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Periparturient and early lactation performance and metabolism of replacement Holstein-Friesian dairy heifers out-wintered on fodder beet or perennial ryegrass compared with winter housing

Running title: Out-wintering replacement heifers

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Abstract

The effect of winter feeding system on the periparturient performance and early milk production and metabolism of pregnant Holstein-Friesian dairy heifers destined for a high output, total mixed ration (TMR) based system was examined. Forty eight, 23 ± 0.4 month old, in-calf Holstein-Friesian heifers were assigned to one of three treatments: out-wintered on perennial ryegrass and grass silage (G); out-wintered on fodder beet and grass silage (F); or housed and fed grass silage and concentrate (H). The study commenced in November 2013, with heifers on treatment for 91 days, then housed from six weeks prior to parturition and fed a dry cow TMR. Post-partum performance and metabolism was monitored for 12 weeks. Pre-partum, average daily gain was lower in heifers receiving G at 0.95 kg d^{-1} cow^{-1} than F or H (1.24 and 1.11 kg d^{-1} cow^{-1} respectively). Body condition score of heifers that received G was also lower compared to treatments F or H both pre- and post-partum. Pre-partum, plasma β-hydroxybutyrate concentrations were lowest in animals receiving treatment H, highest in F and intermediate in G, but did not differ between treatment post-partum. Milk yield averaged 30.7 kg d^{-1} cow^{-1} and was not affected by treatment, but milk fat content was lowest in animals that received F (35.4, 37.1 and 37.9 g kg^{-1} for F, G and H respectively). The results indicate that out-wintering in-calf dairy heifers on fodder beet or perennial ryegrass is a viable alternative to winter housing in high-output TMR based milk production system in a temperate region.

Keywords: dairy heifer, fodder beet, pasture, milk production, out-wintering

Introduction

Out-wintering is the practice of rearing cattle outside through the winter months on a purpose built out-wintering pad, on a 'sacrifice' field, or grazing forage *in-situ* (Barnes *et al*., 2013). The *in-situ* systems commonly use autumn stockpiled pasture (deferred stocking), or a forage crop grown specifically for winter grazing, and usually include grass silage supplementation (Barnes *et al*., 2013). Currently the most popular forages used for out-wintering pregnant heifers within the United Kingdom (UK) are deferred stocking, forage kale or fodder beet, with on average 34% of dry matter intake (DMI) provided as baled grass silage (Atkins *et al*., 2014). Out-wintering replacement dairy heifers has been suggested as a low cost alternative to housing (French *et al*., 2009), and may help facilitate dairy herd expansion. Indeed, farmers that out-winter replacement heifers in the UK generally have a herd size well above average, and the major reason farmers out-winter cattle is reported to be to reduce the cost of rearing (Atkins *et al*., 2014). Improving animal health and welfare is also reported to be a major reason for out-winter replacement heifers (Atkins *et al*., 2014; Barnes *et al*., 2013), although winter weather and soil conditions potentially increase the risk to animal health, welfare and performance (Barnes *et al*., 2013).

Cattle will adapt their behaviour to reduce heat energy loss in cold, wintry conditions, primarily by seeking shelter and reducing the exposed surface area by adopting a lying position (Redbo *et al*., 2001). A reduction in time spent eating has also been reported (Redbo *et al*., 2001), which may have an associated effect of reducing DMI and animal performance. However, previous research has reported little effect of out-wintering on animal performance (Keogh *et al*., 2009; McCarrick and Drennan, 1972; O'Driscoll *et al*., 2010) except when offered a diet exclusively of grazed perennial ryegrass (Keogh *et al*., 2009a). Much of the previous research regarding out-wintering has been undertaken with beef cattle or mature dairy cows that are either kept at maintenance or to achieve low rates of live weight (LW) gain (Keogh *et al*., 2009a; Morgan *et al*., 2009; O'Driscoll *et al*., 2010). Replacement dairy heifers are required to grow at a more rapid rate and consequently have to partition proportionally more energy to LW gain, and usually possess a lower level of body condition than beef cattle or mature cows (Belyea *et al*., 1978). In addition, dairy heifers have a larger surface area to LW ratio than mature cattle (Berman, 2003), and therefore may be more susceptible to greater heat loss and reduced performance in out-wintering systems. Yearling heifers have been reported to have a reduced DMI and LW gain when exposed to outside winter conditions (Boyle *et al*., 2008), and in-calf cross-bred heifers out-wintered in the UK on spring calving herds have been observed to have variable performance, with winter average daily gain (ADG) ranging from acceptable (0.57 kg d^{-1}) to negative (-0.22 kg d^{-1}; Atkins *et al*., 2015). Reduced ADG through the winter as a heifer can negatively impact productivity and longevity as a cow (Le Cozler *et al*., 2010), however, acceptable LW change from out-wintering compared with housing has been reported in pregnant heifers reared for both spring calving (Kennedy *et al*., 2012) and higher-output (Marsh *et al*., 2009) systems.

It has also been reported that there is little subsequent effect of out-wintering on milk performance or fertility in mature dairy cows in spring calving, predominantly pasture-based milk production systems (Kennedy *et al*., 2012; Keogh *et al*., 2009a; O'Driscoll *et al*., 2010). The subsequent effects in first lactation, higher genetic merit, Holstein-Friesian-type heifers within high-output, total mixed ration (TMR), housed herds has not been investigated, nor has the potential of grazing for replacement heifers when used in conjunction with supplementary conserved forage. The objective of this study was to determine the effect of out-wintering replacement Holstein-Friesian heifers on perennial ryegrass or fodder beet compared to indoors on their subsequent production performance when fed a TMR.

