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Parity and housing effects on the behavioural and hypothalamic–pituitary–adrenal axis responses of pregnant ewes

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ABSTRACT

It is common in many countries for sheep to be housed during winter from mid-gestation until lambing to protect ewes and lambs from adverse conditions and improve late gestation nutritional management. Keeping ewes indoors, however, has its own challenges as the animals may be mixed with unfamiliar conspecifics, have limited floor and feeding space, experience changes to their diet and increased handling by humans. Therefore, the objective of this study was to investigate the effect of variation in housing management (space allowance and social stability) on the behaviour and hypothalamic–pituitary–adrenal (**HPA**) axis responses of pregnant ewes from mid-to-late gestation (weeks 11–18 of pregnancy). Seventy-seven ewes (41 primiparous, 36 multiparous) were divided into two groups: 'Control' and 'Restricted space and mixed' (**RS-Mix**), where RS-Mix ewes were allocated half the amount of space (1.27 vs 2.5 m^2 for RS-Mix and Control, respectively) and feedface (concentrate feeder space) allowance (36 vs 71 cm per ewe) given to the Control group and were also subjected to two social mixing events. Aggressive behaviour at the feedface and time spent standing, lying, walking, feeding and ruminating were recorded and faecal samples were collected for assessment of faecal glucocorticoid metabolite (**FGM**) concentrations. Higher aggression was observed in RS-Mix ewes during the first week of observation ($P = 0.044$), which gradually declined to the same level as Control ewes by the end of the study ($P = 0.045$). RS-Mix ewes were significantly less likely to be able to freely join the feedface compared to Controls ($P = 0.022$). No other significant treatment effects on aggressive behaviour or FGM during gestation were found. RS-Mix ewes displayed significantly higher ruminating behaviour at week 18 of gestation compared to Control ewes ($P < 0.001$), but no other effects were seen on general pen behaviour. However, the effect of indoor housing had a significant impact on primiparous ewes, who had lower weight gain ($P = 0.015$) and higher FGM concentrations ($P = 0.014$) compared to multiparous ewes regardless of treatment group. The data suggest that, although no sustained effects on behaviour or HPA axis responses were seen with the differences in space and feeder allowance or social stability at the levels used in this study, inexperienced (primiparous) ewes may find indoor housing more stressful; and are less able to adapt compared to multiparous ewes. These effects may influence the behaviour of the ewe at lambing time, and her offspring.

Implications

Different space allowances and social mixing have different impacts on the behaviour and physiological stress responses of pregnant ewes from different parities. First parity ewes appear to cope less well with indoor housing management in pregnancy compared to experienced ewes, and therefore, greater attention should be given to improve the welfare of this cohort of ewes.

Introduction

In many countries, pregnant sheep are kept indoors for at least part of the production cycle, often during winter from mid-gestation until lambing in order to reduce the risk of lambs dying from exposure to adverse conditions (Piirsalu et al., 2020). This

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can also allow better-individualised management of the nutritional and health status of ewes in the last third of pregnancy to improve fetal lamb growth and the onset of maternal care and lactation (Dwyer et al., 2016). However, relocating from the outdoor pasture to indoor housing requires a period of adaptation from the ewes to cope with a new environment (Miller et al., 2018) and research suggests that sheep prefer to be outside when given the choice (Piirsalu et al., 2020). Apart from the novel environment, indoor housing may involve additional challenges such as mixing with unfamiliar conspecifics at close proximity, limited floor and feeder space allowances, changes to the diet, as well as more human contact and handling. How pregnant sheep respond and adapt to these changes in the environment has relevance for both animal welfare and productivity. In addition, the impact of previous experience of housing on the ewe has not been investigated.

The limited studies on the welfare of housed ewes have focused on stocking density, flooring and bedding, space allowance and pen features. A decrease in space per animal has been shown to reduce activity, increase time spent queuing at the feeder, and to increase positive and negative social interactions (Averós et al., 2014), while increased displacements during feeding were also observed with reduced feeding space (Bøe and Andersen, 2010; Vik et al., 2017). High stocking density and infrequent bedding renewal significantly decreased the pregnancy rate in ewes subjected to artificial insemination (Priskas et al., 2022). Dairy ewes kept at higher stocking density were also found to be less active and have a lower milk yield than ewes at a lower density (Caroprese et al., 2009). These behavioural effects are more related to stocking density rather than group size (Averós et al., 2015), although ewes kept in larger groups were less synchronised in their behaviour (Jorgensen et al., 2009). Indoor-housed ewes may also be subjected to regrouping or relocation multiple times during their pregnancy. Regrouping causes an increase in aggressive interactions, as animals establish new social relationships, which in dairy ewes can affect immune function and milk yield (Sevi et al., 2001). Ewes that have been aversively handled in late pregnancy also tend to have increased concentrations of plasma cortisol compared to ewes handled gently (Hild et al., 2011). In addition, maternal experience has an effect on the behavioural responses to possible stressors. Inexperienced ewes (those which have not given birth before) were significantly more fearful in a surprise effect test as well as to the presence of a human compared to multiparous ewes (Viérin and Bouissou, 2002).

The conditions in which pregnant ewes are kept and handled during indoor housing may potentially also result in negative impacts on their offspring. For example, lambs born to ewes kept at reduced space allowance during gestation and separated from their mother after birth, showed more fearful behaviour during novel arena and social motivation tests compared to lambs whose mothers were kept in larger space allowance (Averós et al., 2015). Ewes which were aversively handled during pregnancy were also shown to produce lambs with increased fearfulness compared to gently handled ewes (Coulon et al., 2011). These show the importance of housing environment not only on the welfare of the pregnant ewes but also on the possible impact it might have on the offspring.

Therefore, the aim of this study was to investigate the impact of indoor housing conditions that mimicked conditions seen on commercial farms and differed in terms of space allowance and social mixing on maintenance behaviour, aggressiveness and stress physiology of primiparous and multiparous pregnant ewes at different stages of gestation. We hypothesised that ewes housed at higher stocking density, with reduced feeder space, and subjected to social mixing during pregnancy would be more active, more aggressive and have greater indicators of physiological stress responses than ewes given more space and maintained with social stability.

Material and methods

Animals, facilities and management

Scottish Mule ewes (Scottish Blackface × Blue-faced Leicester) in their first (primiparous, 2 years old) and second pregnancy (multiparous, 3 years old) were used in this study. Ewes were mated in small groups of 30–40 ewes to each ram for approximately 4–5 weeks. The ram was fitted with a marking harness (raddle) which contained a coloured crayon to identify ewes that had been mated. The colour of the crayon was changed every 10 days to facilitate the estimation of the date of parturition.

