Furthering our understanding of intramammary infections (mastitis) in suckler ewes: the role of chronic infection and udder conformation

Final Report

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INTRODUCTION

There has been very little research on clinical mastitis or intramammary infections (IMI) in suckler ewes. The research published is summarised below.

Incidence rate and prevalence of mastitis and its impact on the suckler sheep industry

Clinical mastitis in sheep can lead to sudden death, loss of an affected udder half, chronic intramammary infection detected as masses (abscesses) in the mammary gland or raised somatic cell count (a measure of immunity), or recovery. Farmers have reported an incidence rate of clinical mastitis of 0 - 5% per year in England and Ireland (Cooper 2011; Onnasch 2000), although the true figure might be higher because it has been suggested that farmers under-report clinical mastitis in dairy ewes (Lafi et al., 2008). In addition, anecdotal reports from farmers indicate that 20 – 30% of ewes culled from the flock at weaning have udder damage from clinical mastitis or chronic mastitis with palpable intramammary masses. This amounts to approximately 8% of the national flock dead / culled because of mastitis each year.

The health, welfare and economic costs of mastitis therefore come from deaths of ewes, premature culling of those that lose the function of one or both glands, culling of ewes with intramammary masses (practised by some farmers) and for ewes with a somatic cell count (SCC) >400,000 cells / ml milk, reduced milk production that causes slower growth rates in lambs (Arsenault et al., 2008; Huntley et al., 2012).

Previously reported risk factors for mastitis in suckler ewes

Larsgard and Vaabenoe (1993) studied 920 ewes in Norway for 6 years. Larger litter size, the Steigar breed, poor udder conformation, and grazing cultivated pasture rather than highland pasture were all associated with a higher risk of mastitis. Waage and Vatn (2008) identified individual animal risk factors for clinical mastitis in suckler sheep in Norway in a case-control study of 1,056 flocks. They identified that larger litter size, older age, breeds other than old Norwegian breeds, dystocia and a previous case of mastitis were associated with an increased risk of mastitis. Arsenault et al. (2008) studied 2,792 ewes in 30 commercial flocks in Quebec. The risk factors for clinical mastitis found were geographical region and litter size of 3 or more lambs, while for subclinical mastitis a litter size of 2 or more lambs, older age of ewes, geographical region and body condition score below 2.5 were associated with a higher risk. Pereira et al. (2014) studied 54 farms in Brazil. Intensive management systems and the

Santa Inês breed were found to have increased risk of clinical mastitis while isolation of affected ewes and weaning after 120 days were associated with lower risk.

Impact of udder conformation on occurrence of mastitis

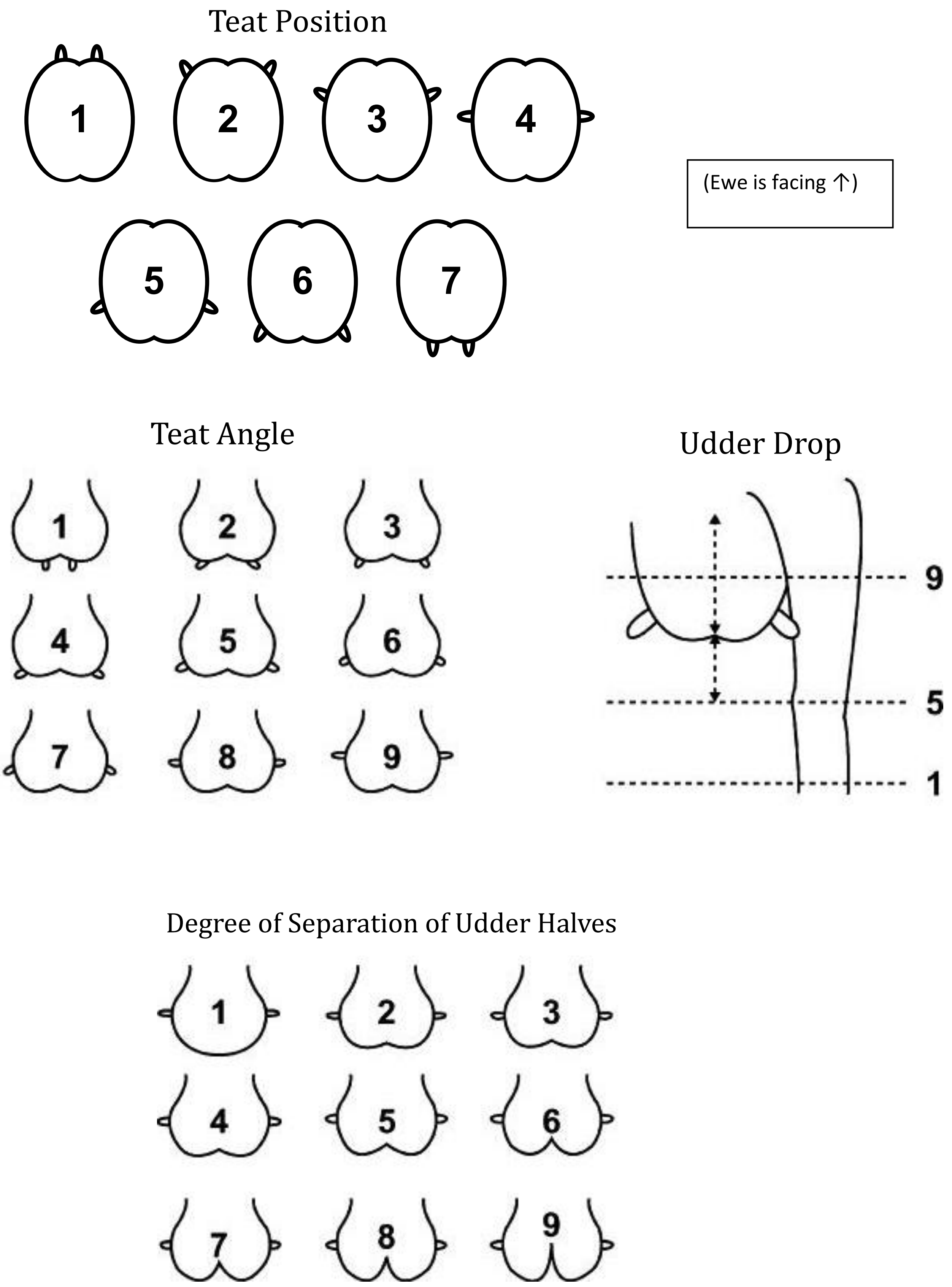
In dairy sheep, good udder conformation is associated with a decreased risk of mastitis (Casu et al. 2010). A number of linear scoring systems of udder traits have been developed in European dairy sheep to assess udder conformation (de la Fuente et al., 1996; Marie-Etancelin et al., 2005; Casu et al., 2006). These measure udder depth, udder attachment, teat placement (or angle), teat length and udder shape (de la Fuente et al., 1996); teat angle, udder cleft and udder depth (Marie-Etancelin et al., 2005); teat placement, udder depth, degree of separation of the two halves of the udder, degree of suspension of the udder (Casu et al., 2006). (See Figure 1: Modified linear scoring system of udder traits).

In some dairy breeds udder traits have been included in breeding programmes (de la Fuente et al., 1996; Marie-Etancelin et al., 2005; Casu et al., 2006). In dairy sheep the aim is to improve machine milking ability (de la Fuente et al., 1996; Marie-Etancelin et al., 2005; Casu et al., 2006) and the ideal udder conformation includes vertically aligned teats (Labussière 1988). A different udder morphology might be suitable for suckling ewes where lambs feed at an oblique angle to the udder. Dairy ewes with pendulous udders and teats placed high on the udder are more prone to poor udder health (Casu et al., 2010). Recent work in suckler ewes indicated that pendulous udders were associated with higher milk somatic cell count. In addition, an optimum teat angle score of 5 (a 45˚ angle on the udder) was associated with greater weight gain in lambs (Huntley et al., 2012). This optimum teat angle was also associated with decreased risk of traumatic teat lesions caused by lambs (Cooper et al., 2013). It has been reported that healthy teats help defend the udder against bacterial infection (Mavrogianni et al., 2006).

Mastitis and nutrition in suckler ewes

Adequate nutrition that meets the ewe's requirements for energy and protein during both pregnancy and lactation is important so that ewes produce healthy lambs and have sufficient milk yield to feed them (Fthenakis et al., 2012; Rooke et al., 2015). Ewes in poor body condition at lambing (below body condition score 3 for lowland ewes) (EBLEX Sheep BRP Manual 4) or with inadequate nutrition may not produce sufficient milk to satisfy their lamb's hunger. Hungry lambs will butt at the udder and perhaps bite the teats in their attempts to satiate themselves, which can lead to udder damage and teat lesions. Ewes with

Figure 1. Modified linear scoring system of udder traits.



low body condition (BCS < 2.5) are more likely to have teat lesions (Cooper et al., 2013) and perhaps an increased risk of mastitis. Well-nourished ewes are also better able to fight infection.

Areas of interest in the UK

It is not currently known whether some breeds of sheep are more susceptible to mastitis than others and whether it is feasible to select for good udder conformation to reduce the risk of mastitis. Mastitis is caused by bacteria, however it is rarely managed as an infectious disease. A common practice among suckler sheep farmers is to check the udder of each ewe at the end of lactation or 6 weeks before the start of the next breeding season. Ewes with damaged or "lumpy" udders (intramammary masses, a type of chronic mastitis) (Bergonier et al., 2003) are often, but not always, culled. The impact of this practice is unknown; it is possible that it reduces onward transmission of bacterial strains causing mastitis, reduces the number of slow growing lambs in a flock, reduces the selection of replacement lambs from ewes with chronic mastitis and slows down the selection of more susceptible offspring.

Aim

The overall aim of this study is to provide advice to the industry on the impact of ewes with good udder conformation, and removal of ewes with damaged / infected udders to improve the health, welfare and economics of the sheep industry by reducing mastitis and improving lamb growth rates.

The objectives are to:

1. Investigate udders with masses of cull ewes to develop a score for udder damage and establish the types of bacteria associated with chronic infection.
2. Develop a scoring system to evaluate the conformation of the udder, including the detection of internal masses.
3. Monitor ewes over time and score both the udder damage (masses in the udder, teat lesions, acute mastitis) and udder conformation.
4. Test the associations between udder damage and udder conformation.
5. Investigate the impact of retaining ewes with masses in the udder into the next lactation (i.e. an increased risk of acute / chronic mastitis or reduced lamb growth rate).
6. Investigate the costs and benefits of scoring udder conformation as part of the selection criteria for replacement ewes.

Objective 1

Investigate udders with masses of cull ewes to develop a score for udder damage and establish the types of bacteria associated with chronic infection.

LAY SUMMARY

Bacterial species and their associations with chronic mastitis in suckler ewes.

There have been no studies of the bacterial species associated with chronic mastitis (intramammary abscesses) in ewes. We examined 24 milk samples and 33 abscesses from 16 udders and isolated 35 bacterial species in total. These did not vary by disease state, though *Staphylococcus aureus* was the most common species, and closely-related strains of *S. aureus* were present across all clinical presentations. Our results suggest that the udders of ewes with chronic mastitis could be reservoirs of *S. aureus*.

Bacterial species and their associations with acute and chronic mastitis in suckler ewes

ABSTRACT

Chronic mastitis, characterised by intramammary abscesses with no systemic disease, is typically detected when ewes are inspected before mating. The aims of this objective were to identify the species and strains of culturable bacteria associated with milk and abscesses of chronically diseased mammary glands to investigate which bacterial species were present and whether species and strains vary by site of isolation. Sixteen ovine udders were obtained from two abattoirs; milk was aspirated from the 32 glands where possible, and the udders were sectioned to expose intramammary abscesses which were swab sampled. All milk and swab samples were cultured aerobically. In total 35 bacterial species were identified; the coincidence index of overlap of species detected in intramammary abscesses and milk was 0.60, reducing to 0.36 within individual glands; indicating a high degree of species overlap in milk and abscesses overall, but less overlap within specific glands. *Staphylococcus aureus* was detected frequently in all sample types, and closely-related strains were detected in milk and abscesses from the same gland.

INTRODUCTION

Intramammary infections (IMI) in suckler ewes are usually caused by bacterial infection of the mammary gland. They have a wide range of presentations from no detectable clinical abnormality (sub-clinical disease), to a hot swollen gland and, occasionally, sudden death (acute mastitis). Intramammary abscesses are a presentation of chronic mastitis and thought to form following an IMI, although not all IMI lead to abscess formation. These abscesses are often only detected when a gland is palpated, typically when farmers inspect ewes at the end of lactation, or when selecting ewes for mating. There are few reports of the prevalence of ewes with intramammary abscesses. Onnasch et al. (2002) [in Ireland] reported a prevalence of chronic mastitis of 2.8 % and Saratsis et al. (1998) [in Greece] reported abnormalities (including nodules, lumps, diffuse hardness, abscesses and cysts) in the udders of 162 / 3529 (4.6 %) ewes. Anecdotally, British farmers indicate that 20 – 30% of ewes culled from the flock (i.e. 4 - 6% of the total flock if 20% are culled) at weaning have intramammary abscesses.

Over 130 bacterial species have been associated with IMI in dairy cows (Watts, 1988) and 20 – 30 with IMI in suckler sheep to date (Mørk et al., 2007, Arsenault et al., 2008, Marogna et al., 2010). In dairy ewes, signs of chronic mastitis defined as nodules, abscesses, sclerosis and atrophy have been significantly associated with isolation of *S. aureus* from milk (Marogna et al., 2010). However there have been no studies on the bacterial species associated with intramammary abscesses in suckler ewes. Abscesses that result from infection with the endogenous flora of a body site are reported to be polymicrobial (Brook, 2002) and it is hypothesised that polymicrobial abscesses persist, in part, because the species act synergistically.

The aims of this objective were to characterise the culturable bacterial species associated with intramammary abscesses (chronic mastitis) and associated milk samples, of suckler ewes, to investigate the bacterial species present and whether species and strains were common or varied between presentations.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

Sixteen ovine udders (32 glands, 2 glands per udder) were obtained from two abattoirs in England. On arrival at the laboratory the surface of each udder was sterilised with 70% ethanol, and one sample of milk was aspirated from each gland where possible, using a sterile 18 gauge needle and syringe. One hundred microliters of each sample was spread across the surface of a brain heart infusion (BHI) agar plate containing 5% sheep blood and incubated at 37 °C for 48 hrs. Growth was observed at 24 and 48 h and the number of colony forming units (cfu) estimated for each morphologically distinct colony type. Where cfu were too numerous to count a figure of 1000 cfu was used for calculations. All morphologically distinct colony types were purified and stored at -80 °C.

Each gland was sliced into parasagittal sections using a sterile blade, sterilised between cuts with 70 % ethanol. The number of abscesses was recorded and each abscess was swabbed using a sterile cotton-tipped swab that was immediately plated and incubated as described above. Morphologically distinct colony types were purified and stored at -80 °C.

Identification of Bacterial Isolates

Matrix-assisted laser desorption ionisation time of flight (MALDI-ToF) mass spectrometry was used to identify isolates (Alatoom et al., 2011). Pure isolates were cultured on BHI agar and a loop of each isolate was suspended in 75% ethanol and pelleted at >20,000 ×*g* for 5 min, the supernatant was discarded and the pellet re-suspended in one volume (equal to pellet size) of 70% formic acid (Sigma-Aldrich Company Ltd., Dorset, England) and one volume of acetonitrile (Sigma-Aldrich Company Ltd., Dorset, England). Samples were then pelleted at >20,000 ×*g* for 2 min, 1 µl of supernatant was placed in a well on a steel target plate (Bruker UK Ltd, Coventry, England) and air-dried. Once dry, 1 µl of α-Cyano-4-hydroxy-cinnamic acid matrix (HCCA; Bruker UK Ltd, Coventry, England) was overlaid on each sample and air-dried.

Target plates were loaded into a Microflex LT instrument (Bruker UK Ltd, Coventry, England) with protein mass profiles obtained using the MALDI Biotyper wizard classification and FlexControl software (Bruker UK Ltd, Coventry, England) with default settings. The MALDI Biotyper compares the spectra obtained with a database of known species (Feb 2014 update; 5,627 isolates, 1,951 species in 388 genera, with 20 - 24 replicates per [predominantly human] isolate) and produces a 'top 10' list of matches for each sample plus a confidence score (range: 0 – 3). Using the recommended cut-off values, a score ≥ 2.30 indicates highly probable species identification, a score of 2.00 – 2.29 indicates probable species identification and scores of 1.70 - 1.99 indicate probable genus identification (Bruker Daltonik GmbH, 2011). Scores < 1.70 are not considered a reliable identification. To overcome the lack of veterinary isolates for some species in the database, the criteria used in the current study were modified for isolates with a score 1.70 – 1.99. If there were ≤ 3 of the identified species in the database, the species designation was accepted. If there were > 3 species in the database and the top ≥ 3 identifications were the same species, then the species designation was also accepted. For all cases outside of these criteria, only the genus designation was accepted.

Where species were not identifiable using MALDI-ToF, DNA was extracted from overnight cultures using the NucleoSpin Tissue Kit (Machery-Nagel GmbH & Co. KG, Düren, Germany) as recommended. The 16S gene was amplified using primers 27F and 1525R and sequenced using the internal primers PSL and PSR (Moore et al., 2008). Sequences were assembled in SeqMan Pro (DNASTAR Inc, Madison, WI, USA), manually trimmed and compared to the NCBI 16S ribosomal RNA sequences database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The species designation was accepted when the identity and query coverage were ≥ 96%.

For the isolates identified by sequencing, no provisional identification was made by the Biotyper because all scores were < 1.70. In total, 11 species were identified, eight of which were represented in the database by 1 – 12 isolates (Supplementary Table S1). One of the species identified by sequencing (*Staphylococcus lentus*) was also identified by the Biotyper in another sample; however the remaining 10 species were only identified by sequencing.

Data Analysis

All data were entered into an Excel spreadsheet and the distribution of abscesses and isolates overall, by ewe (udder) and by gland were calculated. Where more than one bacterial species was detected in a sample, the numerically dominant species was defined as one that had > 50 % cfu. Where this threshold was not reached, no dominant species was identified. Spectral analysis to investigate *S. aureus* diversity was performed in MicrobeMS (Lasch et al., 2014). All spectra were pre-processed, including smoothing, baseline correction and normalisation; peak detection was set to 100 peaks per spectrum, and all spectra were calibrated using an internally generated list of 30 peaks at mass-to-charge-ratios (*m/z*) ranging from 2149.7478 to 8090.5314 (Supplementary Table S2). Spectral peak tables were converted to bar code spectra and analysed using hierarchical cluster analysis. The mass tolerance was set to 200 ppm, and spectra were clustered using Euclidean distances and average linkage (Lasch et al., 2014), with output visualised as a dendrogram. Clusters were defined using an arbitrary spectral distance cut-off value of 22. Strains from the same sample were indicated on the dendrogram. Fisher's exact tests were used to examine the distribution of isolate source by cluster (http://vassarstats.net/tab2x2.html).

The coincidence index of overlap (Dice, 1945) was calculated overall and by ewe and mammary gland to quantify the overlap between two environments [milk and abscesses] using a range from 0, no overlap, to 1, complete overlap of species between environments. This was determined using the formula: $(2b) / ((a + b) + (m + b))$; where b = number of species in both abscess and milk samples, a = number of species in abscess samples and m = number of species in milk samples.

RESULTS

Abscesses

Five udders had no intramammary abscesses, from the remaining 11 there were abscesses in 15 glands (4 ewes had abscesses in both glands), and 33 intramammary abscesses were detected in total (Table 1). Abscess morphologies ranged from 1 - 2 cm diameter pus-filled abscesses to a 6 – 7 cm diameter intramammary void intersected by fibrous strands (Supplementary Images S1 and S2). In total 29 / 33 abscesses were culture positive and 87 morphologically distinct isolates were identified. Six isolates failed to grow on subculture, the remaining 81 were identified using the MALDI Biotyper (n = 63; 77.8 %) or 16S sequencing (n = 18; 22.2 %). In total 26 species were identified in abscesses (Tables 1 and 2).

There was an overdispersed Poisson distribution of number of species within abscesses (mean = 1.9; variance = 3.1; Fig. 1A), the modal number of species detected was one in 16 abscesses, with a maximum of seven species in one abscess. A numerically dominant species was identified in four abscesses with > 1 species present, and there was a weak positive relationship between the numbers of abscesses and number of bacterial species detected within abscesses in a mammary gland. *Staphylococcus aureus* was the most frequently detected species, present in abscesses in seven glands from five udders. *Streptococcus uberis* was detected in abscesses from four glands from four udders; no other species were detected in more than three udders (Table 2). *Staphylococcus aureus* was the only cultured organism detected in four abscesses, and was the dominant species in one further abscess. *Streptococcus ovis* was the only cultured organism detected in two abscesses and *Pseudomonas aeruginosa* dominated two mixed cultures, including one sample from an intramammary void (Supplementary Image S2). No other species was the only cultured organism detected or dominated more than one abscess (Table 2).

Milk samples

Twenty-four milk samples were aspirated from 15 / 16 udders; two milk samples were obtained from nine udders, and one milk sample from six udders (Table 1). Fifteen milk samples from 10 udders were culture positive, yielding 55 bacterial isolates. Forty isolates (72.7 %) were identified using the MALDI Biotyper and 15 (27.3 %) by 16S sequencing. In total 24 species were identified; five samples each contained a single species, and two, four, one, one and two samples each contained two, three, four, five and six species respectively (Fig. 1A).

Staphylococcus chromogenes was the species most frequently isolated from milk, detected in five glands from five udders (Table 2). Numerically dominant species were identified in 12 milk samples; although no one species dominated more than two samples (Table 2). In six mammary glands where a dominant species was identified in milk, abscesses were also present. In four of these, the dominant species in milk was also detected in an abscess from the same gland.

All samples

In total 35 bacterial species were identified, 11 were detected only in abscesses, nine only in milk and 15 in both abscesses and milk (Table 2). The coincidence index of overlap between milk and abscess samples overall, irrespective of ewe and gland was 0.60; by ewe (n = 10 udders) the value was 0.38, and by mammary gland (n = 11) the value was 0.36.

Analysis of *S. aureus* mass spectra (MS) suggested one singleton isolate and two clusters of six and 13 isolates were present within the abscess and aspirated milk samples (Fig. 1B). Both clusters contained isolates from more than one udder. The four isolates detected in milk were in the same clusters as isolates detected in abscesses from the same udders (Udders L and M; Fig. 1B). More than one *S. aureus* strain was isolated from four abscesses and one milk sample, and with the exception of one abscess sample, strains from the same sample were located in separate clusters (Fig. 1B).

DISCUSSION

This is the first study to investigate the culturable bacterial species associated with intramammary abscesses and associated milk samples. A total of 35 bacterial species were cultured from the samples in the study. Whilst some species were more prevalent than others there was no clear difference in species detected in intramammary abscesses or associated milk samples (Table 2). The coincidence index value of 0.60 highlights the strong species overlap between abscesses and milk, and therefore the lack of species niche-specificity to a situation (chronic mastitis, milk). The lower similarity index within individual mammary glands with chronic mastitis might be explained by the fact that the bacterial community in a closed abscess is likely to remain more stable than the bacterial community in milk, where bacteria might leave or arrive via the teat.

The overdispersed Poisson distribution of species from abscesses and associated milk samples suggests a random number of species in any one sample (Fig. 1A). Previous work has suggested that abscesses are generally polymicrobial (Brook, 2002), and that a community of species assist in abscess formation and persistence. This is known as a staphylococcal abscess community (SAC) in abscesses caused by staphylococci (particularly

S. aureus). The SAC is protected by a fibrin-containing pseudocapsule which allows the abscess to mature and evade host defences (Cheng et al., 2010, Cheng et al., 2011). Subsequently the abscess can rupture, releasing the pathogen community to repeat the process. Abscess rupture can result in fibrotic scars (Cheng et al., 2011) and the varying stages of abscess maturation and rupture might explain some of the phenotypic diversity in abscesses in the current study (Supplementary Images S1 and S2).

Whilst 40% of abscesses had polymicrobial communities almost 60 % (17 / 29) of abscesses produced a pure culture. This potentially challenges the hypothesis that abscesses form as polymicrobial communities as a result of introduction of the endogenous flora into a normally sterile body site (Brook, 2002). Samples were, however, only cultured aerobically, and anaerobic bacteria commonly outnumber aerobic species in abscesses (Brook, 2002). Anaerobic species such as *Fusobacterium necrophorum* have been detected in bovine intramammary microbiome studies and human abscesses (Brook, 2002, Oikonomou et al., 2012); so it is possible that these species were present, but not detected by aerobic culture.

Staphylococcus aureus dominated all sample types: swabs of abscesses and associated milk samples. Other authors have reported that *S. aureus* is commonly associated with acute clinical mastitis in suckler ewes (Jones and Watkins, 1998, Mørk et al., 2007), but the current study is the first to report *S. aureus* in chronic mastitis abscesses and associated milk. *S. aureus* strains did not vary by sample type, suggesting that there are no specific strains related to specific disease presentation. The results from the current study suggest that glands from ewes with chronic mastitis might potentially be a reservoir of *S. aureus*.

CONCLUSIONS

A large number of bacterial species were present in intramammary abscesses and milk of suckler ewes; and there was a high degree of overlap between the species detected at different sites. One species of bacteria tended to dominate each abscess and *S. aureus* dominated all sample types. Whilst closely-related strains of *S. aureus* were detected in intramammary abscesses and milk from the same mammary gland, there were no strains specifically linked with diseased states. The results from the current study suggest that glands of ewes with chronic mastitis are a reservoir of *S. aureus*.

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TABLES

Table 1: Numbers of samples collected and bacterial species detected, by udder and mammary gland

Udder	**No. of abscesses sampled**		**No. of species isolated from abscesses**			**No. of species isolated from milk**			**Total number of species in udder**
	Left gland	Right gland	Left gland	Right gland	**Total**	Left gland	Right gland	**Total**	
A	5	5	5	7	**9**	3	6	**7**	**12**
B	0	0	NS[1]	NS	**--**	0	3	**3**	**3**
C	2	1	7	5	**10**	NS	NS	**--**	**10**
D	1	0	7	NS	**7**	1	0	**1**	**7**
E	0	0	NS	NS	**--**	4	6	**9**	**9**
F	1	0	3	NS	**3**	3	5	**7**	**7**
G	0	6	NS	3	**3**	0	0	**0**	**3**
H	1	0	4	NS	**4**	NS	2	**2**	**5**
I	0	1	NS	1	**1**	1	0	**1**	**2**
J	2	2	1	1	**2**	0	NS	**0**	**2**
K	1	0	0	NS	**0**	1	2	**3**	**3**
L	0	3	NS	5	**5**	NS	1	**1**	**5**
M	1	1	1	3	**3**	1	3	**4**	**5**
Total	**33**				**26**			**24**	**35**

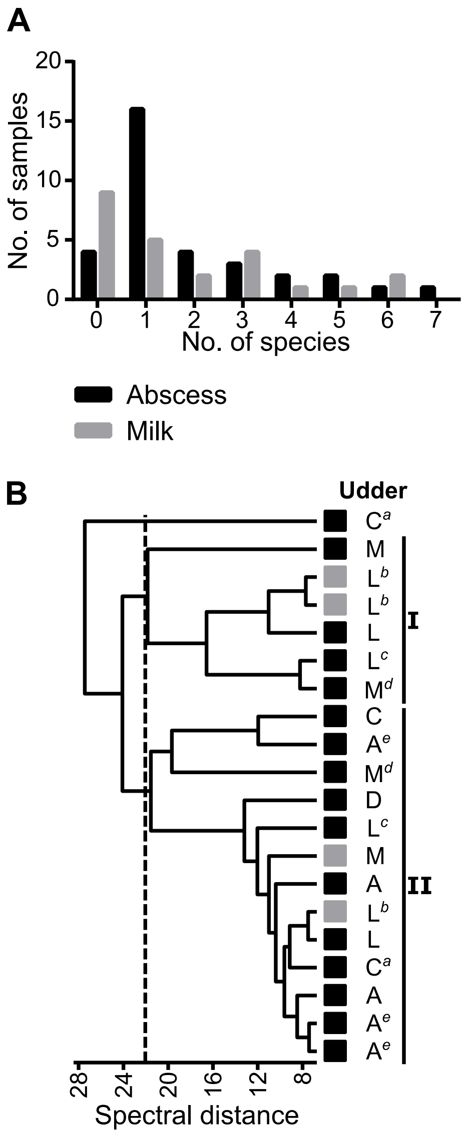
[1]NS = no sample.
Three additional udders contained no visible abscesses, and no bacteria were cultured from aspirated milk samples from these udders;

Table 2: Number of abscesses, glands and udders each species was detected in, from the abscesses and milk of 14 udders. 'Dominant' refers to one organism representing > 50 % of bacterial plate count, based on morphology

Species	Number detected in abscesses				Number detected in milk		
	Abscesses	Glands	Udders	Dominant	Glands	Udders	Dominant
Acinetobacter sp.					1	1	1
Aerococcus viridans	2	2	2		1	1	
Bacillus cereus	1	1	1				
Bacillus clausii	1	1	1				
Bacillus licheniformis	2	2	2		1	1	
Bacillus subtilis					1	1	
Bibersteinia trehalosi	1	1	1		1	1	
Corynebacterium efficiens	1	1	1				
Corynebacterium epidermidicanis					1	1	
Corynebacterium lipophiloflavum	1	1	1	1			
Corynebacterium stationis					1	1	
Corynebacterium striatum	2	2	2		2	2	
Corynebacterium xerosis					1	1	
Enterococcus casseliflavus	2	2	2	1			
Enterococcus hirae	1	1	1				
Macrococcus caseolyticus	4	4	3		3	2	
Mannheimia haemolytica	1	1	1	1			
Mannheimia sp.	1	1	1				
Micrococcus antarcticus					1	1	1
Paenibacillus barengoltzii	1	1	1	1			
Pseudomonas aeruginosa	4	3	2	2	3	2	1
Rothia endophytica					1	1	
Staphylococcus aureus	11	7	5	5	2	2	2
Staphylococcus auricularis					2	2	2
Staphylococcus chromogenes	3	2	2		5	5	2
Staphylococcus cohnii					1	1	
Staphylococcus haemolyticus	5	4	3	1	3	3	
Staphylococcus lentus	3	3	3	1	2	2	1
Staphylococcus simulans	1	1	1		3	2	
Staphylococcus sp.	1	1	1	1	1	1	
Staphylococcus warneri	2	2	2		3	2	1
Streptococcus lutetiensis	1	1	1				
Streptococcus ovis	3	3	2	2	1	1	
Streptococcus uberis	4	4	4	1	1	1	1
Trueperella pyogenes	1	1	1	1			
Unidentified	4	4	4	2			

FIGURE

Figure 1. **A**: Distribution of distinct isolated species from intramammary abscesses and aspirated milk samples; and **B**: Hierarchical cluster analysis of MALDI-ToF mass spectra of *S. aureus* isolated from intramammary abscesses and aspirated milk samples. The indicated clusters (I and II) are defined at a spectral distance cut-off of 22. Isolates with the same superscript were isolated from the same abscess.



APPENDIX

Supplementary Table S1. Species-level 16S rRNA gene sequencing-based identification of bacterial isolates, and the numbers of strains of that species present in the Biotyper database [Feb 2014 update; 5,627 isolates]

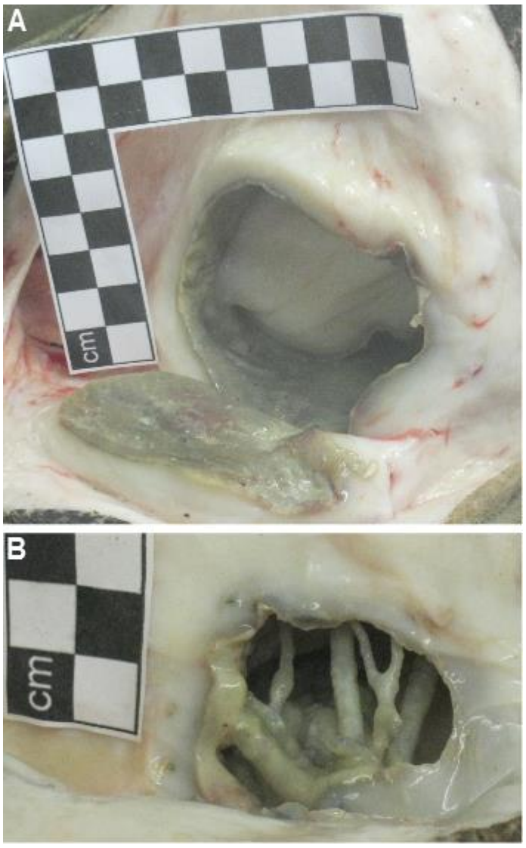
Species	**Isolates sequenced**	**Strains in database**
Bacillus clausii	1	1
Bacillus subtilis	1	9
Corynebacterium efficiens	1	1
Corynebacterium epidermidicanis	1	0
Corynebacterium lipophiloflavum	1	1
Corynebacterium striatum	4	7
Macrococcus caseolyticus	9	7
Micrococcus antarcticus	1	0
Rothia endophytica	2	0
Staphylococcus haemolyticus	11	12
Staphylococcus lentus	1	2

Supplementary Table S2. Mass-to-charge ratios (*m/z*) of internally generated calibration peaks

Mass-to-charge ratio
2149.7478
2747.6499
2965.4903
3211.1400
3236.6319
3279.5048
3303.8380
3306.1554
3409.2820
3410.4407
3444.0437
4047.7395
4304.9765
4448.6584
4817.1330
4939.9578
5054.6716
5494.9872
5875.0490
5930.6678
6237.7300
6264.3807
6421.9673
6572.6015
6613.1569
6703.5375
6745.2516
6817.0925
6887.7748
8090.5314

Supplementary Image S1. Small pus-filled intramammary abscess. The scale ruler is marked in 1 cm^2 blocks

Supplementary Image S2. Large intramammary void (A) intersected with fibrous strands (B). The scale ruler is marked in 1 cm^2 blocks



Objectives 2, 3, 4 and 5

- *Develop a scoring system to evaluate the conformation of the udder, including the detection of internal masses.*

- *Monitor ewes over time and score both the udder damage and udder conformation.*

- *Test the associations between udder damage and udder conformation.*

- *Investigate the impact of retaining ewes with masses in the udder into the next lactation.*

MATERIALS AND METHODS

Study farms

Study farms were identified from farmers with existing relationships with the University of Warwick and from a list of farmers who were interested in participating in research on mastitis provided by EBLEX. Farmers were contacted by telephone or email and the study was explained to them. When farmers were interested in participating they were visited by the research team and the project was explained in full and discussed. Farmers were left with a written description of the project. Once farmers agreed to participate informed consent was obtained; participants were free to withdraw from the project at any stage.

Data collection

Data collection occurred from November 2012 to July 2014. Each flock was visited twice each year for two years, once when ewes were in late pregnancy and once when ewes were in mid - late lactation. Farmers were interviewed to gather information on flock management and nutrition. Data on scanning results, lambing dates, litter size, fostering records, lamb birth and eight-week weights and cases of acute mastitis were obtained from farm records. In the first year, blood samples were collected from a sample of ewes in flocks without Maedi-Visna (MV) accreditation and tested for MV antibodies.

Data collection from sheep

Sheep were either examined upright in the narrowest portion of a race or while held by a clamp, or examined while restrained upright by an assistant in a pen. Udder conformation scores were assessed from a kneeling / crouched position behind the ewe using sight and / or touch. One of two trained researchers examined the ewes. Data were recorded by an assistant into a handheld data-logger (Agrident APR500) programmed with custom designed software (Border Software Ltd). Each ewe was inspected twice each year, once when pregnant approximately 4 weeks before lambing and again when approximately 9 weeks into lactation.

Data collection from pregnant ewes

Ewe identification, body condition score (BCS: 0 – 5 in 0.5 increments. Defra PB1875, undated) and the presence / absence of intramammary masses (IMM) in each udder half were recorded. Masses were defined as a physically detectable mass of abnormal consistency compared with the rest of the glandular tissue. They were graded by size assuming spherical-like shape into 'GRAPE' (approx. ≤ 15 mm diameter), 'PLUM' (approx. >15 - 35 mm diameter), 'KIWI' (approx. >35 – 50 mm diameter), 'APPLE' (approx. >50 - 90 mm diameter), and 'BRAMLEY' (> 90 mm diameter). When several masses or non-spherical masses were detected the total mass was estimated by the researcher and the appropriate size recorded.

Data collection from lactating ewes

Ewe identification, BCS and the presence and size of IMM in each udder half were recorded. In addition, udder conformation (Figure 1) was recorded using a linear scoring system of udder traits adapted from Casu et al. (2006) and reported in Cooper et al. (2013). TEAT POSITION, the placement of the teats on the udder on a horizontal plane, was scored using a seven-point scale from 1 (forward pointing) to 7 (backwards pointing). TEAT ANGLE, the placement of the teats on the udder on a vertical plane, was scored using a nine-point scale from 1 (vertical) to 9 (horizontal). UDDER DROP, the distance from the ventral abdominal wall to the udder cleft when viewed from behind, was scored using a nine-point scale from 1 (below the hock), 5 (level with the hock) to 9 (level with abdominal wall). DEGREE OF SEPARATION of udder halves was scored using a nine-point scale that ranged from 1 (no separation between udder halves) to 9 (clear separation between udder halves). UDDER WIDTH was measured at the widest point of the udder (1 cm increments); and TEAT LENGTH was recorded by measuring the left teat in 0.5cm increments. The presence of wool on the udder was recorded. Teat lesions were recorded as traumatic (broken skin) or non-traumatic (e.g. warts, spots, orf-like lesions).

Collection of milk samples

Farmers were trained to collect milk samples aseptically and provided with milk-sampling kits that also included a laminated reminder of the sampling protocol for easy reference. They were asked to collect milk samples from both udder halves of all pregnant ewes that the researcher had detected with IMM (cases) and from the same number of age-matched ewes with no detectable IMM (controls). In addition, farmers were asked to collect milk samples from both udder halves of ewes that developed acute mastitis at any time during lactation.

At the second examination, when ewes were lactating, researchers collected milk samples from the sheep detected with IMM when pregnant, from their matched control and from ewes with IMM detected for the first time when lactating. Where possible the same cases and controls were resampled in year 2 and any new cases and controls were sampled as necessary. All samples were frozen at -20 °C once collected, were returned to the laboratory at this temperature and are stored at -20˚C for future analysis.

Assessment of nutrition

Representative samples of forage and concentrates were collected from all farms where grass was supplemented and submitted to Sciantec Analytical Services (Selby, Yorkshire, England) for analysis. The metabolisable energy (ME; MJ/kg) and crude protein (%) content of the concentrates were determined and forage was analysed using the typical industry standard approach. The nutritional information on purchased feeds and supplements was recorded. In year 2, an estimate of grass availability to pregnant ewes was made by photographing sward height against a ruler in the field the ewes were in, where this was not possible farmers were asked to estimate sward height.

In the second year, blood samples were collected from six ewes in each flock that were scanned with twins with an average body condition score for the flock. This was used for metabolic profiling: specifically betahydroxybutyrate (BHB), urea and albumen.