Materials and methods

The experiment was conducted at Harper Adams University, Newport, Shropshire, UK (52°780´N, 2°434´W) from November 2013 to August 2014. Weather data was recorded over the study at a UK Meteorological Office automatic climate station that was located approximately 500 m and equidistant between the two forage and the indoor sites. All procedures involving animals were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 (Amended Regulations 2013).

Animals and treatments

Forty eight, (mean ± SE) 23 ± 0.4 month old Holstein-Friesian heifers, due to calve between mid-February and April 2014, were used in a randomized complete block design. Animals were blocked according to their LW (476 ± 8.3 kg) and predicted transmitting ability (Swanson, 1991) for milk fat plus protein yield (18 ± 1.2 kg), and randomly allocated to one of 3 treatments; housed for the winter and fed baled perennial ryegrass silage and concentrate (H), out-wintered on perennial ryegrass and baled perennial ryegrass silage (G) or out-wintered on fodder beet and baled perennial ryegrass silage (F). The baled silage was a first cut of a predominately ryegrass sward and was common for all treatments for the duration of the wintering period. Treatments commenced for the wintering period on 4th November 2013, with the animals remaining on their respective treatments until approximately 6 weeks prior to their expected calving date, and then housed and fed a straw based, dry cow TMR until parturition. Post-parturition the animals were housed in a free stall shed and offered a TMR *ad libitum* for the first 12 weeks of lactation.

Feeding and management

Feed requirements were calculated according to Nicol and Brookes (2007) to achieve an ADG of approximately 0.9 kg d^{-1} cow^{-1} for each group, with an additional 10% of maintenance requirements for the out-wintered groups. Feed requirement was increased every 2 weeks, subsequent to each LW measurement, to account for increasing maintenance requirement and to try to maintain an even ADG between treatments. Trace-minerals were supplemented to each group at the beginning of the wintering period using two CoSeCure (Telsol Ltd, Leeds, UK) rumen mineral boluses per heifer, that contained 13.4 g Cu, 0.3 g Se (as sodium selenite), and 0.5 g Co per bolus.

Fodder beet

For treatment F, a two hectare area of Lactimo (KWS Momont, Mons-en-Pévèle, France) fodder beet (*Beta vulgaris)* was established in early May 2013 on a gently sloping field with Bridgnorth loamy sand soil type comprising of 70% coarse sand, 14% fine sand, 11% silt, 2-2.5% clay and 2-2.5% OM. At the top of the fodder beet area, approximately 0.1 ha of perennial ryegrass (*Lolium perenne*) was established with the intention of providing an area to adapt heifers to the crop and provide a dry lying area. The heifers grazed wide (60 m), thin strips (0.4 -1.2 m) of the fodder beet crop down-hill from the dry lying area. Using temporary electric fencing (Gallagher, Hamilton, NZ), a fresh area of forage crop was offered daily at approximately 0800 h and a daily allocation of round baled perennial rye grass (*Lolium perenne*) silage delivered to a ring feeder with head space to accommodate 20 heifers. During the first week of the study, heifers were transitioned on to the fodder beet by gradually increasing the daily area allocated by 0.5 m^2 from an initial 1.5 m^2 d^{-1} $heifer^{-1}$ to approximately 3.5 m^2 d^{-1} $heifer^{-1}$, and decreasing the quantity of baled silage offered until the amount offered provided approximately 35% of estimated DMI. Feed allocations were adjusted weekly subsequent to measurements of pre and post grazing herbage mass to provide the required intake. If soil conditions became very wet, additional dry lying was supplied by spreading wheat straw bales in an area of the grass strip. A road way ran adjacent to the length of the fodder beet field and was used to deliver the supplementary forage. The ring feeder was moved weekly to a dry area and drinking water was supplied from one large permanent water trough.

Grazing

For treatment G, at the beginning of October 2013, 7.3 ha (of which 6.1 ha was used) of established perennial ryegrass (*Lolium perenne*) that was established in the Spring of 2011 was managed to obtain an average pre-grazing herbage mass of approximately 3500 kg DM ha^{-1} over the wintering period. The major soil type was Salop series clay loam, comprising 20% gravel and coarse sand, 18% fine sand, 22% silt, 30% clay and 5-10% OM. Perennial ryegrass was strip grazed daily using temporary electric fencing (Gallagher, Hamilton, NZ). Fresh pasture was offered at approximately 0800 h each day and the grass back-fenced every 3 d to prevent trampling of the previously grazed area. A daily allocation of round baled perennial ryegrass (*Lolium perenne*) silage was manually fed in the morning. Daily area offered was initially approximately 29 m^2 d^{-1} $heifer^{-1}$ with silage offered to provide 35% of DMI, and was adjusted weekly subsequent to measurements of pre- and post-grazing herbage mass to achieve the required intake. During a period of severe wet weather in the final two weeks of the wintering period, concentrates (containing (g kg^{-1} DM basis); 341 of wheat, 273 molasses (sugar beet), 200 wheat feed, 61 rapeseed meal, 60 maize gluten, 51 wheat distillers dark grains, and 13 feed grade urea) were supplied up to 1.5 kg d^{-1} $heifer^{-1}$ to maintain metabolisable energy (ME) intake and ADG. Prior to the commencement of the study the grass silage bales had been placed in the position they would be fed, with access to the silage (and supplementary feed) via a ring feeder with head space for 20 heifers. The ring feeder was moved daily and drinking water supplied in a trough which was moved in line with the area being grazed.