At approximately 10 weeks of gestation, ewes underwent trans-abdominal ultrasonography for pregnancy determination and to identify the number of lambs they carried. The study animals were drawn from the twin-bearing ewes and 77 ewes were selected comprised of primiparous (n = 41) or multiparous (n = 36) ewes.

Experimental ewes were brought indoors in week 10 of gestation following ultrasonography, when they were weighed and their body condition was scored (measurement of relative fat and muscle over the lumbar vertebrae on a scale from 0 (emaciated) to 5 (obese)) using manual palpation over the lumbar spine.

In order to identify ewes individually, they were also marked with a unique number on their sides using marker spray (Super Sprayline Stock Marker, Ritchey, New Zealand). Ewes were moved to the experimental shed and allocated to straw-bedded pens in groups of seven ewes per pen. Ewes were provided with ad libitum access to hay and water throughout the experiment. Concentrate feeds (18% CP, Premium 18 Nuts, Harbro Ltd., Scotland) were provided from week 14 of gestation (approximately 300 g/ewe) which was given once per day in the morning. The amount of concentrate provided was doubled to 600 g/ewe at week 16 which was given twice a day in the morning and afternoon (300 g/ewe per meal). At week 18, the concentrate feeds were increased to 800 g/ewe which were also given as two feeds in the morning and afternoon. Ewes were vaccinated with Heptavac P Plus (Intervet, Ireland) at week 17 of gestation, to provide maternally derived antibodies to protect the lamb against clostridiosis and pasteurellosis.

Social stress treatment

The ewes were assigned (with multiparous and primiparous ewes balanced within treatment, but otherwise randomly) at week 10 of gestation, to one of two treatment groups, which differed in space allowance per ewe, length of feedface per ewe and occurrence of social mixing. In this study, 'feedface' is defined as the feed trough or container in which concentrate feeds were placed. There were a total of six control pens and five RS-Mix pens with seven ewes in each pen (3–4 primiparous ewes per pen) with treatment details as followed:

- Control **(C):** space allowance of 2.5 m^2/ewe, feedface allowance of 71 cm/ewe, and stable social group (ewes remained within their allocated pen group until lambing),
- Restricted Space and Mix group **(RS-Mix):** space allowance of 1.27 m^2/ewe, feedface allowance of 36 cm/ewe, and subjected to two social mixing events during the experiment.

Ewes in RS-Mix groups were exposed to social mixing on the Monday of weeks 13 and 15 of gestation where new groups consisting of different individuals were established. As there were only five pens of RS-Mix group, each new group was composed of one original member, one pair of ewes from two different pens and another two ewes from the other two pens. Experimental data collection began at week 11 of gestation until week 18 of gestation (as described in Fig. 1).

Weight and body condition score

Body condition score and BW of the ewes were first measured before the ewes were assigned to treatment groups at week 10 of gestation (Fig. 1). Both BW and condition score were measured again on weeks 13, 15 and 17 of gestation, as well as at the end of week 18 of gestation, which was the final day of observation of the ewes before parturition (Fig. 1).

Behavioural observation

Aggressive behaviour at the feedface

Frequency of behaviours and interactions at the feedface were continuously recorded for 30 minutes, using a camcorder (Canon Legria HFM52, Canon Inc., Japan) placed in front of the observed pen, starting at 0800 every morning (immediately after concentrate feed was placed in the feed troughs during the morning feed) during weeks 14, 16, and 18 of gestation. The behaviours observed

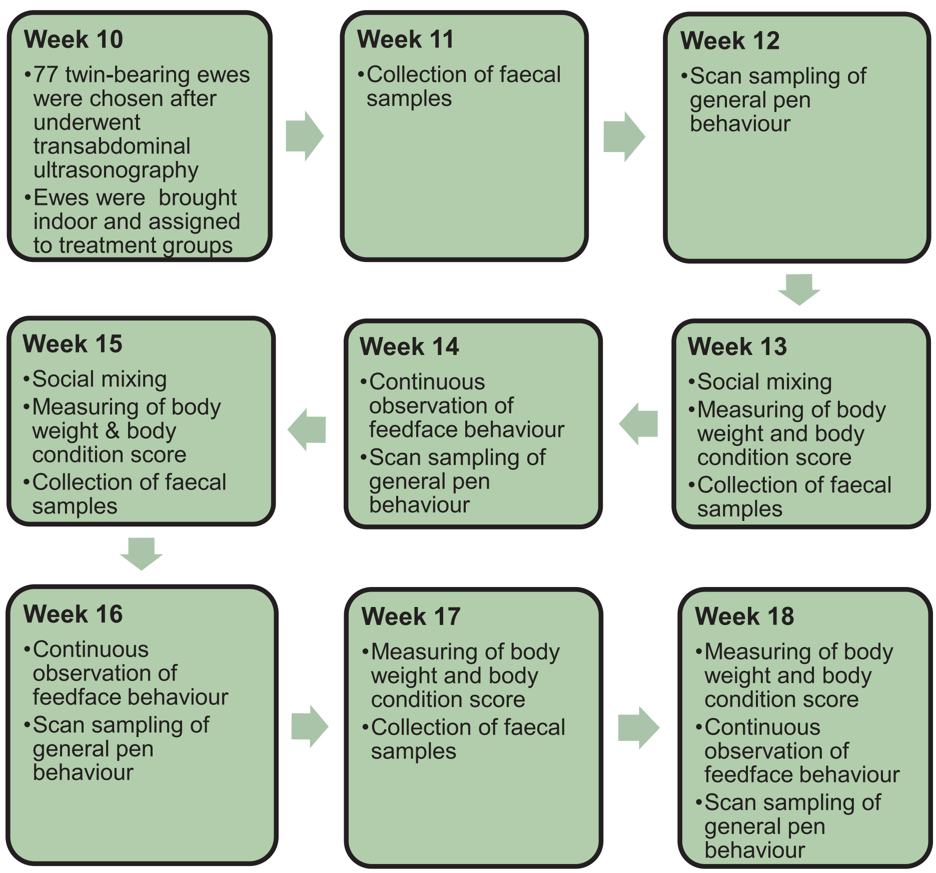

Fig. 1. Timeline of sampling sessions made on primiparous and multiparous pregnant ewes subjected to differences in housing treatments throughout the experiment.

