.0 group were lighter (64.1±0.89 kg, P<0.05,) than ewes in the BCS2.5 (68.5±0.98 kg) and BCS≥3.0 (69.8±1.07 kg) groups, which did not differ (P>0.05). At P114, ewes in the medium (72.3±0.90 kg) feeding treatment were lighter than ewes in the *ad-libitum* treatment (75.5±0.96 kg, P<0.05). The BCS groups did not differ in BCS at P114 (mean BCS 3.1±0.08 kg).

Ewe vocalisation and behaviour

The MBS of the ewe was not (P>0.05) affected by ewe BCS group or feeding treatment (P>0.05) and the average MBS was 2.9. Of the ewes, 91% emitted high-pitched bleats and 89% emitted low-pitched bleats, but the percentage that bleated was not affected by ewe-feeding treatment, BCS group or their interactions (P>0.05, data not shown).

Of those ewes that bleated, there was a significant interaction between ewe-feeding treatment and BCS on the number of high-pitched bleats, such that *ad-libitum* ewes in the medium feeding treatment emitted fewer (P<0.05) high-pitched bleats than BCS≤2.0 ewes in the *ad-libitum* treatment (P<0.05, Table 1). Within the BCS≥3.0 group, ewes in the *ad-libitum* treatment emitted fewer high-pitched bleats than ewes in the medium treatment (P<0.05). No such difference was observed within the BCS2.5 group (P>0.05). The number of low-pitched bleats also varied among groups. Within the BCS2.5 group, ewes in the *ad-libitum* treatment emitted fewer low-pitched bleats than ewes in the medium treatment (P<0.05). Within the BCS≥3.0 group, ewes in the medium treatment emitted fewer low-pitched bleats than ewes in the *ad-libitum* treatment (P<0.05). There was no difference in low-pitched bleats observed within the BCS≤2.0 group (P>0.05).

Lamb vocalisation

Of the lambs, 83% emitted high-pitched bleats and 60% emitted low-pitched bleats, but the percentage that bleated was not affected by ewe-feeding treatment, BCS group or their interactions (P>0.05, data not shown).

There was a significant interaction between ewe-feeding treatment and BCS for the number of bleats emitted, such that in the BCS≤2.0 and ≥3.0 groups, lambs born to ewes in the *ad-libitum* treatment emitted more high-pitched bleats than lambs born to ewes in the medium treatment (P<0.05, Table 1). The opposite effect was observed within the BCS2.5 group (P<0.05). Similarly, for low-pitched bleats, 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-libitum* treatment

Table 1 The number of high- and low-pitched bleats made by ewes, or by lambs born to ewes, that were in body condition score group BCS≤2.0, BCS2.5 and. BCS≥3.0 at P93 and fed medium and *ad-libitum* pasture from P93-P114, during the five minute observation period. The back transformed means (95% confidence limit) are shown.

	n	Number of high-pitched bleats	Number of low-pitched bleats
Feeding x BCS		Ewes	
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-libitum BCS≤2.0	21	20.1^{d} (19.0-21.2)	38.1^{d} (36.6-39.7)
ad-libitum BCS2.5	13	15.1^{b} (13.9-16.3)	31.9^{b} (30.2-33.7)
ad-libitum BCS≥3.0	9	17.4^{c} (15.9-19.1)	24.4^{a} (22.6-26.4)
		Lambs	
Feeding x BCS			
medium BCS≤2.0	42	13.6^{c} (12.5-14.8)	4.2^{cd} (3.7-4.9)
medium BCS2.5	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-libitum BCS≤2.0	63	16.0^{d} (15.0-17.0)	2.6^{a} (2.2-3.0)
ad-libitum BCS2.5	39	10.3^{a} (9.3-11.3)	3.7^{bc} (3.1-4.3)
ad-libitum BCS≥3.0	27	14.9^{cd} (13.5-16.4)	3.2^{ab} (2.6-4.0)

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

(P<0.05, Table 1); the BCS2.5 groups did not differ (P>0.05).

Lamb paddock behaviours

There was no effect (P>0.05) of ewe-feeding treatment or BCS group on the time taken for the lambs to stand, make contact with, suck from or follow their dam and the median times were 70 seconds, 28 seconds, 301 seconds and 301 seconds, respectively.

Maternal-recognition test

Of the lambs in the maternal-recognition test, 55% emitted high-pitched bleats and 98% emitted low-pitched bleats, but the percentage that bleated was not affected by ewe-feeding treatment, BCS group or their interactions (P>0.05, data not shown).

Of lambs that bleated, there was a significant interaction (P<0.05) between 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-libitum* feeding treatment emitted fewer low-pitched bleats than lambs born to ewes in the medium treatment (Table 2). Within the BCS≤ 2.0 group, the opposite was observed (P<0.05).

The number of high-pitched bleats showed an interaction (P<0.05) between 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-libitum* treatment (Table 2). The BCS2.5 and BCS≥3.0 groups did not differ (P>0.05).

The percentage of lambs that sat during the maternal-recognition test was lower 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). 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.

Of the lambs, 92% reached the contact zone and 81% reached their dam, with median times of 26 and 52 seconds, respectively. The percentage, or the time taken, for lambs to reach the contact zone or their dam were not affected (P>0.05) by ewe-feeding treatment or BCS. The median time lambs spent in the contact zone or with their dam were also not affected (P>0.05) by ewe-feeding treatment or BCS.