Inter-rater reliability

Two trained researchers (CG and EMS) carried out the ewe examination and udder characterisation work, so an investigation of the inter-rater reliability (or between observer agreement) was carried out to test whether using the scoring system two people could produce a good level of consistency (e.g. Hewetson et al., 2006; Kaler et al., 2009). On the same day, both researchers scored the same 137 ewes supported by different data entry assistants. Methods and results can be seen in Appendix 2.

Data storage, preparation and analysis

All data were stored on password protected servers at the University of Warwick. Data on the datalogger were downloaded as text files and converted to Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) format.

ADAS carried out nutritional analysis of each farm's diets. Supplemented diets were analysed in the ADAS Sheepfeed rationing program (a computer program based on AFRC (1995)) and grass based diets were analysed using an Excel spreadsheet. Ewes were categorised at the flock level by number of lambs reared as 'OVERFED', 'UNDERFED' or 'OK' for dietary energy during pregnancy, dietary protein during pregnancy, dietary energy during lactation and dietary protein during lactation. See Appendix 1 for the full nutrition report from ADAS. Adequacy of energy and protein levels were assessed using AFRC (1995).

Lamb birth weight and eight-week weight were used to calculate the total litter birth weight (LBW) and litter daily live weight gain (LDLWG) for each ewe. Where lambs were not weighed at birth, but lambs of the same breed were weighed (on the same or another farm), the average of this weight (for lambs born as singles, twins or triplets) was used to calculate LBW and LDLWG where lambing dates and litter size were available.

All the animal, nutrition and management data from each farm for each year were combined in a single workbook for each farm. The workbooks were imported into Access to create one large database. Data checks and corrections were carried out at each stage. Queries were written to extract information as required for analysis. A single spreadsheet containing all the required data from all farms and both years was produced. All ewes were kept in the dataset regardless of missing data, which may have occurred due to incomplete examinations, incomplete records sent by the farmer and / or ewes being present at one exam but not at another, perhaps due to death or sale.

Minitab 17 (Minitab Inc. 2013) was used for preliminary data analysis. Where a category contained small numbers of observations it was grouped with other categories for analysis. Body condition score was grouped as < 3, 3 and > 3 when used as an explanatory variable but was analysed as a continuous outcome variable. Teat angle score, udder drop score and degree of separation of udder halves score were analysed as continuous outcome variables and were occasionally classed as continuous explanatory variables if appropriate (where they showed a linear association with the outcome variable). Teat position score was analysed as a binary outcome, categories 1 - 2 being grouped as forward pointing and 3 - 5 as non-forward pointing. Due to low numbers of observations in each mass size category and udder half, masses in the udder half were re-categorised as masses in the whole udder: "no" and "yes"; and analysed as a binary outcome. Two further reasons for this re-categorisation were: results from the IRR study found good agreement on the presence/absence of masses but less good agreement on size of mass and determining which udder half a mass was in was not always straightforward as masses were often present in the centre portion of the udder.

The following were investigated in mixed effect multivariable models: factors associated with the presence of an IMM (intramammary mass) in pregnancy and during lactation, acute mastitis, traumatic teat lesions, non-traumatic teat lesions, litter daily live weight gain, BCS, and factors associated with each udder conformation variable. Longitudinal analyses used variables that had been recorded earlier in time or measured at the same time as the outcome.

Seven three-level continuous outcome models were used to explore the factors associated with BCS, lamb DLWG, teat angle, udder drop, degree of separation of udder halves, udder width and teat length. These models took the form:

$$y_{ijk} = \beta_0 + \beta x_k + \beta x_{jk} + \beta x_{ijk} + v_k + u_{jk} + e_{ijk}$$

where y is the continuous outcome variable, β_0 is the intercept, and βx is a series of vectors of fixed effects that vary at k (flock), j (sheep) and i (observation) [in the case of lamb DLWG; i (lamb)] with variance estimates at $v_{k,}$, u_{jk} and e_{ijk}.

One two-level continuous outcome model was used to explore the factors associated with BCS in pregnant ewes in year 2. This model took the form:

$$y_{ij} = \beta_0 + \beta x_j + \beta x_{ij} + u_j + e_{ij}$$

where y is the continuous outcome variable, β_0 is the intercept, and βx is a series of vectors of fixed effects that vary at j (sheep) and i (observation), with variance estimates at u_j and e_{ij}.

Five three-level binary logistic models were used to explore the factors associated with teat position, non-traumatic teat lesions, traumatic teat lesions, IMM in lactating ewes and acute mastitis. These models took the form:

$$\text{Logit}(\pi_{ijk}) = \beta_0 + \beta x_k + \beta x_{jk} + \beta x_{ijk} + v_k + u_{jk}$$

where Logit(π_{ijk}) is the log odds of the probability that teat position is forward-pointing, presence of teat lesions, IMM or acute mastitis; β_0 is the constant, βx is a series of vectors of fixed effects that vary k (flock), j (sheep) and i (observation), with variance estimates at $v_{k,}$ and u_{jk}.

One two-level binary logistic model was used to explore the factors associated with IMM in pregnant ewes in year 2. This model took the form:

$$\text{Logit}(\pi_{ij}) = \beta_0 + \beta x_j + \beta x_{ij} + u_j$$

where Logit(π_{ij}) is the log odds of the probability that IMM are present, β_0 is the constant, βx is a series of vectors of fixed effects that vary j (flock) and i (sheep), with variance estimates at u_j.

All models were run in MLwiN version 2.31 (Rasbash et al., 2014) with iterative generalized squares for sample estimation. Forward manual stepwise model building was used to identify the variables that had a significant association ($P < 0.05$) with the outcome variable. All non-significant variables were retested (Cox and Wermuth 1999). Where two highly correlated variables were individually significant in the model the most biologically plausible variable was retained. Flock was tested as a fixed effect in each model in place of breed (most flocks contained 1 breed only) and was retained in cases where it altered the significance of other variables or was more informative than breed.

Latent class analysis (LCA) was conducted to determine the number and composition of subgroups of mammary gland types, using MPlus Editor 7. Six variables relating to mammary gland conformation were used to identify subgroups: teat position score (grouped as 1 – 2, 3, 4 - 5), teat angle score (grouped as 4 and below, 5, 6 and above) udder drop score (grouped as 6 and below, 7, 8 - 9), degree of separation of udder halves score (grouped as 1 - 2, 3, 4, 5 and above), udder width (grouped as 12cm and below, 13 - 14cm, 15cm and above) and teat length (grouped as 2cm and below, 2.5cm, 3cm and above). Latent class models ranging from two-classes to four-classes were obtained using default settings. To ensure that the final solution for each model had converged on the global maximum solution, models were estimated with increasing sets of random start values. When the log likelihood value for each model was replicated several times there was confidence that the solutions obtained were not those of local maxima. Goodness-of-fit statistics (AIC, BIC, Entropy, LMR, BLRT) combined with intuitive reasoning were used to select the final latent class model. Upon reaching a final solution, individuals were allocated to a class using the posterior probabilities.

RESULTS

Summary statistics

In total, 15 farmers were contacted and 11 agreed to take part in the study and these 11 were visited in year 1. Data from 9 of these were included in the final dataset. Two farms had incomplete and inconsistent data and were not visited in year 2. Two further farms declined to participate in year 2 and a fifth agreed to provide data on BCS, lambing details, diet, acute mastitis cases but requested that the researchers not visit the farm in year 2 because of unrelated issues. A further 2 farms volunteered to participate in year 2 but only lactating ewes were examined. One of these farms had sufficient data to be included in the analysis. Data from 10 farms were included in the final dataset. These farms were located throughout Great Britain, and included both pedigree and commercial flocks and indoor and outdoor lambing. Details of these 10 farms are presented in Table 1.

Table 1. Study farm details.

Farm - Location	**Main Breed**	**Lambing**		**No. ewes Yr. 1**	**No. ewes Yr. 2**	**No. ewes present Yr. 1 & 2**
		Month	In / Out			
Cheshire	Charollais	Dec	I	145	155	75
Shropshire	Charollais	Dec/Jan	I	60	56	37
Powys	Charollais	Dec	I	74	93	44
Herefordshire	Texel	Feb/Mar	I	116	89	72
Gwynedd	Texel	Mar/Apr	I	165	**NV**	*NA*
Gloucestershire	Texel	Feb	I	87	**NV**	*NA*
Devon	Texel	Feb/Mar	**I**	**NV**	34	*NA*
Northumberland	Lleyn	Apr/May	O*	1522	1509	1151
Perth and Kinross	Lleyn	Apr/May	O*	321	322	225
West Sussex	Crossbreeds / Lleyn	Mar/Apr	I	1160	**NV** (1113)	(689)
Total number of ewes				3650	3371	2293 (1307 **PFE**)

I: Indoor lambing; O*: Outdoor lambing /small number lambed indoors; NV: Not visited; PFE: Present at all four exams.

Data were collected on 3650 ewes in year 1 and 3371 in year 2 giving a total of 7021 examinations and 4721 individual ewes over the two years. A total of 1604 ewes were present over the two years of the study, and 1307 of those were examined at all four visits. Summary statistics are presented in Tables 2 and 3.

Table 2. Summary statistics for categorical explanatory variables.

Variable	Category	% of observations	Number	Total observations
Intramammary mass at examination in pregnancy	No	95.26	5526	5801
	Yes	4.74	275	
Intramammary mass at examination in lactation	No	89.01	4537	5093
	Yes	10.92	556	
Intramammary mass at examination in lactation the previous year	No	95.57	1533	1604
	Yes	4.43	71	
Acute mastitis year 1	No	97.01	3541	3650
	Yes	2.99	109	
Acute mastitis year 2	No	97.86	3299	3371
	Yes	2.14	72	
Breed	Lleyn	64.30	4437	6901
	Crossbreeds	21.65	1494	
	Charollais	7.43	513	
	Texel	6.62	457	
Body condition score at examination in pregnancy	3	38.00	2606	6857
	Below 3	37.67	2583	
	Above 3	24.33	1668	
Body condition score at examination in lactation	3	25.58	1539	6017
	Below 3	54.28	3266	
	Above 3	20.14	1212	
Woolly udder	No	93.92	4838	5151
	Yes	6.47	313	
Teat position	1 - 2	29.58	1488	5030
	3	51.49	2590	
	4 - 5	18.93	952	
Teat angle	1 - 3	3.67	184	5017
	4	28.80	1445	
	5	40.94	2054	
	6	19.85	996	
	7 - 9	6.74	338	
Udder drop	2 - 5	2.97	149	5018
	6	15.88	797	
	7	65.56	3290	
	8 - 9	15.58	782	
Degree of separation of udder halves	1	7.08	344	4860
	2	17.55	853	

	3	27.04	1314	
	4	21.79	1059	
	5	17.65	858	
	6 - 8	8.89	432	
Non-traumatic teat lesions	None	94.18	4788	5084
	At least 1 teat	5.82	296	
Traumatic teat lesions	None	96.07	4884	5084
	At least 1 teat	3.93	200	
Number of lambs rearing	1	40.18	2370	5898
	≥ 2	59.82	3528	
Dietary energy during pregnancy	OK	94.03	5923	6299
	Overfed	4.54	286	
	Underfed	1.43	90	
Dietary protein during pregnancy	OK	94.62	5960	6299
	Overfed	3.48	219	
	Underfed	1.91	120	
Dietary energy during lactation	OK	51.55	3130	6072
	Overfed	1.35	83	
	Underfed	47.08	2859	
Dietary protein during lactation	OK	79.76	4843	6072
	Overfed	0.51	31	
	Underfed	19.73	1198	
Age at lambing in years	1	7.85	437	5570
	2	25.73	1433	
	3	25.46	1418	
	4	16.59	924	
	5 - 7	23.48	1308	
	> 7	0.90	50	
Number of lambs detected during pregnancy scanning	0	2.31	144	6232
	1	35.62	2220	
	2	54.85	3418	
	≥ 3	7.22	450	
Ewes categorised by latent class analysis of udder variables	1	23.76	1195	5029
	2	34.24	1722	
	3	15.51	780	
	4	26.49	1332	

Table 3. Continuous variables considered in analysis.

Variable	Minimum	Maximum	Mean	SD	N
Udder width (cm)	5.00	26.00	13.14	2.01	5009
Teat length (cm)	1.00	6.00	2.39	0.48	5049
Litter birth weight (kg)	1.00	23.90	6.24	2.37	5250
Litter daily live weight gain (kg)	0.05	1.34	0.52	0.17	5111
Days in milk (at examination in lactation)	4.00	124.00	69.79	17.31	4722
Lamb age when weighed (days)	14.00	96.00	60.98	11.19	5649
Flock % of intramammary masses in pregnancy	1.40	17.60	4.47	2.87	5908
Flock % of intramammary masses in lactation	4.40	41.20	9.42	5.59	5908
Flock number of intramammary masses in pregnancy	3.00	81.00	37.31	27.27	5908
Flock number of intramammary masses in lactation	7.00	164.00	82.35	53.33	5908

Intramammary masses (IMM) and acute mastitis (AM) (Tables 4, 5 6 & 7)

Approximately 5% of pregnant ewes and 11% of lactating ewes had at least one IMM over the course of the study. Table 4 presents IMM in pregnant and lactating ewes by breed and year.

Table 4. Intramammary masses in pregnant and lactating ewes by breed and year.

IMM	All sheep*		Charollais		Texel		Lleyn		Cross-breeds	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Year 1										
IMM pregnancy	147	*4.1*	37	*15.5*	35	*10.5*	46	*2.1*	28	*3.8*
IMM lactation	271	*8.7*	56	*25.8*	38	*16.0*	110	*5.6*	63	*9.7*
Year 2										
IMM pregnancy	128	*5.7*	14	*5.4*	10	*9.4*	103	*5.6*	1	*2.7*
IMM lactation	285	*14.3*	46	*19.4*	30	*33.0*	206	*12.6*	3	*8.6*

% is % of total sheep and of each breed.

Acute mastitis affected 2 - 3% of ewes each year. There were 109 ewes with acute mastitis in year 1. Forty-four of these had an IMM lactation in year 1 and 22 of these IMM were bramley-sized. There were 72 ewes with acute mastitis in year 2. Thirty-three of these had an IMM in lactation in year 2 and 22 of these IMM were bramley-sized. Forty-three of the 109 AM cases in year 1 were from one pedigree flock in Herefordshire. Table 5 presents IMM in pregnancy and lactation and acute mastitis cases each year by farm.

1294 of the 1307 ewes present at all four exams had a full set of IMM observations. These are presented in Table 6.

Table 5. Intramammary masses in pregnancy and lactation and acute mastitis by farm.

Farm	Pregnancy IMM				Lactation IMM				Acute mastitis			
	Year 1		Year 2		Year 1		Year 2		Year 1		Year 2	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Cheshire	17	11.6	8	6.3	34	12.5	21	7.4	3	2.8	13	18.1
Shropshire	4	2.7	4	3.1	11	4.1	9	3.2	3	2.8	1	1.4
Powys	10	6.8	3	2.3	22	8.1	19	6.7	6	5.5	8	11.1
Herefordshire	11	7.5	4	3.1	18	6.6	18	6.3	43	39.4	11	15.3
Gwynedd	16	10.9	NA	NA	13	4.8	NA	NA	9	8.3	NA	NA
Gloucestershire	8	5.4	NA	NA	7	2.6	NA	NA	0	0	NA	NA
Northumberland	22	15.0	81	63.3	71	26.2	164	57.5	15	13.8	18	25.0
Perth and Kinross	11	7.5	21	16.4	14	5.2	40	14.0	5	4.6	5	6.9
West Sussex	38	25.9	NA	NA	81	29.9	NA	NA	25	22.9	14	19.4
Devon	NA	NA	6	4.7	NA	NA	14	4.9	NA	NA	2	2.8
Total	**147**		**128**		**271**		**285**		**109**		**72**	

IMM: Intra-mammary mass. % is % of total IMM or acute mastitis.

Table 6. Intramammary masses (IMM) in pregnancy and lactation in 1294 ewes over 2 years.

Pregnancy Year 1	Lactation Year 1	Pregnancy Year 2	Lactation Year 2
No IMM: 1255 97.0%	No IMM:1202 95.8% (12 AM)	No IMM: 1139 (94.8%)	No IMM: 1001 (87.9%)
			Yes IMM: 138 (12.1%) (7 AM)
		Yes IMM: 63 (5.2%)	No IMM: 42 (66.7%) (1 AM)
			Yes IMM: 21 (33.3%) (1 AM)
	Yes IMM:53 4.2% (9 AM)	No IMM: 44 (83.0%)	No IMM: 28 (63.6%) (3 AM)
			Yes IMM: 16 (36.4%) (1 AM)
		Yes IMM: 9 (17.0%)	No IMM: 1 (11.1%)
			Yes IMM: 8 (88.9%) (1 AM)
Yes IMM: 39 3.0%	No IMM:28 (71.8%) 2 AM	No IMM: 21 (75.0%)	No IMM: 16 (76.2%)
			Yes IMM: 5 (23.8%) (2 AM)
		Yes IMM: 7 (25.0%)	No IMM: 5 (71.4%)
			Yes IMM: 2 (28.6%)
	Yes IMM:11 (28.2%) 2 AM	No IMM: 10 (90.9%)	No IMM: 3 (30%)
			Yes IMM: 7 (70%)
		Yes IMM: 1 (9.1%)	No IMM: 0
			Yes IMM: 1 (100%) (1 AM)

AM: Acute mastitis.

Table 7 tracks the presence and absence of IMM in the 3566 ewes present at the pregnancy examination year 1. It shows how ewes were removed or absent from flocks at various stages and how some IMM can be detected at one exam and absent at the next.

Culls, deaths and sales associated with mastitis (Table 8)

Data on culls, deaths and sales of ewes due to mastitis and udder damage on 3 study farms is presented in Table 8. Data was not received from other farms.

Table 8. Number and percent of ewes culled / sold or dead due to mastitis or udder damage.

Farm	Year 1				Year 2			
	Culls / Sales		Deaths		Culls / Sales		Deaths	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Cheshire	16	11.0			5	3.2		
Herefordshire	16	13.8	4	3.4	7	7.8	3	3.4
Northumberland	11	0.7	2	0.1	1	0.1	1	0.1

% is % of ewes on that farm.

Table 7. Intramammary masses (IMM) in pregnancy and lactation in 3566 ewes over 2 years.

Pregnancy Year 1	**Lactation Year 1**	**Pregnancy Year 2**	**Lactation Year 2**
No IMM: 3419 95.9%	No IMM: 2679 78.4% (35 AM / 1.0%)	No IMM: 1271 / 47.4%	No IMM: 1001 / 78.8% (0 AM)
			Yes IMM: 138 / 10.9% (7 AM)
			R / A: 132 / 10.4% (4 AM)
		Yes IMM: 71 / 2.7%	No IMM: 42 / 59.2% (1 AM)
			Yes IMM: 21 / 29.6% (1 AM)
			R / A: 8 / 11.3% (2 AM)
		Ewe removed / absent: 1337 / 49.9% (991 NV)	(5 AM)
	Yes IMM: 214 6.3% (37 AM / 1.1%)	No IMM: 45 / 21.0%	No IMM: 28 / 62.2% (3 AM)
			Yes IMM: 16 / 35.6% (1 AM)
			R / A: 1 / 2.2%
		Yes IMM: 10 / 4.7%	No IMM: 1 / 10.0% (0 AM)
			Yes IMM: 8 / 80% (1 AM)
			R / A: 1 / 10.0% (1 AM)
		Ewe removed / absent: 159 / 74.3% (80 NV)	
	Ewe removed / absent: 526 15.4% (18 AM / 0.5%)	No IMM: 128 / 24.3%	No IMM: 92 / 71.9% (1 AM)
			Yes IMM: 16 / 12.5% (4 AM)
			R / A: 20 / 15.6% (4 AM)
		Yes IMM: 7 / 1.3%	No IMM: 4 / 57.1% (0 AM)
			Yes IMM: 0
			R / A: 3 / 42.9% (0 AM)
		Ewe removed / absent: 391 / 74.3% (232 NV)	No IMM: 1 / 0.3% (0 AM)
			Yes IMM: 1 / 0.3% (0 AM)
			R / A: 389 / 99.4% (1 AM)
Yes IMM: 147 4.1%	No IMM: 79 53.7% (8 AM / 5.4%)	No IMM: 23 / 29.1%	No IMM: 16 / 69.6% (0 AM)
			Yes IMM: 5 / 21.7% (2 AM)
			R / A: 2 / 8.7% (0 AM)
		Yes IMM: 7 / 8.9%	No IMM: 5 / 71.4% (0 AM)
			Yes IMM: 2 / 28.6% (0 AM)
			R / A: 0
		Ewe removed / absent: 49 / 62.0% (33 NV)	
	Yes IMM: 46 31.3% (6 AM / 4.1%)	No IMM: 10 / 21.7%	No IMM: 3 / 30.0% (0 AM)
			Yes IMM: 7 / 70.0% (0 AM)
			R / A: 0
		Yes IMM: 2 / 4.3%	No IMM: 0
			Yes IMM: 1 / 50.0% (1 AM)
			R / A: 1 / 50.0% (0 AM)
		Ewe removed / absent: 34 / 73.9% (15 NV)	
	Ewe removed / absent: 22 15.0% (2 AM / 1.4%)	No IMM: 1 / 4.5%	No IMM: 0
			Yes IMM: 1 / 100% (0 AM)
			R / A: 0
		Yes IMM: 0	
		Ewe removed / absent: 21 / 95.5% (14 NV)	

AM: Acute mastitis; NV: Not visited – these ewes are from farms not visited year 2; R / A: Removed / absent – these ewes were absent from the examination, probably removed from the flock.

Teat lesions (Table 9)

There were 201 ewes with non-traumatic teat lesions in year 1 (6.5%) and 95 (4.8%) in year 2. Traumatic teat lesions were slightly less common, 107 ewes were found with them in year 1 and 93 in year 2. Teat lesions by year and breed are shown in Table 9.

Table 9. Teat lesions in lactating ewes by breed and year.

Teat lesions	**All sheep**		**Charollais**		**Texel**		**Lleyn**		**Cross-breeds**	
	Count	***%***	***Count***	***%***	***Count***	***%***	***Count***	***%***	***Count***	***%***
Year 1										
Non-traumatic	201	*6.5*	14	*6.5*	14	*5.9*	97	*5.0*	70	*10.8*
Traumatic	107	*3.5*	21	*9.7*	6	*2.5*	70	*3.6*	9	*1.4*
Year 2										
Non-traumatic	95	*4.8*	27	*11.4*	21	*23.1*	46	*2.8*	1	*3.0*
Traumatic	93	*4.7*	20	*8.4*	10	*11.0*	63	*3.9*	0	*0*

Udder conformation variables (Tables 2 & 3)

Teat position

There were 51.5% of observations with teat position score 3. Very few observations were at extreme teat positions (28 were teat position score 1 and 6 were teat position score 5). Of the ewes that were present at both examinations in lactation, 50.3% received the same teat position score in both examinations while 45.6% differed by 1 score.

Teat angle

There were 40.9% of observations with teat angle score 5. There were 4 teat angle scores of 1, 14 of 2, 38 of 8 and 2 of 9 recorded. Of the ewes that were present at both examinations in lactation, 38.6% received the same teat angle score in both examinations, 43.7% differed by 1 score and 11.6% differed by 2 scores.

Udder drop

Udder drop score 7 accounted for 65.6% of the observations. There were 0 udder drop scores of 1 recorded, 1 of 2, 7 of 3, 27 of 4 and 18 of 9 recorded. Of the ewes that were present at both examinations in lactation, 59.2% received the same udder drop score in both examinations while 39.0% differed by 1 score.

Degree of separation of udder halves

Degree of separation score 3 and 4 accounted for 27.0% and 21.8% of the observations respectively. There were 0 degree of separation scores of 9 recorded, 4 of 8 and 61 of 7. Of the ewes that were present at both examinations in lactation, 29.9% received the same degree of separation score in both examinations, 42.8% differed by 1 score and 21.2% differed by 2 scores.

Teat length and udder width

The minimum and maximum teat lengths recorded were 1cm (2 Texels and 6 Lleyns) and 6cm (1 Lleyn). The minimum and maximum udder widths recorded were 5cm (1 Texel) and 26cm (1 Crossbred).

Latent class analysis (LCA) of udder conformation variables

Data from 5029 ewes were included in the LCA. Based on the goodness-of-fit statistics (data not shown) the four class model was chosen as the model that best represented the data. As there were some repeated measures in this dataset (ewes that were present at the post-lambing exam in year 1 and year 2) the model was run again with 1 set of measures for each ewe only giving a total of 3744. Almost identical patterns were found.

23.8% (1195) of ewes were in LC1. In LC1, ewes are most likely to have teat position score 1 & 2, followed closely by 3; teat angle score 4 or less; udder drop score 6 or less and degree of separation score 5 or more. Udder widths of 15cm and above and teat length of 3cm and above are the most likely. The average age of ewes in LC1 was 5 years and average days in milk was 65.

34.2% (1722) of ewes were in LC2. In LC2, ewes are most likely to have teat position score 3, teat angle score 5, udder drop score 7, followed closely by 8 & 9 and degree of separation score 5, with a slightly smaller peak in degree of separation score 2 & 1. Udder widths of 12cm and below and teat lengths of 2cm and below are most common. The average age of ewes in LC2 was 3 years and average days in milk was 71.

15.5% (780) of ewes were in LC3. In LC3, ewes are most likely to have teat position score 3, followed closely by 4; teat angle score 4 or less; udder drop score 7 and degree of separation score 3 and below. Udder widths of 13 – 14cm and teat lengths of 2.5cm are most common. The average age of ewes in LC3 was 3.4 years and average days in milk was 75.

26.5% (1332) of ewes were in LC4. In LC4, ewes were most likely to have teat position score 3, teat angle score 5, udder drop score 7, degree of separation score 5, udder width 13-14cm and teat length 2cm and below. The average age of ewes in LC4 was 3.5 years and average days in milk was 69.

Body condition score (BCS) (Table 10)

The percent of ewes at each BCS by breed and year in pregnancy and lactation is presented in Table 10. BCS tended to be lower in lactating than pregnant ewes. Charollais ewes tended to have higher BCS both in pregnancy and lactation than other breeds, while cross-breeds tended to have lower BCS.

Table 10. Percent of ewes at each body condition score (BCS) by breed and year.

BCS	**All sheep**		**Charollais**		**Texel**		**Lleyn**		**Cross-breeds**	
	1	2	1	2	1	2	1	2	1	2
Pregnant ewes										
1	0.5	0.4	0	0	2.2	0	0.3	0.6	0.5	0
1.5	3.1	0.9	0.4	0	2.9	0	1.9	1.3	7.4	0.3
2	17.4	4.3	6.3	0.8	16.0	10.3	12.0	4.2	36.9	4.9
2.5	36.2	11.1	16.3	3.5	29.5	20.8	38.6	11.9	38.0	10.5
3	30.9	45.7	21.8	8.1	35.3	35.9	37.3	44.6	13.5	63.7
3.5	7.9	23.5	19.7	23.2	9.0	23.6	8.2	25.8	3.0	16.5
4	3.1	11.5	22.6	37.5	4.5	9.4	1.5	11.0	0.8	3.9
4.5	1.0	2.6	13.0	27.0	0.6	0	0.2	0.7	0	0.3
N	*3562*	*3295*	*239*	*259*	*312*	*106*	*2221*	*2177*	*743*	*717*
Lactating ewes										
1	1.4	5.1	0.5	0	0	3.3	1.4	7.3	2.4	0.5
1.5	6.9	7.6	4.6	0.8	3.8	8.8	6.3	10.0	10.6	2.8
2	20.7	23.5	14.7	2.5	15.0	22.0	19.3	23.0	28.2	32.3
2.5	24.6	18.9	17.1	7.2	34.6	19.8	22.8	18.7	28.3	23.3
3	27.0	24.0	28.1	21.1	32.1	23.1	27.5	21.1	23.2	34.8
3.5	11.6	8.6	12.0	24.9	9.0	16.5	14.1	7.6	5.2	4.5
4	6.7	8.2	18.4	24.5	4.7	6.6	7.4	8.6	2.0	1.0
4.5	1.1	4.1	4.6	19.0	0.9	0	1.2	3.7	0.2	0.7
N	*3147*	*2870*	*217*	*237*	*234*	*91*	*1982*	*1910*	*660*	*600*

1: Year 1; 2: Year 2; *N*: Number.

Nutrition

Dietary energy and protein in pregnancy and lactation by farm and year are presented in Table 11. Most ewes were fed appropriately (OK) for energy and protein during pregnancy in year 1 except the Herefordshire flock, where most ewes were underfed for both; and the Cheshire flock, where single bearing ewes were underfed while twin and triplet bearing ewes were overfed. During lactation in year 1 most ewes were underfed energy and protein.

In year 2, most ewes were OK for energy and protein during pregnancy except the Cheshire flock where all ewes were overfed both. There was again more underfeeding in lactation but slightly less than in year 1. Full details are contained in the ADAS report (Appendix 1). Dietary energy and protein tended to be correlated, especially during pregnancy, with ewes either receiving sufficient in both or neither. The results of the blood sample biochemistry in year 2 were largely unremarkable (Appendix 3).

Table 11. Dietary energy and protein in pregnancy and lactation by farm and year.

Farm	P Energy			P Protein			L Energy			L Protein		
	UnF	OK	OvF	UnF	OK	OvF	UnF	OK	OvF	UnF	OK	OvF
Year 1												
Cheshire	35	0	70	35	0	70	103	0	0	48	55	0
Shropshire	7	49	0	0	56	0	34	0	21	34	21	0
Powys	0	39	0	0	39	0	36	31	0	67	0	0
Herefordshire	48	37	0	85	0	0	48	27	0	75	0	0
Gwynedd	0	27	67	0	94	0	27	67	0	27	67	0
Gloucestershire	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Northumberland	0	1493	0	0	1493	0	695	685	0	31	1349	0
Perth and Kinross	0	279	0	0	279	0	171	105	0	171	105	0
West Sussex	0	870	0	0	870	0	548	338	0	548	338	0
% of total ewes	**2.5**	**76.6**	**3.8**	**3.3**	**77.6**	**1.9**	**45.5**	**34.3**	**0.6**	**27.4**	**53.0**	**0**
Year 2												
Cheshire	0	0	149	0	0	149	77	57	0	77	57	0
Shropshire	0	50	0	0	50	0	47	0	0	47	0	0
Powys	0	88	0	0	88	0	0	53	31	53	31	0
Herefordshire	0	76	0	0	76	0	1	38	31	1	38	31
Northumberland	0	1485	0	0	1485	0	861	524	0	19	1366	0
Perth and Kinross	0	317	0	0	317	0	211	92	0	0	303	0
West Sussex	0	1113	0	0	1113	0	0	1113	0	0	1113	0
Devon	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
% of total ewes	**0**	**92.8**	**4.4**	**0**	**92.8**	**4.4**	**35.5**	**55.7**	**1.8**	**5.8**	**86.3**	**0.9**

P: Pregnancy, L: Lactation, UnF: Underfed, Ovf: Overfed.
Note: where scanning data and/or litter size data were unavailable ewes could not be assigned as Unf, OK, or OvF.

Inter-rater reliability of observers scoring udder conformation and body condition

The results of the IRR study on udder conformation and body condition scoring showed moderate to good agreement on all scores. Full results can be found in Appendix 2.

Mixed effect multi-variable models

Factors associated with intramammary masses in pregnant ewes

Data from 1427 ewes were included in the model investigating intramammary masses in pregnant ewes in year 2 (Table 12). Ewes with IMM when lactating in year 1 were 4 times more likely to have IMM when pregnant in year 2 than those without IMM, while ewes with IMM when pregnant year 1 were 4.7 times more likely to have an IMM in year 2. Ewes underfed energy in lactation in year 1 had an almost 3 fold increased risk of IMM when pregnant in year 2. No flock was associated with an increased or decreased risk of IMM in pregnancy.

Table 12. Two-level binary logistic model of factors associated with intramammary masses in 1427 pregnant ewes in year 2.

Variable	**Category**	***N* affected**	**% affected**	**OR**	**Lower 95% CI**	**Upper 95% CI**
Year 1 IMM when lactating	No	80	5.73	Reference		
	Yes	13	18.31	**4.043**	**1.86**	**8.79**
Year 1 IMM when pregnant	No	88	5.73	Reference		
	Yes	9	20.45	**4.688**	**1.85**	**11.87**
Lactation energy year 1	OK	23	3.46	Reference		
	Overfed	1	11.11	5.013	0.21	118.33
	Underfed	71	9.14	**2.691**	**1.63**	**4.45**
Flock	A	10	4.5	Reference		
	B	5	6.8	0.696	0.21	2.37
	C	4	10.8	0.479	0.06	4.12
	D	1	2.3	0.225	0.03	1.97
	E	4	5.6	0.174	0.02	1.55
	F	76	6.6	1.687	0.84	3.38
		Variance	SD			
Random effects	Flock	1.000	1.000			
	Ewe					

N: Number; OR: Odds ratio; CI: Confidence interval; SD: Standard deviation.

Factors associated with intramammary masses in lactating ewes

Data from 3888 exams (3037 ewes) were included in the model investigating intramammary masses in lactating ewes (Table 13). Ewes aged 4 - 7 at lambing were just over 2 times more likely to have an IMM when lactating than ewes aged 1. Those with an IMM when pregnant were almost 4 times more likely to have IMM when lactating than those without an IMM when pregnant, while those with an IMM when lactating the previous year were > 4 times more likely to have an IMM when lactating in year 2 than those without. Ewes that had acute mastitis during lactation were almost 11 times more likely to have an IMM when lactating. Traumatic teat lesions were associated with a higher risk of IMM when lactating. Being underfed protein in lactation was associated with lower risk of an IMM while being overfed protein in lactation and underfed energy in lactation were associated with higher risk. Ewes in ULC 1 had a significantly higher risk of IMM when lactating than ewes in ULC 4. Flock B, C and D were associated with a higher risk of IMM in lactation than flock A. Faster litter daily live weight gain (LDLWG) was associated with decreased risk while as days in milk increased the risk of an IMM increased in the same lactation.

Table 13. Three-level binary logistic model of factors associated with (556) intramammary masses when lactating in 3888 exams (3037 ewes).

Variable	Category	*N* affected	% affected	OR	Lower 95% CI	Upper 95% CI
Age at lambing (yrs.)	1	16	3.66	Reference		
	2	110	7.68	1.385	0.71	2.72
	3	117	8.25	1.728	0.86	3.48
	4	96	10.39	**2.380**	**1.15**	**4.92**
	5 - 7	151	11.54	**2.230**	**1.11**	**4.49**
	> 7	8	16.00	3.062	0.97	9.69
IMM when pregnant	No	456	8.25	Reference		
	Yes	88	32.00	**3.725**	**2.59**	**5.36**
IMM when lactating PY	No	186	12.13	Reference		
	Yes	34	47.89	**4.595**	**2.45**	**8.62**
Acute mastitis	No	479	7.00	Reference		
	Yes	77	42.54	**10.913**	**6.33**	**18.82**
Traumatic teat lesions	None	508	10.40	Reference		
	At least 1 teat	48	24.00	**2.153**	**1.37**	**3.38**
Lactation protein	OK	332	6.86	Reference		
	Overfed	9	29.03	**7.933**	**1.58**	**39.73**
	Underfed	139	11.60	**0.445**	**0.28**	**0.70**
Lactation energy	OK	161	5.14	Reference		
	Overfed	20	24.10	0.404	0.14	1.14
	Underfed	299	10.46	**1.933**	**1.37**	**2.72**
Udder latent class	4	114	8.56	Reference		
	1	180	15.06	**1.709**	**1.23**	**2.35**
	2	181	10.51	1.107	0.80	1.54
	3	66	8.46	0.945	0.62	1.43
Flock	A	54	8.8	Reference		
	B	55	20.8	**2.939**	**1.67**	**5.19**
	C	20	20.0	**4.121**	**1.81**	**9.40**
	D	41	26.8	**8.191**	**3.87**	**17.32**
	E	36	25.4	1.363	0.58	3.21
	F	235	8.9	0.998	0.66	1.50
	G	13	11.1	1.357	0.59	3.12
	H	7	12.7	0.946	0.24	3.74
	I	81	8.2	1.298	0.76	2.23
		Overall mean	**Affected mean**			
Litter DLWG (kg)		0.52	0.51	**0.157**	**0.07**	**0.37**
Days in milk		69.79	68.75	**1.015**	**1.01**	**1.03**
		Variance	SD			
Random effects	Flock	1.000	1.000			
	Ewe	1.088	1.226			
	Year					

N: Number; OR: Odds ratio; CI: Confidence interval; IMM: Intramammary mass; PY: Previous year; DLWG: Daily live weight gain; SD: Standard deviation.

Factors associated with acute mastitis in lactating ewes

Data from 3847 exams (3019 ewes) were included in the model investigating acute mastitis (Table 14). Ewes aged >7 at lambing had a higher risk of acute mastitis than ewes aged 1 at lambing. Ewes rearing 2 or more lambs had a higher risk of acute mastitis than those rearing 1 lamb. Charollais and Texel ewes both had a higher risk than Lleyn ewes. Ewes with teat angle score 4 or below were 4 times more likely to have acute mastitis than ewes with teat angle score 5. Ewes with teat position score 1 or 2 were >2 times more likely to have acute mastitis than those with teat position 3. Non-traumatic teat lesions were associated with increased risk of acute mastitis, as were IMM during lactation the previous year and IMM when pregnant, although the latter two were not significant associations. Being underfed protein in pregnancy was associated with an increased risk of acute mastitis. Higher LDLWG was associated with a decreased risk of acute mastitis.

Factors associated with lamb daily live weight gain

Data from 6453 lambs were included in the model investigating lamb daily live weight gain (DLWG) (kg) (Table 15). Female lambs had slower DLWG than male lambs and lambs that were reared as twins or triplets had slower DLWG than lambs that were reared as singles. Crossbred, Charollais and Texel lambs had higher DLWG than Lleyns. The lambs reared by ewes that had acute mastitis during the lactation had lower DLWG, as did the lambs reared by ewes with IMM in lactation, traumatic teat lesions or non-traumatic teat lesions. The lambs reared by ewes with BCS below 3 in pregnancy had lower DLWG while those reared by ewes with BCS above 3 in pregnancy had greater DLWG than lambs reared by ewes with BCS 3 in pregnancy. The lambs reared by ewes with BCS below 3 in lactation had greater DLWG than lambs reared by ewes with BCS 3 in lactation. Lambs reared by ewes overfed and underfed protein in pregnancy had lower DLWG than lambs reared by ewes fed OK levels of protein in pregnancy. Lambs reared by ewes overfed protein in lactation had greater DLWG than lambs reared by ewes fed OK levels of protein in lactation, while lambs reared by ewes underfed protein in lactation had lower DLWG. Lambs reared by ewes overfed and underfed energy in lactation had lower DLWG than lambs reared by ewes fed OK levels of energy in lactation. Lambs reared by ewes with teat positions 4 - 5 and udder drop 8 had lower DLWG than lambs reared by ewes with teat position 3 and udder drop 7. Lambs reared by ewes with wider udders and longer teats had greater DLWG. Lambs with higher birth weights had lower DLWG. The association between lamb age when weighed and DLWG was non-linear following a typical growth curve. The lambs of older ewes at lambing had greater DLWG. There was significant variation at flock, ewe and lamb level.

Table 14. Three-level binary logistic model of factors associated with acute mastitis (181 cases) in 3847 exams (3019 ewes).