Housing

For treatment H, housing consisted of a 116 m^2 straw bedded area, with a 67 m^2 concrete pad. Fresh straw bedding was provided three times weekly and the concrete pad scraped daily. The concrete area included a rectangular shaped feeder and a large water trough. Concentrates containing the same composition as fed to the out-wintered animals were delivered daily along a feed trough the length of the straw area, initially at 1.5 kg d^{-1} $heifer^{-1}$ and adjusted every two weeks in line with LW and ADG. Forage was offered *ad libitum* and delivered as required. There was sufficient area in both the bale feeder and concentrate trough for all heifers to access feed at once.

Approximately six weeks prior to parturition, heifers were housed in a free stall area for 2 to 3 weeks prior to expected parturition date, and then moved to a straw bedded yard immediately prior to calving. Movement of heifers from their respective winter groups to the dairy housing was undertaken in two batches; the first batch of 33 heifers in week 12 of out-wintering and the remaining 15 heifers two weeks later. Post calving the heifers were housed in a straw yard for the first week before being moved to a free stall area with a roughage intake control (RIC) system (Hokofarm Group BV, Marknesse, the Netherlands) for the remainder of the study. Concrete passageways were scraped using automatic scrapers six times daily, straw bedding applied daily and replaced monthly, and free stalls dusted with sawdust and lime three times weekly. Milking occurred twice daily at approximately 0600 and 1600 h. The dry cow TMR was based on wheat straw, corn silage and alfalfa silage (Table 1) and the lactating cow TMR on corn silage and alfalfa silage, and were formulated according to Thomas (2004). Total mixed rations were offered at approximately 105% of *ad libitum* and were fed once daily at 0700 h with feed removed daily from the dry and fresh cow feeding areas, and 3 times weekly from the RIC feeders.

Experimental routine

Pre-partum period

The heifers were weighed (Trutest, Auckland, NZ) and body condition score (BCS; 1-5 scale where 1 = emaciated and 5 = obese; Lowman *et al.*, 1976) recorded every 2 weeks. Heifers were also assessed for cleanliness every 2 weeks as described by Boyle *et al* (2008), in five areas (front leg, back leg, flank, belly and back) scored on a scale of 1 to 4 where 1 is clean and dry and 4 is very dirty, and summed to provide an overall score. During weeks 0, 4, 8 and 12 of out-wintering, blood samples were collected at 1000 h via the coccygeal vein into vacutainers (Becton Dickinson Vacutainer Systems, Plymouth, UK), containing no anticoagulant, oxylate or lithium heparin, spun at 1200 × g for 10 min, the serum or plasma separated and stored at −20°C before subsequent analysis.

Group intake and forage utilisation were assessed weekly using measurements of herbage mass and the area grazed each week. Pre and post grazing herbage mass was assessed using five 0.9 m^2 quadrats of fodder beet and five 0.1 m^2 quadrats of perennial ryegrass, randomly selected from the next weeks grazing area, and the same from the previous weeks grazing area, excluding the section being grazed on the day of sampling. Pre-grazing fodder beet was plucked from the ground and loose soil knocked off before weighing each quadrat using a digital hand-held scale (Brecknell, Fairmont, MN, USA). A random sub-sample of 5 whole fodder beet were weighed, rinsed to remove soil, patted dry and reweighed to correct for residual soil. The fodder beet leaves were separated from the bulbs and weighed, then each component was chopped, and a sub-sample stored at -20°C prior to subsequent analysis. Pre-grazing perennial ryegrass quadrats were cut to ground level and the soil washed from the base of the plants before oven drying (AOAC, 2012). A separate perennial ryegrass sample cut at the grazing horizon (4 cm above ground level) was retained at -20°C for subsequent analysis. Post grazing herbage mass was cut to ground level, washed, weighed and dried (AOAC, 2012). Each grass silage bale was weighed prior to opening and the weight of the wrap and strings subtracted. The fresh weight of silage offered was recorded for each group with silage refusals weighed and removed weekly. A sub-sample of the silage fed to each group and the concentrate was collected weekly and stored at -20°C for subsequent analysis. During the six week pre-partum period when heifers were housed, a sub-sample of the dry cow TMR was collected according to Sinclair and Atkins (2015) and stored at -20°C until subsequent analysis.

Post-partum period

The birth weight of the calves was recorded within the first 6 h post-partum, and within 24 h of parturition the cows were weighed, assessed for BCS and blood sampled via the coccygeal vein. Subsequently, cows were weighed and BCS recorded during weeks 2, 4, 6, 8, 10 and 12 of lactation, with blood samples collected during weeks 4, 8 and 12 at 1000 h. Blood samples were collected into vacutainers (Becton Dickinson Vacutainer Systems, Plymouth, UK), containing no anticoagulant, oxylate, or lithium heparin, spun at 1200 × g for 10 min, the serum or plasma separated and stored at −20°C prior to subsequent analysis.

Individual feed intake was recorded from week 2 of lactation, and samples of TMR collected weekly according to Sinclair and Atkins (2015). Milk yield was recorded automatically at each milking and a sample collected for milk fat, protein and SCC concentration from two consecutive milkings every 2 weeks.