Table 1
Ethogram of ewes' behaviour recorded during concentrate feed at the feedface.

Behaviour	Definition
Join Feedface	A ewe physically moves in from the back of the pen to join or enter the feedface from approximately one ewe body length away.
Push-In	A ewe forces itself between two other ewes (<ewe body width gap between animals) or between a ewe & barn equipment (fence) at the feedface.
Penetrate	A ewe forces itself between two other ewes (no gap between animals) or between a ewe & barn equipment (fence) at the feedface.
Failed penetrate	A ewe has an unsuccessful attempt to get in between two other ewes or between a ewe & barn equipment (fence) at the feedface.
Displace	Physically forcing another ewe to leave her feeding place by butting, hitting, striking, thrusting, or pushing the receiver with forehead or any other part of the body with a forceful movement resulting in the receiver giving up its position (walking away for at least half an ewe-length or stepping aside for at least one ewe-width).
Half Displace	When a push leads to a ewe moving back approximately half a body length from the feedface, but not being fully displaced.
Leave Feedface	Ewe voluntarily leaves the feedface without any interaction with another ewe.
Push	Forcefully moving another ewe while at the feedface.
Butt	Contact with another ewe either head-to-head or short and forceful contact with the head towards another part of the receiver's body.
Prod	One ewe uses her hoof to tap/prod/kick the back/side of another ewe.
Mount	Jumping on another sheep's back.
Back Press	A ewe rests the jaw or head on the back of another ewe and presses down

include aggressive behaviour (e.g. push, butt, back press), joining and leaving the feedface either voluntarily or by force (scored using the ethogram in Table 1). Two pens were observed each day (1 Control & 1 RS-Mix group) except on Friday where three pens were observed. Observations of ewe behaviour were later made from the recordings using The Observer XT 12.0 software (Noldus Information Technology, Netherlands).

General pen behaviour

Live scan sampling of general pen behaviours (using the ethogram in Table 2) of each individual ewe was conducted on two days per week from week 12 to week 18 of gestation. A total of eight scans at 10-minutes intervals were conducted on each observation day. For week 12 of gestation, which was before concentrate feeding had begun, scan sampling started at 0850 on the observation days. From week 14 of gestation when the concentrate feed was supplied to the ewes, the scan sampling started 15 minutes after the end of the feedface observation on the same pens each day with the addition of two or three other pens (four or five pens were scanned each day) such that each pen was observed twice per week.

Faecal glucocorticoid metabolite assay

Collection of faecal samples

Faecal samples were collected per rectum between 0900 and 1100 on weeks 11, 13, 15 and 17 of gestation from 72 ewes across 11 pens (five ewes were not able to be sampled due to Home Office license constraints) (Fig. 1). Faecal samples were collected from the rectum of the ewes or collected immediately from the ground when an individual ewe was seen to have naturally deposited their faeces during sample collection. Each sample was placed into a labelled plastic bag, homogenised by hand for ease of processing and then frozen at −20 °C until further analysis.

Faeces extraction and enzyme immunoassays

Prior to extraction, the homogenised faecal samples were brought to room temperature for 30 minutes. A 0.5 g sample was transferred into a 15 ml centrifuge tube before 5 ml of 80% methanol was added. Tubes were vortexed for 30 minutes and centrifuged at 2 500g for 15 minutes (Z200A, Hermle, Germany), before 1 ml of the supernatant was transferred to a clean Eppendorf tube.

Faecal extracts were assayed for immunoreactive glucocorticoid metabolites using an 11-oxoaetiocholanolone enzyme immunoassay (**EIA**) (Palme and Mostl, 1997; Morrow et al., 2002; Möstl and Palme, 2002). This method has been successfully validated for the evaluation of adrenal activity in sheep (Palme et al., 1999). The standard, antibody and enzyme label that were used in this study were supplied by Professor Rupert Palme from the University of Veterinary Medicine, Vienna, Austria. The absorbance was measured at 450 nm on a Multiskan FC spectrophotometer (Thermo Scientific, UK) using SkanIT Software 2.5.1. From the assay, CV of intra-plates and inter-plates were shown to be 7.2 and 18.9%, respectively.

Statistical analysis

From the original sample of 77 ewes, only data from 71 ewes were analysed. Six ewes (1 from RS-Mix & 5 from Control group) were excluded from the analysis as one aborted at week 19 of gestation, one died due to prolapse in the middle of gestation (RS-Mix group), one subsequently gave birth to triplets instead of twins, and another three ewes were excluded due to ill thrift throughout the experiment.

Daily BW change was calculated as the difference between two points of weighing divided by the number of interval days. For scan sampling data, to obtain weekly measures of ewe behaviour, the percentages of occurrence of behaviours were calculated individually per ewe for the eight scans conducted each day. The weekly values were obtained from the two observations per pen per week.

Due to the low frequency of each different type of 'aggressive behaviour' during the 30 min observation when concentrates were given, the data for push, butt, prod, mount, backpress, push-in, penetrate, displace and half displace behaviour were combined to make up the total of aggressive behaviour occurring at the feedface. The proportion of 'free join' (from the total of all type of joins to the feedface: join, push-in and penetrate) and 'free leave' (from the total of all types of leaving pen: leave voluntarily and being displaced) were also calculated and used in analysis. However, for general pen, behaviour recorded by scan sampling, feeding, ruminating, idle, standing and lying were the only behaviours analysed since all other behaviours recorded had a very low frequency. After checking for normality, data for aggressive behaviour at the feedface and all general pen behaviour were transformed using log transformation as they were not normally distributed. For all transformed data, the back-transformed means are reported together with 95% Confidence Interval (**CI**) whereas for normally distributed data, the means are reported together with SEM. BW and condition score and concentrations of faecal glucocorticoid metabolites were analysed by linear mixed models using the Restricted Maximum Likelihood procedure.

Table 2
Ethogram of pregnant ewes' behaviour recorded during scan sampling in the pen.