Variable	Category	*N* affected	% affected	OR	Lower 95% CI	Upper 95% CI
Age at lambing (yrs.)	1	6	1.37	Reference		
	2	63	4.40	4.191	0.71	24.60
	3	36	2.54	1.714	0.28	10.67
	4	23	2.49	3.515	0.55	22.40
	5 - 7	34	2.60	3.074	0.50	18.84
	> 7	3	6.00	**13.001**	**1.24**	**136.33**
Number of lambs rearing	1	59	2.49	Reference		
	2 or more	112	3.17	**2.654**	**1.49**	**4.72**
Breed	Lleyn	62	1.40	Reference		
	Crossbreeds	26	1.74	1.093	0.34	3.50
	Charollais	31	6.04	**6.673**	**2.20**	**20.23**
	Texel	62	13.60	**18.746**	**6.04**	**58.20**
Teat angle	5	30	1.46	Reference		
	7 - 9	13	3.85	1.179	0.49	2.86
	6	34	3.41	1.759	0.89	3.47
	4	38	2.63	**3.991**	**2.05**	**7.79**
	3 - 1	9	4.89	**4.683**	**1.36**	**16.16**
Teat position	3	47	1.81	Reference		
	1 - 2	61	4.10	**2.542**	**1.51**	**4.28**
	4 - 5	17	1.79	0.821	0.40	1.69
Non-traumatic teat lesions	None	111	2.32	Reference		
	At least 1 teat	21	7.09	**2.088**	**1.07**	**4.09**
IMM when pregnant	No	138	2.50	Reference		
	Yes	26	9.49	1.822	0.90	3.70
IMM when lactating PY	No	27	1.76	Reference		
	Yes	8	11.27	3.155	0.82	12.15
Pregnancy protein	OK	115	1.93	Reference		
	Overfed	16	7.31	2.646	0.82	8.58
	Underfed	43	35.83	**4.047**	**1.44**	**11.35**
		Overall mean	**Affected mean**			
Litter DLWG (kg)		0.52	0.43	**0.032**	**0.01**	**0.18**
		Variance	SD			
Random effects	Flock	1.217	1.175			
	Ewe	1.000	1.000			
	Year					

N: Number; OR: Odds ratio; CI: Confidence interval; PY: Previous year; DLWG: Daily live weight gain; SD: Standard deviation.

Table 15. Three-level continuous outcome model of factors associated with lamb daily live weight gain (kg) in 6453 lambs.

Variable	**Category**	***N***	**Mean**	**Coefficient**	**Lower 95% CI**	**Upper 95% CI**
Lamb sex	Male	4356	0.35	Reference		
	Female	4325	0.33	**-0.026**	**-0.03**	**-0.02**
Number of lambs rearing	1	2194	0.38	Reference		
	≥ 2	6254	0.32	**-0.050**	**-0.06**	**-0.04**
Lamb breed	Lleyn	4456	0.32	Reference		
	Crossbreeds	3129	0.36	**0.038**	**0.03**	**0.04**
	Charollais	695	0.33	**0.077**	**0.01**	**0.15**
	Texel	329	0.35	**0.062**	**0.01**	**0.11**
Acute mastitis	No	8466	0.34	Reference		
	Yes	215	0.28	**-0.036**	**-0.05**	**-0.02**
Mass at post-lambing exam	No	6058	0.34	Reference		
	Yes	715	0.33	**-0.012**	**-0.02**	**-0.01**
Traumatic teat lesions	No	6485	0.34	Reference		
	Yes	288	0.31	**-0.019**	**-0.03**	**-0.01**
Non-traumatic teat lesions	No	6350	0.341	Reference		
	Yes	422	0.338	**-0.012**	**-0.020**	**-0.004**
BCS at pre-lambing exam	3	3278	0.34	Reference		
	Below 3	2965	0.35	**-0.006**	**-0.009**	**-0.002**
	Above 3	2205	0.33	**0.006**	**0.002**	**0.010**
BCS at post-lambing exam	3	2023	0.34	Reference		
	Below 3	4633	0.34	**0.007**	**0.003**	**0.011**
	Above 3	1440	0.34	0.002	-0.004	0.008
Pregnancy protein	OK	7935	0.34	Reference		
	Overfed	316	0.32	**-0.120**	**-0.15**	**-0.09**
	Underfed	113	0.29	**-0.100**	**-0.13**	**-0.07**
Lactation protein	OK	6453	0.34	Reference		
	Overfed	24	0.56	**0.121**	**0.08**	**0.16**
	Underfed	1929	0.34	**-0.015**	**-0.02**	**-0.01**
Lactation energy	OK	3329	0.35	Reference		
	Overfed	70	0.43	**-0.031**	**-0.05**	**-0.01**
	Underfed	5007	0.33	**-0.011**	**-0.023**	**-0.001**
Teat position	3	3525	0.34	Reference		
	1 - 2	1987	0.35	0.000	-0.003	0.004
	4 - 5	1216	0.34	**-0.004**	**-0.0792**	**-0.0001**

Udder drop	7	4468	0.34	Reference		
	5	240	0.36	-0.007	-0.017	0.003
	6	1211	0.35	-0.002	-0.006	0.002
	8	794	0.33	**-0.017**	**-0.02**	**-0.01**
Udder width		8681		**0.008**	**0.006**	**0.010**
Teat length		8681		**0.008**	**0.004**	**0.012**
Lamb BW (kg)		8667		**-0.003**	**-0.005**	**-0.001**
Lamb age when when weighed (days)	**^ 1**	8681		**0.032**	**0.01**	**0.05**
	^ 2			**-0.0009**	**-0.0015**	**-0.0004**
	^ 3			**0.00001**	**0.000006**	**0.000018**
	^ 4			0.000	0.00	0.00
Age at lambing (yrs.)		7879		**0.004**	**0.002**	**0.006**
		Variance				
Random effects	Flock	0.00215	0.00103			
	Ewe	0.00139	0.00008			
	Lamb	0.00282	0.00007			

N: Number; CI: Confidence interval; BW: Birth weight; BCS: Body condition score; SD: Standard deviation; (Lamb age when weighed (days) was entered as a quadratic term).

Factors associated with teat position in lactating ewes

Data from 3871 exams (3050 ewes) were included in the model investigating teat position (Table 16). Ewes aged > 7 were less likely to have forward pointing teats than ewes aged 1 at lambing. Those with IMM when lactating were more likely to have forward pointing teats as were those that had acute mastitis during lactation. Charollais and Texel ewes were less likely to have forward pointing teats than Lleyns. Ewes with degree of separation score 1 or 2 were less likely to have forward pointing teats than were ewes with degrees of separation 3. Teat angle scores 7 - 9 were more likely to be associated with forward pointing teats than teat angle 5. Ewes overfed protein in pregnancy were more likely to have forward pointing teats as were ewes overfed energy in lactation while those underfed lactation energy were less likely to have forward pointing teats. Those underfed protein in lactation were more likely to have forward pointing teats than those that were OK for lactation protein. Wider udders and longer teats were associated with a higher likelihood of forward pointing teats while more days in milk were associated with a lower likelihood.

Factors associated with teat angle in lactating ewes

Data from 3826 exams (3006 ewes) were included in the model investigating teat angle score (Table 17). Wider udders, longer teats and higher LDLWG were all associated with lower teat angle scores. Longer days in milk were associated with higher teat angle score. Both non-traumatic and traumatic teat lesions in at least 1 teat were associated with lower teat angle score. The presence of IMM when lactating was associated with higher teat angle score. Acute mastitis during the lactation was associated with lower teat angle scores. Teat position scores 4 - 5 were associated with lower teat angle scores when compared to teat position 3. Higher udder drop scores were associated with lower teat angle scores. BCS < 3 when pregnant was associated with lower teat angle scores while > 3 was associated with higher teat angle scores when both were compared to BCS 3. BCS < 3 when lactating was associated with higher teat angle scores when compared to BCS 3. Charollais and Texel breeds were both associated with higher teat angle scores when compared to Lleyns. Ages 5 - 7 years at lambing were associated with lower teat angle scores when compared to age 1 at lambing. Underfed protein in pregnancy was associated with higher teat angle scores while underfed energy in pregnancy was associated with lower teat angle scores when compared to OK protein and energy in pregnancy. Overfed protein in lactation was associated with higher teat angle scores and underfed protein in lactation was associated with lower teat angle scores when compared to OK protein in lactation. Overfed energy in lactation was associated with lower teat angle scores and underfed energy in lactation was associated with higher teat angle scores when compared to OK energy in lactation.

Factors associated with udder drop in lactating ewes

Data from 3901 exams (3067 ewes) were included in the model investigating udder drop score (Table 18). Wider udders, older age at lambing, more days in milk, heavier LBW, longer teat length and higher teat angle score were all associated with lower udder drop score. Teat position scores 1 - 2 were associated with lower udder drop score while 4 - 5 were associated with higher udder drop score when both were compared to teat position score 3. Crossbreeds were associated with higher udder drop score when compared to Lleyns. The presence of IMM during pregnancy was associated with lower udder drop score, as was the presence of IMM during lactation. Overfed pregnancy protein was associated with lower udder drop score when compared to OK pregnancy protein. Overfed protein in lactation was associated with higher udder drop score and underfed protein in lactation was associated with lower udder drop score when both were compared to OK protein in lactation.

Factors associated with the degree of separation of udder halves in lactating ewes

Data from 4426 exams (3295 ewes) were included in the model investigating degree of separation of udder halves score (Table 19). Wider udders and more days in milk were both associated with higher degree of separation of udder halves score. BCS < 3 when pregnant was associated with lower degree of separation of udder halves score while > 3 was associated with higher degree of separation of udder halves score when both were compared to BCS 3. Crossbreeds were associated with lower degree of separation score when compared to Lleyns. Teat position scores 4 - 5 were associated with lower degree of separation score when compared to teat position score 3. The presence of IMM when lactating was associated with lower degree of separation score. Underfed protein in pregnancy was associated with lower degree of separation score when compared to OK protein in pregnancy.

Factors associated with the udder width of lactating ewes

Data from 3749 exams (2922 ewes) were included in the model investigating udder width (Table 20). More days in milk and higher udder drop and teat angle scores were all associated with narrower udder widths. Faster LDLWG, longer teat length and greater degree of separation of udder halves were all associated with wider udders. Teat position scores 1 - 2 were associated with wider udders while teat position scores 4 - 5 were associated with narrower udders when both were compared to teat position score 3. Crossbreeds were associated with wider udders when compared to Lleyns. All ages above 1 year at lambing were associated with wider udders than age 1 year at lambing. Non-traumatic teat lesions were associated with narrower udders, while traumatic teat lesions were associated with wider udders. Underfed protein in pregnancy was associated with wider udders as was overfed protein in lactation.

Factors associated with the teat length of lactating ewes

Data from 4476 exams (3343 ewes) were included in the model investigating teat length (Table 21). Wider udders and older age at lambing were each associated with longer teats. More days in milk and higher teat angle and udder drop scores were all associated with shorter teats. Teat position scores 1 - 2 were associated with longer teats when compared to teat position score 3. BCS < 3 when pregnant was associated with longer teats whereas BCS > 3 was associated with shorter teats when both were compared to BCS 3 when pregnant. BCS < 3 when lactating was associated with longer teats when compared to BCS 3 when lactating. Crossbreeds were associated with longer teats in comparison to Lleyns. Both non-traumatic teat lesions and traumatic teat lesions were associated with longer teats. The presence of IMM when lactating was associated with shorter teats. Underfed protein in pregnancy was associated with longer teats while underfed protein in lactation was associated with shorter teats when both were compared to OK protein in pregnancy/lactation.

Factors associated with non-traumatic teat lesions in lactating ewes

Data from 4721 exams (3486 ewes) were included in the model investigating non-traumatic teat lesions (Table 22). Ewes rearing 2 or more lambs were 4 times more likely to have non-traumatic teat lesions than ewes rearing 1 lamb. Those with IMM when lactating were more likely to have non-traumatic lesions than those with no IMM when lactating. Crossbreeds were more likely than Lleyns to have non-traumatic lesions. Ewes with teat angle score 4 were almost 3 times more likely to have non-traumatic teat lesions than ewes with teat angle score 5. Those underfed energy in lactation were less likely to have non-traumatic teat lesions while those overfed protein in lactation were more likely to have non-traumatic lesions than those that were OK for both. Longer teats were more likely to have non-traumatic lesions while wider udders were less likely to.

Factors associated with traumatic teat lesions in lactating ewes

Data from 4496 exams (3350 ewes) were included in the model investigating traumatic teat lesions (Table 23). Ewes rearing 2 or more lambs were over 1.5 times more likely to have traumatic teat lesions than ewes rearing 1 lamb. Ewes with IMM when lactating were more likely to have traumatic teat lesions than those without IMM when lactating. Crossbred ewes were less likely while Charollais ewes were more likely than Lleyn ewes to be affected. Ewes with teat angle score 4 were 2.5 times more likely to have traumatic teat lesions than ewes with teat angle score 5. Longer teat lengths were associated with increased risk of traumatic teat lesions, while older age at lambing and more days in milk were associated with decreased risk.

Table 16. Three-level binary logistic model of factors associated with forward pointing teat position in 3871 exams (3050 ewes).

Variable	Category	*N* affected	% affected	OR	Lower 95% CI	Upper 95% CI
Age at lambing (yrs.)	1	89	29.18	Reference		
	2	277	27.18	1.075	0.78	1.47
	3	305	30.20	1.183	0.85	1.64
	4	156	30.23	1.028	0.72	1.48
	5 -7	300	31.75	1.147	0.82	1.61
	> 7	1	2.78	**0.064**	**0.008**	**0.504**
IMM when pregnant	No	1008	28.80	Reference		
	Yes	139	34.58	**1.452**	**1.13**	**1.87**
Acute mastitis	No	1102	28.91	Reference		
	Yes	45	47.87	**2.171**	**1.25**	**3.78**
Breed	Lleyn	843	30.65	Reference		
	Crossbreeds	160	29.68	0.890	0.67	1.18
	Charollais	91	22.98	**0.301**	**0.19**	**0.47**
	Texel	52	24.64	**0.571**	**0.36**	**0.91**
Degree of separation of udder halves	3	318	30.78	Reference		
	6 - 8	122	36.31	1.142	0.86	1.52
	5	191	28.17	0.896	0.71	1.13
	4	275	33.29	1.120	0.91	1.38
	2	127	19.07	**0.569**	**0.44**	**0.73**
	1	49	18.63	**0.579**	**0.40**	**0.83**
Teat angle	5	437	27.28	Reference		
	7 - 9	94	35.47	**1.916**	**1.40**	**2.62**
	6	221	28.63	1.138	0.92	1.40
	4	346	30.65	1.023	0.85	1.24
	3 - 1	49	36.57	0.920	0.60	1.41
Pregnancy protein	OK	1044	29.76	Reference		
	Overfed	45	25.57	**1.779**	**1.05**	**3.03**
	Underfed	27	33.75	1.146	0.60	2.21
Lactation energy	OK	485	32.44	Reference		
	Overfed	10	17.24	**2.790**	**1.10**	**7.05**
	Underfed	624	27.53	**0.464**	**0.38**	**0.56**
Lactation protein	OK	821	29.34	Reference		
	Overfed	0				
	Underfed	298	29.80	**2.305**	**1.78**	**2.99**
		Overall mean	**Affected mean**			
Udder width (cm)		13.14	13.62	**1.236**	**1.18**	**1.30**
Teat length (cm)		2.39	2.43	**1.196**	**1.01**	**1.42**
Days in milk		69.79	69.13	**0.989**	**0.983**	**0.995**
		Variance	SD			
Random effects	Flock	1.000	1.000			
	Ewe	1.313	1.112			
	Year					

N: Number; OR: Odds ratio; CI: Confidence interval; IMM: Intramammary mass; SD: Standard deviation.

Table 17. Three-level mixed effect continuous outcome model of factors associated with teat angle in 3826 exams (3006 ewes).

Variable	**Category**	***N***	**Mean**	**Coefficient**	**Lower 95% CI**	**Upper 95% CI**
Udder width (cm)		5009		**-0.033**	**-0.05**	**-0.02**
Teat length (cm)		5049		**-0.346**	**-0.41**	**-0.29**
Days in milk		4722		**0.006**	**0.004**	**0.008**
Litter DLWG (kg)		5111		**-0.224**	**-0.44**	**-0.01**
Non-traumatic teat lesions	None	4717	5.00	Reference		
	At least 1 teat	296	4.56	**-0.310**	**-0.42**	**-0.20**
Traumatic teat lesions	None	4815	4.98	Reference		
	At least 1 teat	198	4.68	**-0.358**	**-0.50**	**-0.22**
IMM when lactating	No	4475	4.95	Reference		
	Yes	539	5.16	**0.140**	**0.05**	**0.23**
Acute mastitis	No	4893	4.97	Reference		
	Yes	124	5.03	**-0.266**	**-0.47**	**-0.06**
Teat position	3	2577	5.03	Reference		
	1 - 2	1488	4.95	-0.010	-0.07	0.05
	4 - 5	948	4.84	**-0.195**	**-0.27**	**-0.12**
Udder drop		5018		**-0.084**	**-0.13**	**-0.03**
BCS when pregnant	3	1637	5.00	Reference		
	Below 3	2022	4.75	**-0.120**	**-0.19**	**-0.05**
	Above 3	1265	5.28	**0.106**	**0.03**	**0.18**
BCS when lactating	3	1225	4.95	Reference		
	Below 3	2663	4.92	**0.113**	**0.05**	**0.18**
	Above 3	1126	5.13	0.030	-0.05	0.11
Breed	Lleyn	3532	4.95	Reference		
	Crossbreeds	678	4.54	-0.020	-0.15	0.11
	Charollais	450	5.57	**0.961**	**0.63**	**1.29**
	Texel	314	5.32	**0.518**	**0.19**	**0.84**
Age at lambing (yrs.)	1	386	5.02	Reference		
	2	1255	5.04	-0.094	-0.21	0.02
	3	1134	4.96	-0.081	-0.20	0.04
	4	627	5.07	-0.015	-0.15	0.12
	5 - 7	1096	4.81	**-0.167**	**-0.29**	**-0.04**
	> 7	42	5.31	0.131	-0.19	0.45
Pregnancy protein	OK	4256	4.92	Reference		
	Overfed	195	5.56	0.055	-0.43	0.54
	Underfed	109	5.66	**0.924**	**0.48**	**1.37**
Pregnancy energy	OK	4221	4.92	Reference		
	Overfed	254	5.47	-0.179	-0.55	0.19
	Underfed	85	5.56	**-0.573**	**-0.99**	**-0.15**
Lactation protein	OK	3347	4.96	Reference		
	Overfed	28	5.18	**0.579**	**0.08**	**1.08**
	Underfed	1121	4.98	**-0.189**	**-0.29**	**-0.09**

Lactation energy	OK	1789	4.96	Reference		
	Overfed	73	5.10	**-0.621**	**-0.92**	**-0.32**
	Underfed	2634	4.96	**0.171**	**0.09**	**0.25**
		Variance	SD			
Random effects	Flock	0.030	0.017			
	Ewe	0.242	0.023			
	Year	0.466	0.022			

N: Number; CI: Confidence interval; DLWG: Daily live weight gain; IMM: Intramammary mass; BCS: Body condition score; SD: Standard deviation.

Table 18. Three-level mixed effect continuous outcome model of factors associated with udder drop in 3901 exams (3067 ewes).

Variable	Category	*N*	Mean	Coefficient	Lower 95% CI	Upper 95% CI
Udder width (cm)		5009		**-0.116**	**-0.13**	**-0.11**
Age at lambing (yrs.)		5570		**-0.126**	**-0.14**	**-0.11**
Days in milk		4722		**-0.003**	**-0.005**	**-0.001**
Litter BW (kg)		5250		**-0.016**	**-0.03**	**-0.01**
Teat length (cm)		5049		**-0.130**	**-0.17**	**-0.09**
Teat angle		5017		**-0.028**	**-0.05**	**-0.01**
Teat position	3	2580	6.96	Reference		
	1 - 2	1488	6.81	**-0.062**	**-0.10**	**-0.02**
	4 - 5	946	7.06	**0.047**	**0.002**	**0.092**
Breed	Lleyn	3533	6.92	Reference		
	Crossbreeds	679	7.05	**0.092**	**0.01**	**0.17**
	Charollais	450	6.82	-0.091	-0.35	0.17
	Texel	314	7.06	0.002	-0.20	0.20
IMM when pregnant	No	4706	6.95	Reference		
	Yes	222	6.69	**-0.138**	**-0.22**	**-0.06**
IMM when lactating	No	4475	6.96	Reference		
	Yes	540	6.73	**-0.158**	**-0.21**	**-0.10**
Pregnancy protein	OK	4258	6.93	Reference		
	Overfed	195	6.77	**-0.295**	**-0.52**	**-0.07**
	Underfed	109	6.88	-0.029	-0.21	0.15
Lactation protein	OK	3347	6.94	Reference		
	Overfed	28	7.43	**0.341**	**0.09**	**0.59**
	Underfed	1123	6.86	**-0.090**	**-0.14**	**-0.04**
		Variance	SD			
Random effects	Flock	0.025	0.013			
	Ewe	0.076	0.009			
	Year	0.202	0.009			

N: Number; CI: Confidence interval; BW: Birth weight; IMM: Intramammary mass; SD: Standard deviation.

Table 19. Three-level mixed effect continuous outcome model of factors associated with the degree of separation of udder halves in 4426 exams (3295 ewes).

Variable	**Category**	***N***	**Mean**	**Coefficient**	**Lower 95% CI**	**Upper 95% CI**
Udder width (cm)		5009		**0.140**	**0.12**	**0.16**
Days in milk		4722		**0.019**	**0.017**	**0.020**
BCS when pregnant	3	1598	3.67	Reference		
	Below 3	1930	3.32	**-0.234**	**-0.32**	**-0.15**
	Above 3	1243	3.66	**0.209**	**0.11**	**0.30**
Breed	Lleyn	3448	3.73	Reference		
	Crossbreeds	639	2.95	**-0.266**	**-0.45**	**-0.08**
	Charollais	434	2.86	0.078	-0.36	0.52
	Texel	297	3.40	0.280	-0.24	0.80
Teat position	3	2534	3.55	Reference		
	1 - 2	1389	3.73	0.078	-0.004	0.16
	4 - 5	934	3.12	**-0.235**	**-0.33**	**-0.14**
IMM when lactating	No	4345	3.57	Reference		
	Yes	512	3.13	**-0.333**	**-0.45**	**-0.21**
Pregnancy protein	OK	4130	3.59	Reference		
	Overfed	189	3.14	0.140	-0.34	0.62
	Underfed	104	3.15	**-0.405**	**-0.74**	**-0.07**
		Variance	SD			
Random effects	Flock	0.284	0.134			
	Ewe	0.756	0.043			
	Year	0.836	0.034			

N: Number; CI: Confidence interval; BCS: Body condition score; IMM: Intramammary mass; SD: Standard deviation.

Table 20. Three-level mixed effect continuous outcome model of factors associated with udder width (cm) in 3749 exams (2922 ewes).

Variable	**Category**	***N***	**Mean**	**Coefficient**	**Lower 95% CI**	**Upper 95% CI**
Days in milk		4722		**-0.024**	**-0.03**	**-0.02**
Litter DLWG (kg)		5111		**2.649**	**2.34**	**2.96**
Teat length (cm)		5049		**0.261**	**0.16**	**0.36**
Udder drop		5018		**-0.890**	**-0.97**	**-0.81**
Teat angle		5017		**-0.190**	**-0.24**	**-0.14**
Degree of separation of udder halves		4860		**0.203**	**0.17**	**0.24**
Teat position	3	2575	13.1	Reference		
	1 - 2	1481	13.6	**0.324**	**0.22**	**0.43**
	4 - 5	948	12.5	**-0.203**	**-0.32**	**-0.08**
Breed	Lleyn	3534	13.4	Reference		
	Crossbreeds	678	12.9	**0.514**	**0.29**	**0.74**
	Charollais	440	12.2	-0.977	-2.13	0.18
	Texel	314	12.0	-0.208	-1.14	0.72
Age at lambing (yrs.)	1	386	12.0	Reference		
	2	1257	12.8	**0.582**	**0.39**	**0.77**
	3	1132	13.3	**0.669**	**0.47**	**0.87**
	4	626	13.7	**0.807**	**0.59**	**1.03**
	5 - 7	1092	13.7	**0.672**	**0.46**	**0.88**
	> 7	40	13.0	**0.660**	**0.15**	**1.17**
Non-traumatic teat lesions	None	4711	13.2	Reference		
	At least 1 teat	296	12.6	**-0.292**	**-0.49**	**-0.10**
Traumatic teat lesions	None	4810	13.1	Reference		
	At least 1 teat	197	13.5	**0.246**	**0.01**	**0.48**
Pregnancy protein	OK	4257	13.3	Reference		
	Overfed	189	12.4	-0.248	-0.96	0.46
	Underfed	108	11.8	**0.776**	**0.17**	**1.38**
Lactation protein	OK	3343	13.4	Reference		
	Overfed	28	11.8	**1.316**	**0.56**	**2.07**
	Underfed	1118	12.8	-0.098	-0.25	0.05
		Variance	SD			
Random effects	Flock	0.546	0.265			
	Ewe	0.242	0.066			
	Year	1.671	0.073			

N: Number; CI: Confidence interval; DLWG: Daily live weight gain; SD: Standard deviation.

Table 21. Three-level mixed effect continuous outcome model of factors associated with teat length (cm) in 4476 exams (3343 ewes).

Variable	**Category**	***N***	**Mean**	**Coefficient**	**Lower 95% CI**	**Upper 95% CI**
Udder width (cm)		5009		**0.026**	**0.02**	**0.03**
Age at lambing (yrs.)		5570		**0.034**	**0.02**	**0.04**
Days in milk		4722		**-0.0012**	**-0.0021**	**-0.0002**
Teat angle		5017		**-0.087**	**-0.10**	**-0.05**
Udder drop		5018		**-0.078**	**-0.10**	**-0.05**
Teat position	3	2579	2.36	Reference		
	1 - 2	1486	2.42	**0.044**	**0.02**	**0.07**
	4 - 5	949	2.39	-0.027	-0.06	0.01
BCS when pregnant	3	1654	2.33	Reference		
	Below 3	2028	2.48	**0.079**	**0.05**	**0.11**
	Above 3	1668	2.30	**-0.073**	**-0.11**	**-0.04**
BCS when lactating	3	1227	2.36	Reference		
	Below 3	2673	2.42	**0.032**	**0.003**	**0.061**
	Above 3	1146	2.34	0.013	-0.02	0.05
Breed	Lleyn	3565	2.32	Reference		
	Crossbreeds	677	2.57	**0.126**	**0.06**	**0.19**
	Charollais	450	2.54	0.17	-0.13	0.47
	Texel	314	2.56	-0.019	-0.21	0.17
Non-traumatic teat lesions	None	4751	2.37	Reference		
	At least 1 teat	296	2.65	**0.174**	**0.12**	**0.23**
Traumatic teat lesions	None	4848	2.38	Reference		
	At least 1 teat	199	2.55	**0.071**	**0.01**	**0.13**
IMM when lactating	No	4498	2.38	Reference		
	Yes	548	2.42	**-0.048**	**-0.09**	**-0.01**
Pregnancy protein	OK	4288	2.36	Reference		
	Overfed	195	2.56	0.045	-0.15	0.24
	Underfed	109	2.69	**0.204**	**0.06**	**0.35**
Lactation protein	OK	3376	2.35	Reference		
	Overfed	28	2.43	-0.064	-0.26	0.13
	Underfed	1121	2.50	**-0.049**	**-0.09**	**-0.01**
		Variance	SD			
Random effects	Flock	0.040	0.019			
	Ewe	0.069	0.005			
	Year	0.111	0.004			

N: Number; CI: Confidence interval; BCS: Body condition score; IMM: Intramammary mass; SD: Standard deviation.

Table 22. Three-level binary logistic model of factors associated with (296) non-traumatic teat lesions in 4721 exams (3486 ewes).

Variable	Category	*N* affected	% affected	OR	Lower 95% CI	Upper 95% CI
Number of lambs rearing	1	85	4.45	Reference		
	2 or more	194	7.06	**4.047**	**2.26**	**7.26**
IMM when lactating	No	246	5.44	Reference		
	Yes	50	8.99	**1.527**	**1.06**	**2.20**
Breed	Lleyn	143	4.00	Reference		
	Crossbreeds	71	10.46	**1.542**	**1.05**	**2.26**
	Charollais	41	9.03	1.568	0.94	2.63
	Texel	35	10.67	1.550	0.88	2.73
Teat angle	5	73	3.56	Reference		
	7 - 9	15	4.45	0.887	0.48	1.66
	6	33	3.32	0.829	0.53	1.29
	4	158	10.93	**2.875**	**2.09**	**3.95**
	3 - 1	17	9.24	1.804	0.98	3.34
Lactation energy	OK	88	4.86	Reference		
	Overfed	12	16.22	0.874	0.24	3.18
	Underfed	170	6.39	**0.359**	**0.20**	**0.64**
Lactation protein	OK	144	4.24	Reference		
	Overfed	9	32.14	**8.654**	**1.74**	**43.09**
	Underfed	117	10.42	1.445	0.99	2.11
		Overall mean	**Affected mean**			
Teat length (cm)		2.39	2.65	**2.499**	**1.93**	**3.24**
Udder width (cm)		13.14	12.62	**0.839**	**0.783**	**0.898**
		Variance	SD			
Random effects	Flock	1.000	1.000			
	Ewe	1.662	1.335			
	Year					

N: Number; OR: Odds ratio; CI: Confidence interval; IMM: Intramammary mass; SD: Standard deviation.

Table 23. Three-level binary logistic model of factors associated with (200) traumatic teat lesions in 4496 exams (3350 ewes).

Variable	**Category**	***N* affected**	**% affected**	**OR**	**Lower 95% CI**	**Upper 95% CI**
Number of lambs rearing	1	62	3.25	Reference		
	2 or more	134	4.88	**1.662**	**1.19**	**2.32**
IMM when lactating	No	152	3.36	Reference		
	Yes	48	8.63	**2.691**	**1.85**	**3.92**
Breed	Lleyn	133	3.72	Reference		
	Crossbreeds	9	1.33	**0.365**	**0.16**	**0.82**
	Charollais	41	9.03	**2.438**	**1.59**	**3.74**
	Texel	16	4.88	1.752	0.98	3.14
Teat angle	5	63	3.07	Reference		
	7 - 9	10	2.97	0.548	0.26	1.15
	6	23	2.31	0.646	0.39	1.07
	4	98	6.78	**2.537**	**1.79**	**3.60**
	3 - 1	4	2.17	0.726	0.22	2.43
		Overall mean	**Affected mean**			
Teat length (cm)		2.39	2.55	**1.621**	**1.20**	**2.19**
Age at lambing (yrs.)		3.4	3.1	**0.766**	**0.68**	**0.87**
Days in milk		69.79	61.87	**0.984**	**0.98**	**0.99**
		Variance	SD			
Random effects	Flock	1.000	1.000			
	Ewe	1.174	1.420			
	Year					

N: Number; OR: Odds ratio; CI: Confidence interval; SD: Standard deviation.

Factors associated with body condition score in pregnant ewes

Data from 1361 ewes were included in a model investigating factors associated with BCS in pregnant ewes examined in year 2 (Table 24). Key results were that BCS < 3 when pregnant and when lactating in year 1 was associated with lower BCS when pregnant in year 2 while BCS > 3 when pregnant and when lactating in year 1 was associated with higher BCS when pregnant in year 2. When compared to ewes age 2, ewes aged 3 or 4 at lambing in year 2 had higher BCS when pregnant while ewes aged 5 or above had lower BCS when pregnant. The presence of IMM when pregnant in year 2 was associated with a lower BCS at the same examination

Table 24. Two-level mixed effect continuous outcome model of factors associated with body condition score in 1361 pregnant ewes in year 2.

Variable	**Category**	***N***	**Year 2 Mean pregnant BCS**	**Coefficient**	**Lower 95% CI**	**Upper 95% CI**
Year 1 BCS when pregnant	3	657	3.24	Reference		
	Below 3	647	2.99	**-0.433**	**-0.56**	**-0.31**
	Above 3	267	3.57	**0.296**	**0.11**	**0.48**
Year 1 BCS when lactating	3	443	3.22	Reference		
	Below 3	626	3.05	**-0.182**	**-0.32**	**-0.05**
	Above 3	400	3.39	**0.270**	**0.12**	**0.42**
Age year 2 (yrs.)	2	249	3.19	Reference		
	3	440	3.28	**0.338**	**0.16**	**0.52**
	4	329	3.36	**0.394**	**0.20**	**0.58**
	5 - 7	440	2.99	**-0.277**	**-0.46**	**-0.10**
	> 7	14	3.11	-0.439	-1.07	0.19
Year 2 IMM when pregnant	No	1498	3.20	Reference		
	Yes	100	3.10	**-0.292**	**-0.52**	**-0.06**
		Variance	SD			
Random effects	Flock	0.561	0.334			
	Ewe	1.103	0.042			

BCS: Body condition score; *N:* Number; IMM: Intramammary mass; CI: Confidence interval.

Factors associated with body condition score in lactating ewes

Data from 4599 exams (3410 ewes) were included in the model investigating BCS in lactating ewes in years 1 and 2 (Table 25). For every day longer in lactation BCS was higher. Ewes rearing 2 or more lambs had lower BCS than ewes rearing 1 lamb. Ewes aged 5 or above at lambing had lower BCS than ewes ages 1 at lambing. BCS below 3 when pregnant was associated with lower BCS in lactation while above 3 was associated with higher BCS in lactation. Overfed pregnancy energy was associated with higher BCS when compared to OK pregnancy energy. Overfed lactation protein was associated with lower BCS while underfed was associated with higher BCS when both were compared to OK. Overfed lactation energy was associated with higher BCS while underfed was associated with lower BCS when both were compared to OK.

Table 25. Three-level mixed effect continuous outcome model of factors associated with body condition score during lactation in 4599 exams (3410 ewes).

Variable	**Category**	***N***	**Mean**	**Coefficient**	**Lower 95% CI**	**Upper 95% CI**
Days in milk		4722		**0.020**	**0.016**	**0.024**
Number of lambs rearing	1	2169	2.80	Reference		
	2 or more	3301	2.57	**-0.330**	**-0.608**	**-0.052**
Age at lambing (yrs.)	1	395	2.90	Reference		
	2	1294	2.72	-0.057	-0.23	0.12
	3	1318	2.73	0.154	-0.03	0.33
	4	855	2.71	0.135	-0.06	0.33
	5 - 7	1207	2.46	**-0.310**	**-0.49**	**-0.13**
	> 7	47	2.63	**-0.501**	**-0.99**	**-0.02**
BCS when pregnant	3	2239	2.65	Reference		
	Below 3	2182	2.44	**-0.387**	**-0.49**	**-0.29**
	Above 3	1461	3.02	**0.460**	**0.35**	**0.57**
Pregnancy energy	OK	5192	2.63	Reference		
	Overfed	257	3.31	**0.499**	**0.03**	**0.97**
	Underfed	85	2.89	-0.376	-0.94	0.19
Lactation protein	OK	4294	2.61	Reference		
	Overfed	28	2.57	**-1.365**	**-2.14**	**-0.59**
	Underfed	1138	2.82	**1.262**	**1.11**	**1.42**
Lactation energy	OK	2708	2.68	Reference		
	Overfed	74	3.14	**1.597**	**1.11**	**2.08**
	Underfed	2678	2.62	**-0.463**	**-0.76**	**-0.17**
		Variance	SD			
Random effects	Flock	0.504	0.235			
	Ewe	0.308	0.058			
	Year	1.727	0.064			

BCS: Body condition score; *N:* Number; CI: Confidence interval; SD: Standard deviation

DISCUSSION

Objective 1

The results from the study of intramammary masses (IMM) indicated that they were all abscesses with one or more species of bacteria present and many species of bacteria cultured from milk in affected glands. We can be confident that 'lumps' palpated in live sheep are intramammary abscesses.

Objectives 2 and 3

Development of udder conformation scoring system and monitoring of udders over time

The udder conformation scoring system we developed drew on previous work in dairy ewes and studies in suckler ewes (Huntley et al., 2012; Cooper et al., 2013). Teat angle (or placement), udder drop (or depth), degree of separation of udder halves, udder width and teat length were part of the scoring systems used in dairy sheep. In the current study teat position (direction of the teats from anterior to posterior) was included after the previous studies when considerable variation among ewes was noted and one farmer stated that they were specifically selecting for forward pointing teats. This attribute has not been included in recent research on udder traits of dairy ewes (e.g. Casu et al, 2006), but was included in earlier work (Fernadez et al., 1995; Kretschmer and Peters, 2002; Labussière et al., 1981) and in a study looking at the suitability of the Rouge de L'Ouest as a dual purpose breed (dairy / suckler) (Malher and Vrayla-Anesti, 1994). However, the scores used were slightly different from that used in the current study and so cannot be directly compared.

The udder scoring system was simple and reliable between two observers and we think that it would be possible for farmers to implement as part of their routine udder checks, especially if using the pictures guidance. There is some between observer variability in the scoring system. This was highlighted in our IRR study (Appendix 2). Part of this might be explained by the difficulty in observing the udder of suckler sheep in a race as the udder is not at eye-level, the sheep is relatively free to move about and is not used to having the udder palpated as would be the case for dairy sheep. Therefore for farmer use, it may be acceptable to look for extremes in udder conformation and modify the scoring system to a 5 point scale. Clearly if there is a 5 point scale it must identify the phenotypes likely to be avoided for future breeding.

For ewes that were present at examinations in lactation in both years, udder conformation did not change markedly from year to year. Where scores were different they were minor and might be due to rater error (due to the difficultly in applying the rating scales accurately, mentioned above) as well as some actual change from ageing, differences in stage of lactation and / or udder damage. In dairy ewes, udder traits have been measured at a number of time points within lactations and across lactations. Stage of lactation and parity number have significant effect on udder morphology, with udders getting wider and lower and teats longer with age while teat angle gets smaller (Fernadez et al., 1995; de la Fuente et al., 1996; Makovický et al., 2013). Others have found high repeatability of measures within and across lactations (Casu et al., 2006), though repeatability across lactation was not as high. Therefore some age-related change in udder conformation is likely and is supported by the results of our mixed effect, multi-variable models, where the age of ewe was associated with teat position, teat angle and udder drop, (but not degree of separation of udder halves). The direction of the association suggests that the conformation generally gets poorer with age, though the coefficients and therefore the changes are small. Our models also show that udders get wider and teats longer as ewes age, though again the effects are small. Days in milk was also associated with each udder conformation variable, including degree of separation of udder halves, adding to the evidence that udder conformation varies within lactations.

The majority of ewes in our study had teat position scores of 3, teat angle scores of 5, udder drop scores of 7 and degree of separation scores of 3 – 4. These are in line with our hypothesised optimum udder conformation scores, except the degree of separation of udder halves score which is 1 – 2 scores below the optimum hypothesised. A study of 894 dairy sheep found degrees of separation of udder half of 5 -7 were most common (Casu et al., 2010). Degree of separation scores may be higher in dairy ewes than suckler ewes because they have been bred for milk production. The breed of ewe did not have a huge effect on udder conformation but was most notable in teat position score, where Charollais ewes and Texel ewes were 3 times and 1.8 times less likely to have forward pointing teats than were Lleyns.