Chemical analysis

Forage, concentrate and TMR samples were bulked by month and analysed (AOAC 2012) for dry matter (DM; 934.01), crude protein (CP; 990.03) and ether extract (2003.05), with neutral detergent fibre (NDF) concentration determined according to the method of Van Soest *et al*. (1991), and water soluble carbohydrate (WSC) according to (Thomas, 1977). For perennial ryegrass and big bale grass silage, the ME content was predicted from the modified acid detergent fibre (MADF) content (Givens *et al*., 1990), and the ME concentration of the concentrate and TMR estimated from their composition. In addition, forages were analysed for Ca, K, Mg, Na, P, S, Co, Cu, Fe, Mn, Mo and Se using the DigiPREP digestion system (Qmx Laboratories, Essex, UK) and analysis by inductively coupled plasma mass spectroscopy (ICP-MS; Thermo Fisher Scientific Inc., Hemel Hempstead, UK) as described by Sinclair and Atkins (2015). Blood serum samples were analysed for blood urea nitrogen (BUN) and β-hydroxybutyrate (BHB), and blood plasma for glucose (Randox Laboratories, County Antrim, UK; kit catalogue no. UR221, RB1007 and GL2623, respectively) using a Cobas Mira Plus autoanalyser (ABX Diagnostics, Bedfordshire, UK). Milk fat, protein and somatic cell count (SCC) were determined at National Milk Laboratories (NML, Wolverhampton, UK).

Statistical analysis

Data were analysed using Genstat version 16. Continuous variables were tested for normality and analysed using repeated measures ANOVA. The model included main effects of treatment and time, and their interaction according to:

$$y_i = \mu + B_i + w_j + t_k + w.t_{jk} + \epsilon_{ijkw}$$

where y_i = dependent variable; μ = overall mean; B_i = random effect of block; w_j = effect of winter treatment (j = fodder beet, perennial ryegrass, housed); t_k= effect of time (k = week 0 to 12 in either the out-wintering or post-partum periods); $w\ .\ t_{jk}$ = interaction between variables; ε_{ijkw} = residual error. Milk SCC was right skewed and transformed to $\log_{10}$ prior to analysis by ANOVA. Average daily gain was predicted by linear regression from each LW measurement prior to analysis by ANOVA. Differences were considered significant at $P < 0.05$ and least significant difference tests conducted post hoc. Means and pooled standard error of the mean are presented in figures and tables.

Results

The mean maximum temperature was 9.6 ± 0.38, 9.8 ± 0.37 and 8.7 ± 0.36°C respectively and the number of days where air temperature was below 0°C was 7, 3 and 4 in November, December and January respectively. Rainfall for November, December and January was 66.1, 64.6 and 103.9 mm and it rained on 12, 13 and 21 days respectively. The average length of the wintering period was 91 d for animals receiving G or F treatments and 92 d for H treatment group (SED 3.1, P = 0.928). Days between housing in the dry cow area and parturition was 48, 42 and 40 for G, F and H treatment groups respectively (SED 8.8, P = 0.633).

Crop yield and chemical composition

Fodder beet yield was 19,900 ± 580 kg DM ha^{-1} and the proportion of the crop utilised was 0.82 ± 0.037. The mean area of fodder beet offered was 4.3 ± 0.17 m^2 d^{-1} $heifer^{-1}$, which equated to 8.5 ± 0.43 kg DM d^{-1} $heifer^{-1}$ offered and 7.0 ± 0.47 kg DM d^{-1} $heifer^{-1}$ consumed. Herbage mass of perennial ryegrass was 3410 ± 128 and 1960 ± 100 kg DM ha^{-1} pre and post grazing respectively. The mean area of perennial ryegrass pasture offered was 41.2 ± 1.86 m^2 d^{-1} $heifer^{-1}$, which equated to 14.1 ± 0.53 kg DM d^{-1} $heifer^{-1}$ offered and 6.0 ± 0.58 kg DM d^{-1} $heifer^{-1}$ consumed. The fodder beet and perennial ryegrass had the lowest DM content which was approximately 240 g kg^{-1} lower than the grass silage (Table 2). The NDF content was greatest in the perennial ryegrass and grass silage and lowest in the fodder beet, whereas the WSC was 280 g kg^{-1} DM greater in the fodder beet than the perennial ryegrass, with the grass silage having the lowest value. The quality of the perennial ryegrass was high, with a mean ME content 0.8 MJ kg^{-1} DM greater than the baled silage.

Live weight, body condition score and cleanliness

Group DMI during the wintering period was numerically similar between heifers receiving F or G treatments, however it was estimated to be 1.1 kg d^{-1} $heifer^{-1}$ lower in animals receiving treatment H (Table 3 and Figure 1). Mean LW increased by 96 kg $heifer^{-1}$ over the 91 d wintering period, and no difference ($P > 0.05$) was observed between treatments at housing or parturition. There was however, an interaction ($P < 0.001$) between treatment and time, with heifers receiving F or H treatments increasing in LW throughout the wintering period, but there was no change in treatment G between weeks 13 to 11 and weeks 9 to 7 pre-partum (Figure 2). This was reflected in ADG over the wintering period, which was lower ($P < 0.05$) in treatment G than either F or H treatments. The birth weight of calves was not affected by treatment ($P = 0.281$), with a mean of 39.2 kg. Live weight decreased ($P < 0.001$) from parturition to week 4 of lactation and increased from week 6 onwards, but there was no effect of treatment or treatment x time interaction ($P > 0.05$).