Level	Category	Definition
Posture	Standing	Ewe is standing on all four legs; body clear of the ground.
	Walk	Ewe in motion, moving from one location to another.
	Lying	Ewe's body (ventral or lateral surface) is in contact with the ground.
	Sitting	Ewe has rear end in contact with ground but supporting some weight on straight front legs.
	Kneeling	Ewe is supporting BW on knees of front legs, supporting some weight on back legs.
Behaviour	Idle	No activity, motionless, head up.
	Head down idle	No activity, motionless, head down (resting on substrate while lying).
	Feed	Ewe in front of the feeder biting, chewing or pulling on hay.
	Feed Sub	Ewe is biting, chewing or pulling on substrate material, not normal allocated food.
	Drink	Ewe standing in front of the drinker and is seen to consume water or with its nose within 10 cm of from the drinker.
	Ruminate	Ewe making lateral chewing movements with its mouth while lying or standing.
	Lick (self)	Ewe licking or scratching a part of the body with tongue or teeth.
	Rub	Rubbing any part of the head or body against pen fixtures.
	Groom (other)	Ewe licking or pulling feed from the wool of other animals.
	Agonistic	Includes all forms of aggression towards another ewe (pushing; mounting, kicking, butting, threat, block; displacement).

Aggressive behaviour at feedface was analysed using a Generalised Linear Mixed Model (**GLMM**), fitting a Poisson distribution with a Logarithm function. The proportion of free joins and free leaves (voluntarily joined and left the feedface without any aggression) which occurred at the feedface, and general pen behaviour by scan sampling were also analysed using a GLMM, fitting a binomial distribution with a Logit function. Gestation week, parity and treatment as well as the interactions were fitted as the fixed effects whereas pen and individual ewe were fitted as random effects to account for repeated measures during data collection over the gestation period, and possible non-independence of the behaviour of ewes in the same pen. Where differences were found, posthoc comparisons were made using Fishers' LSD tests. All analyses were conducted in GenStat (16th edition) software.

Results

Weight and body condition score

Throughout the experiment, there were no significant differences in weight change by treatment group (mean daily weight (kg) change (SEM): Control: 0.24 kg (0.012), RS-Mix: 0.25 kg (0.012); $F_{1,12.5}$ = 0.12, P = 0.731). However, Multiparous ewes were significantly heavier than Primiparous ewes when they were weighed at week 10 of gestation prior to being assigned to either one of the two treatment groups (mean weight (SEM): Multiparous: 77.98 kg (0.92), Primiparous: 72.52 kg (0.90); $F_{1,71.8}$ = 26.06, P < 0.001). Primiparous ewes gained less weight in weeks 13 and 15 of gestation, and some ewes lost weight in week 13 of gestation, compared to multiparous ewes whose BW increased in all weeks (Fig. 2; $F_{3,224.7}$ = 3.58, P = 0.015).

Overall body condition score (**BCS**) was not significantly affected by either treatment or parity. However, there were significant interactions between gestation week and treatment as well as between gestation week and parity on BCS (Table 3). For Control ewes, BCS at week 18 of gestation was significantly lower compared to weeks 13, 15 and 17 of gestation. Primiparous ewes also showed a similar trend: BCS at week 18 of gestation was significantly lower compared to weeks 13, 15 and 17 of gestation. However, in RS-Mix ewes, the BCS was low in week 13 of gestation and significantly increased in weeks 15 and 17 of gestation before it started to decline in the week 18 of gestation. The BCS of multiparous ewes was also observed to have a similar trend as RS-Mix ewes (Table 3).

Aggressive behaviour at feedface

Multiparous ewes displayed significantly more aggression at the feedface (frequency/30 mins) over the treatment period than primiparous ewes (mean frequency (CI range): Multiparous: 2.69 (2.01–3.6), Primiparous: 1.77 (1.32–2.01); $F_{1,70.8}$ = 4.2, P = 0.044). Aggressive behaviour performed by ewes from the RS-Mix group declined over the three observation periods compared to the Control group, which did not change over time (Fig. 3; $F_{2,145.4}$ = 3.17, P = 0.045). Posthoc testing also revealed that significantly more aggressive behaviour was displayed by RS-Mix ewes compared to control ewes at week 14 of gestation.

Overall, ewes from the RS-Mix group had a significantly lower proportion of free join to the feedface (out of all types of joins: join, push-in and penetrate) compared to Control ewes (mean proportion (CI range): Control: 0.91 (0.88–0.93), RS-Mix: 0.76 (0.71–0.81); $F_{1,11.7}$ = 30.4, P < 0.001). Multiparous ewes also displayed a significantly lower proportion of free join compared to Primiparous ewes (mean proportion (CI range): Multiparous: 0.81 (0.77–0.85), Primiparous: 0.88 (0.84–0.91); $F_{1,59.6}$ = 5.5, P = 0.022). However, no effect of treatment and parity were found on the proportion of free leave by the ewes.

General pen behaviour

There was no significant effect of treatment alone on any of the general pen behaviours, but a significant interaction between behaviour and week of gestation was observed for some behaviours.

For ruminating behaviour, there was a significant interaction between treatment and gestation week with ewes from both treatments displaying significantly higher ruminating behaviour at week 12 of gestation before declining to week 16 (Fig. 4; $F_{3,290.1}$ = 7.26, P < 0.001). However, at week 18 of gestation, ewes from RS-Mix group displayed significantly higher frequency of ruminating than Control ewes.

Multiparous ewes were observed to feed more frequently compared to primiparous ewes throughout the experiment (Percentage scans observed feeding (95% CI): Multiparous: 30.6% (26.6–35), Primiparous: 26.1% (22.5–30); $F_{1,71.7}$ = 5.56, P = 0.021).

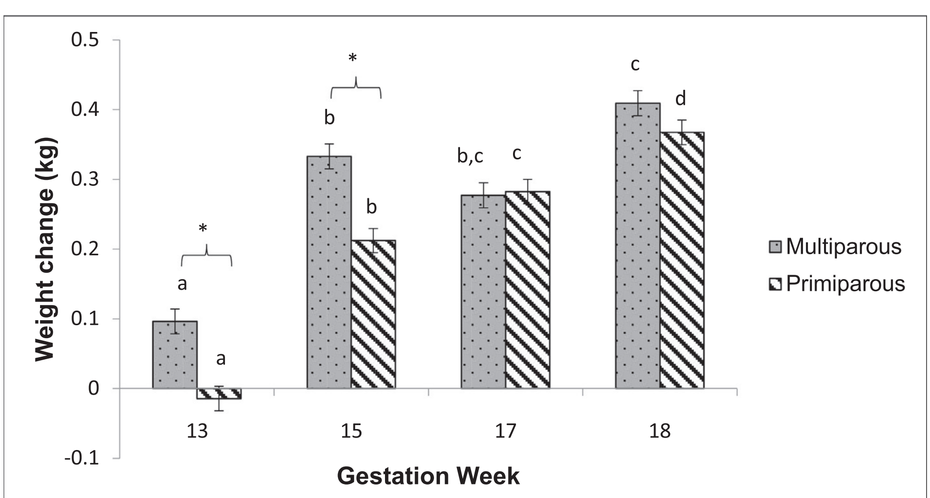

Fig. 2. Change in daily weight (kg) with increasing gestational week for multiparous and primiparous ewes. Data presented are mean weight change with SEM as error bars. Bars with different letter superscripts and * indicate significant difference between gestation week and parity respectively at P < 0.05 level.