We believe extremely forward and sideways pointing teats, represented by teat position scores 1 and 5 are least desirable. Less than 1% of ewes had teat position scores of 1 and 5. Downward pointing teats, represented by teat angle scores 1-3, found in almost 4% of ewes, are less desirable than teat angle score 5. Udder drop scores of 5 and below, which would mean level with or below the hock, are less desirable than udder drop score 7 but affected only 3% of ewes. Degree of separation of udder half scores might be affected by the presence of IMM, previous udder damage or level of milk production more so than the other udder conformation variables. It might be more appropriate to say that asymmetrical udders are less desirable than any particular degree of separation score. In some cases of extreme udder conformation or udder damage there was no score in our scale that could be attributed to that udder shape. This was especially the case for degree of separation of udder halves, where an udder half could be missing or atrophied. Two hundred and ninety one ewes (5.6%) examined in lactation are missing a degree of separation of udder half score. Thirty-eight of these (13.1%) had acute mastitis and 44 (15.1%) had IMM in lactation. In retrospect it would have been useful to have a score in each udder conformation trait for "extreme udder conformation that does not fit elsewhere in the scale".

The latent class analysis of udder conformation variables identified four sub-groups of ewes. LC1 was formed of older ewes with less than optimum udder conformation while LC3 was formed of younger ewes with less than optimum udder conformation. This highlights how sub-optimum udder conformation can be present in flocks and is not simply related to ageing.

Teat position score 3, teat angle score 5 and udder drop score 7 might be the optimum udder conformation for suckling lambs, providing best access for them and thus helping to keep the udder and teats free from damage. This might also be the optimum udder conformation for suckling lambs to empty the cistern effectively. The associations between udder damage and udder conformation are discussed further under Objective 4.

Objective 4

Associations between udder damage and udder conformation

Teat position, teat angle and udder drop were the udder conformation traits that most impact on udder health. Forward pointing teats (1 & 2) were associated with a higher risk of IMM in lactation and with acute mastitis. Teat angle scores of < 5 were associated with a higher risk of acute mastitis. Udder drop scores < 7 were associated with an increased risk of IMM in lactation (included in the model as ULC 1: teat position 1 & 2, teat angle < 5 and udder drop < 5). Teat angle score 4 was also associated with a higher risk of traumatic and non-traumatic teat lesions, as were longer teat lengths. Both traumatic and non-traumatic teat lesions were associated with higher risk of IMM in lactation while non-traumatic teat lesions were associated with a higher risk of acute mastitis.

Forward pointing teats might be less protected by the non-woolly skin in the flank, more likely to come into contact with the wool on the underside of the ewe and more exposed to climate and soil environments and bacteria such as *Escherichia coli*. Udders with udder drop scores < 7, but particularly 5 and below, are closer to the ground and have a larger surface area. Therefore they are more likely to come into contact with environmental bacteria both when the ewe is standing and lying down. Teat angles scores < 5 are more downward pointing, towards the dirt. Bacteria can enter the mammary gland through the teat canal (Bergonier and Berthelot, 2003), thus leading to IMI. Dairy ewes with low udder drop (at or below the hock) are also more prone to intra-mammary infections and udder damage, explained by udders being closer to the ground and therefore bacterial contamination, as well as the difficulty of milking these low udders by machine (Casu et al. 2010).

Teat angle scores < 5, pendulous udders and long teats are also more likely to be difficult for lambs to suckle effectively (Huntley et al., 2012), resulting in repeated suckling attempts and possibly increased trauma to the teats and udder. This could explain the association with teat lesions (Cooper et al., 2013). Repeated suckling attempts could also explain the association with IMI. It has been suggested that increased frequency of suckling bouts (due to poor udder conformation where lambs have difficulty accessing the teats, low milk yield, or large litter sizes) increase the chances of bacteria entering the udder and could lead to an increase in mastitis (Gougoulis et al., 2008). If teats are damaged, their defence mechanisms will not be as effective and even the resident bacteria can cause IMI in these cases (Fragkou et al. 2007). In healthy teats, increased bacterial teat duct infections, which persisted up to two hours after suckling, have been found immediately after lamb suckling bouts. However, it seems that healthy teats have sufficient defence mechanisms to prevent the spread of infection into the gland as no increase in IMI was found (Gougoulis et al., 2008).

Objective 5

Impact of retaining ewes with intramammary masses into the next lactation

Ewes that had acute mastitis during lactation were almost 11 times more likely to have IMM when lactating, this suggests that the presence of IMM is a good indicator that a lactating ewe with an IMM is likely to have had acute mastitis. In addition, ewes with previously detected IMM were approximately 4 times more likely to have IMM at a later date. IMM in pregnancy (the same year) or lactation (the previous year) were not significantly associated with higher risk of acute mastitis, although there was a tendency towards such an association. Because farmers removed some ewes with IMM from their flocks (Table 7), the association might be stronger than that detected. IMM in lactation (the same year) were significantly associated with acute mastitis, but were not included in the model results as the examination in lactation took place towards the end of lactation, after the ewe had suffered acute mastitis in most cases. We can hypothesise that IMM are a result of an acute disease event and are themselves chronically persistent infection.

No particular size of IMM in pregnancy or lactation was more likely to be associated with IMM at a later date, therefore when examining udders for masses, presence / absence might be sufficient. Tables 6 and 7, which track IMM over the 2 years of the study, show how IMM can be present at 1 examination and then absent at the next. Further research is necessary to determine what is happening to IMM in these cases.

Bramley sized masses (the largest in our mass scale) in lactation were found to dominate in ewes that had acute mastitis in that lactation. Seventy-seven of the 181 ewes with acute mastitis over the 2 years of the study had IMM in that lactation and while the IMM recorded were of all sizes, 57% were bramley sized. It is likely that farmers select to cull ewes on the basis of size of IMM and if so culling those with the largest IMM is possibly a good idea as they are likely to have had acute mastitis. However, small masses could also be associated with acute mastitis and all mass sizes appear to be equally associated with chronic mastitis.

As IMM do not always lead to acute mastitis, farmers may be tempted to retain ewes with IMM in the flock. However, IMM when lactating impact negatively on lamb daily live weight gain, as shown in the Table 15. This is in line with previous findings (e.g. Arsenault et al., 2008; Huntley et al., 2012).

Flock did not have a significant impact on IMM in pregnancy (Table 12), but did on IMM in lactation (Table 13). Flock percentage of IMM was tested in the models for IMM in pregnancy and lactation (data not shown) and a higher flock percentage of IMM in pregnancy was associated with a higher risk of IMM in lactation in individual ewes. This suggests that IMM can be a source of infection to other ewes in the flock. The flocks with the highest flock percentage of IMM in pregnancy were the smaller flocks. It could be that in these smaller flocks, affected ewes and their lambs are more likely to come into contact with other ewes in the flock as they are grazing smaller areas or lambing indoors and housed together.

Flock did have a significant impact on acute mastitis. The Shropshire, Powys, Herefordshire and Gwynedd flocks had significantly higher risk of acute mastitis when compared to the Perth & Kinross flock (data not shown). However as breed, non-traumatic teat lesions and dietary protein in pregnancy were highly correlated with flock the decision was made to include the latter three variables in the model rather than flock as they were more informative.

Flock percentage IMM was also tested in the model for acute mastitis. A higher flock percentage of IMM in pregnancy or lactation was not associated with an increased risk of acute mastitis. However, higher flock number of IMM both in pregnancy and lactation were separately associated with a reduced risk of acute mastitis. This again could be a flock size effect, as the larger flocks had a higher flock number of IMM (they had more IMM because they had more sheep) but healthy ewes may have less chance of coming into contact with affected ewes and their lambs (grazing larger areas or separate fields, lambing outdoors, farmers less likely to spread infection from ewe to ewe). Charollais and Texel ewes (the main breeds of the Shropshire, Powys, Herefordshire and Gwynedd flocks) which were the breeds kept in the smaller flocks in our study, were both more likely to suffer acute mastitis.

It is possible that subclinical and acute mastitis are two different diseases. In a study investigating the association between non-clinical intramammary infections and clinical mastitis in dairy ewes, there was little similarity between the bacteria isolated the milk of ewes with sub-clinical mastitis (determined by level of Colony Forming Units/ml of milk (CFU/ml)) and the milk from ewes prior to a case of clinical mastitis. Despite a high rate of subclinical mastitis, there was a very low rate of clinical mastitis. The authors concluded that subclinical mastitis in sheep is not an early stage of clinical mastitis (Bor et al., 1989). Vautor et al. (2009) isolated *Staphylococcus aureus* from 6 subclinical cases of mastitis and one fatal clinical mastitis case in a flock of 80 dairy ewes. The isolate from the clinical mastitis case was genomically different to the other isolates and more virulent. Subclincal mastitis as determined by level of CFU/ml or raised SCC is likely to be different to subclinical mastitis as determined by IMM, which may be more correctly referred to as chronic mastitis (Marogna et al., 2010) and as we hypothesise is a result of an acute disease event.

IMM may in some way protect the ewe against acute mastitis. If different bacteria or bacterial strains are responsible for IMM than for acute mastitis as has been suggested, perhaps the

SCC is raised and so protects the gland from other invading bacteria by raised innate immunity.

Studies of subclinical mastitis often report that the IMI had spontaneously resolved itself at some stage during the lactation or at weaning (e.g. Bor et al., 1989; Contreras et al., 2007; Kirk et al., 1996). Usually subclinical mastitis is determined by level of CFU/ml or SCC and the presence of IMM is not mentioned. If we assume that in at least some of these cases IMM were present as well as elevated SCC counts or level of CFU/ml, then our findings that IMM were present at one examination and then absent at the next are in line with previous research. Abscesses are generally thought to be polymicrobial (Brook, 2002) and they are not static, they develop and rupture as part of their maturation cycle, rupture facilitates spread of bacteria which subsequently form abscesses elsewhere within their environment. On rupture they can leave behind fibrotic scars (Cheng et al. 2011). IMM are abscesses and so would rupture and reform as described. This might explain why IMM were present at one examination and not at another. Where a ewe was reported with a mass of different sizes at different examinations, this could be because the mass was at different stages of reformation. In some cases abscesses may not reform as the bacteria are killed by the host's immune system or possibly treatment with antibiotics.

Associations with protein and energy in diets

Dietary energy and protein tended to be correlated, especially during pregnancy, with ewes either receiving sufficient in both or neither. Eighty-three ewes were underfed both protein and energy in pregnancy and 23 (28%) of these had an IMM in lactation while 31 (37%) had acute mastitis.

Levels of energy and protein in pregnancy and lactation impacted significantly on most of the udder conformation variables, IMM and acute mastitis and the associations between underfeeding, adequate feeding and overfeeding were statistically associated with these factors when BCS was not. Ewes underfed energy in lactation had an increased risk of IMM both in that lactation and in the subsequent pregnancy. Ewes overfed protein in lactation had an increased risk of IMM in that lactation while those underfed protein in lactation had a decreased the risk. Ewes underfed protein in pregnancy had an increased risk of acute mastitis though this was mainly a single flock effect in year 1.

Under-feeding could be a “trigger factor” that causes bacteria normally resident in the mammary gland to become pathogenic (Fragkou 2007). Control of mastitis in heifers by nutritional means has been explored, with the feeding of energy and protein in the correct balance highlighted as key to a healthy immune system and especially important at the onset of lactation. Cows in negative energy balance have been found to have depressed immune systems and increased risk of IMI (Heinrichs et al., 2009).

Dietary protein at recommended levels (metabolisable protein between ≈ 75g and 115g daily depending on ewe weight and litter size)(EBLEX Sheep BRP Manual 12) before lambing is important to ensure adequate milk for lambs once they are born (Fthenakis et al., 2012). Inadequate milk supply leads to hungry lambs and pressure on the udder, hence the link between underfeeding of protein in pregnancy and acute mastitis. Studies have shown that if energy in the diet is adequate, increasing protein beyond requirements has no benefit for lamb growth rate (Van Emon et al., 2014). With this in mind, sheep farmers may be neglecting to ensure the quality of the forages they are feeding and focusing on supplementing the diet with high protein concentrate which promises higher milk yield. In doing this they may be denying their ewes the energy they need in their diet to remain healthy and not actually improving lamb growth rate. A complexity of studies of nutrition in suckler ewes is the impact of forage - both grass and conserved, however, further work is urgently needed on diet and its links with mastitis to identify the feed requirements of pregnant and lactating ewes.

This study is limited by the small number of flocks that took part and this should be considered when drawing conclusions from the results and making recommendations to the sheep industry.

Objective 6

Investigate the costs and benefits of scoring udder conformation as part of the selection criteria for replacement ewes.

To help inform a cost/benefit analysis, population attributable fractions (AFp) of selected factors that were associated with an increased risk of acute mastitis and IMM in lactation were calculated using the formula:

$$AFp = AFe\ (a1/m1)$$

where AFe (attributable fraction) = RD/(a1/n1); a1 = number of ewes diseased and exposed to risk, n1= number ewes exposed to risk; RD (risk difference) = (a1/n1) - (a0/n0); a0 = number of ewes diseased and non-exposed; n0 = total non-exposed and m1 = total diseased (a1 + a0).

Twenty-four percent of the acute mastitis cases in the population were attributable to ewes underfed protein in pregnancy, while 25% of the IMM in lactation were attributable to ewes underfed energy in lactation.

Six and four percent of acute mastitis cases in the population were attributable to ewes with teat position 1 and teat angle < 4 respectively. Teat position 1 was observed in 0.8% of Charollais ewes, 1.5% of Texel ewes, 0.8% of Crossbred ewes and 0.6% of Lleyn ewes in year 1 while teat angle < 4 was found in 0.4% of Charollais ewes, 1.2% of Texel ewes, 9.5% of Crossbred ewes and 3.3% of Lleyn ewes in year 1.

Eleven percent of IMM in lactation and 19% of the acute mastitis cases in the population in year 2 were attributable to ewes with an IMM in lactation the previous year; 22.67% of Charollais ewes, 11.52% of Texel ewes, 8.41% of Crossbred ewes and 4.90% of Llyen ewes were detected with IMM in lactation in year 1.

There are significant benefits to reducing the risk of acute and chronic mastitis by feeding ewes appropriately in pregnancy and lactation. In addition, ewes with IMM contribute a large risk to future acute mastitis. A target should be to reduce the percentage of ewes with IMM in a flock. Where IMM is common then this might be best initiated by separating affected and unaffected ewes into two separate flocks, one for ewes with IMM and the other for ewes without, to reduce the infection to ewes without IMM and slowly reduce the prevalence of IMM over time.

There are minimal benefits to selecting for good udder conformation. This is because the udder conformation of the vast majority of ewes in our study was appropriate for ewes suckling lambs and for maintaining udder health, preventing teat lesions, udder lumps and mastitis. Poor udder conformation tended to be in older ewes, so this could help inform on decisions to cull older ewes. Given that the vast majority of ewes had good udder conformation we do not think genetic selection for the traits we measured would be cost effective and that the best advice is to cull or not select offspring from ewes with extreme udder and teat conformation as necessary.

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Appendix 1

Assessment of the adequacy of ewe nutrition on study farms

Kate Phillips and Karen Wheeler, ADAS

Assessment of the adequacy of ewe nutrition on mastitis case study farms

Introduction

Correct formulation of rations to meet energy and protein requirements of ewes pre-lambing has a large influence on the successful delivery of healthy, strong lambs, colostrum production and subsequent milk yield. Rations should aim to maintain ewes in good condition at lambing (for lowland ewes about CS 3.0) without excessive weight loss or gain. The effects of poor nutrition and poor ewe body condition have been well documented. Under-nutrition has been shown to affect maternal behaviour – with underfed ewes grooming and bleating to their lambs less, ewes needing more birth assistance and lambs being of low birth weight with poor vigour (Dwyer *et al* 2003[1]) Likewise – over-feeding can lead to oversized lambs (particularly in single bearing ewes) and difficult births, a higher risk of pregnancy toxaemia and prolapse. Recent EBLEX funded work[2] led by the University of Nottingham and Lesley Stubbings has demonstrated the impact of body condition on lamb performance, and hence milk yield, on three commercial farms with strong correlations between ewe body condition at lambing with growth rate in lambs to 8 weeks and weaning; ewes maintained in good condition to lambing producing higher growth rates in their lambs than thinner ewes. Poor nutrition could therefore, potentially have an influence on mastitis as hungry lambs butt and bite the udder in an attempt to stimulate more milk production – typically this is around 3 to 4 weeks post lambing around ewe peak milk yield. The aim of this element of the project was to check the diets offered to the case study flocks to see if this has any correlation with the level of mastitis.

Methodology

Information on the diets offered to pregnant and lactating ewes was collected by Warwick University (Ed Smith/Claire Grant) from 8 case study farms (Year 1) and 6 case study farms (Year 2) by a standard questionnaire and included recording the feeds offered and the timing and quantities fed. Samples of conserved forage and concentrate feeds were analysed by Sciantec Laboratories (unless the farmer was able to supply a recent analysis). For other feeds such as feed blocks/buckets the manufacturers declared analysis was used. Spring grass quality was assumed to be 12.3 MJ/kgDM and 19% CP unless otherwise advised and was assumed to be in sufficient supply to meet the appetite of the ewes in combination with any supplementary feeds offered.

Diets were checked either by inputting information into the ADAS Sheepfeed rationing program (a computer program based on AFRC (1995). The program takes forage analysis and predicts ewe dry matter intake in pregnancy and lactation from forage and then calculates the amount of supplement required to meet requirements for Metabolisable Energy (ME MJ/kgDM) and Metabolisable Protein (MP g/day)). Intake for grass based diets, was calculated using the same prediction equations from AFRC (1995) in an Excel spreadsheet. In each case the energy and protein supplied by the diet was compared with the standard requirements (AFRC 1995: Energy and Protein Requirements of Ruminants: an advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB INTERNATIONAL, Wallingford, UK).

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[1] Dwyer, C. M., Lawrence, A. B., Bishop, S. C. and Lewis, M. (2003) Ewe-lamb bonding behaviours are affected by maternal undernutrition in pregnancy. British Journal of Nutrition **89**, 123-136

[2] Sheep KPI Validation Project http://www.eblex.org.uk/research/animal-nutrition/animal-nutrition-sheep/sheep-kpi-validation-project/

Year 1

Lambing 2012 – 2013

Data from 8 farms

Farm 1 Pedigree Charollais sheep – Shropshire

Assumptions:
Ewe weight 82 kg, Condition score 4.0, Lambing from 1 Dec 2012 indoors
Overall litter size: 1.82 (18 singles, 32 twins, 7 multiples)

Pregnancy rations

Table 1. Reported amounts fed on fresh basis kg/hd/day – same for all groups

	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*			
Haylage			*Ad-lib*	*Ad-lib*	*Ad-lib*
Compound		0.25	0.50	0.75	1.00

Table 2 Analysis of feeds offered

	Haylage	**Concentrate**
Report type	DM basis	DM basis
Dry matter (g/kg)	647	871
Crude protein (g/kg)	110	206
Oil-B (g/kg)	26	61
Ash (g/kg)	60	90
NCGD g/kg)		798
NDF (g/kg)	555	
ADF (g/kg)	333	
Sugar (g/kg)	114	
D value (%)	43	
ME (MJ/kg)	6.9	12.7

General comment – the forage is very poor (very low ME) and not really suitable for pregnant ewes. Ewes also had access to 'Coxi clear ewe tubby', but at 20g/hd/day predicted intake this has negligible impact on energy and protein intake.

Tables below were compiled using the Sheepfeed rationing program:

Single bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			1.5	1.6	1.7
ME supplied (MJ)			12.5	14.5	16.9
ME required* (MJ)			12.2	13.9	14.9
MP supplied (g)			102	117	133
MP required (g)			102	109	112
MP excess (g)			0	8	21
Weight change (g)			-10	1	17
CP in diet (%)			13.9	15.0	15.9

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Twin bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			1.5	1.6	1.8
ME supplied (MJ)			12.5	14.8	17.1
ME required* (MJ)			14.7	17.6	19.2
MP supplied (g)			108	130	149
MP required (g)			113	125	131
MP excess (g)			-5	5	18
Weight change (g)			-70	-76	-53
CP in diet (%)			13.9	14.9	15.8

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Triplet bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			1.5	1.6	1.8
ME supplied (MJ)			12.5	14.8	17.1
ME required* (MJ)			16.0	19.5	21.5
MP supplied (g)			111	134	154
MP required (g)			119	134	141
MP excess (g)			-8	0	13
Weight change (g)			-101	-121	-107
CP in diet (%)			13.9	14.9	15.8

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Comments

All ewes were fed the same irrespective of number of lambs expected/reared.
Hence singles tended to be overfed in pregnancy, twins were predicted to lose an acceptable amount of weight (and hence were fed slightly below requirements), but triplets would be considered to be underfed in terms of energy in particular.

Lactation rations

Tables below were compiled using the Sheepfeed rationing program. The feeds on offer were as for pregnancy.

Table 3. Reported amounts fed on fresh basis kg/hd/day – same for all groups

	Weeks post-lambing				
	1	3	6	9	10
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Compound	1.50	1.50	1.50	1.00	0.50

Single rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.7	2.7	2.7	2.4	2.1
Pred milk yield (kg)	2.1	2.1	1.8	1.4	1.2
ME supplied (MJ)	26.5	26.5	26.5	21.9	17.3
ME required* (MJ)	22.0	22.0	22.0	18	16
MP supplied (g)	226	229	239	204	168
MP required (g)	209	212	203	182	174
MP excess (g)	17	17	36	22	-6
Weight change (g)	70.0	59.0	92.0	24.0	-89
CP in diet (%)	15.6	15.6	15.6	14.4	13.0

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 2 kg/d for weeks 1-6 inclusive (Ref: Energy and protein requirements of ruminants)

Twin rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.7	2.7	2.7	2.4	2.1
Pred milk yield (kg)	*3.4*	*3.4*	*2.8*	*2.2*	*2.0*
ME supplied (MJ)	26.5	26.5	26.5	26.5	17.3
ME required* (MJ)	30.2	30.2	30.2	22.0	22.0
MP supplied (g)	260	262	255	255	177
MP required (g)	299	305	268	268	220
MP excess (g)	-39	-43	-12	-16	-43
Weight change (g)	-268	-295	-157	-164	-261
CP in diet (%)	15.6	15.6	15.6	14.4	13.0

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 3 kg/d for weeks 1-6 inclusive (Ref: Energy and protein requirements of ruminants)

Triplet rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.7	2.7	2.7	2.4	2.1
Pred milk yield (kg)	*4.2*	*4.3*	*3.5*	*2.7*	*2.5*
ME supplied (MJ)	26.5	26.5	26.5	21.9	17.3
ME required* (MJ)	*40*	*40*	*34.4*	28.0	26.0
MP supplied (g)	267	267	266	222	179
MP required (g)	359	367	319	270	255
MP excess (g)	-93	-100	-53	-48	-77
Weight change (g)	-474	-506	-337	-297	-377
CP in diet (%)	15.6	15.6	15.6	14.4	13.0

* ME required is based on allowing ewes to lose 100g/day and extrapolating from information in the reference book (Ref: Energy and protein requirements of ruminants)

Singles were predicted to gain weight in lactation and have sufficient MP to meet requirements. Twins would have been deficient in MP and energy and losing >200g/day in early lactation. Triplets were predicted to lose large amounts of body weight and be very deficient in MP – *but it is unlikely that any ewes actually reared triplets.* The poor forage quality had a major influence on the adequacy of the diet for this flock.

Farm 2 Pedigree Texel sheep –Herefordshire

Assumptions:
Ewe weight 79 kg, Condition score 3.0, Lambing from 4 Feb 2013 indoors
Overall litter size: 1.63 (33 singles, 42 twins, 4 multiples)

Pregnancy rations

Table 4. Reported amounts fed on fresh basis kg/hd/day – same for all ewes

	Weeks pre-lambing				
	8	6	4	2	1
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Mix PW	0	0	0.20	0.20	0.25

Note: forage reported as 0.75 kg/hd/day but seems very low – so have assumed available to appetite in the worked examples below

Table 5 Analysis of feeds offered

	Haylage	**Mix PW**	**Mix PG**
Report type	DM basis	DM basis	DM basis
Dry matter (g/kg)	667	856	847
Crude protein (g/kg)	80	227	230
Oil-B (g/kg)		48	28
Ash (g/kg)	26	97	100
NCGD g/kg)	750	834	852
NDF (g/kg)	661	306	124
Fibre (g/kg)		160	59
Starch (g/kg)		244	406
Sugar (g/kg)	30	102	58
D value (%)	64.9		
ME (MJ/kg)	10.4	12.86	12.63
pH	5.9		
Ammonia N (% tot N)	1		
Lactic acid (g/kg)	5		
VFAs (g/kg)	2		

Haylage was of average quality and the purchased mixes were high energy and digestibility. Tables below were compiled using the Sheepfeed rationing program.

Single bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	1.2	1.2	1.3	1.3	1.4
ME supplied (MJ)	12.7	12.7	14.3	14.3	14.7
ME required* (MJ)	10.3	10.9	12.2	13.9	14.9
MP supplied (g)	50	51	70	71	76
MP required (g)	90	93	99	106	108
MP excess (g)	-39	-43	-29	-35	-33
Weight change (g)	54	30	39	11	2
CP in diet (%)	8.0	8.0	9.9	9.9	10.3

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Twin bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	1.2	1.2	1.3	1.3	1.4
ME supplied (MJ)	12.7	12.7	14.3	14.3	14.7
ME required* (MJ)	11.6	12.5	14.7	17.6	19.2
MP supplied (g)	51	51	71	71	77
MP required (g)	95	101	110	122	127
MP excess (g)	-44	-50	-39	-50	-51
Weight change (g)	39	5	0	-66	-95
CP in diet (%)	8.0	8.0	9.9	9.9	10.3

Triplet bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	1.2	1.2	1.3	1.3	1.4
ME supplied (MJ)	12.7	12.7	14.3	14.3	14.7
ME required* (MJ)	12.3	13.4	16.0	19.5	21.5
MP supplied (g)	51	51	71	72	77
MP required (g)	97	105	116	130	137
MP excess (g)	-46	-54	-45	-58	-60
Weight change (g)	31	-11	-29	-108	-145
CP in diet (%)	8.0	8.0	9.9	9.9	10.3

Comments

All ewes were fed the same irrespective of lambs expected/reared.

Singles were predicted to gain weight in late pregnancy but were deficient in MP. Twins and triplets were short of energy and would have lost some weight in the last couple of weeks but would have been very deficient in MP.

ERDP:FME (rumen degradable protein: fermentable energy) ratio for all groups was between 6.1-7.4 (target range 10-12) i.e. much more ERDP/protein required.

Lactation rations

Table 6. Reported amounts fed on fresh basis kg/hd/day – same for all ewes

	Weeks post-lambing				
	1	3	6	9	10
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Mix PG	0.5	0.5	0.5	0.5	0.25
Supalyx (red)	*0.1*	*0.1*	*0.1*	*0.1*	*0.1*

The intake of Supalyx is reported by the manufacturer in the range of 20-150g/day – have assumed 100g/day for this example.

Tables below are compiled using the Sheepfeed rationing program.

Single rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.3	2.3	2.3	2.3	2.1
Pred milk yield (kg)	2	2.1	1.7	1.3	1.2
ME supplied (MJ)	25.1	25.1	25.1	25.1	23.2
ME required* (MJ)	22.0	22.0	22.0	16.4	15.6
MP supplied (g)	139	139	140	140	114
MP required (g)	201	205	196	176	168
MP excess (g)	-62	-66	-56	-36	-53
Weight change (g)	36	24	79	148	120
CP in diet (%)	11.1	11.1	11.1	11.1	9.8

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 2 kg/d for weeks 1-6 inclusive (Ref: Energy and protein requirements of ruminants)

Twin rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.3	2.3	2.3	2.3	2.1
Pred milk yield (kg)	*3.2*	*3.3*	*2.7*	*2.1*	*1.9*
ME supplied (MJ)	25.1	25.1	25.1	25.1	23.2
ME required* (MJ)	30.2	30.2	30.2	22.0	22.0
MP supplied (g)	141	142	141	141	115
MP required (g)	289	294	259	223	212
MP excess (g)	-147	-153	-117	-82	-97
Weight change (g)	-239	-263	-137	1	-17
CP in diet (%)	11.1	11.1	11.1	11.1	9.8

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 3 kg/d for weeks 1-6 inclusive and 2 kg/d wks 9/10 (Ref: Energy and protein requirements of ruminants)

Triplet rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.3	2.3	2.3	2.3	2.1
Pred milk yield (kg)	*4*	*4.1*	*3.4*	*2.6*	*2.4*
ME supplied (MJ)	25.1	25.1	25.1	25.1	23.2
ME required* (MJ)	*39*	*39*	*33*	26	26
MP supplied (g)	142	142	142	142	116
MP required (g)	347	354	308	260	247
MP excess (g)	-204	-211	-165	-119	-131
Weight change (g)	-426	-456	-298	-123	-129
CP in diet (%)	11.1	11.1	11.1	11.1	9.8

* ME required is based on allowing ewes to lose 100g/day and extrapolating from information in the reference book for twin rearing ewes (Ref: Energy and protein requirements of ruminants)

Singles would have generally gained weight in lactation but would have been deficient in MP. Twins were very deficient in MP and energy, losing >200g/day in early lactation.

Triplets were predicted to lose large amounts of body weight and would have been very deficient in MP – *only 4 ewes were scanned as carrying triplets so it is unlikely that any lambs were reared as triplets but were fostered onto singles.*

The diet checks above have identified large deficits of protein in this flock for all groups in both pregnancy and lactation – these will have impacted on colostrum supply and quality and milk production of the ewes.

Farm 3 Commercial flock – West Sussex

Assumptions:
Ewe weight 70 kg (for rationing purposes), Condition score – mean 2.5 (range 0.5 – 4.0), Lambing from 5 February 2013 indoors – ewes winter shorn. TMR rations fed.
Overall litter size: 1.75 (339 singles, 641 twins, 77 multiples)

Pregnancy rations

Table 7. TMR ration formulation and energy and protein supplied

	Diet 1	Diet 2	Diet 3	Diet 4
Forage 1	BM1 3kg	CR1 2kg	CR1 2 kg	CR 2 kg
Forage 2		BH1 2kg	BH1 1.5kg	BH1 1.2kg
Regumaize	100g	50g		
Molasses	50g	100g	200g	200g
Hipro soya	-	-	150g*	250g
Minerals	20g	20g	20g	20g
Ration details				
Total kg/head fresh	3.25	4.25	4	3.7
ME supplied (MJ/hd)	15	16	17	18
Metabolisable protein (g/hd)	95	100	120	135

Table 8 Analysis of feeds offered in pregnancy (dry matter basis)

	BM1 baled silage	CR1 haylage	BH1 haylage	CR2 baled silage
Dry matter (g/kg)	466	496	420	349
Crude protein (g/kg)	101	90	117	104
Oil-B (g/kg)		23	24	
Ash (g/kg)	74	84	97	75
NDF (g/kg)	579	594	607	554
ADF (g/kg)		346	351	
Sugar (g/kg)	56	80	78	66
D value (%)	63	58	58	66
ME (MJ/kg)	10.1	9.3	9.2	10.5
DE (MJ/kg)		8.6	8.2	
FME (MJ/kg)	8.2			8.5
Oil-A (g/kg)	23			34
Potential intake (FiM) (g/kgW 0.75)	97			104
pH	4.8			4
Ammonia N (% total N)	9.3			1.3
Pot. Acid loading (FiM) (meq/kg)	1071			786
ERDP (FiM) (g/kg)	69			84
DUP (FiM) (g/kg)	10.6			10.8
Acetic acid (g/kg)	10.1			8.7
n Butyric acid (g/kg)	6.2			0.3

Tot. Ferm. Acids (FiM) (g/kg)	70.6			76.3
Lactic acid (g/kg)	51.9			67.3
Nitrogen solubility (N)	0.66			0.66
(a)	0.69			0.69
(b)	0.26			0.26
(c; per hour)	0.084			0.084
Dry matter solubility (S)	0.31			0.27
(a)	0.31			0.34
(b)	0.69			0.57
(c; per hour)	0.038			0.035

Forages were of average to moderate quality.

Table 9. TMR diet feeding plan by group

Group	**Last 2 weeks January**	**First 2 weeks Feb**	**Weeks 3 & 4 Feb**	**Weeks 1 and 2 March**	**Weeks 3 and 4 March**	**First half April**
	10-8	8-6	6-4	4-2	2-lambing	
Fit Triplets (+ lean early twins + lean later triplets)	1	2	3	4	4	4
Lean earlier triplets	2	2	3	4	4	-
Fit early Twins and lean later twins	1	1	2	3	4	4
Fit later twins	1	1	2	2	3	4
Fit Singles and lean later singles	1	1	1	1	2	2
Fit Later Singles	1	1	1	1	1	2

Tables below were compiled using the declared ME and MP content of the TMR rations and the recommendations in reference book: Energy and protein requirements of ruminants

Fit early single bearing ewes (and lean late singles)

	Weeks pre-lambing				
	8	6	4	2	1
TMR diet	**1**	**1**	**1**	**2**	**2**
DMI (reported) kg	1.5	1.5	1.5	1.9	1.9
ME supplied (MJ)	15	15	15	16	16
ME required* (MJ)	10.2	10.7	11.9	13.5	14.4
MP supplied (g)	95	95	95	100	100
MP required (g)	81	85	90	96	99
MP excess (g)	14	10	5	4	1

* ME required is based on allowing ewes to maintain weight (Ref: Energy and protein requirements of ruminants)

Ration is calculated to meet (or slightly exceed) the MP requirements and will allow ewes to gain weight in late pregnancy.

Fit late single bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
TMR diet	**1**	**1**	**1**	**1**	**1**
DMI (reported) kg	1.5	1.5	1.5	1.5	1.5
ME supplied (MJ)	15	15	15	15	15
ME required* (MJ)	10.2	10.7	11.9	13.5	14.4
MP supplied (g)	95	95	95	95	95
MP required (g)	81	85	90	96	99
MP excess (g)	14	10	5	-1	-4

* ME required is based on allowing ewes to maintain weight (Ref: Energy and protein requirements of ruminants)

Ration is calculated to meet the MP requirements and will allow ewes to gain weight in late pregnancy.

Fit early twin bearing ewes (and lean late twins)

	Weeks pre-lambing				
	8	6	4	2	1
TMR diet	**1**	**2**	**3**	**4**	**4**
DMI (reported) kg	1.5	1.9	1.9	1.6	1.6
ME supplied (MJ)	15	16	17	18	18
ME required* (MJ)	11.4	12.3	14.2	16.8	18.3
MP supplied (g)	95	100	120	135	135
MP required (g)	86	92	100	111	116
MP excess (g)	9	8	20	24	19

* ME required is based on allowing ewes to maintain weight (Ref: Energy and protein requirements of ruminants)

Energy requirements from 8-2 weeks pre-lambing exceed requirements allowing ewes to gain weight, MP requirements are met throughout.

Fit late twin bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
TMR diet	**1**	**2**	**2**	**3**	**3**
DMI (reported) kg	1.5	1.9	1.9	1.9	1.9
ME supplied (MJ)	15	16	16	17	17
ME required* (MJ)	11.4	12.3	14.2	16.8	18.3
MP supplied (g)	95	100	100	120	120
MP required (g)	86	92	100	111	116
MP excess (g)	9	8	0	9	4

* ME required is based on allowing ewes to maintain weight (Ref: Energy and protein requirements of ruminants)

Energy requirements from 8-4 weeks pre-lambing exceed requirements so ewes were predicted to gain weight, and were close to requirements in the final two weeks. MP supplied was closely matched to requirements throughout.

Triplet bearing ewes (plus lean early twins)

	Weeks pre-lambing				
	8	6	4	2	1
TMR diet	**2**	**3**	**4**	**4**	**4**
DMI (reported) kg	1.9	1.9	1.6	1.6	1.6
ME supplied (MJ)	16	17	18	18	18
ME required* (MJ)	12	13	15.4	18.5	20.3
MP supplied (g)	100	120	135	135	135
MP required (g)	88	95	105	118	125
MP excess (g)	12	25	30	17	10

* ME required is based on allowing ewes to maintain weight (Ref: Energy and protein requirements of ruminants)

Energy requirements from 8-4 weeks pre-lambing exceeded requirements allowing ewes to gain weight only falling below requirements just before lambing where predicted liveweight loss would have been just over 100g/day. MP supplied was closely matched to requirements throughout. (Leaner triplets started on ration 2 at 10 weeks pre-lambing and later lambing ewes remained on diet 4 for an additional two weeks.)

Lactation diets

Table 10. Reported amounts fed in lactation kg/hd/day (DMI reported 2.0 kg/d)

Singles	Weeks post-lambing				
	1	3	4	6	9
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Concentrates	0.5	0.5	0	0	0
Early (lean) twins	Weeks post-lambing				
	1	3	5	6	9
Silage BM2	*Ad-lib*	*Ad-lib*			
Grass			*Ad-lib*	*Ad-lib*	*Ad-lib*
Concentrates	1.0	1.0	0.5	0	0
Late twins	Weeks post-lambing				
	1	3	5	6	9
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Concentrates	0	0	0	0	0

Table 11 Analysis of feeds offered in lactation to lean twins (dry matter basis)

	BM2 clamp silage	**Concentrate**
Dry matter (g/kg)	245	880
Crude protein (g/kg)	123	205
Ash (g/kg)	67	80
NDF (g/kg)	577	
Sugar (g/kg)	4	
D value (%)	66	
ME (MJ/kg)	10.6	12.5
FME (MJ/kg)	6.4	10.2
Oil-A (g/kg)	47	

Oil B (g/kg)		45
NCGD (g/kg)		800
Potential intake (FiM) (g/kgW 0.75)	119	
pH	4.2	
Ammonia N (% total N)	16.3	
Pot. Acid loading (FiM) (meq/kg)	922	
ERDP (FiM) (g/kg)	97	120
DUP (FiM) (g/kg)	16.8	40
Acetic acid (g/kg)	47.5	
n Butyric acid (g/kg)	12.7	
Tot. Ferm. Acids (FiM) (g/kg)	173.7	
Lactic acid (g/kg)	113.6	
Nitrogen solubility (N)	0.58	
(a)	0.68	
(b)	0.23	
(c; per hour)	0.079	
Dry matter solubility (S)	0.22	
(a)	0.32	
(b)	0.51	
(c; per hour)	0.036	

This silage had a relatively high level of ammonia which could have depressed intake, but was of above average ME.

Single rearing ewes

	Weeks post-lambing			
	1-3	4	6	9
DMI (predicted) (kg)	2.0	2.0	2.0	2.0
Pred milk yield (kg)	*1.8*	*1.8*	*1.5*	*1.2*
ME supplied (MJ)	24.7	24.6	24.6	24.6
ME required* (MJ)	23.3	23.3	19.2	16.8
MP supplied (g)	232	236	230	225
MP required (g)	222	222	184	161
MP excess (g)	10	14	46	64
CP in diet (%)	19.3	19.0	19.0	19.0

* ME required is based on allowing ewes to lose 50g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

Diet met energy and MP requirements in the first month of lactation so ewes should not have lost more than 50g live weight/day. From the second month of lactation MP and ME supplied was in excess of requirements and ewes were likely to have been maintaining or increasing in live weight.