Heifers receiving treatment H had a greater gain in BCS by the end of the wintering period compared to G ($P = 0.002$), and at parturition the BCS of heifers receiving G was lower ($P < 0.05$) than treatment F or H (Figure 3). Post-partum, there was a main effect of time on BCS ($P < 0.001$), which reduced between week 0 to 2 and then increased from week 4 to 12 of lactation. Heifers receiving treatment H tended ($P = 0.052$) to have a greater BCS loss over the first 12 weeks post-partum compared to treatment G. Heifers began the study with a mean total cleanliness score of 8.1, 8.3 and 8.3 (SED 0.30, $P = 0.685$), and for the wintering period were dirtier in F, scoring 14.5, 11.7 and 12.0 (SED 0.50, $P < 0.001$) for F, G and H respectively.

Blood metabolite concentrations

Heifers receiving treatment F had a lower ($P < 0.05$) mean serum BUN concentration during the wintering period than those receiving treatment G or H, whereas post-partum no difference ($P = 0.715$) was observed between treatments (Table 3). During the wintering period a time x treatment interaction ($P < 0.001$) was observed (Figure 4); BUN concentration in heifers receiving treatment G or H increased at each successive pre-partum measurement, while in treatment F, concentrations decreased over the first four weeks of out-wintering (weeks 19 to 15 pre-partum), increased over the subsequent four weeks (weeks 15 to 11 pre-partum) before decreasing during the final four weeks (weeks 11 to 7 pre-partum). Post-partum, a main effect of time on BUN concentration was observed ($P < 0.001$), which decreased between calving and week 4 then increased between week 4 to 8 of lactation, and there was a tendency ($P = 0.094$) for concentrations to be lower between parturition and week 4 of lactation in treatment F than G.

Mean serum BHB concentration was affected by treatment during the wintering period ($P < 0.001$), with BHB being greatest in animals receiving treatment F, followed by G and H. There was a time x treatment interaction ($P < 0.001$) in serum BHB concentration over the wintering period (Figure 5) with concentrations in heifers receiving treatment H increasing gradually over the wintering period (weeks 19 to 7 pre-partum), but in those receiving either treatment F or G concentrations increased rapidly between week 0 to 4 of out-wintering (weeks 19 to 15 pre-partum), then decreased between week 4 to 8 (weeks 15 to 11 pre-partum). Post-partum a main effect of time on serum BHB was observed ($P < 0.001$), with concentrations increasing in all treatments over the 12 week period, but there was no effect ($P > 0.05$) of treatment.

Mean blood plasma glucose concentration were affected by treatment during the wintering period, and was greatest (P < 0.001) in heifers receiving treatment F and lowest in G, with treatment H being intermediate. There was a time x treatment interaction (P = 0.001) during the wintering period (Figure 6) with a decrease in plasma glucose concentration across all treatments between week 0 to 4 of out-wintering (weeks 19 to 15 pre-partum), with concentrations being higher in heifers receiving treatment F than G or H. Post-partum, a main effect of time on plasma glucose was observed (P = 0.003), with concentrations decreasing between calving and week 4 of lactation and then remained unchanged, but there was no effect (P > 0.05) of dietary treatment, although there was a tendency (P = 0.085) for concentrations to be lower in treatment G than F.

Intake and milk production

There was no effect (P = 0.389) effect of treatment on mean DMI over the 12 weeks of lactation, with an average of 18.4 kg DM d^{-1}. However, a time x treatment interaction was observed (P = 0.013), with a greater DMI recorded during week 2 and 3 of lactation for animals receiving treatment G, than F or H (Figure 1). Mean milk yield was 30.7 kg $d^{-1}$and was not affected by treatment (P > 0.05; Table 4). The lowest milk fat concentration and yield was recorded in treatment F compared to H, with G being intermediate (P < 0.05). In contrast, heifers receiving F had the highest milk protein concentration (P < 0.05), although milk protein yield did not differ between treatments (P > 0.05). On a milk-solids (kg fat + protein) basis, heifers that received treatment F produced approximately 0.12 kg d^{-1} less (P < 0.05) over the first 12 weeks of lactation than treatment G or H, which did not differ. Heifers which had been out-wintered on perennial ryegrass had a lower milk SCC than treatment F or H (P < 0.05), although SCC was generally low in all treatments.

Discussion

Pre-partum performance

Cattle, particularly beef and lactating animals, are generally considered to be very cold hardy and in still, dry conditions, rarely experience temperature below their lower critical temperature (LCT**;** the temperature below which an animal must increase its rate of heat production to maintain homethermy), unless weather is extreme (Young, 1981). However, mild cold stress can increase resting metabolic rate, increasing the energy requirement for maintenance and rate of passage of digesta, resulting in reduced digestive efficiency (Christopherson and Kennedy, 1983). Adaptations to cold weather include an increase in the thickness of the hide and length of coat, as well as behavioural changes such as seeking shelter and lying to reduce the animals exposed surface area (Redbo *et al*., 2001). The winter conditions experienced in 2013/2014 during this study were slightly warmer than the long term regional average, but the latter half of the winter was considerably wetter than typical. For instance, the long term regional average (1981-2010) for January is 74.0 mm of rain on 12.9 wet days (Met Office, 2012), however there was 40% more rain and an additional 8 wet days during the January of the study. Cold experienced by cattle is a function of temperature, solar radiation, wind and rainfall (O'Driscoll *et al*., 2010; Redbo *et al*., 2001; Tucker *et al*., 2007); therefore, due to the wet conditions experienced during the latter half of the winter, the heifers which were out-wintered are likely to have been under some degree of climatic challenge.