Table 3
Mean body condition score observed in pregnant ewes based on the interaction of gestation week with treatment groups and parity.

Item	Gestation week				SEM	Interaction *P*-value
	13	15	17	18		
Treatment						
Control	3.23[a]	3.28[a]	3.30[a]	3.08[b]	Control = 0.056;	$F_{3,196.9}$ = 4.08, P = 0.008
RS-Mix	3.08[a]	3.18[bc]	3.24[b]	3.14[ac]	RS-Mix = 0.059	
Parity						
Multiparous	3.09[a]	3.24[bc]	3.33[b]	3.17[c]	Multiparous = 0.059;	$F_{3,196.7}$ = 8.51, P < 0.001
Primiparous	3.22[a]	3.23[a]	3.21[a]	3.05[b]	Primiparous = 0.055	

Abbreviation: RS-Mix = Restricted space and mixed.
Values within a row with different letter superscripts differ significantly at P < 0.05.

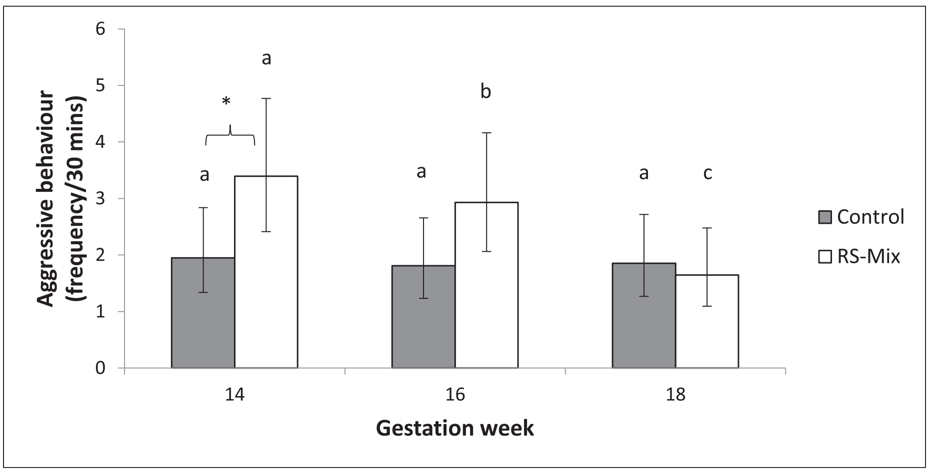

Fig. 3. Means (±95% confidence interval) frequency of aggressive behaviour displayed by Control and RS-Mix pregnant ewes in 30 minutes at the feedface at weeks 14, 16 and 18 during gestation. Bars with different letter superscripts and * indicate significance different between gestation week and treatment respectively at P < 0.05 level. Abbreviation: RS-Mix = Restricted space and mixed.

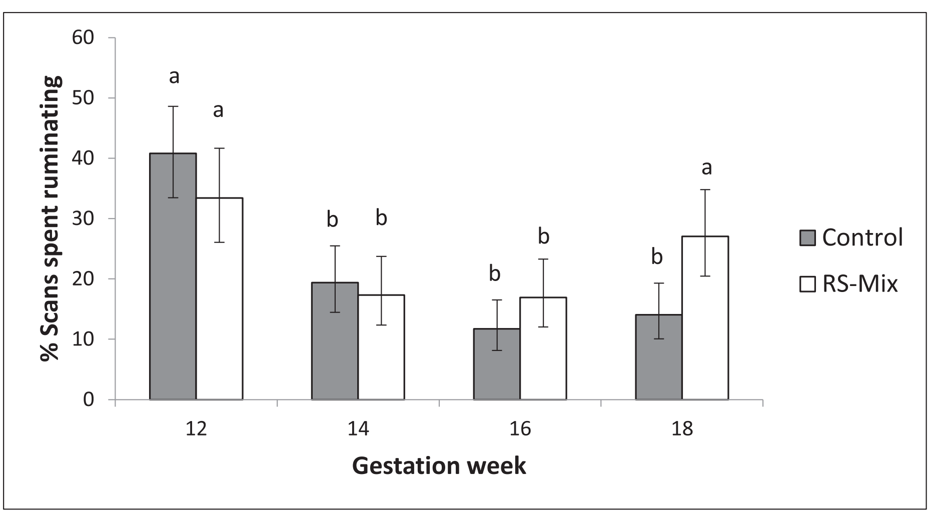

Fig. 4. Mean percentage (±95% confidence interval) of observation spent ruminating by Control and RS-Mix ewes with increasing gestation week. Bars with different letter superscripts indicate significant difference within treatment group and between gestation week at P < 0.005. Abbreviation: RS-Mix = Restricted space and mixed.

However, primiparous ewes overall spent significantly more time ruminating compared to multiparous ewes over the experimental period (Percentage scans observed ruminating (95% CI): Multiparous: 18.7% (15.5–25.4), Primiparous: 24.1% (20.4–28.3); $F_{1,299.0}$ = 9.54, P = 0.002). Control ewes spent less time idle at 12 weeks of gestation than in other weeks and idling time linearly increased before remaining constant at the end of the observation period (Fig. 5; $F_{3,219.5}$ = 4.59, P = 0.004). RS-Mix ewes also spent less time idle at 12 weeks of gestation, but increased at week 14 of gestation and remained constant throughout the rest of the observation period.

Primiparous ewes were observed to be idle more frequently compared to Multiparous ewes at week 12 of gestation but did not differ thereafter (Fig. 6); $F_{3,223.7}$ = 4.58, P = 0.004).

There were no significant differences by treatment or parity in the frequency with which ewes were observed standing and lying.