Twin rearing ewes (indoors for first 4 weeks) (early leaner ewes)

	Weeks post-lambing				
	1	3	5	6	9
DMI (predicted) (kg)	2.0	2.0	2.0	2.0	2.0
Pred milk yield (kg)	*2.7*	*2.7*	*2.4*	*2.4*	*1.9*
ME supplied (MJ)	22.9	22.9	24.7	24.6	24.6
ME required* (MJ)	26.9	26.9	24.8	24.8	21.4
MP supplied (g)	161	162	235	238	233
MP required (g)	240	244	245	245	215
MP excess (g)	-79	-83	-10	-7	18
Weight change (g)	-144	-158	Not calc	Not calc	Not calc
CP in diet (%)	15.9	15.9	19.3	19.0	19.0

* ME required is based on allowing ewes to lose 100g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

Diet struggled to meet the requirements of ewes, particularly in the first 4 weeks of lactation when ewes were indoors.

Later twin rearing ewes

	Weeks post-lambing			
	1-3	4	6	9
DMI (predicted) (kg)	2.0	2.0	2.0	2.0
Pred milk yield (kg)	*2.9*	*2.9*	*2.4*	*1.9*
ME supplied (MJ)	24.6	24.6	24.6	24.6
ME required* (MJ)	29.8	29.8	24.8	21.4
MP supplied (g)	243	243	238	233
MP required (g)	291	291	245	215
MP excess (g)	-48	-48	-7	18
CP in diet (%)	19.0	19.0	19.0	19.0

* ME required is based on allowing ewes to lose 100g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

Weeks 1-4 ewes predicted to lose in excess of 150 g/day and MP in deficit. Ration close to requirements by week 6. These ewes did not receive any concentrates in early lactation and were at grass (assumes a level of protein and ME from grass that may not be entirely accurate).

Farm 4 Lleyn Northumberland

Assumptions:
Ewe weight 70 kg, Condition score 2.7, Lambing from 20th April outdoors
Overall litter size: 1.60 (656 singles, 741 twins, 67 multiples)

Pregnancy rations

Table 12. Reported amounts fed on fresh basis kg/hd/day – based on appetite of 2.5% of live weight (1.75kgDM). Hay and silage intakes reported as 1.5 kg DM – which may be an overestimate. We have no indication of grass availability.

Singles	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Hay	0	0	1.8	1.8	1.8
Supalyx	0	0	0.1	0.1	0.1
Twins and triplets	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Silage	0	0	4.9	*4.9*	4.9
Supalyx	0	0	0.1	0.1	0.1

Table 13 Analysis of feeds offered

	Silage	**Hay**
Report type	DM basis	DM basis
Dry matter (g/kg)	304	848
Crude protein (g/kg)	115	82
Oil (g/kg)	35	26
Ash (g/kg)	74	62
NDF (g/kg)	558	602
ADF (g/kg)		349
Sugar (g/kg)	13	96
D value (%)	70	49
ME (MJ/kg)	11.2	7.8
FME (MJ/kg)	8.2	
Potential intake (FiM) (g/kgW 0.75)	108	
pH	4.3	
Ammonia N (% total N)	13.3	
Pot. Acid loading (FiM) (meq/kg)	902	
ERDP (FiM) (g/kg)	93	
DUP (FiM) (g/kg)	12.6	
Acetic acid (g/kg)	23.8	
n Butyric acid (g/kg)	7.3	
Tot. Ferm. Acids (FiM) (g/kg)	114.4	
Lactic acid (g/kg)	83.3	
Nitrogen solubility (N)	0.66	
(a)	0.69	
(b)	0.26	

(c; per hour)	0.084	
Dry matter solubility (S)	0.31	
(a)	0.31	
(b)	0.69	
(c; per hour)	0.038	

Supalyx, assume similar protein quality to molasses:
DM 900g/kg ME 11.5MJ, FME 11.4 MJ, CP 13.3.

Grass silage of high digestibility and ME, marginally high ammonia. Hay moderate to poor quality.

Grass: reported as 10.2 MJ/kgDM, CP 16-17 % CP – have used 11.6 MJ/kgDM, 17%CP for pregnancy for tables below as grass quality should be improving closer to lambing.

Single bearing ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	20.3	20.3	14.3	14.3	14.3
ME required* (MJ)	10.2	11.1	12.3	13.9	14.8
MP supplied (g)	166	166	99	99	99
MP required (g)	88	92	97	103	105
MP excess (g)	78	74	2	-4	-6
CP in diet (%)	17.0	17.0	9.2	9.2	9.2

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants)

Diet is oversupplying energy and protein up to 4 weeks pre-lambing but the shift to hay reduces energy and protein intakes from 4 weeks close to requirements for single-bearing ewes with a small deficit of MP in the final 2 weeks.

Twin bearing ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	20.3	20.3	19.7	19.7	19.7
ME required* (MJ)	11.8	12.6	14.6	17.2	18.7
MP supplied (g)	166	167	118	120	122
MP required (g)	93	98	107	117	122
MP excess (g)	73	69	11	3	0
CP in diet (%)	17.0	17.0	11.8	11.8	11.8

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants)

Diet is predicted to meet/oversupply energy and protein requirements throughout late pregnancy.

Triplet bearing ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	20.3	20.3	19.7	19.7	19.7
ME required* (MJ)	12.4	13.4	15.7	19.0	20.6
MP supplied (g)	166	169	117	120	122
MP required (g)	95	102	112	125	131
MP excess (g)	71	67	5	-5	-9
CP in diet (%)	17.0	17.0	11.8	11.8	11.8

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants)

Energy supplied is close to requirements throughout; there is a small MP deficit in the last two weeks which may impact on colostrum supplies and milk yield.

Lactation rations

Assume dry matter intake 3.0% of live weight. Assume Supalyx is fed for first month of lactation – but no information provided. Assume high quality grass in lactation (12.3 ME and 19% CP).

Table 14. Reported amounts fed on fresh basis kg/hd/day

	Weeks post-lambing			
	1	3	6	9
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Supalyx	0.1	0.1	0	0

Single rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*1.8*	*1.8*	*1.5*	*1.2*
ME supplied (MJ)	25.8	25.8	25.8	25.8
ME required* (MJ)	23.3	23.3	19.2	16.8
MP supplied (g)	242	242	241	237
MP required (g)	222	222	184	161
MP excess (g)	20	20	57	76
CP in diet (%)	18.7	18.7	19.0	19.0

* ME required is based on allowing ewes to lose 50g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

Energy levels above requirements so ewes likely to be maintaining or gaining weight. MP in excess throughout.

Twin rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*2.9*	*2.9*	*2.4*	*1.9*
ME supplied (MJ)	25.8	25.8	25.8	25.8
ME required* (MJ)	29.8	29.8	24.8	21.4
MP supplied (g)	251	251	246	240
MP required (g)	291	291	245	215
MP excess (g)	-40	-40	1	25
CP in diet (%)	18.7	18.7	19.0	19.0

* ME required is based on allowing ewes to lose 100g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

ME below requirements for first month so ewes predicted to lose more than 100g/day in this period. MP deficit for the first month.

Triplet rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*3.6*	*3.7*	*3*	*2.3*
ME supplied (MJ)	25.8	25.8	25.8	25.8
ME required* (MJ)	34.6	35.4	29.8	24.0
MP supplied (g)	251	251	256	249
MP required (g)	337	344	291	238
MP excess (g)	-86	-93	-35	11
CP in diet (%)	18.7	18.7	19.0	19.0

* ME required is based on allowing ewes to lose 100g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

ME below requirements for first 6 weeks so ewes predicted to lose more than 100g/day in this period. MP deficit for the first six weeks of lactation but this is probably not relevant given that it is unlikely that any ewes reared triplets.

Farm 5 Lleyn Perth and Kinross

Assumptions:
Ewe weight 70 kg, Condition score 3.0, Lambing from 18th April outdoors
Overall litter size: 1.85 (84 singles, 183 twins, 29 multiples)

Pregnancy rations

Table 15. Reported amounts fed on fresh basis kg/hd/day – based on appetite of 2.5% of live weight (1.75kgDM). Hay only fed during snow – 1 bale/day – grass supply assumed to be adequate to meet remainder of appetite.

Singles and twins	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Hay	0.65	0.65	0	0	0
Concentrates	0.25	0.25	0.25	0.25	0.25
Triplets	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Hay	0.65	0.65	0	*0*	0
Concentrates	0.7	0.7	0.7	0.7	0.7

Plus Rumenco 'Lifeline' buckets from 8 weeks pre-lambing – estimated intake of 160 g/hd/day

Table 16 Analysis of feeds offered

	Hay	**Concentrate**
Report type	DM basis	DM basis
Dry matter (g/kg)	830	867
Crude protein (g/kg)	92	202
Oil (g/kg)	24	39
Ash (g/kg)	60	122
NDF (g/kg)	601	
ADF (g/kg)	349	
Sugar (g/kg)	89	
NCGD (g/kg)		691
D value (%)	51	
ME (MJ/kg)	8.2	10.7

Lifeline buckets fed -8wks to +4weeks. assume:
DM 900g/kg ? ME 13.0 MJ, FME 12.6? MJ, CP 12.0.

Moderate quality hay and low energy concentrate.

Single bearing ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	19.2	19.2	21.3	21.3	21.3
ME required* (MJ)	10.2	11.1	12.3	13.9	14.8
MP supplied (g)	167	170	189	192	193
MP required (g)	88	92	97	103	105
MP excess (g)	79	78	92	89	88
CP in diet (%)	15.9	15.9	18.7	18.7	18.7

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants)

Twin bearing ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	19.2	19.2	21.3	21.3	21.3
ME required* (MJ)	11.8	12.6	14.6	17.2	18.7
MP supplied (g)	168	171	193	197	199
MP required (g)	93	98	107	117	122
MP excess (g)	75	73	86	80	77
CP in diet (%)	15.9	15.9	18.7	18.7	18.7

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants)

Triplet bearing ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	18.6	18.6	20.7	20.7	20.7
ME required* (MJ)	12.4	13.4	15.7	19.0	20.6
MP supplied (g)	166	168	190	195	197
MP required (g)	95	102	112	125	131
MP excess (g)	71	70	78	70	66
CP in diet (%)	16.1	16.1	18.9	18.9	18.9

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants)

Diet offered met requirements of all ewes with energy and protein in excess of requirements (although this assumes good quality grazing). This suggests that ewes would have been gaining weight between 8 and 3 weeks before lambing.

Lactation rations

Assume dry matter intakes 3.0% live weight. Assumes that sufficient grass is available to meet appetite.

Table 17. Reported amounts fed on fresh basis kg/hd/day (all ewes)

	Weeks post-lambing			
	1-2	3-4	6	9
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Concentrates	0.25	0.25	0	0

Plus lifeline buckets up to week 4

Single rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*1.8*	*1.8*	*1.5*	*1.2*
ME supplied (MJ)	25.6	25.6	25.8	25.8
ME required* (MJ)	23.3	23.3	19.2	16.8
MP supplied (g)	244	244	241	237
MP required (g)	222	222	184	161
MP excess (g)	22	22	57	76
CP in diet (%)	18.7	18.7	19.0	19.0

* ME required is based on allowing ewes to lose 50g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

Energy levels above requirements throughout so ewes likely to be maintaining or gaining weight. MP in excess throughout.

Twin rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*2.9*	*2.9*	*2.4*	*1.9*
ME supplied (MJ)	25.6	25.6	25.8	25.8
ME required* (MJ)	29.8	29.8	24.8	21.4
MP supplied (g)	251	251	250	246
MP required (g)	291	291	245	215
MP excess (g)	-40	-40	5	31
CP in diet (%)	18.7	18.7	19.0	19.0

* ME required is based on allowing ewes to lose 100g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

ME below requirements for first month so ewes are predicted to lose more than 100g/day body-weight in this period. MP deficit for the first month but close to requirements from week 6. Again – unlikely that any ewes reared triplets.

Farm 6 Texel Gwynedd

Assumptions:
Ewe weight 79 kg, Most ewes CS 2.7, some thin ewes 1.7
Lambing from 10th March indoors – housed 1-2 weeks before lambing
Overall litter size: not known as did not scan

Pregnancy rations

Table 18. Reported amounts fed on fresh basis kg/hd/day – same for all groups

Most ewes	Weeks pre-lambing				
	8	6	4	2	1
Haylage		*Ad-lib*	*Ad-lib*	*Ad lib*	*Ad lib*
Easy lamber nuts		0.25	0.25	0.50	0.75
Crystalyx Hi E block		*Ad-lib*	*Ad-lib*	*Ad lib*	*Ad lib*
Thin ewes	Weeks pre-lambing				
	8	6	4	2	1
Haylage		*Ad-lib*	*Ad-lib*	*Ad lib*	*Ad lib*
Easy lamber nuts		0.25	0.50	0.75	1.00
Crystalyx Hi E block		*Ad-lib*	*Ad-lib*	*Ad lib*	*Ad lib*

Crystalyx intake estimated at 85g/hd/day – providing 1.4 MJ energy (if blocks are 16MJ/kg DM)

Table 19 Analysis of feeds offered

	Haylage	**Concentrate***
Report type	DM basis	DM basis
Dry matter (g/kg)	771	870
Crude protein (g/kg)	144	194
Oil-B (g/kg)	24	55
Ash (g/kg)	80	102
NCGD g/kg)		724
NDF (g/kg)	595	
ADF (g/kg)	346	
Sugar (g/kg)	84	
D value (%)	56	
ME (MJ/kg)	9.0	11.5

* mean of two samples

Haylage quite poor quality and compound has a disappointing ME.

Tables below were compiled using the Sheepfeed rationing program:

Most ewes (fitter) - Single bearing

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			1.5	1.6	1.7
ME supplied (MJ)			14.3	16.1	17.9
ME required* (MJ)			12.2	13.9	14.9
MP excess (g)			27	36	45
Weight change (g)			33	39	58
CP in diet (%)			15.1	15.7	16.3

* ME required is based on allowing ewes to lose 50g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

Most ewes (fitter) - Twin bearing

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			1.4	1.6	1.7
ME supplied (MJ)			14.3	16.1	17.9
ME required* (MJ)			14.7	17.6	19.2
MP excess (g)			24	31	39
Weight change (g)			-6	-24	-17
CP in diet (%)			15.2	15.7	16.3

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

For the fitter ewes the singles were overfed whilst the twins were slightly underfed energy close to lambing (typical of normal practice – allowing ewes to mobilise some body fat close to lambing). The additional feed offered to the thinner ewes provides an adequate diet pre-lambing with virtually no weight loss for twin-bearing ewes.

Lactation rations

Tables below were compiled using the Sheepfeed rationing program. The feeds on offer are as for pregnancy.

Table 20. Reported amounts fed on fresh basis kg/hd/day – same for all groups

	Weeks post-lambing				
	1-2	3-4	5		
Grass			*Ad-lib*		
Haylage	*Ad-lib*	*Ad-lib*			
Easy lamber nuts	0.75	0.75			
Crystalyx Hi E block	*Ad-lib*	*Ad-lib*			

Assume intakes of crystalyx similar to above at 85g/hd/day

Single rearing ewes

	Weeks post-lambing			
	1-2	3-4	5-6	9
DMI (predicted) (kg)	2.5	2.5		
Pred milk yield (kg)	2.0	2.1	1.7	
ME supplied (MJ)	23.8	23.8		
ME required* (MJ)	23.8	24.6	21.4	
MP excess (g)	30	28		
Weight change (g)	-24	-40		
CP in diet (%)	15.8	15.8		

* ME required is based on allowing ewes to lose 50g/day and assuming milk yield of 2 kg/d for weeks 1-6 inclusive (Ref: Energy and protein requirements of ruminants)

Twin rearing ewes

	Weeks post-lambing			
	1-2	3-4	6	9
DMI (predicted) (kg)	2.5	2.5		
Pred milk yield (kg)	*3.2*	*3.3*	*2.7*	
ME supplied (MJ)	23.8	23.8		
ME required* (MJ)	31.8	32.7	27.7	
MP excess (g)	-35	-40		
Weight change (g)	-307	-331		
CP in diet (%)	15.8	15.8		

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 3 kg/d for weeks 1-6 inclusive (Ref: Energy and protein requirements of ruminants)

Energy and protein levels were met for single rearing ewes allowing a body weight loss of up to 50g/day. Twin rearing ewes were deficient in energy and protein and predicted to be losing a lot of weight in the first 4 weeks of lactation. The thinner ewes (CS 2.0) would have been even more compromised by underfeeding in early lactation as they had little weight to lose. Energy supplied by Crystalyx blocks has not been included in the tables above and might provide an additional 1.4 MJ of energy assuming an intake of 85g/hd/day.

Farm 7 Charollais Powys

Assumptions:
Ewe weight 90 kg, Condition score 3.0, Lambing from 8 Dec 2012 indoors
Overall litter size: 1.66 (25 singles, 37 twins, 3 multiples)

Pregnancy rations

Table 21. Reported amounts fed on fresh basis kg/hd/day – same for all groups

Singles	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*		
Hay				*Ad-lib*	*Ad-lib*
Mega ewe nuts					0.5
Crystalyx blocks			*Ad-lib*	*Ad-lib*	*Ad-lib*
Twins	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*		
Hay				*Ad-lib*	*Ad-lib*
Mega ewe nuts			0.5	0.5	0.5
Crystalyx blocks					
		Ad-lib	*Ad-lib*	*Ad-lib*	*Ad-lib*
Triplets	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*		
Hay				*Ad-lib*	*Ad-lib*
Mega ewe nuts			0.5	1.0	1.0
Crystalyx blocks		*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*

Table 22 Analysis of feeds offered

	Silage	**Hay**	**Compound**
Report type	DM basis	DM basis	DM basis
Dry matter (g/kg)	237	874	856
Crude protein (g/kg)	99	103	208
Oil (g/kg)	67	25	56
Ash (g/kg)	100	78	85
NCGD g/kg)			756
NDF (g/kg)	658	590	
ADF (g/kg)		345	
Sugar (g/kg)	4	93	
D value (%)	52	54	
ME (MJ/kg)	8.3	8.6	12.0
FME (MJ/kg)	7.0		
ERDP	69		
DUP	19.2		
pH	4.6		
Ammonia N (% tot N)	15.1		
Acetic acid	17.1		
n butyric acid (g/kg)	9.4		
Lactic acid (g/kg)	5.0		

The silage was of particularly poor quality. Tables below were compiled using the Sheepfeed rationing program:

Single bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)				1.3	1.6
ME supplied (MJ)				11.1	15.0
ME required* (MJ)				15.3	16.4
MP excess (g)				-24	4
Weight change (g)				-88	-6
CP in diet (%)				10.3	13.2

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Single bearing ewes would not have received enough protein from 2 weeks before lambing. The ERDP:FME ratio was low indicating that more protein was required.

Twin bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)				1.7	1.7
ME supplied (MJ)				16.1	16.1
ME required* (MJ)				20.4	22.1
MP excess (g)				-2	-6
Weight change (g)				-73	-116
CP in diet (%)				13.2	13.2

* ME required is based on ewes maintaining weight and is extrapolated from: (Ref: Energy and protein requirements of ruminants)

Twin bearing ewes were underfed energy and protein in the last two weeks of pregnancy and are predicted to lose moderate amounts of weight.

Triplet bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)				2.0	2.0
ME supplied (MJ)				20.0	20.0
ME required* (MJ)				22.5	24.7
MP excess (g)				23	19
Weight change (g)				-18	-70
CP in diet (%)				14.9	14.9

- ME required is based on ewes maintaining weight and is extrapolated from: (Ref: Energy and protein requirements of ruminants)

Triplet bearing ewes were fed less than full energy requirements in the last 2 weeks before lambing.

Lactation rations

Tables below were compiled using the Sheepfeed rationing program.

Table 23. Reported amounts fed on fresh basis kg/hd/day – same for single and twin rearing ewes.

	Weeks post-lambing				
	1	3	6	8	10
Silage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Mega ewe nuts	1.7	1.7	1.7	1.7	

Single rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.7	2.7	2.7	
Pred milk yield (kg)	2.3	2.4	1.9	
ME supplied (MJ)	27.8	27.8	27.8	
ME required* (MJ)	28.1	28.9	24.8	
MP excess (g)	-23	-25	-6	
Weight change (g)	37	20	74	
CP in diet (%)	15.9	15.9	15.9	

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Twin rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.7	2.7	2.7	
Pred milk yield (kg)	*3.7*	*3.8*	*3.1*	
ME supplied (MJ)	27.8	27.8	27.8	
ME required* (MJ)	40.5	41.4	35.6	
MP excess (g)	-93	-98	-63	
Weight change (g)	-310	-338	-190	
CP in diet (%)	15.9	15.9	15.9	

* ME required is based on allowing ewes to maintain weight (Ref: Energy and protein requirements of ruminants)

Ewes rearing singles have adequate energy but would have been deficient in protein in early lactation. Twin rearing ewes were short of protein and predicted to lose a lot of weight in early lactation.

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Farm 8 Charollais Cheshire

Assumptions:
Ewe weight 110 kg, Condition score 3.4 (Triplets 2.7), Lambing from 26 Nov 2012 indoors
Overall litter size: 1.78 (35 singles, 59 twins, 11 multiples)

Pregnancy rations

Table 24. Reported amounts fed on fresh basis kg/hd/day

Singles	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*		
Haylage				*Ad-lib*	*Ad-lib*
Ewe nuts		0	0	0	0
Lifeline*		*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Twins	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*		
Haylage				*Ad-lib*	*Ad-lib*
Ewe nuts	1.0	1.0	2.0	2.0	2.0
Lifeline*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Triplets	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*			
Haylage			*Ad-lib*	*Ad-lib*	*Ad-lib*
Ewe nuts	1.0	2.0	2.0	2.0	2.0
Lifeline*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*

*Intake of Lifeline has been assumed to be 0.2 kg/ewe/day but could be significantly more or less.

Table 25 Analysis of feeds offered

	Haylage	**Ewe nuts**	**Northern Gold**
Report type	DM basis	DM basis	DM basis
Dry matter (g/kg)	778	867	250
Crude protein (g/kg)	87	196	280
Oil-B (g/kg)	25	63	44
Ash (g/kg)	60	81	108
NCGD g/kg)		748	812
NDF (g/kg)	609		
ADF (g/kg)	351		
Sugar (g/kg)	89		
D value (%)	48		
ME (MJ/kg)	7.6	12.1	12.5

Haylage was of poor quality with low energy and protein typical of poor hay. The ewe nuts were of average energy content.

Tables below were compiled using the Sheepfeed rationing program:

Single bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	**Assumed to be adequate at grass			1.8	1.8
ME supplied (MJ)				11.6	11.6
ME required* (MJ)				19.1	20.4
MP excess (g)				-60	-63
Weight change (g)				-173	-203
CP in diet (%)				9.0	9.0

*ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

** Grass quality and quantity unknown

Twin bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	Assumed to be adequate at grass			2.7	2.7
ME supplied (MJ)				29.2	29.2
ME required* (MJ)				22.1	25.9
MP excess (g)				68	65
Weight change (g)				132	104
CP in diet (%)				16.0	16.0

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Triplet bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	Assumed to be adequate at grass			2.7	2.7
ME supplied (MJ)				29.2	29.2
ME required* (MJ)				23.2	24.7
MP excess (g)				66	62
Weight change (g)				106	58
CP in diet (%)				16.0	16.0

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Singles may have been underfed close to lambing unless they were eating a lot of 'Lifeline'. For twins and triplets the predicted amount of long forage in the diet was too low given the very high level of compound feed and was estimated at only 29% of the diet. Twins and triplets appear to have been overfed concentrates in late pregnancy.

Lactation rations

Tables below were compiled using the Sheepfeed rationing program.

Table 26. Reported amounts fed on fresh basis kg/hd/day

Singles	Weeks post-lambing				
	1	3	6	9	10
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Ewe nuts	0.5	0.5	0.5	0.5	0.5
Northern Gold	2.0	2.0	2.0	2.0	2.0
Twins					
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Ewe nuts	2.0	2.0	2.0	2.0	2.0
Northern Gold	2.0	2.0	2.0	2.0	2.0
Triplets					
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Ewe nuts	2.0	2.0	2.0	2.0	2.0
Northern Gold	2.0	2.0	2.0	2.0	2.0

Single rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.9	2.9	2.9	2.9	2.9
Pred milk yield (kg)	2.8	2.9	2.4	1.8	1.7
ME supplied (MJ)	27.7	27.7	27.7	27.7	27.7
ME required* (MJ)	*34.9*	*35.7*	*31.7*	*26.9*	*26.1*
MP excess (g)	-39	-42	-17	8	16
Weight change (g)	-159	-183	-91	23	55
CP in diet (%)	14.5	14.5	14.5	14.5	14.5

* ME required is based on allowing ewes to maintain weight (Ref: Energy and protein requirements of ruminants)

Post-lambing ewes rearing singles were low in protein up to week 6 and deficient in energy assuming the predicted milk yields are achieved in practice.

Twin rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	3.9	3.9	3.9	3.9	3.9
Pred milk yield (kg)	*4.5*	*4.6*	*3.8*	*2.9*	*2.6*
ME supplied (MJ)	40.9	40.9	40.9	40.9	40.9
ME required* (MJ)	*48.5*	*49.3*	*42.9*	*35.7*	*33.3*
MP excess (g)	-11	-16	24	64	76
Weight change (g)	-121	-162	30	188	229
CP in diet (%)	16.7	16.7	16.7	16.7	16.7

* ME required is based on allowing ewes to maintain weight - requirements extrapolated from: (Ref: Energy and protein requirements of ruminants)

Twins were deficient in energy and protein in early lactation (assuming the very high milk yields are achieved in practice) but predicted weight loss was not excessive. By weeks 9-10 when milk yields are predicted to fall ewes are likely to have been gaining a lot of weight. Requirements for triplet rearing ewes are high in the first month of lactation and the diet is unlikely to have fully met energy and protein requirements.

Overview of farm diets, Year 1

			Last 4 weeks pregnancy		First four weeks lactation	
Farm no.	**Farm name**		**Energy**	**Protein**	**Energy**	**Protein**
1	**Char Shrop.**	Single	Slightly overfed	OK	Overfed	OK
		Twin	OK *	OK	Deficient ***	Deficient
		Triplet	Deficient **	Deficient	Deficient ***	Deficient
2	**Tex Heref.**	Single	OK	Deficient	OK	Deficient
		Twin	Deficient *	Very deficient	Deficient ***	Very deficient
		Triplet	Deficient **	Very deficient	Deficient ***	Very deficient
3	**Comm W. Suss**	Single	OK	OK	OK	OK
		Twin	OK	OK	Deficient **	Deficient
		Triplet	OK	OK	N/A	N/A
4	**Lleyn Nor**	Single	OK	Slightly deficient	OK	OK
		Twin	OK	OK	Deficient **	Deficient
		Triplet	OK	Deficient	Deficient ***	Deficient
5	**Lleyn Per.**	Single	Overfed	Overfed	OK	OK
		Twin	Slightly overfed	Overfed	Deficient **	Deficient
		Triplet	OK	Overfed	N/A	N/A
6	**Tex Gwy.**	Single	Overfed	OK	OK *	OK
	(Most ewes)[1]	Twin	Slightly deficient *	OK	Deficient ***	Deficient
7	**Char Powys**	Single	OK *	Deficient	OK	Deficient
		Twin	Slightly deficient *	Slightly deficient	Deficient ***	Deficient
		Triplet	OK *	OK	N/A	N/A
8	**Char Ches.**	Single	Deficient **	Deficient	Deficient **	Below req
		Twin	Overfed	Overfed	Deficient **	Below req
		Triplet	Overfed	Overfed	Deficient ***	Deficient

[1] Farm 6 did not scan ewes – flock split by condition into fitter and thinner ewes. Thinner ewes well fed.

Note that ewes can mobilise a lot of body weight in early lactation if they lamb down in good condition. Early lactation diets would rarely meet the ewe's full theoretical requirements for energy and protein. As a guide to the degree of energy deficiency diets have been coded as follows:

* Predicted weight loss up to 100g/day
** Predicted weight loss 101 – 200 g/day
*** Predicted weight loss >200 g/day

100 to 200 g/day body weight loss would in practice be acceptable.

Year 2

Lambing 2013 – 2014

Data from 6 farms

Farm 1 Pedigree Charollais sheep – Shropshire

Assumptions:
Ewe weight 82 kg, Condition score 4.0, Lambing from 1 Dec 2013 indoors
Overall litter size: 1.80 (17 singles (CS 4.29), 25 twins (CS 4.34), 7 multiples (CS 4.14)
Lambs creep fed from 3 weeks, I'ansons 18% ad lib until 20 weeks

Grass: Sward composition - clover mix
Grass height 3 months to lambing: (approx. 5 cm)

Pregnancy rations

Table 1. Reported amounts fed on fresh basis kg/hd/day

Singles	Weeks pre-lambing					
	8	6	4	3	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*			
Haylage				*Ad-lib*	*Ad-lib*	*Ad-lib*
Compound		0.23	0.23	0.45	0.45	0.68
Twins & triplets	Weeks pre-lambing					
	8	6	4	3	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*			
Haylage				*Ad-lib*	*Ad-lib*	*Ad-lib*
Compound		0.23	0.45	0.68	0.68	0.91

Table 2 Analysis of feeds offered

	Haylage	**Concentrate**
Report type	DM basis	DM basis
Dry matter (g/kg)	648	876
Crude protein (g/kg)	106	201
Oil-B (g/kg)	26	59
Ash (g/kg)	75	82
NCGD g/kg)		793
NDF (g/kg)	605	
ADF (g/kg)	350	
Sugar (g/kg)	90	
D value (%)	54	
ME (MJ/kg)	8.7	12.6

Ewes also had access to 'Coxi clear ewe tubby' from 2 weeks pre-lambing to 10 weeks after lambing, but at 20g/hd/day predicted intake this has a negligible impact on energy and protein intake.

Tables below were compiled using the Sheepfeed rationing program:

Single bearing ewes

	Weeks pre-lambing				
	8	6	3	2	1
DMI (predicted) (kg)			1.6	1.6	1.7
ME supplied (MJ)			15.1	15.1	17.0
ME required* (MJ)			13.1	13.9	14.9
MP supplied (g)			119	121	134
MP required (g)			105	109	112
MP excess (g)			13	12	23
Weight change (g)			28	13	33
CP in diet (%)			13.0	13.0	14.0

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Twin bearing ewes

	Weeks pre-lambing				
	8	6	3	2	1
DMI (predicted) (kg)			1.7	1.7	1.8
ME supplied (MJ)			17.0	17.0	18.9
ME required* (MJ)			16.2	17.6	19.2
MP supplied (g)			140	143	160
MP required (g)			119	125	131
MP excess (g)			21	18	28
Weight change (g)			16	-13	-2
CP in diet (%)			14.0	14.0	14.8

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Triplet bearing ewes

	Weeks pre-lambing				
	8	6	3	2	1
DMI (predicted) (kg)			1.7	1.7	1.8
ME supplied (MJ)			17.0	17.0	18.9
ME required* (MJ)			17.8	19.5	21.5
MP supplied (g)			144	145	164
MP required (g)			126	134	141
MP excess (g)			18	11	23
Weight change (g)			-13	-58	-56
CP in diet (%)			14.0	14.0	14.8

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Comments

Single bearing ewes: Generally fine – energy supply above requirements in the last 3 weeks, protein levels OK (program shows small MP surplus). Weight change OK

Twin bearing ewes: Diet is close to requirements in last 3 weeks pre-lambing, program shows small MP excess. Weight change minimal.

Triplet bearing ewes: Diet fairly close to requirements for protein. Slightly underfed energy in last 2 weeks resulting in small (but acceptable) weight loss.

Lactation rations

Tables below were compiled using the Sheepfeed rationing program. The feeds on offer were as for pregnancy.

Table 3. Reported amounts fed on fresh basis kg/hd/day

Singles	Weeks post-lambing				
	1	3	6	9	10
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Compound	0.68	0.68	0.68	0.68	0
Twins	Weeks post-lambing				
	1	3	6	9	10
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Compound	1.36	1.36	1.36	1.36	1.36

Single rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.2	2.2	2.2	2.2	1.8
Pred milk yield (kg)	2.1	2.1	1.8	1.4	1.2
ME supplied (MJ)	21.1	21.1	21.1	21.1	13.2
ME required* (MJ)	22.0	22.0	22.0	18	16
MP supplied (g)	152	154	160	159	102
MP required (g)	209	212	203	182	174
MP excess (g)	-56	-58	-43	-23	-71
Weight change (g)	-124	-140	-68	17	-134
CP in diet (%)	13.2	13.2	13.2	13.2	10.6

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 2 kg/d for weeks 1-6 inclusive (Ref: Energy and protein requirements of ruminants)

Twin rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	2.6	2.6	2.6	2.6	2.6
Pred milk yield (kg)	*3.4*	*3.4*	*2.8*	*2.2*	*2.0*
ME supplied (MJ)	27.0	27.0	27.0	27.0	27.0
ME required* (MJ)	30.2	30.2	30.2	22.0	22.0
MP supplied (g)	222	222	220	215	214
MP required (g)	299	305	268	231	220
MP excess (g)	-77	-83	-48	-16	-6
Weight change (g)	-230	-256	-121	21	57
CP in diet (%)	15.0	15.0	15.0	15.0	15.0

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 3 kg/d for weeks 1-6 inclusive (Ref: Energy and protein requirements of ruminants)

Singles slightly short of metabolisable protein (MP) in theory but close to requirements for energy. Twins deficient in MP and energy and potentially losing >200g/day in early lactation. As ewes were fit this may not have had any significant effects on performance with ewes able to mobilise body fat.

A change to a slightly higher protein compound for lactation and or better forage is likely to have proved beneficial.

Lamb performance, Year 2

Birth type	Number of lambs	Av. [range] birth wt (kg)	Av. [range] 8wk wt (kg)	Av. [range] DLWG (kg)
Single	18	6.0 [3.0 – 7.5]	30.7 [16.0 – 37.0]	0.41 [0.23 – 0.50]
Twin	52	5.4 [3.0 – 7.5]	25.1 [16.2 - 35.0]	0.34 [0.23 – 0.45]
Triplet	9	4.6 [4.0 – 6.0]	30.5 [26.0 – 33.6]	0.40 [0.34 – 0.45]

Note: 8wk wt = 8 week weight, DLWG = Daily live weight gain

Average lamb birth weights were acceptable for all types of lamb and performance to 8 weeks seems good for singles, marginally below expectations for twins and good for the one set of triplets. Whilst performance of some individuals at 230g/day seems disappointing for pedigree lambs, the twin rearing ewes were slightly short of energy and protein in early lactation which might help to explain the lamb growth rate.

Farm 2 Pedigree Texel sheep – Herefordshire

Assumptions:

Ewe weight: 79 kg, Condition score 3.3, Lambing from 6 Feb 2014 to 25 Apr 2014 indoors (early lambers housed 2-6 weeks pre-lambing, late lambers > 6 weeks pre-lambing)
Overall litter size: 1.66 (34 singles (CS 3.2), 34 twins (CS 3.3), 8 multiples (CS 3.3). Condition scored 8 January 2014
Lambs creep fed from 3-4 weeks, "GLW lamb creep" max 0.5kg/day until 21 weeks

Grass: Sward composition – traditional ley, little clover
Grass height 3 months to lambing: 8cm falling to <1cm in 2 weeks

Pregnancy rations

Table 4. Reported amounts fed on fresh basis kg/hd/day – same for all ewes

		Weeks pre-lambing				
		8	6	4	2	1
Singles	Hay	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
	Concentrates	0.1	0.1	0.4	0.4	0.4
Twins	Hay	*Ad-lib*	*Ad-lib*	*Ad-lib*		
	Haylage				*Ad-lib*	*Ad-lib*
	Concentrates	0.25	0.25	0.6	0.6	0.6
Triplets	Hay	*Ad-lib*	*Ad-lib*	*Ad-lib*		
	Haylage				*Ad-lib*	*Ad-lib*
	Concentrates	0.3	0.3	0.8	0.8	0.8

Table 5. Analysis of feeds offered

	Hay (mean of 2 samples)	**Haylage**	**Concentrate (GLW feeds)**
Report type	DM basis	DM basis	DM basis
Dry matter (g/kg)	848	719	871
Crude protein (g/kg)	102	161	220
Oil-B (g/kg)	26	30	66
Ash (g/kg)	75	94	95
NCGD g/kg)			823
NDF (g/kg)	614	591	
ADF (g/kg)	353	345	
Sugar (g/kg)	88	107	
D value (%)	53	55	
ME (MJ/kg)	8.5	8.9	13.2

Haylage CP as analysed is high relative to the D value. Purchased concentrate feed was good quality.

Tables below were compiled using the Sheepfeed rationing program.

Single bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	1.3	1.3	1.5	1.5	1.5
ME supplied (MJ)	11.4	11.4	14.1	14.1	14.1
ME required* (MJ)	10.3	10.9	12.2	13.9	14.9
MP supplied (g)	83	84	106	110	111
MP required (g)	90	93	99	106	108
MP excess (g)	-7	-9	7	4	2
Weight change (g)	22	1	29	3	-19
CP in diet (%)	8.0	8.0	9.9	9.9	10.3

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Twin bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	1.4	1.4	1.6	1.6	1.6
ME supplied (MJ)	12.7	12.7	15.9	16.6	16.6
ME required* (MJ)	11.6	12.5	14.7	17.6	19.2
MP supplied (g)	95	98	126	164	167
MP required (g)	95	101	110	122	127
MP excess (g)	0	-3	16	42	40
Weight change (g)	32	1	25	-11	-50
CP in diet (%)	12.1	12.1	14.1	18.0	18.0

Triplet bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)	1.4	1.4	1.7	1.7	1.7
ME supplied (MJ)	13.2	13.2	17.7	18.3	18.3
ME required* (MJ)	12.3	13.4	16.0	19.5	21.5
MP supplied (g)	99	103	143	182	186
MP required (g)	97	105	116	130	137
MP excess (g)	2	-2	27	52	49
Weight change (g)	33	-6	40	-7	-55
CP in diet (%)	12.4	12.4	15.1	18.5	48.5

All ewes were fed close to requirements until the last week before lambing when all ewes appeared to be underfed (3.2MJ for triplets and 2.6 MJ for twins) compared to full requirements but received excess protein. The haylage did have a very unusually high level of protein.

Lactation rations

Summary of rearing litter size - 27 rearing singles (CS 2.6), 29 twins (CS 2.3) and 1 triplet (CS 2.0). Condition scored 30 April 2014.

Table 6. Reported amounts fed on fresh basis kg/hd/day – same for all ewes

	Weeks post-lambing				
	1	2	4	6	8
Hay & Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Concentrates	1.5	1.5	1.5	0	0

The relative proportions of hay and haylage in the lactation diet are not specified so have assumed that forage was split 50:50 (as fed).
Forage as fed: weeks 1-4 1.0 kg each of hay and haylage
weeks 6-8 1.3 kg each of hay and haylage

Tables below are compiled using the Sheepfeed rationing program.