Redbo *et al*. (2001) observed a reduction in time spent feeding in periods of high climatic energy demand. A reduction in the time spent feeding may have a number of effects on the animal, including reduced feed intake, lower ADG and mobilisation of body condition, unless compensated for by an increased rate of feed intake or energy density of the feed. Individual DMI pre-partum was not measured in this study, however, mean DMI was numerically lower in heifers which had been housed during the winter on treatment H; 9.5 kg DM d^{-1} heifer^{-1} compared with 10.6 kg DM d^{-1} heifer^{-1} in the out-wintered groups. Despite the heifers that were out-wintered on perennial ryegrass having a lower daily growth rate (0.95 kg d^{-1} heifer^{-1}), the mean for this group was still above the 0.76-0.83 kg d^{-1} required by Holstein-Friesian heifers to reach an optimum LW at first calving (Hoffman, 1997). Achieving optimum LW at first parturition by 22 to 24 months of age reduces the cost of heifer rearing and decreases the period of time required before heifers return the investment on their rearing (Bach, 2011; Boulton *et al.*, 2015). These results indicate that out-wintering replacement heifers need not threaten this aim. Furthermore, out-wintering systems have been reported to reduce the cost of heifer rearing (Atkins *et al.*, 2014; Barnes *et al.*, 2013), providing greater economic advantage to both dairy and beef farmers.

The daily growth rate that we recorded for the heifers on perennial ryegrass was sufficient only to maintain initial BCS by the end of gestation, but heifers that were housed or out-wintered on fodder beet increased by approximately 0.25 of a BCS by parturition. Reasons for the lower performance of heifers offered perennial ryegrass are unclear. Keogh *et al*. (2009a) also reported that multiparous non-lactating dairy cows offered perennial ryegrass *in-situ* lost LW and BCS through the winter, although unlike this study, supplementary silage was not provided. Pasture quality in this study was high, and remained so throughout the temperate winter. The mean ME concentration of fodder beet was not determined, but values reported in the literature are generally high but variable. For example, Clark *et al*. (1987) conducted a series of six balance studies using wether sheep and reported the ME content of fodder beet to be 11.8 MJ kg DM^{-1}, although other studies have estimated the value to be substantially higher at approximately 13 MJ kg DM^{-1} (Chakwizira *et al*., 2014; Matthew *et al*., 2011). An average grass silage is reported to contain approximately 10.3 MJ ME kg DM^{-1} (Park *et al*., 1997), and in comparison the quality of the grass silage used in this study was high at 11.0 MJME kg DM^{-1}, but lower than that of the perennial ryegrass. As a consequence, as the proportion of grass silage in the diet of heifers receiving G increased over time the overall energy content of the diet decreased. Low DM forages or those with surface water can reduce the voluntary feed intake of dairy cows (Butris and Phillips, 1987), and soiling of pasture may also make grass less palatable. Multiparous non-lactating dairy cows offered perennial ryegrass *in-situ* without silage supplementation have been reported to loose LW and BCS through the winter (Keogh *et al*. 2009a). In light of this, high pasture allocations were a deliberate strategy during the study to achieve high intakes, despite compromising grass utilisation. The performance of heifers on perennial ryegrass matched those of the housed and out-wintered on fodder beet for the first six weeks of the winter period. After this LW change and BCS change was lower for G. The onset of very wet weather from mid-December onwards may explain the reduced ADG in heifers fed G. It is also possible that heifers on the fodder beet did not show the same fluctuation in performance because fodder beet access and quality was more consistent and a dry lying area was provided, whereas field conditions and surface water may have increased standing time and subsequent energy expenditure, which in combination with a low grass DM content and soiling may have had a greater effect on DMI.

Elevated blood ketone levels are indicative of catabolism of adipose tissue, and in this study mean BHB concentration over the wintering period was greatest in heifers receiving F and lowest in H, even though the BCS of animals fed F was intermediate between H and G. Fodder beet is typically low in structural carbohydrates and high in soluble carbohydrates which have a rapid rate of disappearance from the rumen (Sabri *et al*., 1988). Grass silage was included to increase dietary fibre and CP concentration in F, but the composition of the resulting diet nevertheless had a low NDF and high WSC content. Rapidly fermented carbohydrates are associated with a reduction in the molar proportion of acetic acid and an increase in the proportions of propionic and butyric acid in the rumen, the latter being converted to BHB by the rumen epithelium (Huhtanen *et al*., 1993). Increasing the quantity of fodder beet in the diet has also been shown to increase the concentration of butyric acid in the rumen fluid of fistulated cattle and increase blood BHB (Vérité *et al*., 1973), and may therefore explain high BHB levels in heifers fed fodder beet. However, in studies conducted using sheep, Sabri *et al*. (1988) did not observe an increase in the ruminal proportion of butyric acid when fodder beet was compared with barley/maize or sugarbeet pulp based diets. Similarly, Keogh *et al*. (2009b) using non-lactating pregnant dairy cows offered fodder beet in-situ during the winter observed no increase in blood BHB.