Table 2 The number of high- and low-pitched bleats made by lambs in the maternal-recognition test born to ewes that were in body condition score group BCS≤2.0, BCS2.5 and BCS≥3.0 at P93 and fed medium and *ad-libitum* pasture from P93-P114, during the five minute observation period. The back transformed means (95% confidence limit) are shown.

	n	High-pitched bleat	Low-pitched bleats
Feeding x BCS group			
medium BCS≤2.0	27	40.3^{d} (38.0-42.8)	2.4^{a} (1.9-3.1)
medium BCS2.5	21	23.3^{a} (21.3-25.4)	8.0^{c} (6.9-9.3)
medium BCS≥3.0	33	27.3^{b} (25.6-29.2)	4.6^{b} (3.9-5.4)
*ad-libitum*BCS≤2.0	33	35.5^{c} (33.6-37.6)	4.2^{b} (3.6-5.0)
*ad-libitum*BCS2.5	18	25.2^{ab} (23.0-27.6)	4.3^{b} (3.5-5.4)
*ad-libitum*BCS≥3.0	18	27.1^{b} (25.6-29.2)	2.2^{a} (1.6-3.2)

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

Table 3 The the percentage of lambs that walked and sat and the time (seconds) spent walking and sitting (for those exhibited the behaviours) in the maternal-recognition test by lambs born to ewes that were in body condition score group BCS≤2.0, BCS2.5 and BCS≥3.0 at P93 and fed medium and *ad-libitum* pasture from P93-P114, during the five minute observation period. The back transformed means (95% confidence limit) are shown.

	n	Walk[1]		Sit[1]	
		Percentage	Time	Percentage	Time
Feeding treatment					
medium	81	95.6 (87.1-98.6)	30	20.9 (12.5-32.8)	10
ad-libitum	67	92.9 (84.7-96.9)	40	9.7 (4.8-18.6)	75
BCS group					
BCS≤ 2.0	59	95 (85.6-98.4)	50.0^{b}	13.6^{ab} (7.0-24.9)	70
BCS2.5	38	95 (82.1-98.8)	30.0^{a}	25.4^{b} (14.0-41.5)	15
BSC≥ 3.0	51	92.8 (81.5-97.5)	30.0^{a}	8.2^{a} (3.1-20.4)	10

[1]Walk and Sit refers to the percentage of lambs that walked and sat during the maternal-recognition test and the time in seconds that those lambs walked and sat. [abc] Different superscripts within columns and main effects indicate values that significantly differ (P<0.05).

Discussion

An ewe-lamb bond is an exclusive relationship and ewes will only offer maternal care to lambs with which they have bonded (Nowak et al. 2011). Ewe MBS is considered an indicator of the strength of the ewe-lamb bond (O'Connor 1996). Ewe-feeding treatments have, in earlier studies, been shown to affect ewe MBS (Everett-Hincks et al. 2005b; Corner et al. 2010), however, in the present experiment this was not the case. Everett-Hincks et al. (2005b) and Corner et al. (2010) subjected ewes to lower feeding levels compared to the present experiment, and for longer periods, which may explain the lack of an effect on MBS. There was also no effect of ewe BCS on MBS in the current experiment. The results indicate that, for ewes of BCS≤2.0-3.0 in mid-pregnancy, offering feeding levels above pregnancy-maintenance requirement in mid- to late-pregnancy is not a useful management option to increase MBS.

High-pitched bleats from both ewe and lambs are often emitted when the ewe and lambs are separated and may indicate a degree of need (Poindron et al. 1994; Dwyer et al. 1998). Low-pitched bleats help strengthen 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). 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-libitum*-treatment, however, the number of low-pitched ewe bleats were similar for these groups. Being of a lower BCS and on lower feed intake during mid-pregnancy, compared to ewes in BCS group 2.5 or ≥3.0 and in the *ad-libitum*-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 in response to bleats from the other littermates. Everett-Hincks et al. (2005a) showed that triplet-bearing ewes on a restricted diet during pregnancy emitted more high-pitched bleats than did well-fed ewes. A similar association was not, however, seen in the current experiment. As indicated previously, the feeding levels used in the current experiment met or were above pregnancy requirements, which may explain the differences. The effect of ewe mid-pregnancy BCS and feeding on lamb vocalisation in the maternal-recognition test differed from that recorded after tagging. This may indicate a different response to the different stressors, or that bleating is not a repeatable indicator of bonding.

Lambs born to ewes offered *ad-libitum* feeding followed their dam more quickly than lambs born to ewes offered medium feeding. This supports the finding of Everett-Hincks et al. (2005a) who reported a positive relationship between feeding levels of triplet-bearing ewes and the proportion of lambs that located the dams' udder and followed her when she moved away. In the current experiment, there was no effect on the time for the lamb make contact with the dam or suck from her. Likewise, in the maternal-recognition test there was little effect of ewe BCS and feeding treatment on the behaviour of the lamb. With the exception that lambs born to ewes of BCS≤2.0 spent less time walking, perhaps suggesting that they may have been weaker, no other behaviours were affected by ewe BCS or nutrition. Overall, the results indicate that feeding triplet-bearing ewes above pregnancy requirements has little or no effect on lamb behaviours. It is also possible that the feeding treatments in the current experiment were implemented too early in pregnancy to influence ewe and lamb behaviour, as the majority of fetal growth occurs later in pregnancy (Rattray et al. 1974), and the ewes would not have been under nutritional stress during late-pregnancy.

Conclusion

While previous studies have indicated that poor maternal nutrition can negatively affect ewe and lamb behaviours, there was little effect of nutrition on behaviour of ewes and lambs in the present study. This is most likely explained by the fact that ewes were offered feeding levels that either met, or were above pregnancy-maintenance requirements. This was possibly compounded by the fact that feeding treatments ended well before lambing and all ewes had reached similar body condition score at day 114 of pregnancy. There is, however, some evidence to suggest that lambs born to ewes in the BCS≤ 2.0 group offered the medium treatments may have experienced a weaker ewe-lamb bond.

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