Single rearing ewes

	Weeks post-lambing				
	1	2	4	6	8
DMI (predicted) (kg)	2.9	2.9	2.9	2.0	2.0
Pred milk yield (kg)	2	2.1	2.0	1.7	1.4
ME supplied (MJ)	30.7	30.7	30.7	17.4	17.4
ME required* (MJ)	22.0	22.0	22.0	16.4	15.6
MP supplied (g)	286	290	285	173	173
MP required (g)	215	222	211	200	185
MP excess (g)	72	68	75	-27	-13
Weight change (g)	130	117	134	-169	-111
CP in diet (%)	17.0	17.0	17.0	12.9	12.9

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 2 kg/d for weeks 1-6 inclusive (Ref: Energy and protein requirements of ruminants)

Twin rearing ewes

	Weeks post-lambing				
	1	2	4	6	8
DMI (predicted) (kg)	2.9	2.9	2.9	2.0	2.0
Pred milk yield (kg)	*3.2*	*3.4*	*3.1*	*2.7*	*2.3*
ME supplied (MJ)	30.7	30.7	30.7	17.4	17.4
ME required* (MJ)	30.2	30.2	30.2	22.0	22.0
MP supplied (g)	308	308	308	176	175
MP required (g)	302	313	295	268	241
MP excess (g)	6	-5	12	-93	-66
Weight change (g)	-88	-129	-76	-400	-307
CP in diet (%)	17.0	17.0	17.0	12.9	12.9

* ME required is based on allowing ewes to lose 100g/day and assuming milk yield of 3 kg/d for weeks 1-6 inclusive and 2 kg/d wks 9/10 (Ref: Energy and protein requirements of ruminants)

Triplet rearing ewes (note only 1 ewe reported to be rearing triplets)

	Weeks post-lambing				
	1	2	4	6	8
DMI (predicted) (kg)	2.9	2.9	2.9	2.0	2.0
Pred milk yield (kg)	*4.0*	*4.2*	*3.9*	*3.4*	*2.9*
ME supplied (MJ)	30.7	30.7	30.7	17.4	17.4
ME required* (MJ)	*39*	*39*	*33*	26	26
MP supplied (g)	310	311	310	177	176
MP required (g)	360	374	352	317	282
MP excess (g)	-50	-63	-42	-140	-106
Weight change (g)	-284	-335	-269	-555	-438
CP in diet (%)	17.0	17.0	17.0	12.9	12.9

* ME required is based on allowing ewes to lose 100g/day and extrapolating from information in the reference book for twin rearing ewes (Ref: Energy and protein requirements of ruminants)

Singles would have generally gained weight in the first month of lactation and were well supplied with energy and protein. From week 6 post-lambing losing weight and deficient in protein.
Twins predicted to lose an acceptable amount of body weight in the first month and protein supply close to requirements. From 6 weeks post-lambing deficient in protein and predicted to lose large amounts of body weight (although will depend on whether ewes were at grass by this point or lambs had been weaned off).

Farm 2. Texel, Herefordshire. Year 2

	Number of lambs	Average birth weight (kg)	Range of birth weights (kg)
Born as 1	31	6.5	3.7 – 8.8
Born as 2	66	5.3	2.6 – 7.3
Born as 3	17	4.4	3.1 – 6.2

Farm 2. Texel, Herefordshire. Year 2

	Average 8wk wgt	Range 8wk wgt	Average DLWG	Range DLWG
Born as 1	32.6kg	22.0 – 42.0kg	0.56kg	0.39 – 0.83kg
Born as 2	29.0kg	13.5 – 39.5kg	0.48kg	0.23 – 0.63kg
Born as 3	28.8kg	24.0 – 35.0kg	0.44kg	0.34 – 0.57kg

Lamb performance: Births weights were on average good with a typical range about the mean. Growth rates to 8 weeks were exceptionally good showing that ewes were milking well and feeding their high genetic merit lambs to reach potential.

Farm 4 Lleyn sheep - Northumberland

Assumptions:

Ewe weight 70 kg, Condition score 3.1, Lambing from 29 March – 28 May 2014 mainly outdoors except for 330 early lambers
Overall litter size: 1.66 (579 singles (CS 3.2), 790 twins (CS 3.1), 82 multiples CS 3.0))
Condition scored 13-14 March 2014
No creep feed for lambs

Grass: Sward composition ryegrass/white clover and some old mixed pasture. Cocksfoot and tall fescue being added.
Grass height 3 months to lambing: 7cm at best, not falling below 3cm.

Pregnancy rations

Table 7. Reported amounts fed on fresh basis kg/hd/day – grass intake figures in pregnancy based on appetite of 2.0% of live weight for single bearing ewes (1.4kgDM) and 2.5% of live weight for twin/triplet bearing ewes (1.75kgDM).

	Weeks pre-lambing					
All outdoor lambers	8	6	4	3	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Indoor early lambers						
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*			
Silage				*Ad-lib*	*Ad-lib*	*Ad-lib*
Soya expeller mix				0.18	0.18	0.18

Table 8 Analysis of feeds offered

	Silage	**Soya expeller***
Report type	**DM basis**	**DM basis**
Dry matter (g/kg)	249	907
Crude protein (g/kg)	179	493
Oil (g/kg)	51	77
Ash (g/kg)	90	68
NDF (g/kg)	507	111
Sugar (g/kg)	4	
D value (%)	67	
ME (MJ/kg)	10.8	14.7
FME (MJ/kg)	6.5	(12.7) HiPro
Potential intake (FiM) (g/kgW 0.75)	113	
pH	4.8	
Ammonia N (% total N)	17.5	
Pot. Acid loading (FiM) (meq/kg)	929	
ERDP (FiM) (g/kg)	140	
DUP (FiM) (g/kg)	27.7	
Acetic acid (g/kg)	43.7	
n Butyric acid (g/kg)	19.1	
Tot. Ferm. Acids (FiM) (g/kg)	182.6	

	Silage	Soya expeller*
Report type	**DM basis**	**DM basis**
Lactic acid (g/kg)	110.7	
Nitrogen solubility (N)	0.58	
(a)	0.68	
(b)	0.23	
(c; per hour)	0.079	
Dry matter solubility (S)	0.27	
(a)	0.34	
(b)	0.57	
(c; per hour)	0.035	

Grass silage of high digestibility and ME, but rather high ammonia indicating some protein breakdown. Quite high pH indicating relatively poor stability of the fermentation.
* Note: the soya expeller was actually a high protein blend containing a range of ingredients and therefore the analysis is likely to differ from that in the table above (Soya expeller analysis from www.feedipedia.org/).

For the 2014 lambing season have assumed grass supply quality to be ME 10.8 and CP of 17% - (Ref Grass 3/4 from Energy and Protein requirements).

Single bearing ewes – have assumed a slightly lower DMI than for twins

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.40	1.40	1.40	1.40	1.40
ME supplied (MJ)	15.1	15.1	15.1	15.1	15.1
ME required* (MJ)	10.2	11.1	12.3	13.9	14.8
MP supplied (g)	163	163	163	163	163
MP required (g)	88	92	97	103	105
MP excess (g)	75	71	66	60	58
CP in diet (%)	17.0	17.0	17.0	17.0	17.0

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants). Feeding level is assumed to be 1.8 throughout (single ewe at 1 week pre-lambing)

If grass quality assumptions are correct then this diet fully meets both energy and protein to single-bearing ewes in late pregnancy.

Twin bearing ewes – assumed higher DMI than single ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	18.9	18.9	18.9	18.9	18.9
ME required* (MJ)	11.8	12.6	14.6	17.2	18.7
MP supplied (g)	166	166	166	166	166
MP required (g)	93	98	107	117	122
MP excess (g)	73	68	59	49	44
CP in diet (%)	17.0	17.0	17.0	17.0	17.0

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants). Feeding level is assumed to be 2.3 throughout (twin-bearing ewe at 1 week pre-lambing). Book value suggests feeding level increases from 1.5 at seven weeks pre-lambing to 2.3 at 1 week pre-lambing.

Diet is predicted to fully meet the energy and oversupply protein requirements throughout late pregnancy.

Triplet bearing ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	18.9	18.9	18.9	18.9	18.9
ME required* (MJ)	12.4	13.4	15.7	19.0	20.6
MP supplied (g)	166	166	166	166	166
MP required (g)	95	102	112	125	131
MP excess (g)	71	64	54	41	35
CP in diet (%)	17.0	17.0	17.0	17.0	17.0

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and protein requirements of ruminants). Have assumed feeding level as for twin ewes.

Diet is predicted to fully meet the energy requirements until one week pre-lambing and oversupply protein requirements throughout late pregnancy.

Additional diet check for early lambing ewes housed for last 3 weeks pre-lambing (assume 70 kg liveweight) silage plus soya expeller mix (at 0.18 kg/day). Diet below produced with ADAS Sheepfeed program.

Single bearing ewes

	Weeks pre-lambing				
	8	6	3	2	1
DMI (predicted) (kg)			1.2	1.2	1.2
ME supplied (MJ)			13.2	13.2	13.2
ME required* (MJ)	10.3	10.9	12.2	13.9	14.9
ERDP:FME			20.6	20.4	20.3
MP supplied (g)			95	97	98
MP required (g)			93	96	99
MP excess (g)			2	1	0
Weight change (g)			31	16	0
CP in diet (%)			22.2	22.2	22.2

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Twin bearing ewes

	Weeks pre-lambing				
	8	6	3	2	1
DMI (predicted) (kg)			1.2	1.2	1.2
ME supplied (MJ)			13.2	13.2	13.2
ME required* (MJ)	11.6	12.5	14.7	17.6	19.2
ERDP:FME			19.8	19.5	19.2
MP supplied (g)			103	107	109
MP required (g)			105	111	116
MP excess (g)			-2	-4	-7
Weight change (g)			-21	-53	-89
CP in diet (%)			22.2	22.2	22.2

Triplet bearing ewes

	Weeks pre-lambing				
	8	6	3	2	1
DMI (predicted) (kg)			1.2	1.2	1.2
ME supplied (MJ)			13.2	13.2	13.2
ME required* (MJ)	12.3	13.4	16.0	19.5	21.5
ERDP:FME			19.4	19.1	18.8
MP supplied (g)			107	110	113
MP required (g)			111	118	125
MP excess (g)			-4	-8	-11
Weight change (g)			-53	-91	-134
CP in diet (%)			22.2	22.2	22.2

Above rations show very high ERDP:FME ratio since silage had very high level of protein. Dry matter intake predicted is below actual and the balance of the diet would have been improved by providing some rumen available carbohydrate with this very high protein silage. Worked well in practice since silage intakes better than predicted.

Lactation rations

Assumptions: Ewe weight 70 kg.
Post-lambing condition score 435 singles (CS 2.9), 180 twins (CS 2.4), 16 triplets (CS 2.8)
Condition scored 24-26 June and 8-11 July 2014 (approximately 3 months post-lambing)

Assume dry matter intakes 3.0% of live weight. Assume high quality grass in lactation (12.3 ME and 19% CP).

Table 9. Reported amounts fed on fresh basis kg/hd/day - all ewes

	Weeks post-lambing			
	1	3	6	9
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*

Single rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*1.8*	*1.8*	*1.5*	*1.2*
ME supplied (MJ)	25.8	25.8	25.8	25.8
ME required* (MJ)	23.3	23.3	19.2	16.8
MP supplied (g)	242	242	241	237
MP required (g)	222	222	184	161
MP excess (g)	20	20	57	76
CP in diet (%)	19.0	19.0	19.0	19.0

* ME required is based on allowing ewes to lose 50g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

Energy levels above requirements so ewes likely to be maintaining or gaining weight. MP in excess throughout.

Twin rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*2.9*	*2.9*	*2.4*	*1.9*
ME supplied (MJ)	25.8	25.8	25.8	25.8
ME required* (MJ)	29.8	29.8	24.8	21.4
MP supplied (g)	255	255	250	250
MP required (g)	291	291	245	215
MP excess (g)	-36	-36	5	35
CP in diet (%)	19.0	19.0	19.0	19.0

* ME required is based on allowing ewes to lose 100g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

ME below requirements for first month so ewes predicted to lose more than 100g/day in this period. MP deficit for the first month but by week six close to (and then exceeding requirements).

Triplet rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*3.6*	*3.7*	*3*	*2.3*
ME supplied (MJ)	25.8	25.8	25.8	25.8
ME required* (MJ)	34.6	35.4	29.8	24.0
MP supplied (g)	257	257	255	255
MP required (g)	337	344	291	238
MP excess (g)	-80	-87	-36	17
CP in diet (%)	19.0	19.0	19.0	19.0

* ME required is based on allowing ewes to lose 100g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

16 ewes reported as rearing triplets. ME below requirements for first 6 weeks so ewes predicted to lose more than 100g/day in this period. Large MP deficit in the first month and a lower deficit to 6 weeks of lactation. A small amount of an energy supplement may well have improved MP supplied.

Farm 5 - Lleyn Perth and Kinross

Assumptions:
Ewe weight 70 kg, Condition score 3.1, Lambing from 16th April – 9th June 2014 outdoors (except for 75 singles housed 1 week before lambing)
Overall litter size: 1.85 (77 singles (CS 3.0), 205 twins (CS 3.2), 29 multiples (CS 3.1))
Condition scored 20/03/14
No creep feed for lambs

Grass sward composition: Permanent pasture, ryegrass, white clover and meadow grasses. Sward height in 3 months up to lambing 3.75 cm (1.5 inches)

Pregnancy rations

Table 10. Reported amounts fed on fresh basis kg/hd/day

Singles and twins	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	
Hay					*Ad-lib*
Concentrates	0.25	0.25	0.25	0.25	0.25
Twins & triplets	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Concentrates	0.25	0.25	0.25	0.25	0.25

Plus Rumenco 'Lifeline' buckets– (800 kg fed from 3 weeks pre-lambing to end of lambing) estimated intake based on only pregnant ewes receiving Lifeline and average lambing date of 15th May (i.e. approx. 50 days on average for 311 ewes in lamb) Approximately 50g/hd/day fed.

Table 11 Analysis of feeds offered

	Hay	**Concentrate**
Report type	DM basis	DM basis
Dry matter (g/kg)	838	874
Crude protein (g/kg)	87	195
Oil (g/kg)	24	44
Ash (g/kg)	71	82
NDF (g/kg)	604	
ADF (g/kg)	350	
Sugar (g/kg)	82	
NCGD (g/kg)		773
D value (%)	54	
ME (MJ/kg)	8.7	11.9

Lifeline buckets fed from 3 wks pre-lambing to end lambing
DM 900g/kg ME 13.0 MJ/kgDM, FME 12.6 MJ/kgDM MJ, CP 12.0 %

Moderate quality hay and moderate energy concentrate.

Single bearing ewes grass intake figures in pregnancy based on appetite of 2.0% of live weight for single bearing ewes (1.4kgDM) but increased to 1.5 kg because some concentrate fed – ewes housed 1 week pre-lambing.

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.5	1.5	1.5	1.5	1.5
ME supplied (MJ)	18.4	18.4	18.4	18.4	14.0
ME required* (MJ)	10.2	11.1	12.3	13.9	14.8
MP supplied (g)	151	151	151	151	94
MP required (g)	88	92	97	103	105
MP excess (g)	63	59	54	48	-11
CP in diet (%)	188	188	188	188	104

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and Protein Requirements of Ruminants)

Twin bearing ewes DMI of 2.5% of live weight for twin/triplet bearing ewes (1.75kgDM)

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	21.4	21.4	21.4	21.3	21.3
ME required* (MJ)	11.8	12.6	14.6	17.2	18.7
MP supplied (g)	169	169	169	169	169
MP required (g)	93	98	107	117	122
MP excess (g)	76	71	62	52	47
CP in diet (%)	191	191	191	18.9	18.9

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and Protein Requirements of Ruminants)

Triplet bearing ewes

	Weeks pre-lambing				
	7-8	5-6	3-4	2	1
DMI (predicted) (kg)	1.75	1.75	1.75	1.75	1.75
ME supplied (MJ)	21.4	21.4	21.4	21.3	21.3
ME required* (MJ)	12.4	13.4	15.7	19.0	20.6
MP supplied (g)	169	169	169	169	169
MP required (g)	95	102	112	125	131
MP excess (g)	74	71	57	44	38
CP in diet (%)	191	191	191	18.9	18.9

* ME required is based on allowing ewes to maintain live weight (Ref: Energy and Protein Requirements of Ruminants)

Diet offered met requirements of all ewes with energy and protein in excess of requirements. The only exception to this is the single bearing ewes in the week before lambing when they are housed on hay and concentrate - these ewes are slightly deficient in energy and protein. This suggests that ewes would have been gaining weight particularly between 8 and 3 weeks before lambing.

Lactation rations

Assumptions: Ewe weight 70 kg.
Post-lambing condition score 91 singles (CS 2.8), 205 twins (CS 2.4), 4 triplets (CS 2.3)
Condition scored 2 July 2014 (approximately 6 weeks post-lambing)

Assume dry matter intakes of 3.0% of live weight. Assume high quality grass in lactation (12.3 ME and 19% CP). Assumes that sufficient grass is available to meet appetite.

Table 12. Reported amounts fed on fresh basis kg/hd/day (all ewes)

	Weeks post-lambing			
	1-2	3-4	6	9
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Concentrates	0.5	0	0	0

Have assumed lifeline not offered to lactating ewes

Single rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*1.8*	*1.8*	*1.5*	*1.2*
ME supplied (MJ)	25.7	25.8	25.8	25.8
ME required* (MJ)	23.3	23.3	19.2	16.8
MP supplied (g)	250	242	241	237
MP required (g)	222	222	184	161
MP excess (g)	28	20	57	76
CP in diet (%)	19.1	19.0	19.0	19.0

* ME required is based on allowing ewes to lose 50g/day (extrapolated from Ref: Energy and protein requirements of ruminants)

Energy levels above requirements throughout so ewes likely to be maintaining or gaining weight. MP in excess throughout.

Twin rearing ewes

	Weeks post-lambing			
	1-2	3	6	9
DMI (predicted) (kg)	2.1	2.1	2.1	2.1
Pred milk yield (kg)	*2.9*	*2.9*	*2.4*	*1.9*
ME supplied (MJ)	25.7	25.8	25.8	25.8
ME required* (MJ)	29.8	29.8	24.8	21.4
MP supplied (g)	259	255	250	250
MP required (g)	291	291	245	215
MP excess (g)	-32	-36	5	35
CP in diet (%)	19.0	19.0	19.0	19.0

* ME required is based on allowing ewes to lose 100g/day (extrapolated from Ref: Energy and protein requirements of ruminants

ME below requirements for first month so ewes are predicted to lose more than 100g/day in this period. MP deficit for the first month but close to requirements from week 6.
Only 4 ewes reported to rear triplets.

Farm 5.Lleyn, Perth and Kinross. Year 2

	Number of lambs	Average birth weight	Range of birth weights
Born as 1	83	4.3	3.0 – 6.0kg
Born as 2	395	3.8	2.5 – 6.0kg
Born as 3 / 4	77	3.3	2.0 – 4.0kg

Farm 5. Lleyn, Perth and Kinross. Year 2

	Average 8wk wt	Range 8wk wt	Average DLWG	Range DLWG
Born as 1	26.6kg	12.5 – 34.5kg	0.35kg	0.28 – 0.43kg
Born as 2	24.9kg	9.5 – 36.0kg	0.31kg	0.19 – 0.43kg
Born as 3 / 4	24.2kg	15.5 – 32.0kg	0.33kg	0.24 - 0.40kg

Farm 7 Charollais - Powys

Assumptions:
Ewe weight 90 kg, Condition score 3.6, Lambing from 9 Dec 2013 indoors
Overall litter size: 1.92 (22 singles 50 twins, 15 multiples)
Lambs fed creep from 10 – 14 days, "Bibby's" ad lib until weaning and beyond

Grass: Sward composition: Permanent pasture/parkland, couch/meadow grass "rubbish".
Grass height in 3 months to lambing: 8 inches when introduced, 2 inches left after 2 months. (20cm reducing to 5cm 1 month pre-lambing)
This year ewes were weaned onto grass two days after visit [12/2/2014], with about 2" of grass cover, and then moved onto a thin crop of regrown fodder rape [21/02/2014] (on maintenance only).

Pregnancy rations

Table 13. Reported amounts fed on fresh basis kg/hd/day – same for all groups

Singles	Weeks pre-lambing				
	8	6	4	2	1
Hay				*Ad-lib*	*Ad-lib*
Concentrates					0.46
Fodder beet	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	
Crystalyx blocks	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Twins	Weeks pre-lambing				
	8	6	4	2	1
Hay				*Ad-lib*	*Ad-lib*
Mega ewe nuts					0.46
Fodder beet	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	
Crystalyx blocks	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Triplets	Weeks pre-lambing				
	8	6	4	2	1
Silage			*Ad-lib*	*Ad-lib*	*Ad-lib*
Concentrates			0.46	0.91	0.91
Crystalyx blocks	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*

Assume a daily intake of Crystalyx of 53g/ewe. Note this is 16 MJ/kgDM, CP 12% with urea (protein equivalent 5%)

Information provided by the farmer on fodder beet intakes pre-lambing:
Ewes had access to fodder beet ad lib at 90% of dry matter intake (assumed to be 3% body weight and an average body weight of 85 kg) Note ewes were reported to be 90 kg in year 1.

This would equate to total DMI of 2.55 kg – taking off 0.05kg for Crystalyx leaves 2.5 kg from other feeds of which 90% is fodder beet (2.25 kg DM). This equates to approx. 12 kg of fodder beet as fed. Assume that in week 2 pre-lambing this drops to 8 kg as hay is introduced.
Presume the remaining 0.25 kg DM is grazed grass? To get a rough idea of how this might affect diet have allowed 0.3 kg of hay in week 4 but this probably underestimates ME from grass.

Table 14 Analysis of feeds offered

	Silage	Hay	Compound *
Report type	DM basis	DM basis	DM basis
Dry matter (g/kg)	655	849	874
Crude protein (g/kg)	142	112	203
Oil (g/kg)	32	27	59
Ash (g/kg)	85	78	90
NCGD g/kg)			797
NDF (g/kg)	561	623	
ADF (g/kg)		356	
Sugar (g/kg)	31	90	
D value (%)	66	52	
ME (MJ/kg)	10.5	8.4	12.6
FME (MJ/kg)	8.4		
ERDP	97		
DUP	17.2		
pH	5.3		
Ammonia N (% tot N)	10.6		
Acetic acid	10.5		
n butyric acid (g/kg)	6.4		
Lactic acid (g/kg)	46.4		

* Compound values are mean of two samples. Tables below were compiled using the Sheepfeed rationing program:

Single bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			2.5	2.2	1.7
ME supplied (MJ)			28.6	24.2	16.2
ME required* (MJ)			14.3	15.3	16.4
MP supplied (g)			99	109	128
MP required (g)			110	117	120
MP excess (g)			-11	-8	7
Weight change (g)			133	81	-3
CP in diet (%)			6.9	8.1	13.4

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Single bearing ewes: if assumptions on DMI of fodder beet are correct this group. is oversupplied with energy when outside on fodder beet but diet is low in protein. ERDP:FME ratio very low (4.5 and 5.4 for weeks 4 and 2 respectively). Ration at 1 week pre-lambing fairly well matched to requirements.

Twin bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			2.5	2.2	1.7
ME supplied (MJ)			28.6	24.2	16.2
ME required* (MJ)			16.1	19.4	21.1
MP supplied (g)			100	111	139
MP required (g)			122	135	141
MP excess (g)			-23	-25	-3
Weight change (g)			129	48	-112
CP in diet (%)			6.9	8.1	13.4

* ME required is based on allowing ewes to lose 50g/day and is extrapolated from: (Ref: Energy and protein requirements of ruminants)

Twin bearing ewes: as above if DMI intakes of fodder beet are correct this group. is oversupplied with energy when outside on fodder beet but diet is low in protein. ERDP:FME ratio very low (4.4 and 5.2 for weeks 4 and 2 respectively). Ration at 1 week pre-lambing is below requirement in energy and has a small MP deficit.

Triplet bearing ewes

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			1.7	1.9	1.9
ME supplied (MJ)			18.6	22.0	22.0
ME required* (MJ)			17.6	21.5	23.7
MP supplied (g)			130	165	168
MP required (g)			129	144	152
MP excess (g)			2	21	16
Weight change (g)			30	32	-10
CP in diet (%)			15.6	16.7	16.7

- ME required is based on allowing ewes to lose 50g/day and is extrapolated from: (Ref: Energy and protein requirements of ruminants)

Triplet bearing ewes – diet pretty well matched to requirements in last 4 weeks

Lactation rations

Tables below were compiled using the Sheepfeed rationing program.

Table 15. Reported amounts fed on fresh basis kg/hd/day – same rates fed for single and twin rearing ewes.

	Weeks post-lambing				
	1	3	6	8	10
Silage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Fodder beet	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	
Concentrates	0.91	0.91	0.91	0.91	

For tables below have assumed that concentrates are fixed and then remaining DMI is split roughly two thirds from silage and one third from fodder beet. (Assume 4.5kg fodder beet). Actual quantities consumed could be very different.

Single rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	3.1	3.1	3.1	
Pred milk yield (kg)	2.3	2.4	1.9	
ME supplied (MJ)	35.9	35.9	35.9	
ME required* (MJ)	28.1	28.9	24.8	
MP supplied (g)	246	247	248	
MP required (g)	227	231	221	
MP excess (g)	19	15	27	
Weight change (g)	222	215	292	
CP in diet (%)	13.7	13.7	13.7	

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Twin rearing ewes

	Weeks post-lambing			
	1	3	6	9
DMI (predicted) (kg)	3.1	3.1	3.1	
Pred milk yield (kg)	*3.7*	*3.8*	*3.1*	
ME supplied (MJ)	35.9	35.9	35.9	
ME required* (MJ)	38.5	39.4	33.6	
MP supplied (g)	250	250	250	
MP required (g)	327	333	292	
MP excess (g)	-77	-83	-43	
Weight change (g)	-33	-62	69	
CP in diet (%)	13.7	13.7	13.7	

* ME required is based on allowing
requirements of ruminants)

Single rearing ewes predicted to gain a lot of weight if assumed fodder beet intakes are anywhere near actual. ERDP:FME ratio lower than ideal (9.2-9.4). MP levels show small excess.
Twins rearing ewes very low in protein but reasonable energy so ewes predicted to lose modest amount of weight in first month of lactation. ERDP:FME ration lower than ideal at 8.9 -9.0. Need to check ration balance with forage quality and perhaps feed a higher protein compound if feeding large amounts of fodder beet.

Lamb performance, Year 2

Birth type	**Number of lambs**	**Av. [range] birth wt (kg)**	**Av. [range] 8wk wt (kg)**	**Av. [range] DLWG (kg)**
Single	30	4.8 [3.5 – 6.0]	29.8 [19.0 – 40.0]	0.34 [0.18 – 0.49]
Twin	80	4.3 [3.0 – 5.5]	28.8 [19.0 – 39.5]	0.33 [0.20 – 0.46]
Triplet	36	4.0 [3.0 – 5.0]	29.1 [20.0 - 47.5]	0.33 [0.22 – 0.56]

Note: 8wk wt = 8 week weight, DLWG = Daily live weight gain

Low protein supply up to 1 week pre-lambing may have affected lamb birth weight as weights for singles and twins rather lower than expected. DLWG disappointing for singles in particular.

Farm 8 Charollais - Cheshire

Assumptions:
Ewe weight 110 kg, Condition score 3.8 (Triplets 3.4), Lambing from 28 Nov 2013 indoors. Housed 1-2 weeks pre-lambing
Overall litter size: 1.83 (51 singles, 67 twins, 26 multiples)
Lambs creep fed from >3weeks, ad lib

Grass: Sward composition: high sugar ryegrass 80%, 5-20% white clover
Grass height 3 months to lambing: 3 – 4 inches (7-10 cm)

Pregnancy rations

Table 16. Reported amounts fed on fresh basis kg/hd/day

Singles	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	
Hay					*Ad-lib*
Concentrate			1.0	1.0	1.0
Twins	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	
Hay					*Ad-lib*
Concentrate		1.0	1.5	2.0	2.0
Triplets	Weeks pre-lambing				
	8	6	4	2	1
Grass	*Ad-lib*	*Ad-lib*	*Ad-lib*		
Haylage				*Ad-lib*	*Ad-lib*
Ewe nuts	1.0	1.5	1.5	2.0	2.0
Lifeline*			*Ad-lib*	*Ad-lib*	*Ad-lib*

*Intake of Lifeline has been assumed to be 0.2 kg/ewe/day but could be significantly more or less. 10 buckets/45 ewes from 4 weeks equivalent to 180 g/head/day.

Table 17 Analysis of feeds offered

	Haylage	**Ewe nuts**
Report type	DM basis	DM basis
Dry matter (g/kg)	617	834
Crude protein (g/kg)	122	199
Oil-B (g/kg)	23	79
Ash (g/kg)	95	85
NCGD g/kg)		804
NDF (g/kg)	605	
ADF (g/kg)	350	
Sugar (g/kg)	74	
D value (%)	60	
ME (MJ/kg)	9.7	13.1

Tables below were compiled using the Sheepfeed rationing program:

Single bearing ewes (housed 1 week pre-lambing)

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)				2.4	2.4
ME supplied (MJ)				26.5	26.5
ME required* (MJ)				19.1	20.4
MP supplied (g)				213	214
MP required (g)				138	141
MP excess (g)				75	73
Weight change (g)				180	156
CP in diet (%)				14.6	14.6

*ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

** Grass quality and quantity unknown

Twin bearing ewes (housed 1 week pre-lambing)

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)				3.0	3.0
ME supplied (MJ)				34.8	34.8
ME required* (MJ)				22.1	25.9
MP supplied (g)				286	289
MP required (g)				158	165
MP excess (g)				128	123
Weight change (g)				254	228
CP in diet (%)				16.2	16.2

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Triplet bearing ewes (housed 2 weeks pre-lambing)

	Weeks pre-lambing				
	8	6	4	2	1
DMI (predicted) (kg)			2.8	3.1	3.1
ME supplied (MJ)			32.1	36.2	36.2
ME required* (MJ)			19.0	23.2	24.7
MP supplied (g)			259	295	296
MP required (g)			151	170	179
MP excess (g)			107	125	117
Weight change (g)			266	242	210
CP in diet (%)			15.4	16.1	16.1

* ME required is based on allowing ewes to lose 50g/day (Ref: Energy and protein requirements of ruminants)

Long forage is low in triplet diet at 38% in last two weeks (should ideally not be less than 60%). All of the ewes appear to be overfed in terms of energy and protein in late pregnancy suggesting concentrate levels are higher than necessary.

Lactation rations

Tables below were compiled using the Sheepfeed rationing program.

Table 18. Reported amounts fed on fresh basis kg/hd/day

Singles	Weeks post-lambing				
	1	3	6	9	10
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	
Ewe nuts	0.5	0.5	0.5	0.5	
Twins					
Haylage	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*	*Ad-lib*
Ewe nuts	1.5	1.5	1.5	1.5	

Single rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	3.3	3.3	3.3	3.3	3
Pred milk yield (kg)	2.8	2.9	2.4	1.8	1.7
ME supplied (MJ)	33.3	33.3	33.3	33.3	29.2
ME required* (MJ)	*34.9*	*35.7*	*31.7*	*26.9*	*26.1*
MP supplied (g)	277	278	280	280	245
MP required (g)	274	279	267	239	227
MP excess (g)	3	-1	13	41	18
Weight change (g)	8	-11	70	165	87
CP in diet (%)	13.1	13.1	13.1	13.1	12.2

* ME required is based on allowing ewes to maintain weight (Ref: Energy and protein requirements of ruminants)

Post-lambing energy supply for ewes rearing singles is close to requirements with only small weight change in first month of lactation.

Twin rearing ewes

	Weeks post-lambing				
	1	3	6	9	10
DMI (predicted) (kg)	3.8	3.8	3.8	3.8	3.0
Pred milk yield (kg)	*4.5*	*4.6*	*3.8*	*2.9*	*2.6*
ME supplied (MJ)	41.6	41.6	41.6	41.6	29.2
ME required* (MJ)	*48.5*	*49.3*	*42.9*	*35.7*	*33.3*
MP supplied (g)	353	354	352	351	247
MP required (g)	395	403	354	304	289
MP excess (g)	-42	-50	-2	47	-42
Weight change (g)	-112	-148	27	186	-120
CP in diet (%)	14.5	14.5	14.5	14.5	12.2

* ME required is based on allowing ewes to maintain weight - requirements extrapolated from: (Ref: Energy and protein requirements of ruminants)

Post-lambing energy supply for ewes rearing singles is close to requirements with only a small weight change in the first month of lactation.

Twins were deficient in energy and protein in the first month of lactation (assuming high milk yields are achieved) but predicted weight loss is acceptable and ewes received very generous feeding in late pregnancy. By week 6 ewes are starting to gain weight and MP is much closer to requirements.

- It is unusual to reduce concentrate feeding levels post lambing when energy and protein requirements increase.

Lamb performance, Year 2

Birth type	Number of lambs	Av. [range] birth wt (kg)	Av. [range] 8wk wt (kg)	Av. [range] DLWG (kg)
Single	52	5.7 [3.0 – 7.8]	26.5 [9.6 – 38.2]	0.37 [0.11 – 0.53]
Twin	144	4.9 [2.5 – 6.8]	22.5 [12.4 – 32.6]	0.31 [0.10 – 0.48]
Triplet	67	4.1 [2.6 - 6.0]	22.7 [10.8 – 30.4]	0.32 [0.11 – 0.45]

Note: 8wk wt = 8 week weight, DLWG = Daily live weight gain

Lamb birth weights were satisfactory, but below expectations given the amount of concentrates fed, and the high body weight of the ewes. High energy intake pre-lambing may simply have added to ewe condition rather than to lamb growth.
Lamb growth rate was disappointing for twins but in line with low ewe energy and protein intake in early lactation. Some very low gains for some lambs of 0.1 kg/day.

Overview of farm diets, Year 2

			Last 4 weeks pregnancy		First four weeks lactation	
Farm no.	**Farm name**		**Energy**	**Protein**	**Energy**	**Protein**
1	**Char Shrop.**	Single	OK	OK	Deficient **	Deficient **
		Twin	OK	OK	Deficient ***	Deficient ***
		Triplet	Deficient *	OK	N/A	N/A
2	**Tex Heref.**	Single	OK	OK	Over	Over
		Twin	Deficient *	OK	Deficient *	OK
		Triplet	Deficient *	OK	Deficient ***	Deficient **
4	**Lleyn Nor.**	Single	OK	OK	OK	OK
	(Grass fed)	Twin	OK	OK	Deficient **	Deficient *
		Triplet	OK	OK	Deficient **	Deficient **
5	**Lleyn Per.**	Single	OK	OK	OK	OK
		Twin	OK	OK	Deficient **	Deficient*
		Triplet	OK	OK	N/A	N/A
7	**Char Powys**	Single	OK	Deficient *	Overfed	OK
		Twin	Deficient *	Deficient *	Deficient*	Deficient ***
		Triplet	OK	OK		
8	**Char Ches.**	Single	Over	Over	OK	OK
		Twin	Over	Over	Deficient **	Deficient **
		Triplet	Over	Over		

Note that ewes can mobilise a lot of body weight in early lactation if they lamb down in good condition. Early lactation diets would rarely meet the ewe's full theoretical requirements for energy and protein. As a guide to the degree of energy and protein deficiency diets have been coded as follows:

Coded	**Energy (based on weight loss)**	**Protein (MP excess)**
Overfed	Mod to high gain	Over 25g/day
OK	No loss to mod gain	0 – 25 g/day
*	Predicted weight loss up to 100g/day	-20 to -1 g/day
**	Predicted weight loss 101– 200 g/day	-50 to -21 g/day
***	Predicted weight loss >200 g/day	Deficit > 50g/day

100 to 200 g/day body weight loss would, in practice, be acceptable.

Current thinking suggests that the theoretical figures may underestimate MP requirements by 10-20% and hence an 'adequate' MP supply would require a small MP excess (assumed here to be up to 25g/day).

<u>Follow up with farmers</u>

All the case study farmers were contacted by ADAS to discuss the outcomes of the diet reviews. Most of the farmers were happy to discuss their rationing plans and make amendments where deficiencies were apparent. There were generally more issues on farms that used conserved forage and housed ewes for lambing than for outdoor lambing flocks at grass. Most of the concerns related to the quality of forage and the balance of forage to concentrate feeding.

Appendix 2

Inter-rater reliability of observers scoring udder conformation and body condition

Introduction

Reliability studies can be used to assess the level of variability between raters in the measurement procedures to be used in the acquisition of data in cases where more than 1 rater will be involved in data acquisition. Over the course of the 2 year study "Furthering our understanding of intramammary infections in meat ewes – the role of chronic infection and udder conformation", 2 separate researchers were involved in examining ewes to acquire data on body condition score, intramammary masses and udder conformation. To ensure that there was good agreement between the researchers on all measures involved in ewe examinations, an inter-rater reliability study was carried out.

Materials and methods

On the same day, both researchers scored the same 137 ewes supported by different data entry assistants. The ewes were part of the main study and due to undergo the examination in lactation.

The IRR study was analysed in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) Minitab (Minitab Inc. 2013) and SPSS 22 (IBM SPSS Statistics 2013). The percentages of exact agreements and one-point, two-point, three-point and four-point disagreements (where applicable) between the two raters were calculated for the ordinal data (BCS, teat position, teat angle, udder drop, degree of separation, left udder masses and right udder masses) and nominal data (teat lesions and woolly udder) where possible. The formula used was:

$$\text{Percentage agreement} = \frac{(100)\,(X)}{\text{Total number of observations}}$$

Where X is the number of exact agreements (or one-point / two-point / three-point / four-point disagreements) (Hewetson *et al*., 2006).

Kendall's coefficient of concordance (W), which does not treat all disagreements equally but takes ordering into consideration, was calculated. Kendall's W ranges from 0 (no agreement) to 1 (complete agreement) (Minitab). Cohen's Kappa and the intra-class correlation coefficient (ICC) were also calculated (data not shown). To measure pairwise correlation between the two raters, Spearman's rank correlation coefficients (ordinal data) and Pearson's product – moment correlation coefficients (scale data) were calculated. It was hypothesised that the relationship between the measures taken by both raters would be significant, fairly strong (above 0.4) and positive. To assess whether one rater was rating attributes consistently higher or lower than the other rater (bias), the Mann - Whitney U Test (ordinal data) or T - test (scale data) was used.

Results (Table 1 and 2)

Of the 137 sheep examined as part of the IRR study, 120 had complete ratings from both raters and these 120 were included in the analysis.

The % exact agreement between the two raters on BCS was 24.17%, the % 1 point disagreement was 35.00%, the % 2 point disagreement was 36.67, and the % 3 point disagreement was 4.17%. Kendall's coefficient of concordance was high, W = 0.87. Spearman's rank correlation coefficient was significant, positive and strong ($r_s = 0.75$, N= 120, *P*=0.000). The Mann-Whitney U test found significant difference between the ratings given by both raters (U = 12070.0, N = 120, $P < 0.05$).

The % exact agreement between the two raters on woolly udder was 94.17%, equalling 113 out of 120 exact agreements. Rater 1 (CG) found 7 woolly udders whereas rater 2 (EMS) found 0.