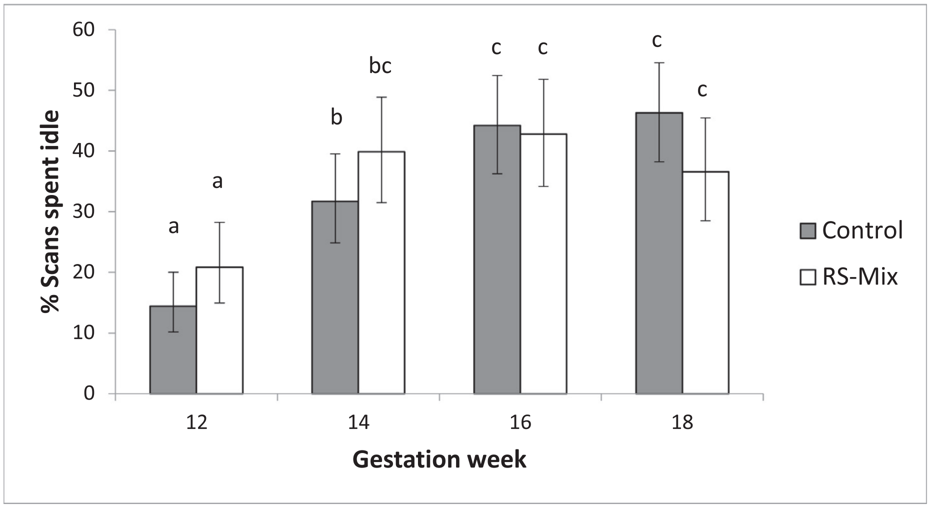

Fig. 5. Mean percentage (±95% confidence interval) of observation spent idle by Control and RS-Mix ewes with increasing gestation week. Bars with different letter superscripts indicate significant difference within treatment group and between gestation week at $P < 0.005$. Abbreviation: RS-Mix = Restricted space and mixed.

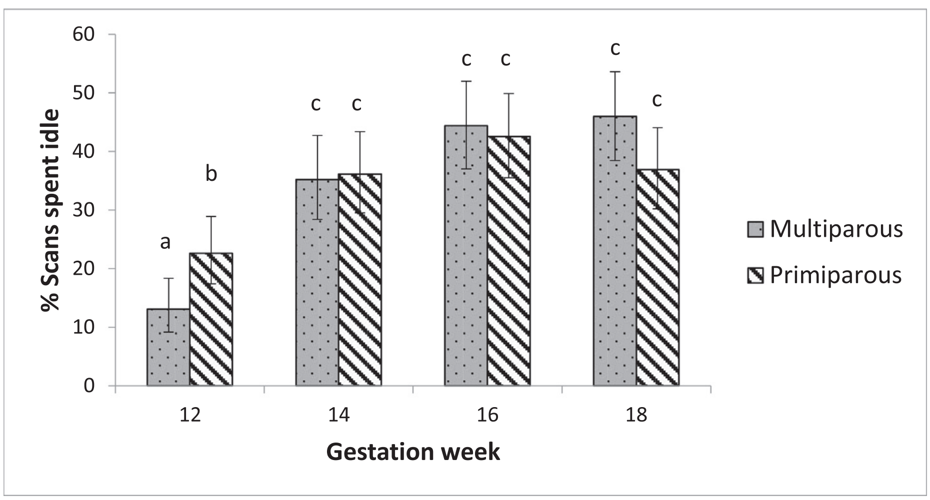

Fig. 6. Mean percentage (±confidence interval) of observation where multiparous and primiparous ewes spent time idle with increasing gestation week. Bars with different letter superscripts indicate significant difference within treatment group and between gestation week at $P < 0.005$.

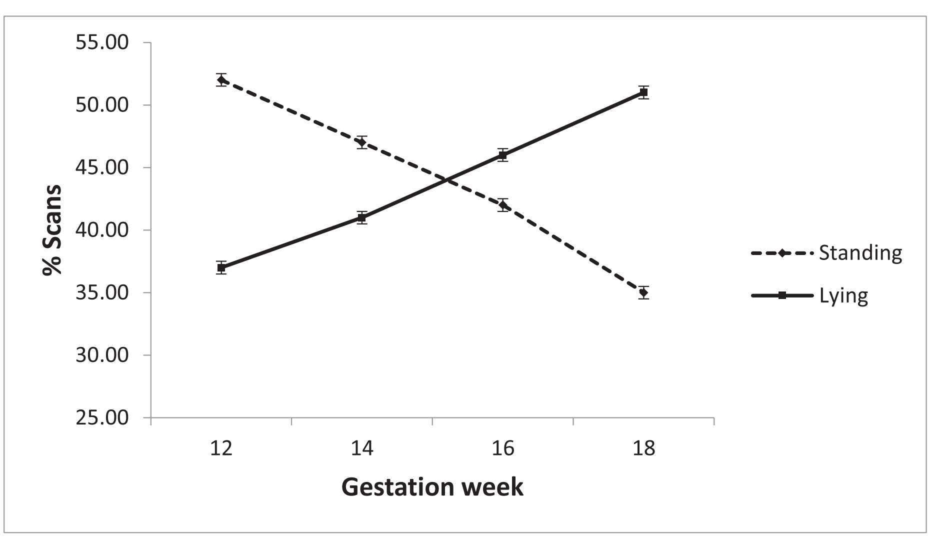

Fig. 7. Mean percentage (±95% confidence interval) of observations where ewes were observed to lie and stand with increasing gestation week.

However, as pregnancy progressed, ewes decreased the frequency of standing and were seen to lie more frequently (Fig. 7; $F_{3,218.6}$ = 9.38, P < 0.001).

Faecal glucocorticoid metabolites

There was no effect of treatment on the concentration of faecal glucocorticoid metabolites (**FGM**) throughout gestation (Concentration (ng/ml) (SEM): Control: 53.14 (3.88), RS-Mix: 57.22 (3.86); $F_{1,21.9}$ = 0.52, P = 0.48). However, there was a significant interaction between gestation stage and parity in the concentration of FGM with primiparous ewes having higher FGM concentration in weeks 11, 13 and 15 of gestation compared to multiparous ewes (Fig. 8; $F_{3,178.7}$ = 3.64, P = 0.014).