According to NRC (2001), a diet containing 14.5% CP is required for growing heifers, and the high CP content of the pasture in this study may have resulted in an additional energy cost associated with detoxifying excess ammonia to urea (Twigge and van Gils, 1984). Fodder beet is generally low in protein, particularly in the bulb (Chakwizira *et al*., 2014; Clark *et al*., 1987), and in heifers fed fodder beet, BUN concentrations during the wintering period were low at 2.37 mmol L^{-1}, which may indicate a deficiency in rumen available N and consequently impair animal performance. The high ADG of the heifers fed fodder beet indicates that this was not necessarily the case. Ruminants are able to recycle N extensively during periods of protein deficiency (Reynolds and Kristensen, 2008), and overall N use efficiency is increased in low protein diets (Sinclair *et al*., 2014). Although fodder beet has a low CP content, rumen microbial protein synthesis is primarily determined by the fermentation of carbohydrates (Reynolds and Kristensen, 2008; Sinclair *et al*., 2014), and microbial protein supply may therefore have been greater in heifers fed F than H or G.

Post-partum performance

The intake of TMR was observed to be similar across all treatments over the first 12 weeks of lactation, although heifers that had been out-wintered on perennial ryegrass had a greater DM intake in weeks 2 and 3 of lactation compared with those out-wintered on fodder beet or housed. The most likely explanation for this difference is the lower BCS at parturition in heifers receiving G, with increased DMI as a response to lower levels of adipose tissue at calving being well documented (Roche *et al*., 2009).

Milk yield (kg d^{-1}) during early lactation in Holstein-Friesian animals in the current study when fed a high quality TMR was not affected by winter treatment, a finding consistent with that of similar studies that have examined the effect of out-wintering systems for dairy cows destined for a low-input, pasture-based milk production system (Kennedy *et al*., 2012; Keogh *et al*., 2009a, 2009b). Despite the same diet being fed post-partum, heifers that had grazed fodder beet during the winter period had the lowest milk fat and the greatest milk protein content. Keogh *et al*. (2009b) reported a similar result in mature dairy cows, with a greater milk protein content in animals that had been out-wintered on fodder beet or kale in comparison with those that were housed and fed grass silage. Dairy cows that have been fed fodder beet as part of a ration during lactation exhibit variable effects on milk constituents. Roberts (1987) observed that milk fat and protein concentration increased with fodder beet inclusion whereas Fisher *et al*. (1994) and Ferris *et al*. (2003) found no effect on milk fat but an increase in milk protein content, and Sabri and Roberts (1988) reported no effect on milk fat or protein content following the inclusion of fodder beet. A reduced ruminal acetate concentration in animals fed fodder beet has been reported (Vérité *et al*., 1973), which would be expected to reduce milk fat synthesis (Roche *et al*., 2009), but it would not be expected for this effect to carry-over into lactation. Similarly, Ferris *et al*. (2003) attributed the greater milk protein levels in cows fed fodder beet during lactation to a greater ME intake, but the findings from this study demonstrate that feeding fodder beet during the rearing period can also have a major effect on milk composition during early lactation, even following a 6 week dry-period when heifers were all fed the same basal ration. The heifers that were housed during the winter period in this study tended to lose more body condition during the first 12 weeks of lactation, which may also account for their greater milk fat concentration compared to the out-wintered heifers as has been reported in dairy cows (Garnsworthy and Topps, 1982). The lower SCC in heifers receiving G was unexpected. This may have been a response to cleanliness, as the heifers on fodder beet were dirtier than G over the wintering period, although the heifers that were housed during the wintering period had a similar mean cleanliness score to G.

The differences in blood metabolite concentrations observed pre-partum were not apparent post-partum, although cows receiving G tended to have a lower plasma glucose concentration during early lactation as well as the wintering period. The lack of an effect of treatment on blood metabolites may have been due to the six week period prior to calving when all heifers were housed and fed a common TMR. However, our findings are consistent with Keogh *et al*., (2009b), who reported that plasma BHB and glucose concentrations were not affected post-partum despite out-wintering treatment diets continuing right up to parturition.

Conclusions

Fodder beet or perennial ryegrass that is grazed *in-situ* along with grass silage supplementation is able to support high levels of performance of pregnant Holstein-Friesian heifers during winter in a temperate region. Out-wintering performance can also be similar to housing and feeding a grass silage and concentrate based diet, although it may be more difficult to manage ADG and BCS when grazing perennial ryegrass in the wet conditions experienced during the latter half of this study. Subsequent milk yield of animals offered a high quality ration will not be affected by out-wintering treatment, although milk fat content may be lower and milk protein content greater in animals that are out-wintered on fodder beet.

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Figure 1 DM intake of Holstein-Friesian heifers during 12 weeks of out-wintering on fodder beet with grass silage (■), perennial ryegrass with grass silage (▲), or housed for the winter with grass silage and concentrates (●), and in the first 12 weeks of lactation. Bars are standard error of the mean during the lactation period.