The % exact agreement between the two raters on teat position was 54.17%, the % 1 point disagreement was 44.17%, and the % 2 point disagreement was 1.67%. Kendall's coefficient of concordance was moderately high, W = 0.68. Spearman's rank correlation coefficient was significant and positive ($r_s = 0.36$, N= 120, *P*=0.000). The Mann-Whitney U test found no significant difference between the ratings given by both raters (U = 14298.0, N = 120, $P > 0.05$).

The % exact agreement between the two raters on teat angle was 45.00%, the % 1 point disagreement was 49.17%, and the % 2 point disagreement was 5.83%. Kendall's coefficient of concordance was high, W = 0.71. Spearman's rank correlation coefficient was significant, moderately strong and positive ($r_s = 0.42$, N= 120, *P*=0.000). The Mann-Whitney U test found no significant difference between the ratings given by both raters (U = 13820.0, N = 120, $P > 0.05$).

The % exact agreement between the two raters on udder drop was 54.17%, the % 1 point disagreement was 45.00%, and the % 2 point disagreement was 0.83%. Kendall's coefficient of concordance was high, W = 0.73. Spearman's rank correlation coefficient was significant, moderately strong and positive (r_s = 0.47, N= 120, P=0.000). The Mann-Whitney U test found no significant difference between the ratings given by both raters (U = 13490.0, N = 120, P > 0.05).

The % exact agreement between the two raters on degree of separation was 36.67%, the % 1 point disagreement was 36.67%, the % 2 point disagreement was 22.50% and the % 3 point disagreement was 4.17%. Kendall's coefficient of concordance was high, W = 0.88. Spearman's rank correlation coefficient was significant, strong and positive (r_s = 0.76, N= 120, P=0.000). The Mann-Whitney U test found significant difference between the ratings given by both raters (U = 16752.0, N = 120, P < 0.05).

For udder width, Kendall's coefficient of concordance was high, W = 0.78. Pearson's product moment correlation coefficient was significant, moderately strong and positive (r = 0.48, P = 0.000). The T – test found significant difference between the udder widths given by both raters (T = 4.02, P < 0.05).

For teat length, Kendall's coefficient of concordance was high, W = 0.76. Pearson's product moment correlation coefficient was significant, moderately strong and positive (r = 0.58, P = 0.000). The T – test found no significant difference between the teat lengths given by both raters (T = 0.17, P > 0.05).

The % exact agreement between the two raters on left udder lumps was 88.33%, the % 1 point disagreement was 5.00%, the % 2 point disagreement was 3.33%, the % 3 point disagreement was 0.83% and the % 4 point disagreement was 2.50%. Kendall's coefficient of concordance was moderately high, W = 0.61. Spearman's rank correlation coefficient was significant, moderately strong and positive (r_s = 0.41, N= 120, P=0.000). The % exact agreement between the two raters on right udder lumps was 88.33%, the % 1 point disagreement was 3.33%, the % 2 point disagreement was 2.50%, the % 3 point disagreement was 3.33% and the % 4 point disagreement was 2.50%. Kendall's coefficient of concordance was moderately high, W = 0.67. Spearman's rank correlation coefficient was significant, moderately strong and positive (r_s = 0.48, N= 120, P=0.000).

The % exact agreement between the two raters on left teat lesions was 98.33%, the % 1 point disagreement was 0.83% and the % 2 point disagreement was 0.83%. Kendall's coefficient of concordance was high, W = 0.88. Spearman's rank correlation coefficient was significant, strong and positive (r_s = 0.75, N= 120, P=0.000). The % exact agreement between the two raters on right teat lesions was 97.50% and the % 2 point disagreement was 2.50%. Kendall's coefficient of concordance could not be calculated as there were only two different recorded values for the variable. Spearman's rank correlation coefficient was significant, strong and positive (r_s = 0.70, N= 120, P=0.000).

Discussion

Overall the results for all udder conformation scores and BCS showed significant correlation between the ratings of both raters and moderate to good agreement. We are confident that using the scoring system the raters were producing a good level of consistency.

The finding of moderate to good agreement rather than 100% agreement could be explained by the fact that though both raters scored the same sheep, the same sheep may have been standing differently for both raters. The sheep were relatively free to move about (in the confined space) and lift/place back legs in different positions which does alter the appearance of the udder and can make assessments difficult, even for udder width/teat length which were measured with measuring tape. Therefore, significant correlation and moderate agreement could be considered a very good result under the circumstances. Ideally, an intra-observer reliability study should have been carried out at the same time as the inter-observer reliability study, which would better inform us as to how repeatable the udder conformation scoring is.

The fact that there was some disagreement over the size of udder lumps suggests that the rating scale may be being interpreted/applied differently by the raters. This could be due to inadequate training or it could be a problem with the rating scale itself. Some of the difference could be rater error due to the difficulty in assessing moving sheep. Feeling for udder lumps comes towards the end of the udder examination by which stage some ewes have become very agitated and can be difficult to assess. Udder scores that involve presence/absence such as woolly udders, teat lesions and udder lumps can be particularly difficult to spot if the rater is rushing/the sheep is agitated, whereas a value for teat position (for example) can be given even if it is a rushed "guesstimate".

The results for BCS suggest that though there is significant correlation between the ratings of both raters and moderate to good agreement there may be some bias with one rater rating attributes consistently higher or lower than the other rater. A 1 point disagreement in BCS would mean one rater rated a ewe as BCS 3 while the other rated it as 2.5 or 3.5 while a 2 point disagreement would mean that one rater rated a ewe as BCS 3 while the other rated it as

2 or 4. It would seem from the results in this case that rater 1 (CG) (median 2) rated ewes consistently 1 point lower than rater 2 (ES) (median 2.5). This may be explained by the fact that rater 1 (CG) had been scoring ewes from richer grazing (which therefore may have has higher BCSs) over the previous 2 days, whereas rater 2 (ES) had not scored any ewes in the previous 6 months.

Conclusion

The inter-rater reliability of observers scoring udder conformation and body condition was good considering the nature of the examinations.

Table 1. Levels of agreement between raters (ordinal data)

	BCS	Teat position	Teat angle	Udder drop	Degree of Separation	Left udder lumps	Right udder lumps	Left teat lesions	Right teat lesions
% exact agreement	24.17	54.17	45.00	54.17	36.67	88.33	88.33	98.33	97.50
% 1 point disagreement	35.00	44.17	49.17	45.00	36.67	5.00	3.33	0.83	N/A
% 2 point disagreement	36.67	1.67	5.83	0.83	22.50	3.33	2.50	0.83	2.50
% 3 point disagreement	4.17	N/A	N/A	N/A	4.17	0.83	3.33	N/A	N/A
% 4 point disagreement	N/A	N/A	N/A	N/A	N/A	2.50	2.50	N/A	N/A
Kendall's coefficient of concordance (W)	0.87	0.68	0.71	0.73	0.88	0.61	0.67	0.88	N/A
Spearman's rank correlation coefficient (r_s)	0.75 N=120 P = 0.000	0.36 N=120 P = 0.000	0.42 N=120 P = 0.000	0.47 N=120 P = 0.000	0.76 N=120 P = 0.000	0.41 N=120 P = 0.000	0.48 N=120 P = 0.000	0.75 N=120 P = 0.000	0.70 N=120 P = 0.000
Mann-Whitney U (U)	12070.0 N=120 P < 0.05	14298.0 N=120 P > 0.05	13820.0 N=120 P > 0.05	13490.0 N=120 P > 0.05	16752.0 N=120 P < 0.05				

Table 2. Levels of agreement between raters (continuous data)

	Udder width	Teat length
Kendall's coefficient of concordance (W)	0.78	0.76
Pearson's product moment correlation coefficient (r)	0.48 P = 0.000	0.58 P = 0.000
T – test (T)	4.02 P < 0.05	0.17 P > 0.05

Appendix 3

Are udder and teat conformation heritable traits that affect mastitis in Texel sheep?

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Final report

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Executive Summary

A total of 968 records were collected from 10 Texel flocks over a period of 3 years (2012-2014) from England, Wales and Scotland to assess whether udder and teat conformation were heritable traits associated with higher levels of chronic mastitis. Data collection occurred mid-lactation and included scoring and recording udder conformation, teat lesions, intramammary masses, litter size and individual ewe and lamb IDs. Pedigree data were obtained through BASCO.

Univariate quantitative genetic parameters were estimated using individual animal and sire models. The heritabilities for teat length and teat placement were greatest (0.42 and 0.35, respectively). The remaining traits (traits that generally describe the volume of the udder) were of moderate to low heritability.

Univariable logistic regression was used to identify the phenotypic association between udder traits and chronic mastitis. All udder conformation variables were significantly correlated with each other apart from teat placement.

1. Introduction

The average prevalence of clinical mastitis in sheep is <5% (data not shown). However, the prevalence of subclinical mastitis is likely to be much higher, ranging from 10-50% in small ruminants (Bergonier et al., 2013).

Using parallels with cattle, it was estimated that mastitis was likely to cost the Texel sheep breed alone approximately £2.7 million per annum (Conington et al., 2008). There are many negative economic costs associated with mastitis including treatment, poor lamb growth rate, ewe replacement and carcass disposal costs (Huntley et al., 2012).

Mastitis, although caused by bacteria, is likely to be influenced by both genetic and environmental factors. Over the past few decades research on the genetic element of mastitis in cattle has accumulated, however, a previous SPARK award indicated that there was a dearth of information on the causes of mastitis and the heritable traits that are likely to be associated with udder health in ewes (Conington et al., 2008).

Two reports have shown an association between genetic and phenotypic factors (specifically udder conformation) and mastitis in dairy ewes (Casu et al., 2010; Legarra & Ugarte 2005). The factors associated with udder health in suckler ewes, and in particular the role udder and teat conformation plays in mastitis, has been researched at the University of Warwick for several years through two EBLEX funded projects. Results from these studies include that poor udder and teat conformation, scored using an amended version of a published protocol (Casu et al., 2006) (Figure 1), were associated with:

i. Decreased lamb growth rate (Huntley et al., 2012);
ii. Increased levels of subclinical mastitis (measured by somatic cell count) (Huntley et al., 2012), and;
iii. The occurrence of traumatic teat lesions (caused during suckling) (Cooper et al., 2013).

Low lamb weight was associated with high somatic cell count, an incident case of a teat lesion, sub-optimal teat position, rearing in more than one lamb and ewe age. High udder half somatic cell count (indicative of sub clinical mastitis) was associated with pendulous udders, and large cross-sectional area of the teats (Huntley et al., 2012). Traumatic teat lesions were associated with heavier total litter weight, body condition score, udder skin condition, sub-optimal teat position and udder depth (Cooper et al., 2013).

The lowest somatic cell count, fewest traumatic teat lesions and greatest lamb growth rate was associated with teat position '5', where the teats are at the 4 and 8 o'clock position (Figure 1a). This is in direct contrast to studies of dairy ewes where position 1 is thought to be the optimum, and highlights the potential pitfalls of comparing results from analyses of dairy and non-dairy ewes.

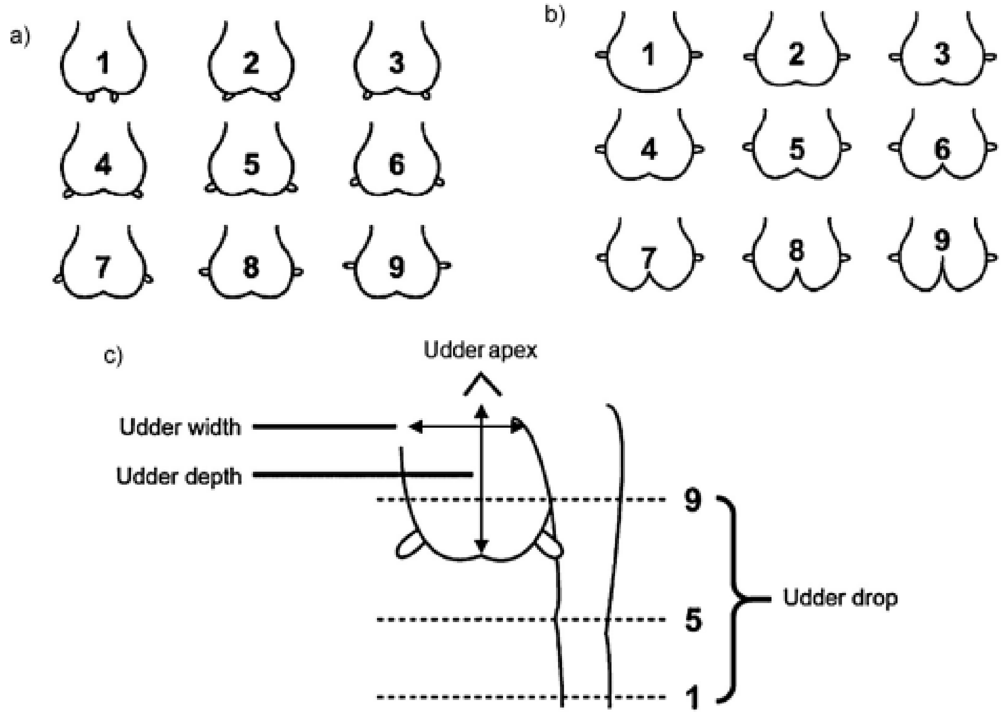

Figure 1: Scoring methods for a) teat placement, b) degree of separation and c) udder drop

Improving ewe resilience to mastitis through selective breeding of heritable traits could ultimately reduce mastitis. However, the traits in question would need to be repeatable within and across lactations in order to be useful. Previous studies have indicated some udder conformation traits (in particular teat placement) have high repeatability within (Fernandez et al., 1995; de la Fuente et al., 2011) and across lactation (de la Fuente et al., 2011) whilst others relating to udder size and therefore milk yield were affected by lactation and flock (Serrano et al., 2002). These traits also had lower heritability (Serrano et al., 2002). The repeatability and heritability of udder conformation traits in suckler ewes in England has not been explored.

2. Aims

The aims of this project were to record udder conformation scores of ewes in mid-lactation, along with cases of chronic clinical mastitis (detected by palpation of mammary gland abscesses), offspring and pedigree data in order to:

i. assess whether udder and teat conformation were heritable traits; and
ii. investigate whether udder and teat conformation were phenotypically associated with higher levels of chronic mastitis.

The results of this project could ultimately lead to Estimated Breeding Values (EBV) for some udder and teat conformation traits, which could provide an objective way for Texel society members to assess the genetic potential of rams selected for breeding and female replacements in terms of mastitis resilience. This could eventually contribute to a reduction in mastitis, thus improving ewe and lamb welfare and increasing the net return of meat producing sheep.

3. Methods

3.1 Flock and ewe selection

Ten performance recorded pedigree Texel flocks with individual ewe identification were convenience selected based on recommendations and farmer interest. Flocks included representatives from England, Scotland and Wales (Figure 2).

Figure 2: A map of study flock locations

3.2 Data collection

All Texel ewes from each flock were included in the study, which took place between 2012 and 2014. Two flocks were visited over several years. All ewes lambed between January and May of each year. Data collection occurred mid-lactation and included 10 udder and teat conformation traits, and individual ewe and lamb IDs.

3.2.1 Variable definitions

Udder traits

All ewes were scored for 10 udder and teat traits using a combination of linear scores, measurements, udder palpation and visual examination. Linear scores were used to characterise teat placement, udder drop, and the degree of separation of the udder halves as described previously (Casu et al., 2006; Marie-Etancelin et al., 2005). A tape measure was used to record udder width from the rear at the widest point and the left teat length only because the initial study indicated a strong positive correlation between left and right udder teat length and width. Udder masses were detected by manual palpation of each udder half, and teat lesions were observed by visual inspection. All measurements were performed by two researchers and data recorded on custom-designed data recording sheets.

Binary variables

Chronic mastitis was defined as a palpable mass in either udder half.

Teat lesions (left and/or right) were defined as any lesion present on the teat at the observation. These could include bites, tears and grazes, which tended to be attributed to suckling; or spots, warts and/or proliferative scabs on the teat.

3.3 Data summary and analyses

All data were entered into Microsoft Excel spreadsheets. Individual ewe data were matched to flock book numbers via BASCO or by the farmers. Flock level pedigree data including sire and dam IDs were obtained from the BASCO database (BASCO). Data were moved into Microsoft Access. Queries were used to select and link data from Microsoft Access for statistical analysis. Ewe age in years at the time of observation was calculated as the difference between the date of birth and the observation date. Number of days in milk was calculated as the difference between the ewes' lambing date and the observation date.

Measures of dispersion and central tendency were used to investigate the data (R Development Core Team). Obvious errors were corrected where possible.

3.4 Univariate variance component estimation for udder traits

3.4.1 Data and pedigree

The udder trait data file contained 968 records with BASCO identifier and associated pedigree information. Some data edits were performed sequentially to remove:

1. All Blue Texels (leaving 953 records);
2. Records with either conformation scoring or lambing date missing (876);
3. Records with unknown sire (833); and
4. Records for one year old ewes (817).

The 817 udder trait records were on 740 ewes; 665, 73 or 2 having been recorded on one, two or three occasions.

All pedigree records (associated with estimated breeding value reports) for each recorded flock were extracted from the BASCO database and these were concatenated to provide an overall pedigree file. The 817 ewe records were matched to these pedigree data. The resulting pedigree contained the recorded animals plus all known ancestors. Pruning was performed to remove redundant members of the pedigree leaving a 2270 animal pedigree for inclusion in the animal model analyses. There were 188 sires and maternal grandsires within this pedigree (i.e. the records used to create the sire-maternal grandsire relationship matrix for sire model analyses). Recorded ewes were the offspring of 145 sires with an average of 5.6 records associated with their offspring but 39 had only a single recorded offspring in the data set excluding repeated records on ewes.

The recorded traits were either objective measurements on a continuous scale or subjective observations with either two or several categories. Those traits recorded on a continuous scale or with many categories were treated as if they were normally distributed, and are referred to as such in this document. Traits with only two categories (referred to variously as binary, or binomial or categorical in this document) were treated as binomial data.

A data set that included only one record per ewe was summarised, the latest observation on any ewe was retained to create this set. Means, standard deviations and number of missing records of the normally distributed traits are given in Table 1. The incidence of the different phenotypic observations for the binary traits retained for analysis is in Table 2. The Woolly Udder binary trait was dropped due to its low incidence (only 16 cases out of 817 records), while the left and right chronic mastitis traits were dropped because it could be difficult to differentiate between udder halves in the field.

	Mean	**Standard deviation**	**Missing**
Teat Placement	5.87	1.16	4
Udder Drop	6.93	0.86	4
Degree of Separation	3.23	1.38	54
Udder Width	13.45	2.09	5
Left Teat Length	2.50	0.44	4

Table 1: Summary of traits assumed to be normally distributed from the single observation per ewe data set

	No	**Yes**	**P(x=1)**	**Missing**
Chronic Mastitis	516	224	0.30	0
Left Teat Lesion	652	88	0.12	0
Right Teat Lesion	639	101	0.14	0
Any Teat Lesion	601	139	0.20	0

Table 2: Incidence for binary traits in the single observation per ewe data set

3.4.2 Fixed effects

The primary fixed effects considered were:

- A flock-by-scoring date (FSD) factor,
- The ewe age (EA) factor has 5 levels (2,3,4,5, and >5 years old); and
- A linear regression on days in milk (DIM).

In addition, some information on litter size was available. For records taken subsequent to the preliminary analyses, the number of lambs born was recorded. An approximation to this could also be made by counting the number of registered offspring of a ewe in the BASCO database, and this was used where the actual number of lambs born was not available. There were still a number of ewe records where no litter size information was available, and so litter size was considered in a series of separate analyses using a subset of the overall data. The two types of litter size record were fitted within the source of the record; such that there was a level for single and multiple births within each of 'observed' and 'BASCO-derived' (Table 3).

Source	**Birth type**	**Data set (ewe records)**	
		Single[1]	**All**
Observed	Single	123	124
	Multiple	127	128
BASCO-derived	Single	189	234
	Multiple	274	301
No litter size information		27	30
Total		740	817

[1] Latest observation retained on each ewe. The subset of these records with litter size information was used in the litter size analyses.

Table 3: Summary of litter size information used in analyses.

3.4.3 Data sets

For the different analyses, three data sets were used:

- all 817 records (when permanent environmental effects were considered to account for repeated measures);
- 740 single records per ewe, retaining the latest observation for each ewe, and
- 713 single records per ewe which also had litter size information (for use in analyses considering the impact of litter size).

3.4.3 Analyses

Univariate quantitative genetic analyses were performed using individual animal models (equation 1) and sire models (for binomial traits, equation 3). Repeated records were considered in individual animal model analyses including permanent environmental effects associated with ewes, equation 2.

When permanent environmental effects were excluded, the data used was the latest record on ewes recorded. This data set contained 740 records of 740 ewes rather than the 817 in the full data set.

The Bayesian Integrated Nested Laplace Approximation (INLA) method Rue et al. (2009) as implemented in the inla R package (http://r-inla.org) was used for both the animal and sire model analyses.

Comparison of fixed effect models

Quantitative genetic analysis was carried out for each trait with various combinations of the three primary fixed effects. The individual animal model (equation 1) was used for the normally distributed traits and the sire model (equation 3) for binary traits. For any trait, the model with the lowest Deviance Information Criterion (Spiegelhalter et al., 2002, DIC) was selected for use in all subsequent analyses of that trait.

Inclusion of litter size

The litter size variable had four levels; single or multiple births for each of two data sources ('recorded' or 'BASCO-derived'). The subset of single ewe records with litter size information was analysed both including and excluding litter size category as an additional fixed effect in the model. Either an individual animal model (equation 1) or a sire model (equation 3) was used depending on the nature of the trait.

Sensitivity to priors

Bayesian analysis utilises prior information. The priors used in the main analyses are described in section 3.5. When there is sufficient information from the data the influence of the prior information upon the analysis results is negligible. In order to assess the sensitivity of our analyses to the chosen priors, analyses were repeated with various priors defined with respect to the heritability: the priors varied in terms of the mode and the width (and hence informativeness) of the prior distribution of the heritability. The range of prior distributions considered for the heritability is shown in Figure 3. The sensitivity analyses used the individual animal model for the normally distributed traits and the sire model for the binary traits. Repeated records were excluded from these analyses.

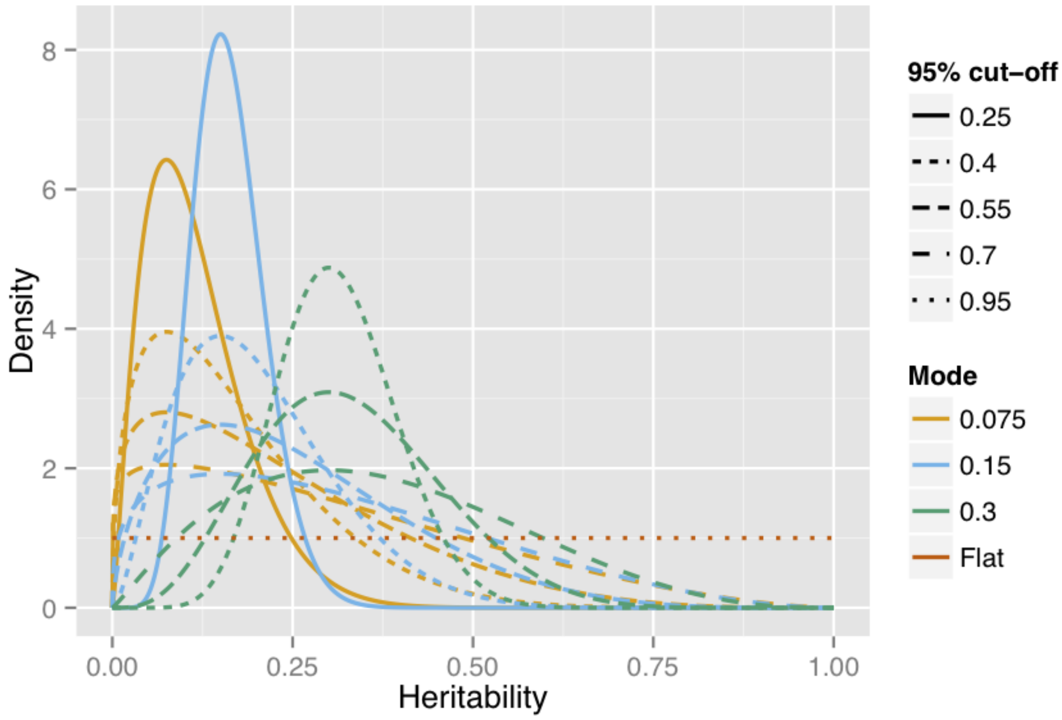

Figure 3: Prior distributions of the heritability considered in sensitivity analyses. The prior distribution was defined in terms of the mode and the 95% cut-off point of the heritability.

3.4.4 The individual animal model

The following individual animal models were considered:

$$\mathbf{y = Xb + Za + e} \qquad (1)$$

$$\mathbf{y = Xb + Za + Zu + e} \qquad (2)$$

Where:

y is a vector of phenotypic observations;

b is a vector of fixed effects as selected from the analyses in section 3.4.3;

a is a vector of random animal effects;

u is a vector of random permanent environmental effects associated with ewes;

X and **Z** are design matrices relating observations to fixed effect levels or animals, respectively; and

e is a vector of random residual effects.

The variance of **a** is $var(\boldsymbol{a}) = \mathbf{A}\sigma_a^2$, where **A** is the numerator relationship matrix; the variance of **u** is $var(\boldsymbol{u}) = \mathbf{I}\sigma_{pe}^2$ and the variance of **e** is $var(\boldsymbol{e}) = \mathbf{I}\sigma_e^2$.

The heritability is $h^2 = \frac{\sigma_a^2}{\sigma_p^2}$, where the phenotypic variance is $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$ without permanent environmental effects and $\sigma_p^2 = \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2$ for analyses including permanent environmental effects. The repeatability is $r = \frac{\sigma_a^2+\sigma_{pe}^2}{\sigma_p^2}$.

For the binomial traits, the analysis was carried out using a binomial generalised linear mixed model (GLMM) with a logit link function. In these analyses, the residual variance has a fixed value, $\sigma_e^2 = \frac{\pi^2}{3}$.

3.4.5 The sire model

There is evidence that for categorical traits, particularly with non-intermediate frequencies, sire models provide better estimates of variance components than the individual animal model. To this end, a sire model with relationships was also considered for the binomial traits.

The data set used excluded repeated records, using the same 740 ewe records as in the non-repeatability individual animal model analyses.

The following sire model was used:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zs} + \mathbf{e} \qquad (3)$$

Where:

y is a vector of phenotypic observations;

b is a vector of fixed effects as selected from the analyses in section 3.4.3;

s is a vector of random sire effects;

X and **Z** are design matrices relating observations to fixed effect levels or sires, respectively; and

e is a vector of random residual effects

The variance of **s** is $var(\mathbf{s}) = \mathbf{A_s}\sigma_s^2$ (where $\sigma_s^2 = 0.25\sigma_a^2$), where $\mathbf{A}_s$ is the sire-maternal grandsire numerator relationship matrix pertaining to 145 sires or recorded animals and their ancestors (a 188 animal sire-maternal grandsire pedigree).

A binomial GLMM with logit link function was used and the heritability was estimated as $h^2 = 4 \times \frac{\sigma_s^2}{\sigma_s^2 + \frac{\pi^2}{3}}$.

3.5 Priors

In the previous report, the default priors used in the code developed by Holand et al. (2013) were used. These priors were specified in terms of precisions, since the INLA analyses are parameterised in terms of these rather than variances. While these parameters were uninformative with regards to the precision to which they were being applied, they would not be completely uninformative with regard to the heritability and nor did they take account of the overall variation for the trait (they did not relate to the phenotypic or residual variance of the trait).

For these revised analyses, prior distributions for heritabilities were generated. A beta distribution was assumed with a mode of 0.15 and in which 95% of the distribution fell below a heritability of 0.7. The same prior distribution of heritability was assumed for all traits and priors for the variance components were derived for each trait as follows:

Continuous traits

The heritability of a trait is $h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} = \frac{\sigma_a^2}{\sigma_p^2}$ where σ_a^2, σ_e^2 and σ_p^2 are the additive direct genetic, residual and phenotypic variances for the trait. The heritability relates to the additive genetic and residual precisions thus: $\tau_a = \frac{1}{\sigma_a^2} = \frac{1}{h^2\sigma_p^2}$ and $\tau_e = \frac{1}{\sigma_e^2} = \frac{1}{(1-h^2)\sigma_p^2}$. Random samples from the prior distribution of the heritability may be converted into compatible random samples of τ_a and τ_e assuming that $\sigma_p^2 = 1$. Gamma distributions were then fitted to these samples. The rate parameter of the gamma distribution generated was then scaled by an estimate of $\sigma_p^2$to give priors specific to each continuous trait. The residual mean squares from multiple linear regression analyses fitting FSD, DIM and EA were used to approximate σ_p^2 for each continuous trait.

Binomial traits

The binomial traits were subject to a generalised linear mixed model with a logit link. Under the logit link function, the residual variance is fixed at $\sigma_e^2 = \frac{\pi^2}{3}$. Rearranging the equation for the heritability, the additive genetic and sire precisions are $\tau_a = \frac{1-h^2}{h^2\sigma_e^2}$ and $\tau_s = \frac{4-h^2}{h^2\sigma_e^2}$, respectively. As above, the prior distributions for τ_a and τ_s were derived from a random sample from the prior distribution of the heritability. Rather than fitting a gamma distribution the R 'logspline' procedure (Kooperberg, 2013) was used to approximate the distribution taking account of truncation, for the sire model $\tau_s \geq \frac{9}{\pi^2}$, to give a more realistic prior distribution.

Repeated records

To take some account of the presence of repeated observations on ewes, a repeatability model was considered. This requires the estimation of a permanent environmental variance component, σ_{pe}^2. In this model $\sigma_p^2 = \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2$ and the repeatability, $r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_p^2}$. The methods described above to generate priors for continuous and binomial traits were extended to produce consistent priors for τ_a, τ_{pe} and τ_e.

4. Results

4.1 Descriptive analyses

The average flock size across the 10 flocks in the study was 92 ewes (range 34-165). There were 873 ewes in the study, of which two year olds were the most frequent (Figure 4A). Body condition score ranged from 1-4.5 with the majority of ewes (190 of 313 ewes scored) being a score of 2.5-3 (Figure 4B). Ewes were scored on average at 65 days in lactation (range 5-123 days, Figure 5). Very few ewes were scored in the first 3 weeks of lactation.

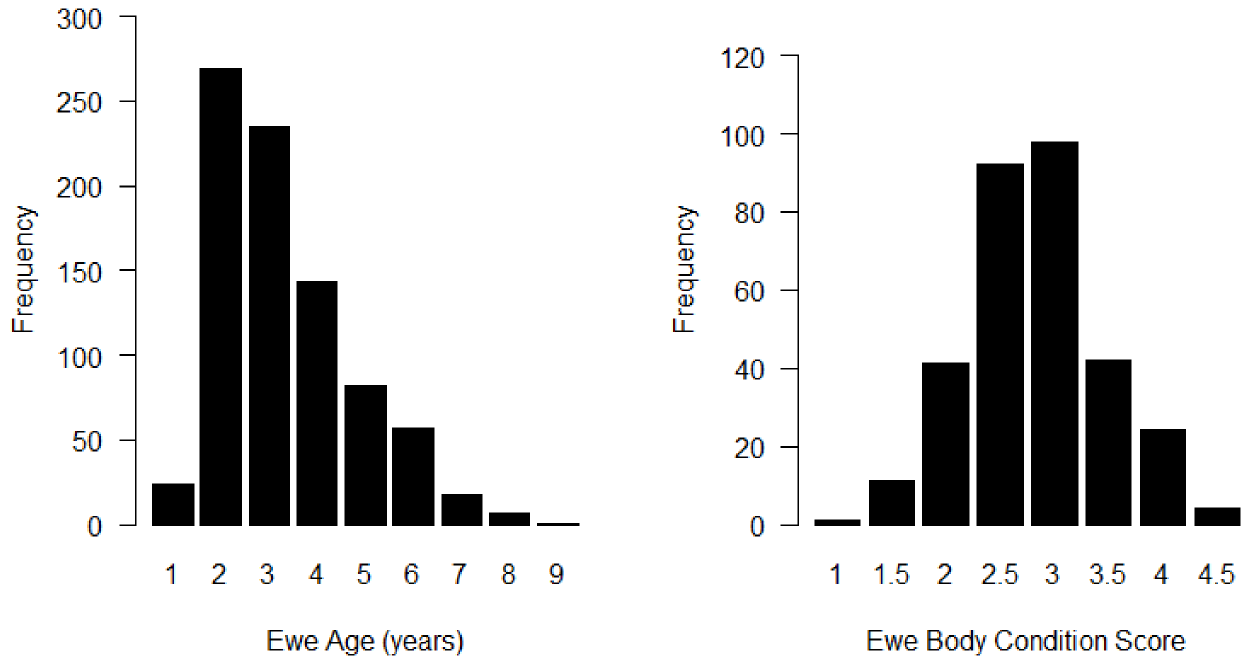

Figure 4: Distribution of A) ewe age and B) Body condition score

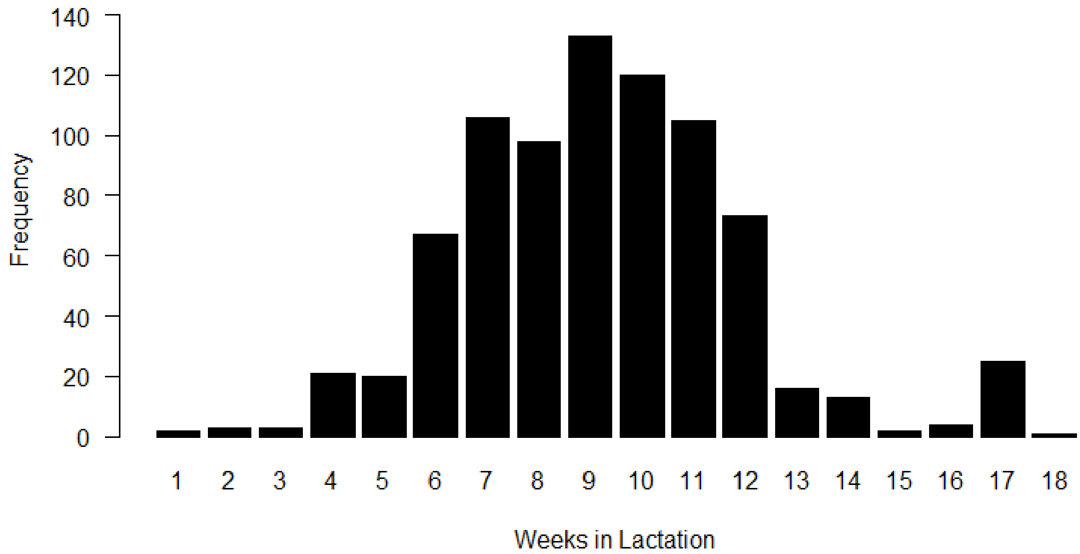

Figure 5: Distribution of weeks in milk when scored

Two methods of litter size were included in the study to minimise missing data: Method 1- number of lambs per ewe recorded by researcher (believed to be more accurate) and method 2- number of lambs per ewe obtained through BASCO (increased number of records). The ewes included in the initial pilot study did not have litter size recorded by a researcher; this variable was added later during the full study hence the lower frequencies for method 1. On average, the litter size for researcher-recorded records were 1.59 lambs per ewe, and 1.66 lambs per ewe for records obtained through BASCO (Table 4).

	Recorded	BASCO
Number of ewes with litter size data	287	765
Number of ewes with missing litter size data	586	108
Average litter size per ewe	1.59	1.66

Table 4: The number of ewes included and excluded in litter size analyses and the average litter size per ewe for two methods

From this point on in the analyses, distributions are based on record rather than ewe due to repeated measurements over multiple years for some ewes. There were 968 observations for 873 ewes.

Teat placement and udder drop scored were normally distributed around scores of 6 (range 2-9) and 7 (range 1-8) respectively whereas degree of separation was positively skewed around score 7 (range 3-9) (Figure 7).

Due to the consistency and correlation between left teat length, left teat width, right teat length and right teat width in the pilot study, the only teat measurement included in the full study was left teat length (Figure 8A). Udder width was negatively skewed around 14cm (range 5-20cm) (Figure 8B). This variable in particular has been linked in the literature to point of lactation in which the ewe was scored. The summary of traits is shown in Table 5.

	Mean	Standard deviation	Missing
Teat Placement	5.85	1.15	6
Udder Drop	6.92	0.85	6
Degree of Separation	3.18	1.37	63
Udder Width	13.47	2.05	7
Left Teat Length	2.49	0.43	6

Table 5: Summary of continuous traits assumed to be normally distributed

Alongside teat and udder conformation variables, ewe udder half health and teat lesion presence were also recorded (Table 6).