Discussion

The housing condition treatments with multiple potential stressors (a reduction in pen and feeder space, and two mixing events over the last half of pregnancy compared to ewes with more space and a stable social group) had only a limited impact on the expression of aggressive behaviour between ewes, and no effect on physiological stress measures (FGM) or weight gain. However, parity differences were observed in terms of weight change, behavioural responses, and physiological stress, with primiparous ewes gaining less weight, having higher FGM and being less likely to feed and more likely to ruminate or be idle than multiparous ewes, especially in the earlier stages of the study.

Higher total aggressive interactions during concentrate feeding were recorded when the feeding space was restricted (RS-Mix ewes), and these ewes also had a significantly lower frequency of being able to freely join the feedface without having to use aggressive behaviour. This observation was consistent with previous studies which reported an increase in displacement behaviour by ewes with reduced space allowance per ewe (Marsden and Wood-Gush, 1986; Silveira et al., 2018). The number of displacements was also found to be high with reduced feeding space for ewes provided with hay (Bøe and Andersen, 2010). However, the difference between RS-Mix and Control groups was only significant during the first feedface observation at week 14 of gestation, although RS-Mix ewes still displayed high aggressive behaviour at week 16 before it declined considerably at week 18 of gestation. The decrease in aggressive behaviour shown by RS-Mix ewes may be due to the progression of gestation, which decreased overall activity probably as the fetus gets heavier, and the gravid uterus restricts organ space. However, in this study, the proportion of free leave (voluntary leaving the feedface rather than being pushed out or displaced) was not affected by treatment group or parity, which indicates there was also no difference in performing displacements at this stage in pregnancy. In contrast, the RS-Mix ewes showed significantly lower free join compared to Control ewes, indicating ewes from RS-Mix groups had to apply some form of physical effort on other ewes in order to access the feed trough due to smaller feeding space available. Besides displaying higher total aggression, multiparous ewes were more likely to display forced entry to get to the feed trough regardless of the treatment group even though the Control group had twice the length of feed trough compared to the RS-Mix group. The Department for Environment, Food & Rural Affairs (**DEFRA**) in the UK has recommended approximately 45 cm of trough space for lowland ewes to prevent competition and aggression which might be detrimental to sheep welfare (DEFRA, 2000). The feeding space allowance recommended by DEFRA is more than that provided to RS-Mix ewes and far less than Control ewes in this study, but high aggression was still observed in multiparous ewes in the Control treatment, which suggests that feedface allowance may not be the only reason for the high aggression, or that the recommended feeder space allowances are not sufficient to prevent feeding aggression. Previous studies have suggested that group size does not influence aggression (Jorgensen et al., 2009), but the delivery of a small amount of very palatable feed (as in this study) may still induce aggression at the feedface, even when there is sufficient space for all animals to feed together. Several studies have reported that older domestic as well as wild sheep are more aggressive compared to younger individuals (Hass, 1991; Favre et al., 2008; Gorecki and Dziwinska, 2014). However, in general, female sheep tend to interact less with other females and perform shorter bouts of aggressive behaviour compared to the rams (Fisher and Matthews, 2001). The ewes also rarely show clash, mount or threat-jump type of antagonistic behaviour unlike their male counterparts (Fisher and Matthews, 2001). The majority of aggressive behaviour performed by the ewes in this study was pushing other ewes during concentrate feeding at the feed trough in order to get more access to feed.

The effects of parity on the parameters tested were more pronounced in this study than the effects of housing treatment. Primiparous ewes had a lower weight gain than multiparous ewes at weeks 13 and 15 of gestation. The primiparous ewes were first introduced to the indoor environment during this study while mul-

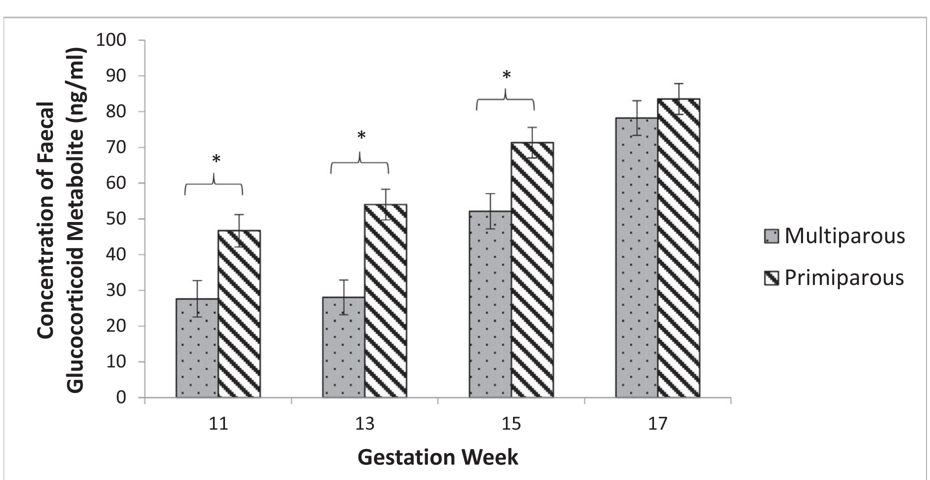

Fig. 8. Concentration of faecal glucocorticoid metabolites (FGM) over the gestation weeks 11–17 for multiparous and primiparous ewes. Values are means (±SEM). Bars with * indicate significant difference in concentration of FGM between parity.

tiparous ewes had been exposed to living indoors during late pregnancy until a few days after lambing in the previous year. A study investigating sheep transferred from pasture to indoor crates recorded withdrawal behaviour in weeks two and three of confinement (Fordham et al., 1991). Exposure to confinement in addition to a novel environment may also lead to disruption of feeding behaviour. Sheep have been observed to refuse feeding on novel food in an unfamiliar location and consumed more of a familiar but aversive food, which they have been conditioned to avoid prior to relocation (Burritt and Provenza, 1997). These animals might display what is called 'neophobia', also referred to as 'shy feeder' (Savage et al., 2008), which is more pronounced in unfamiliar than familiar environments (Burritt and Provenza, 1997). Feeding behaviour observed by scan sampling in this study showed that multiparous ewes displayed more feeding behaviour compared to primiparous ewes throughout the study. Therefore, apart from not having adjusted to being housed indoor for the first time, it may also be possible that the low weight gain achieved by the Primiparous ewes was due to the competition with multiparous ewes for feeding space at the hay rack although this parameter was not recorded in this study. Special attention should be given to the weight loss or minimal weight gain on primiparous ewes in gestation since it has been demonstrated that ewes exposed to low nutrition during mid-gestation give birth to low birthweight in lambs (Muñoz et al., 2009; Rooke et al., 2010). The birth weight of lambs from ewes giving birth for the first time had been reported to be significantly less compared to a second pregnancy (Gardner et al., 2007). This may be due to a greater blood volume expansion caused by increased vascularisation as a result of the first pregnancy which may promote a greater fetal growth in the following pregnancies (Gardner et al., 2007). As the uterine blood flow is a major regulator of transplacental fetal nutrient supply (Wallace et al., 2008), multiparous ewes may need to increase their nutrient uptake to meet their larger fetal burden and maintenance needs, compared to primiparous ewes, which could explain the higher display of forced entry in accessing the concentrate feed. In addition, inadequate food intake may also cause an adverse effect on the establishment of the ewe-lamb bond since lambs from under-nourished ewes take longer to suck and vocalise more while the ewes show reduced expression of maternal behaviour which could compromise lamb survival (Corner et al., 2010; Dwyer et al., 2003). First parity ewes are known to be more likely to show aberrant or disturbed maternal behaviour and have higher lamb mortality (Dwyer and Smith, 2008), which may be partly related to differing nutritional needs and further argues for more focused care for primiparous ewes in gestation.

Ewes spent more time ruminating at week 12 of gestation before this declined at week 14 regardless of treatment groups and parities. The ewes may have been displaying withdrawal behaviour as a result of moving to a new environment. Done-Currie et al. (1984) also reported a similar outcome where newly confined sheep were seen to ruminate more compared to long-term confined sheep. At week 18 of gestation, RS-Mix ewes displayed a significantly higher frequency of ruminating than Control ewes. This difference may have occurred by chance or ruminating may act as a coping mechanism for chronically stressed RS-Mix ewes. Since stereotypic behaviours (repetitive and functionless behaviour) are not often performed by ruminants including sheep (Lawrence and Rushen, 1993), it is postulated that rumination may play a role in alleviating the impact of stress condition in a similar way to stereotypies (Broom and Fraser, 2007). As opposed to feeding behaviour, primiparous ewes ruminated significantly more frequently than multiparous ewes. This is consistent with the rest of the outcomes in this study, indicating primiparous ewes may have had a more difficult time to adjust to the new environment: being pregnant for the first time as well as competing with multiparous ewes in the same pen at close proximity compared to when at pasture. Idling behaviour was negatively correlated with ruminating behaviour as the ewes displayed a low frequency of idling in week 12 of gestation before increasing at week 14 and remained constant until the end of observation at week 18 of gestation. This is similar to the increased lying recorded in this study as gestation progresses regardless of the treatment group or parity. Rumination in pregnant sheep is mainly observed while they're lying down (Dwyer, 2021) which may be due to the decrease in space for the uterus and rumen in late gestation especially in twin-bearing ewes as observed in this study.

Concentrations of FGM were found to be higher in primiparous than multiparous ewes in weeks 11, 13 and 15 of gestation, but no difference was found between treatment groups. The higher level of FGM concentration in Primiparous ewes may be due to the physiological alteration associated with first pregnancy, which may have been related to increased stress experienced in first-time mothers. Speculatively, it may also be a possibility that higher concentrations of FGM in primiparous ewes were due to an increased metabolic rate in preparing the body for first pregnancy, as similar responses have been seen in primiparous primate mothers (Carrera et al., 2020). It has been argued that glucocorticoids should more properly be considered as metabolic hormones since they mediate a number of routine metabolic activities, including reproduction (Dantzer et al., 2016). Late pregnancy was also associated with increased cortisol level in the present study regardless of the parity, as previously reported (McMillen et al., 1987; Keller-Wood and Wood, 2008; Sawyer et al., 2019) and this may have masked subsequent signals of stress in primiparous ewes, or reflect a common metabolic responses to impending parturition. Hild et al. (2011) reported a higher salivary cortisol in pregnant ewes after being handled aversively but showed no difference between parities. This was perhaps due to the time of the handling test and salivary sample collection which were conducted at late pregnancy (beginning from week 5 before birth), may have masked the metabolic response in primiparous ewes to prepare them for parturition. No difference between treatment in the concentration of FGM in this study suggests that different types of stressful event may produce different impacts on the behaviour and physiology of the animals (Blanchard et al., 2001). It has been shown that neuroendocrine response to stressors may be attenuated during pregnancy in many species including humans (Young and Rose, 2002). Therefore, ewes in the RS-Mix group may have displayed hyporesponsiveness towards the stressors presented in this study, hence no significant differences between groups were seen. Hyporesponsiveness to stressors is important in pregnant mothers since it is hypothesised to offer a protective mechanism to the fetus (Brunton et al., 2008). This is crucial as increased maternal hypothalamo-pituitary-adrenal activation has been associated with behavioural and physiological alterations in the offspring (Coulon et al., 2011; Roussel-Huchette et al., 2008; Weinstock, 1997).

In this study, we chose to apply a number of concurrent potential stressors (reduced space allowance, reduced feeder allowance, social stress through mixing), rather than focus on each possible stressor individually. This was designed to replicate the typical variation in management (where these stressors are often applied together, Rutherford, unpublished observation) for pregnant ewes as a first step to assessing the welfare impacts of pregnancy housing. Our data suggest that the stressors do not induce a large change in behaviour in housed ewes, and primiparity may be a more important factor in the response of the ewes to housing. However, there was a small increase in aggressive behaviour in all ewes through housing and future research should investigate if this is related to space allowance or social mixing to improve advice that can be given to farmers.

Conclusion

In conclusion, a reduction in space and social mixing resulted in only a small increase in aggressive behaviour in RS-Mix ewes at the feeding trough compared to Control ewes with more space, and no impact on physiological stress responses. However, primiparous ewes appeared to be more affected by the housing conditions as they had lower weight gain, higher concentration of FGM along with altered feeding and ruminating behaviour compared to multiparous ewes. These differences may be due to the exposure that multiparous ewes had to indoor housing in their previous lambing compared to primiparous ewes, or a combined effect of housing on the extra-biological effort required of ewes pregnant for the first time. Therefore, ewe welfare could be improved by giving extra consideration to housing conditions for gestating ewes, especially those pregnant for the first time.

Ethics approval

The study was granted ethical approval from SRUC (ED-AE 4-2015) and was conducted under UK Home Office regulations, complying with EU directive 2010/63/EU.

Data and model availability statement

The data/models were not deposited in an official repository. Data are available upon request to the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration of interest

None.

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