	No	Yes	P(x=1)	Missing
Chronic mastitis	573	242	0.30	2
Left teat abnormality	713	102	0.13	2
Right teat abnormality	693	122	0.15	2
Teat abnormality on either half	650	165	0.20	2

Table 6: Incidence of binary traits

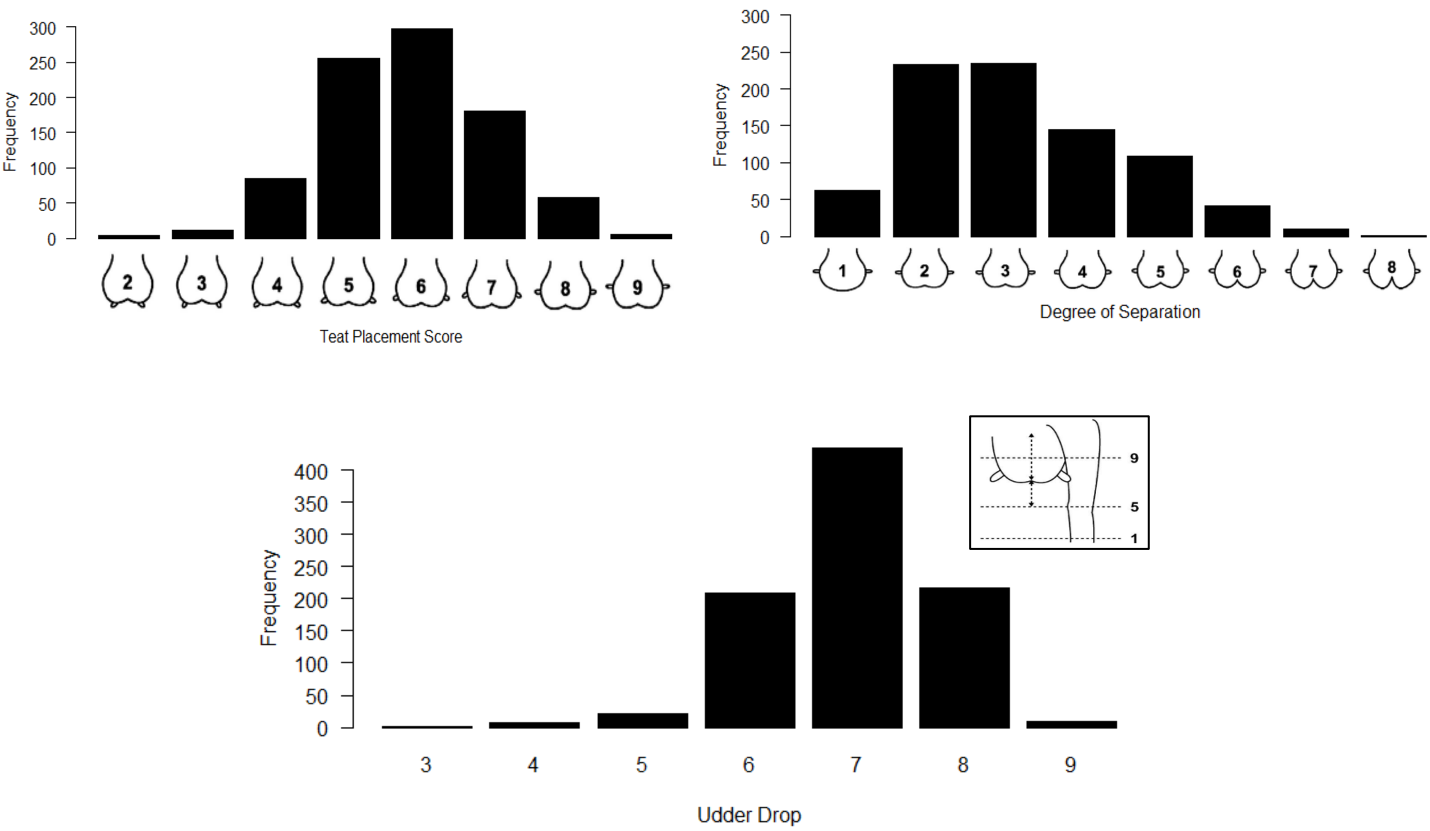

Figure 6: Distribution of categorical variables A) Teat Placement, B) Degree of separation and c) Udder Drop

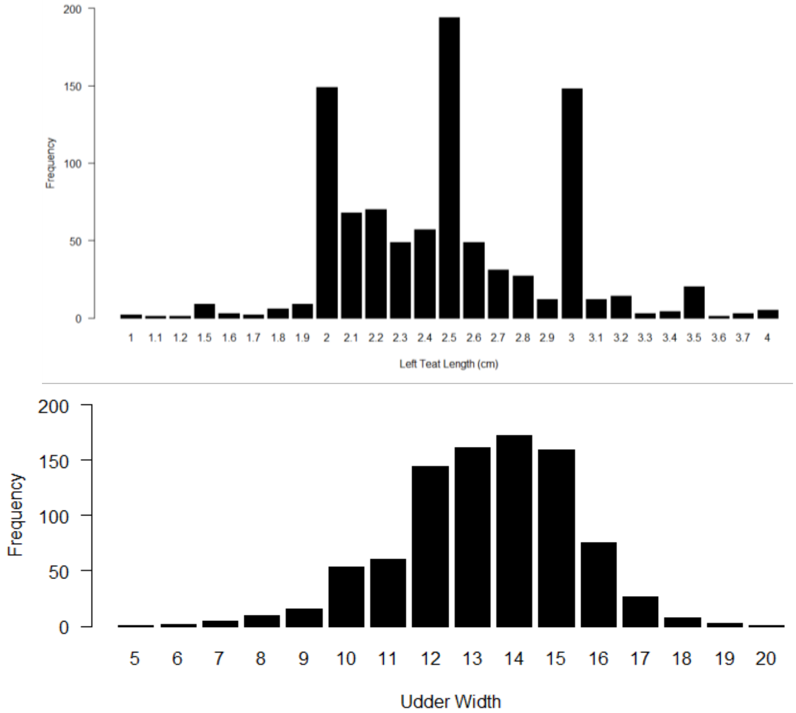

Figure 7: Distribution on continuous variables a) left teat length (scored to 0.5cm in year 2) and b) udder width

4.2 Phenotypic correlations between variables

As indicated in the previous report, several of the variables were phenotypically correlated (Table 7). Age was significantly correlated with all udder conformation variables and body condition score, which is to be expected. Unusually, however, teat placement was correlated with both methods used for litter size calculation. Days in lactation was correlated with all udder conformation variables apart from teat placement. Body condition score was also significantly correlated with udder drop, degree of separation and left teat length. Both methods of litter size were significantly correlated, despite there being significantly fewer records in method 1.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1. Age									
2. Teat Placement	**-0.066 (0.047)**								
3. Udder Drop	**-0.268 (0.000)**	-0.027 (0.409)							
4. Degree of Separation	**-0.075 (0.030)**	-0.011 (0.754)	-0.062 (0.065)						
5. Udder Width	**0.075 (0.023)**	0.042 (0.199)	**-0.432 (0.000)**	**0.114 (0.001)**					
6. Left Teat Length	**0.209 (0.000)**	-0.048 (0.136)	**-0.124 (0.000)**	**0.067 (0.048)**	0.033 (0.314)				
7. Days in Lactation	0.031 (0.357)	0.019 (0.566)	**0.108 (0.001)**	**0.124 (0.000)**	**-0.291 (0.000)**	**0.156 (0.000)**			
8. Litter size method 1	0.012 (0.837)	**0.118 (0.048)**	-0.280 (0.066)	0.113 (0.066)	0.096 (0.108)	0.098 (0.101)	0.012 (0.836)		
9. Litter size method 2	-0.029 (0.415)	**0.126 (0.000)**	-0.168 (0.060)	0.069 (0.060)	**0.183 (0.000)**	**0.123 (0.000)**	0.057 (0.103)	**0.946 (0.000)**	
10. Body Condition Score	**-0.164 (0.004)**	0.074 (0.194)	**0.168 (0.013)**	**0.145 (0.013)**	0.006 (0.910)	**-0.117 (0.041)**	0.025 (0.672)	-0.083 (0.160)	-0.079 (0.194)

Table 7: Spearman correlation values between the continuous variables. P values are given in parentheses; pairs with a p value of ≤ 0.05 are highlighted.

4.4 Udder trait heritability

The principal quantitative genetic parameter results are in Tables 8 and 10, which contain summaries of the marginal distributions of variance components and heritabilities for normally distributed and binomial traits, respectively.

The normally distributed traits are generally reasonably heritable with modes of the posterior distributions ranging from 0.10 (for Udder Width) to 0.42 (Left Teat Length), see Table 8. The heritability estimates of the binomial traits were lower, with the mode of sire model estimates being higher than animal model estimates for three out of four traits, see Tables 10 and 9. The modal sire model estimates of the heritability of Left and Right Teat Lesions were 0.04 and 0.14, respectively, but with large credible intervals.

Trait	**Parameter**	**Mean**	**sd**	**Percentiles**			**Mode**
				2.5%	**50%**	**97.5%**	
Teat Placement	σ_e^2	0.67	0.08	0.53	0.67	0.82	0.67
	σ_a^2	0.36	0.09	0.20	0.35	0.55	0.34
	h^2	0.35	0.04	0.27	0.35	0.43	0.35
Udder Drop	σ_e^2	0.50	0.05	0.40	0.50	0.59	0.50
	σ_a^2	0.13	0.05	0.04	0.12	0.24	0.11
	h^2	0.21	0.04	0.13	0.21	0.30	0.21
Degree of Separation	σ_e^2	1.32	0.15	1.05	1.31	1.60	1.32
	σ_a^2	0.47	0.16	0.21	0.45	0.81	0.42
	h^2	0.27	0.04	0.19	0.27	0.36	0.27
Udder Width	σ_e^2	2.61	0.20	2.22	2.61	3.00	2.62
	σ_a^2	0.36	0.18	0.09	0.33	0.79	0.25
	h^2	0.12	0.04	0.05	0.11	0.20	0.10
Left Teat Length	σ_e^2	0.11	0.01	0.08	0.11	0.14	0.11
	σ_a^2	0.08	0.02	0.05	0.08	0.12	0.08
	h^2	0.42	0.04	0.35	0.42	0.50	0.42

Table 8 Summaries of marginal distributions of variance components and heritabilities from individual animal model INLA analyses of normally distributed traits.

Trait	Parameter	Mean	sd	Percentiles			Mode
				2.5%	50%	97.5%	
Chronic Mastitis	σ_a^2	0.33	0.22	0.03	0.29	0.85	0.18
	h^2	0.09	0.05	0.01	0.08	0.20	0.06
Left Teat Lesion	σ_a^2	0.41	0.30	0.03	0.33	1.07	0.12
	h^2	0.10	0.07	0.01	0.09	0.25	0.04
Right Teat Lesion	σ_a^2	0.44	0.34	0.03	0.36	1.31	0.16
	h^2	0.11	0.07	0.01	0.10	0.28	0.06
Any Teat Lesion	σ_a^2	0.33	0.24	0.02	0.27	0.88	0.11
	h^2	0.09	0.06	0.01	0.07	0.21	0.04

Table 9: Summaries of marginal distributions of heritabilities from individual animal model INLA analyses of binomial traits.

Trait	Parameter	Mean	sd	Percentiles			Mode
				2.5%	50%	97.5%	
Chronic Mastitis	σ_s^2	0.16	0.11	0.02	0.14	0.43	0.10
	σ_a^2	0.65	0.44	0.07	0.56	1.71	0.39
	h^2	0.18	0.12	0.02	0.16	0.46	0.12
Left Teat Lesion	σ_s^2	0.12	0.08	0.01	0.11	0.29	0.03
	σ_a^2	0.49	0.33	0.03	0.44	1.16	0.13
	h^2	0.14	0.09	0.01	0.13	0.32	0.04
Right Teat Lesion	σ_s^2	0.23	0.17	0.02	0.20	0.67	0.11
	σ_a^2	0.94	0.69	0.07	0.79	2.70	0.45
	h^2	0.26	0.17	0.02	0.23	0.68	0.14
Any Teat Lesion	σ_s^2	0.15	0.10	0.01	0.13	0.35	0.04
	σ_a^2	0.60	0.41	0.04	0.51	1.42	0.16
	h^2	0.17	0.11	0.01	0.15	0.39	0.05

Table 10: Summaries of marginal distributions of heritabilities from sire model INLA analyses of binomial traits.

4.4.1 **Fixed effect models**

Table 11 contains the deviance information criteria from individual animal model analysis of various combinations of the primary fixed effects. The inclusion of flock by scoring date (FSD) effects was enforced, and combinations of ewe age (EA) and days in milk (DIM) were included alongside it. The combination of fixed effects corresponding to the lowest value of DIC for each trait was used in all other analyses reported here.

Trait	**Included fixed effects**			
	FSD	FSD, EA	FSD, DIM	FSD, EA, DIM
Teat Placement	2022.61	2019.92	2022.49	**2019.37**
Udder Drop	1718.41	**1708.25**	1719.69	1708.94
Degree of Separation	**2290.07**	2291.51	2291.11	2292.41
Udder Width	2907.05	2894.59	2899.37	**2884.11**
Left Teat Length	725.71	725.93	**719.12**	722.22
Chronic Mastitis	**902.48**	904.66	904.30	906.46
Left Teat Lesion	**531.30**	533.24	531.97	533.87
Right Teat Lesion	**572.57**	573.20	574.07	574.71
Any Teat Lesion	**699.28**	702.33	701.04	704.08

Table 11: Deviance information criteria (DIC) for individual animal models with various combinations of fixed effects included. The lowest (best) value of DIC for each trait is in bold type.

4.4.2 **Inclusion of repeated records**

Tables 12 and 13 contain summaries of posterior distributions of variance components from analyses including repeated records. Only for Udder Drop, Degree of Separation and Udder Width was the estimate of the permanent environmental variance sufficient to make an estimate of the repeatability noticeably greater than the heritability.

Trait	Parameter	Mean	s.d.	Percentiles			Mode
				2.5%	50%	97.5%	
Teat Placement	σ_e^2	0.6340	0.0634	0.5180	0.6320	0.7600	0.6280
	σ_a^2	0.4220	0.0819	0.2760	0.4180	0.5880	0.4110
	σ_{pe}^2	0.0003	0.0015	0.0000	0.0001	0.0017	0.0000
	h^2	0.4010	0.0369	0.3300	0.4010	0.4740	0.4010
	r	0.4010	0.0369	0.3300	0.4010	0.4750	0.4020
Udder Drop	σ_e^2	0.3070	0.0415	0.2300	0.3050	0.3870	0.2980
	σ_a^2	0.1560	0.0532	0.0664	0.1530	0.2670	0.1500
	σ_{pe}^2	0.1660	0.0544	0.0798	0.1620	0.2750	0.1510
	h^2	0.2440	0.0439	0.1620	0.2430	0.5970	0.5170
	r	0.5170	0.0418	0.4340	0.5180	0.5970	0.5170
Degree of Separation	σ_e^2	1.0400	0.1200	0.8100	1.0400	1.2700	1.0400
	σ_a^2	0.5260	0.1500	0.2510	0.5190	0.8280	0.5030
	σ_{pe}^2	0.2010	0.1410	0.0461	0.1600	0.5260	0.0447
	h^2	0.2720	0.0443	0.1890	0.2700	0.3620	0.2680
	r	0.4200	0.0465	0.3290	0.4200	0.5100	0.4240
Udder Width	σ_e^2	2.3200	0.2110	1.8800	2.3300	2.6900	2.3600
	σ_a^2	0.4220	0.1790	0.1440	0.3970	0.8230	0.2780
	σ_{pe}^2	0.1870	0.1940	0.0295	0.0968	0.6980	0.0294
	h^2	0.1290	0.0394	0.0621	0.1260	0.2140	0.1150
	r	0.2340	0.0494	0.1410	0.2320	0.3340	0.2390
Left Teat Length	σ_e^2	0.0992	0.0111	0.0793	0.0988	0.1210	0.0980
	σ_a^2	0.0850	0.0153	0.0577	0.0843	0.1160	0.0833
	σ_{pe}^2	0.0002	0.0005	0.0000	0.0001	0.0015	0.0000
	h^2	0.4620	0.0360	0.3910	0.4620	0.5330	0.4720
	r	0.4630	0.0360	0.3920	0.5330	0.4720	0.4630

Table 12: Summaries of marginal posterior distributions of variance components, heritabilities, and repeatabilities of continuous traits estimates with an individual animal model and including repeated records.

Trait	Parameter	Mean	s.d.	Percentiles			Mode
				2.5%	50%	97.5%	
Chronic mastitis	σ_a^2	0.36278	0.23669	0.04078	0.31901	0.92031	0.21607
	σ_{pe}^2	0.00050	0.00242	0.00001	0.00007	0.00174	0.00002
	h^2	0.09666	0.05219	0.02226	0.08840	0.21917	0.05832
	r	0.09671	0.05219	0.02230	0.08844	0.21919	0.05925
Left Teat Lesion	σ_a^2	0.48234	0.35379	0.04008	0.40653	1.39118	0.19756
	σ_{pe}^2	0.00038	0.00165	0.00001	0.00007	0.00166	0.00002
	h^2	0.12342	0.07273	0.02300	0.11073	0.29431	0.06695
	r	0.12346	0.07273	0.02306	0.11076	0.29435	0.06692
Right Teat Lesion	σ_a^2	0.56167	0.38060	0.05980	0.48630	1.48308	0.33203
	σ_{pe}^2	0.00035	0.00152	0.00001	0.00007	0.00162	0.00002
	h^2	0.14128	0.07426	0.03278	0.13040	0.31263	0.10039
	r	0.14132	0.07425	0.03283	0.13045	0.31267	0.10042
Any Teat Lesion	σ_a^2	0.37653	0.26795	0.03245	0.32027	1.06284	0.16672
	σ_{pe}^2	0.00044	0.00203	0.00001	0.00007	0.00167	0.00002
	h^2	0.09919	0.05885	0.01841	0.08897	0.23909	0.05199
	r	0.09923	0.05884	0.01846	0.08900	0.23918	0.06561

Table 13: Summaries of marginal posterior distributions of variance components of binary traits estimated with an individual animal model and including repeated records.

4.4.3 **Inclusion of litter size**

Litter size was included in analysis of single ewe record data using an animal model for normally distributed traits and a sire model for the binomial traits. The results of these analyses are summarised in Tables 14 and 15.

Comparing the modes of the posterior distributions of heritability estimates for normally distributed and binary traits with and without litter size included (Tables 14 and 15), the modal heritability was generally slightly lower when litter size was included in the model. It was only higher for Teat Placement and Chronic Mastitis.

Trait	Litter size?	Parameter	Mean	sd	Percentiles			Mode
					2.5%	50%	97.5%	
Teat Placement	Yes	σ_e^2	0.714	0.078	0.571	0.712	0.868	0.710
		σ_a^2	0.302	0.089	0.150	0.296	0.487	0.284
		h^2	0.303	0.042	0.223	0.302	0.387	0.303
	No	σ_e^2	0.713	0.078	0.571	0.711	0.867	0.710
		σ_a^2	0.302	0.089	0.150	0.296	0.486	0.284
		h^2	0.303	0.042	0.224	0.303	0.387	0.299
Udder Drop	Yes	σ_e^2	0.494	0.048	0.403	0.494	0.586	0.497
		σ_a^2	0.108	0.051	0.029	0.102	0.218	0.084
		h^2	0.187	0.044	0.108	0.185	0.278	0.189
	No	σ_e^2	0.496	0.050	0.403	0.496	0.591	0.499
		σ_a^2	0.122	0.053	0.039	0.117	0.237	0.100
		h^2	0.206	0.044	0.126	0.204	0.297	0.199
Degree of Separation	Yes	σ_e^2	1.292	0.150	1.020	1.290	1.583	1.295
		σ_a^2	0.504	0.171	0.228	0.490	0.862	0.454
		h^2	0.288	0.044	0.205	0.288	0.377	0.284
	No	σ_e^2	1.285	0.150	1.014	1.283	1.575	1.286
		σ_a^2	0.513	0.171	0.235	0.499	0.870	0.465
		h^2	0.293	0.044	0.209	0.292	0.381	0.294
Udder Width	Yes	σ_e^2	2.533	0.193	2.152	2.535	2.910	2.544
		σ_a^2	0.323	0.172	0.085	0.292	0.732	0.213
		h^2	0.106	0.038	0.043	0.103	0.191	0.097
	No	σ_e^2	2.583	0.204	2.187	2.584	2.982	2.594
		σ_a^2	0.368	0.189	0.094	0.336	0.810	0.260
		h^2	0.116	0.039	0.051	0.113	0.203	0.106
Left Teat Length	Yes	σ_e^2	0.111	0.014	0.085	0.111	0.139	0.111
		σ_a^2	0.076	0.018	0.046	0.075	0.113	0.072
		h^2	0.405	0.040	0.328	0.405	0.484	0.405
	No	σ_e^2	0.110	0.014	0.084	0.110	0.138	0.110
		σ_a^2	0.080	0.018	0.049	0.079	0.117	0.077
		h^2	0.421	0.039	0.344	0.421	0.498	0.423

Table 14: Summaries of marginal distributions of variance components and heritabilities from individual animal model INLA analyses of normally distributed traits when litter size was included in or excluded from the model.

Trait	Litter size?	Parameter	Mean	sd	Percentiles			Mode
					2.5%	50%	97.5%	
Chronic Mastitis	Yes	σ_s^2	0.158	0.109	0.015	0.136	0.428	0.090
		σ_a^2	0.632	0.435	0.061	0.544	1.712	0.359
		h^2	0.180	0.117	0.019	0.159	0.460	0.113
	No	σ_s^2	0.148	0.105	0.013	0.126	0.416	0.079
		σ_a^2	0.593	0.420	0.054	0.504	1.664	0.315
		h^2	0.169	0.113	0.016	0.148	0.449	0.100
Left Teat Lesion	Yes	σ_s^2	0.114	0.075	0.007	0.102	0.267	0.030
		σ_a^2	0.454	0.300	0.030	0.407	1.067	0.121
		h^2	0.132	0.084	0.009	0.120	0.300	0.038
	No	σ_s^2	0.114	0.075	0.008	0.105	0.265	0.031
		σ_a^2	0.458	0.298	0.030	0.418	1.059	0.123
		h^2	0.133	0.084	0.009	0.123	0.298	0.040
Right Teat Lesion	Yes	σ_s^2	0.221	0.165	0.016	0.182	0.636	0.098
		σ_a^2	0.882	0.660	0.065	0.728	2.543	0.392
		h^2	0.243	0.168	0.020	0.210	0.648	0.127
	No	σ_s^2	0.229	0.170	0.018	0.191	0.661	0.108
		σ_a^2	0.918	0.679	0.071	0.764	2.645	0.432
		h^2	0.253	0.171	0.022	0.220	0.669	0.139
Any Teat Lesion	Yes	σ_s^2	0.113	0.075	0.007	0.100	0.266	0.030
		σ_a^2	0.452	0.300	0.030	0.401	1.065	0.120
		h^2	0.131	0.084	0.009	0.118	0.299	0.038
	No	σ_s^2	0.130	0.086	0.008	0.114	0.304	0.034
		σ_a^2	0.514	0.345	0.034	0.455	1.216	0.138
		h^2	0.149	0.096	0.010	0.134	0.338	0.044

Table 15: Summaries of marginal distributions of sire and additive genetic variances and heritabilities from sire model INLA analyses of binomial traits when litter size was included in or excluded from the model

4.5 **Sensitivity to priors**

Figures 8 and 9 summarise the results of the analyses with varying priors for normally distributed and binomial traits, respectively. The graphs show the mode and 95% confidence interval of the posterior distribution of the heritability for each trait under each set of priors.

Where observed data provide little information, it would be expected that the posterior distribution would resemble the prior distribution: the modes and would be similar and the credible interval would reflect the shape of the prior distribution. From the figures it can be seen that where the priors were informative (low 95% cut-off point for the prior distribution of the heritability), the posterior mode was closer to the mode of the prior distribution than where the prior was less informative. For the normally distributed traits (Figure 8), the credible interval of the heritability was reasonably constant across priors. For the binomial traits (Figure 9) the 95% credible interval varies with the width of the prior distribution.

Our priors for this study were derived from a prior distribution of the heritability with a mode of 0.15 and a 95% cut-off point at 0.7. For both the normally distributed and binary traits this does not appear to have unduly influenced the results; with the mode and 95% credible intervals for our chosen prior being similar to those obtained when a uniform prior for the heritability was used.

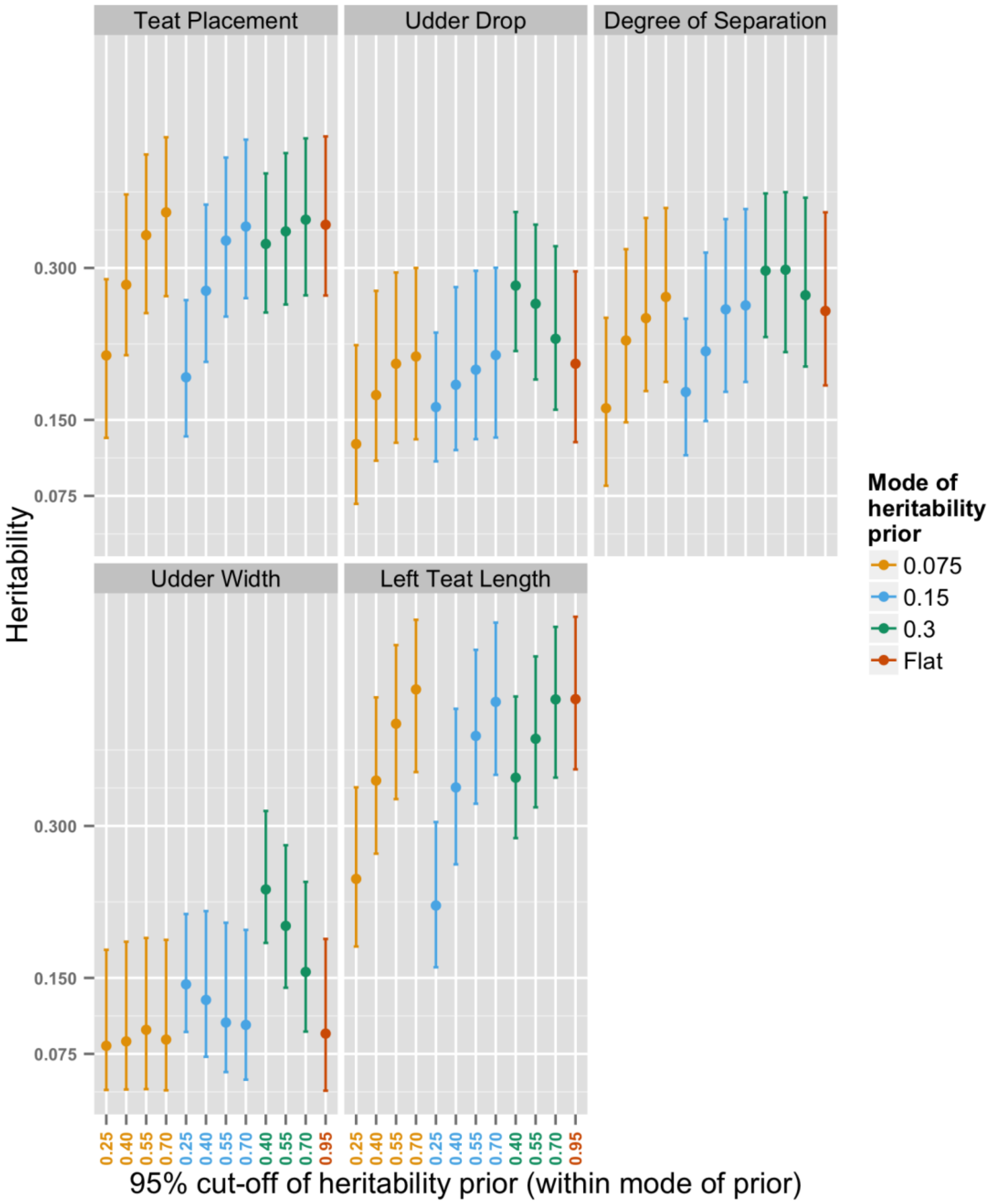

Figure 8: Mode and 95% confidence interval of posterior marginal distribution of heritability of continuous traits as the mode and informativeness of the prior distribution of heritability varies.

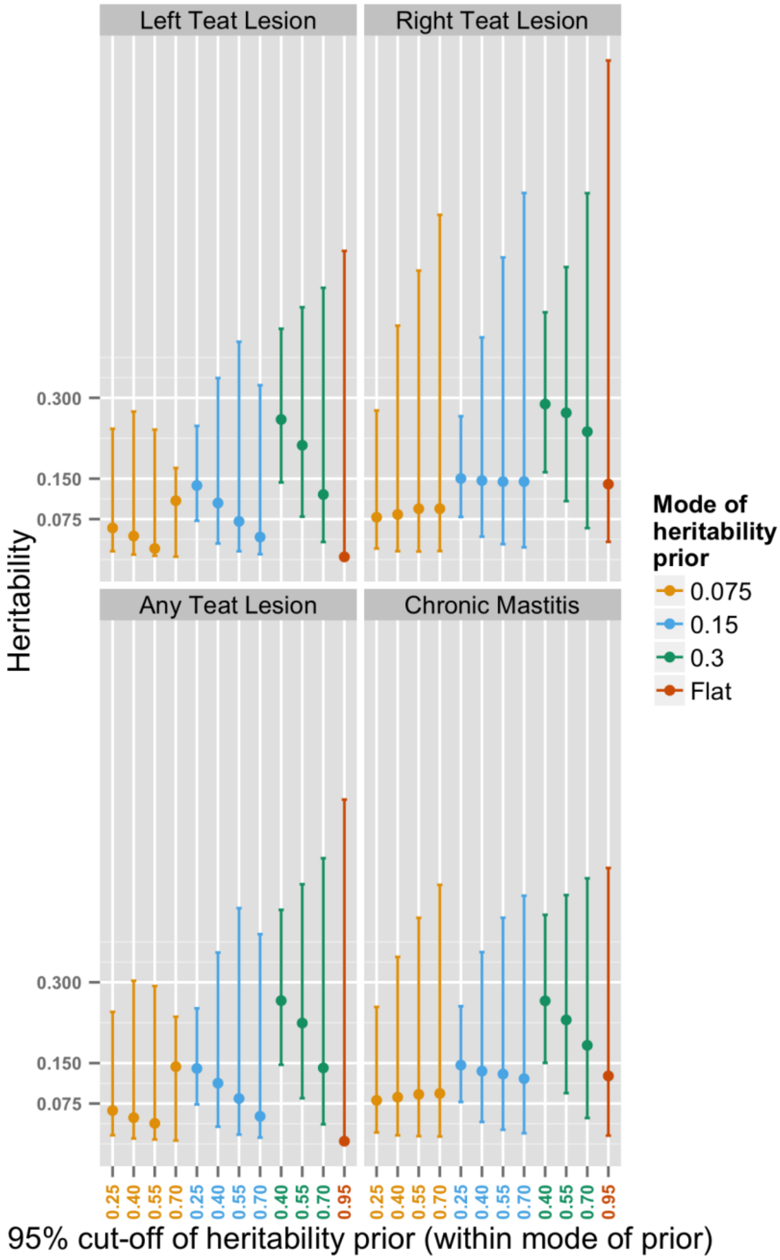

Figure 9: Mode and 95% confidence interval of posterior marginal distribution of heritability of binary traits as the mode and informativeness of the prior distribution of heritability varies.

5. Discussion

This analysis initially included 968 observations for 873 ewes. After removing duplicate values and ewes without pedigree data for the genetic modelling, a total of 817 records for 740 ewes remained in the quantitative genetic analysis of udder trait data of Texel ewes.

Ewe age was phenotypically correlated with all udder conformation traits, indicating the importance of accounting for ewe age in statistical analyses. The number of days into lactation at which ewes were scored was also correlated with all udder conformation traits bar teat placement, indicating the other traits may be linked to milk yield and/or udder volume. This is further supported by the significant correlation of body condition score with these traits. All udder trait variables apart from teat placement were significantly correlated with each other. All udder conformation traits but degree of separation were significantly associated with chronic mastitis in the univariable analyses.

Heritabilities of binary traits were slightly higher under a sire model, with the posterior distributions more skewed towards higher values but the modes similar to those from animal model analyses. Simulation and previous analyses in the literature would indicate that the sire model estimates may be better estimates of the true h^2.

The normally distributed traits generally show good levels of heritability, with only Udder Width (h^2 = 0.10) having a h^2 less than 0.2. This would indicate that selection on any of the normally distributed traits should be feasible given sufficient data recording.

The binomial traits had estimated heritabilities on the underlying scale of between 4% and 14% (Table 10). The levels of heritability for Chronic Mastitis and Right Teat Lesions would imply that selection could be feasible in these traits, given sufficient recording to ensure reasonable levels of accuracy of selection (many related animals recorded). The difference of 0.10 between the modal estimates of the heritability of Left and Right Teat Lesions, when they would be expected to be highly similar traits, may indicate the influence of random sampling in our small data set. This would show the danger of over-interpreting the results here, in particular for binary traits where each observation is less informative than an observation on a normally distributed trait.

While there were more repeated observations on ewes in these data than there were in the preliminary data set, the number was not great (75 ewes with more than one record). Non-zero estimates of the permanent environmental variance were only observed for Udder Drop, Degree of Separation and Udder Width. Given the data structure, permanent environmental variances may be detected for other traits when there are considerably more ewes with repeated observations. The binary nature of some traits makes estimation of this effect more problematic.

There are considerable differences between the heritability estimates presented in this report and those in the preliminary report. The two principle reasons for this are a shift in emphasis to focus on the mode of the posterior distribution rather than the mean and, more importantly, better selection of priors. In the preliminary analyses the default priors used in the code of Holand et al. (2013) were used (although the code itself was not run, only used as a guide). These priors do not appear to have been uninformative, particularly with respect to the heritability. This problem was greater for the binomial traits for which the data set contains less information. An analysis of one trait using the priors used in the preliminary analysis was used to confirm the large impact.

Inclusion of litter size in the model has some small impact upon the heritability estimates of the normally distributed traits. This is consistent with these traits being genetically correlated with litter size such that the genetic variance includes some variation due to litter size.

These analyses have provided estimates of the heritabilities of udder and teat conformation traits and Chronic Mastitis in Texel ewes that are better than those from the preliminary analysis, due to the increased amount of data and an improved analysis. Also, some degree of consideration was given to the relationship between litter size and these traits.

For inclusion of any of these traits in a selection programme further information would be required. Without knowledge of the genetic correlations among these traits and with other traits, it is not possible to predict the genetic progress that would be made in these traits or the correlated genetic progress in other traits. In particular, there is no information on the genetic relationship between these traits and the tendency of ewes to suffer from mastitis.

Acknowledgements

We are grateful to all the farmers involved in this project for their time, and for allowing us access to their flocks and data records. We would also like to thank Amy KilBride for technical assistance and Libby Henson for permitting access to the Grassroots database.

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Appendix 4

Blood sample biochemistry results

Submission reference: **S010260** **Date received:** 05/12/2013
Your reference: Ed Smith
Owner: Smith Ed School of Life Sciences University of Warwick Gilbert Hill Rd CV4 7AL
1084040 x54 183.60 **Charge: £** 183.60

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LABORATORY REPORT

VETERINARY CENTRE MANAGER

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Scotland UK
Tel: (0131) 535 3130
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Email: vcedinburgh@sac.co.uk

Practice: Ed Smith
Research Fellow
School Of Life Sciences
University of Warwick
Coventry
CV4 7AL

Clinician: Ed Smith

Your reference: Ed Smith

Owner: Smith Ed School of Life Sciences
University of Warwick Gilbert Hill Rd
CV4 7AL

Species: OVINE
Breed: Not stated
Age: X X
Sex: FEMALE
Sample ID: WAL001 02920504
Main Specimen Type: BLOOD

Submission reference: S010260
Date received: 05/12/2013
Last reported on: 17/12/2013
Status : FINAL
Previous Reference :

TESTING SUMMARY

Determination:	Urea	Albumin	BOHB
Units:	mmol/l	g/l	mmol/l
Limits:	4.0 - 8.0	30 - 40	<= 1.2
Sample ID			
WAL001 02920504	4.8	31	0.4
WAL002 02920528	4.2	**29**	0.4
WAL003 02920542	**9.0**	33	0.3
WAL004 02920559	**9.5**	**30**	0.4
WAL005 02920573	7.2	32	0.4
WAL006 02920597	4.1	31	0.5
SHR001 02920610	5.4	32	0.6
SHR002 02920634	5.7	32	1.0
SHR003 02920658	6.0	34	0.6
SHR004 02920672	5.6	31	**1.3**

F Howie

Reported by: Fiona Howie, V.I.O. Reported on: 17/12/2013 at 09:58

Methods as agreed 01/04/2013

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SHR005 02920696	5.7	32	**1.2**
SHR006 02920719	5.0	30	0.7
CHE001 02920733	4.8	34	0.5
CHE002 02920757	5.5	**29**	1.1
CHE003 02920771	5.6	34	0.5
CHE004 02920795	6.5	35	0.6
CHE005 02920818	4.3	32	0.4
CHE006 02920832	4.0	31	0.4

Biochemistry fairly unremarkable.
FH 5/12/13

F Howie

Reported by: Fiona Howie, V.I.O.

Reported on: 17/12/2013 at 09:58

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Methods as agreed 01/04/2013

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Submission reference: **S010486** **Date received:** 18/03/2014

Your reference: School of Life

Owner: Smith Ed School of Life Sciences University of Warwick Gilbert Hill Rd CV4 7AL

1084040 x18 61.20 **Charge: £** 61.20

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LABORATORY REPORT

VETERINARY CENTRE MANAGER

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Coventry
CV4 7AL

Clinician: Claire Grant

Your reference: School of Life

Owner: Smith Ed School of Life Sciences
University of Warwick Gilbert Hill Rd
CV4 7AL

Species: OVINE

Breed: Ovine

Age: X X

Sex: FEMALE

Sample ID: 6674

Main Specimen Type: BLOOD

Submission reference: S010486

Date received: 18/03/2014

Last reported on: 18/03/2014

Status : FINAL

Previous Reference :

TESTING SUMMARY

Determination:	Urea	Albumin	BOHB
Units:	mmol/l	g/l	mmol/l
Limits:	4.0 - 8.0	30 - 40	<= 1.2
Sample ID			
6674	6.2	**29**	0.3
3852	7.7	31	0.6
1121408	**9.2**	**26**	0.7
01382	**8.1**	**29**	0.8
3579	**8.7**	**30**	0.5
6074	5.3	**29**	0.4

No evidence of energy deficit. There is a tendency to hypoalbuminaemia but the urea results do not support a suggestion of inadequate protein intake and in fact concentrations are slightly high in most samples.

MGK 18-3-14

Reported by: Morag Kerr, V.I.O. Reported on: 18/03/2014 at 17:07

Methods as agreed 01/04/2013

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Submission reference: **S010507** **Date received:** 24/03/2014
Your reference: PO00053021
Owner: School of Life Sciences University of Warwick CV4 7AL
1084040 x18 61.20 **Charge: £** 61.20

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LABORATORY REPORT

VETERINARY CENTRE MANAGER

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Practice: School of Life Sciences
University of Warwick
Coventry

Clinician: C Grant
Your reference: PO00053021
Owner: School of Life Sciences University of Warwick CV4 7AL

Species: OVINE
Breed: Sheep
Age: X X
Sex: FEMALE
Sample ID: 00120
Main Specimen Type: BLOOD

Submission reference: S010507
Date received: 24/03/2014
Last reported on: 26/03/2014
Status : FINAL
Previous Reference :

TESTING SUMMARY

Determination:	Urea	Albumin	BOHB
Units:	mmol/l	g/l	mmol/l
Limits:	4.0 - 8.0	30 - 40	<= 1.2
Sample ID			
00120	**11.7**	32	0.5
00268	**10.6**	33	0.7
07163SH28718	**10.5**	31	0.9
2826	**11.3**	32	0.7
01770	**12.9**	31	0.8
010135	**10.1**	**29**	0.8

All urea concentrations are somewhat elevated. It's unlikely this is related to renal dysfunction, or has a circulatory cause, given that the sheep are well. It is possible it is related to excessive or poor quality protein intake. No evidence of any energy deficit and albumin results are unremarkable.

MGK 26-3-14

Morag G. Kerr

Reported by: Morag Kerr, V.I.O. Reported on: 26/03/2014 at 17:41

Methods as agreed 01/04/2013

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Submission reference: **S010314** **Date received:** 10/01/2014

Your reference: Leom Bloods

Owner: Smith Ed School of Life Sciences University of Warwick Gilbert Hill Rd CV4 7AL

1084040 x18 61.20 **Charge: £** 61.20

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LABORATORY REPORT

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Clinician: Ed Smith

Your reference: Leom Bloods

Owner: Smith Ed School of Life Sciences
University of Warwick Gilbert Hill Rd
CV4 7AL

Species: OVINE

Breed: Sheep

Age: X X

Sex: UNKNOWN

Sample ID: L11458

Main Specimen Type: BLOOD

Submission reference: S010314

Date received: 10/01/2014

Last reported on: 10/01/2014

Status : FINAL

Previous Reference :

TESTING SUMMARY

Determination:	Urea	Albumin	BOHB
Units:	mmol/l	g/l	mmol/l
Limits:	4.0 - 8.0	30 - 40	<= 1.2
Sample ID			
L11458	7.1	32	0.4
L263	4.2	32	0.5
CFP10099	5.1	33	0.8
I33	4.5	33	0.5
L375	6.6	34	0.5
L467	4.8	34	0.5

Biochemistry within normal limits.
AC 10/01/14

Reported by: Alistair Cox BVMS MSc MRCVS FRCPath

Reported on: 10/01/2014 at 17:16

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Methods as agreed 01/04/2013

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