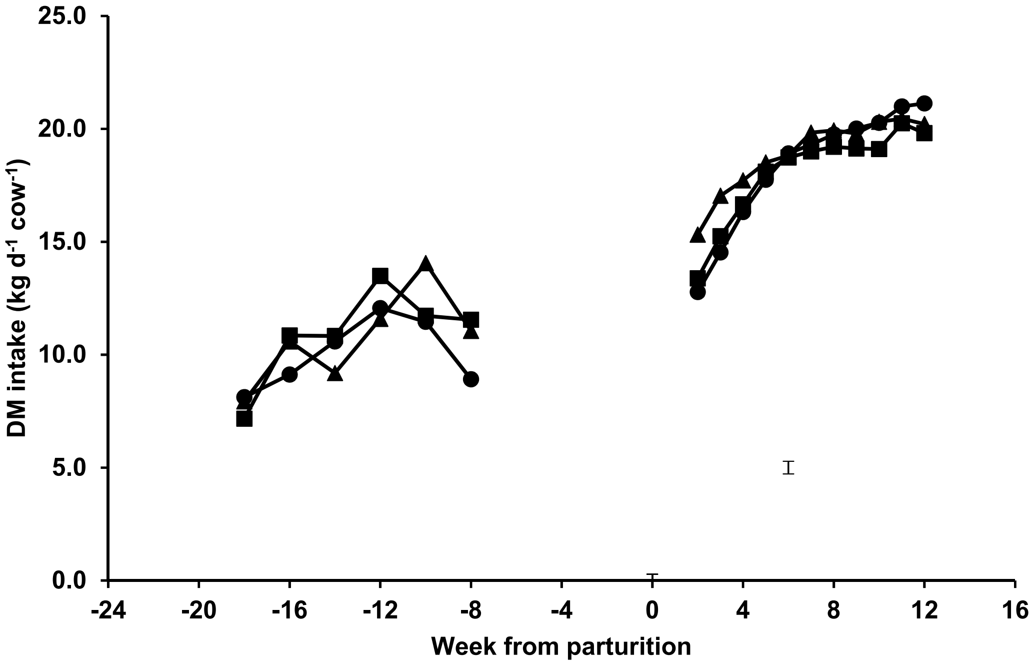
Figure 2 LW of Holstein-Friesian heifers during 12 weeks of out-wintering on fodder beet with grass silage (■), perennial ryegrass with grass silage (▲), or housed for the winter with grass silage and concentrates (●), and in the first 12 weeks of lactation. Bars are standard error of the mean during the out-wintering and lactation period respectively.

Figure 3 BCS of Holstein-Friesian heifers during 12 weeks of out-wintering on fodder beet with grass silage (■), perennial ryegrass with grass silage (▲), or housed for the winter with grass silage and concentrates (●), and in the first 12 weeks of lactation. Bars are standard error of the mean during the out-wintering and lactation period respectively.

Figure 4 BUN concentration of Holstein-Friesian heifers during 12 weeks of out-wintering on fodder beet with grass silage (■), perennial ryegrass with grass silage (▲), or housed for the winter with grass silage and concentrates (●), and in the first 12 weeks of lactation. Bars are standard error of the mean during the out-wintering and lactation period respectively.

Figure 5 Blood serum BHB concentration of Holstein-Friesian heifers during 12 weeks of out-wintering on fodder beet with grass silage (■), perennial ryegrass with grass silage (▲), or housed for the winter with grass silage and concentrates (●), and in the first 12 weeks of lactation. Bars are standard error of the mean during the out-wintering and lactation period respectively.

Figure 6 Blood plasma glucose concentration of Holstein-Friesian heifers during 12 weeks of out-wintering on fodder beet with grass silage (■), perennial ryegrass with grass silage (▲), or housed for the winter with grass silage and concentrates (●), and in the first 12 weeks of lactation. Bars are standard error of the mean during the out-wintering and lactation period respectively.



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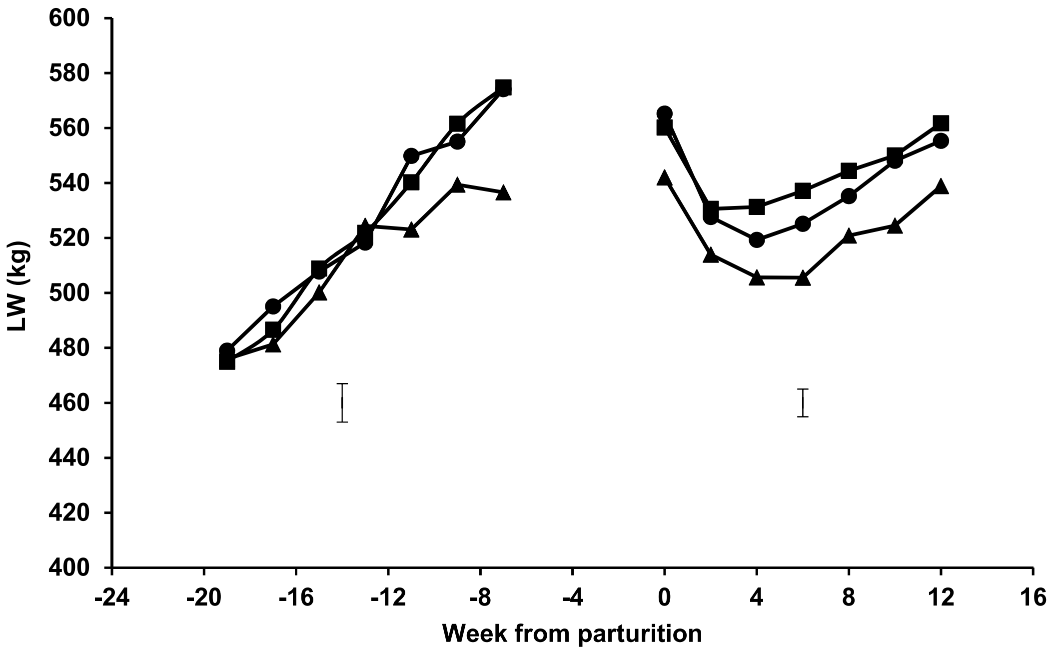

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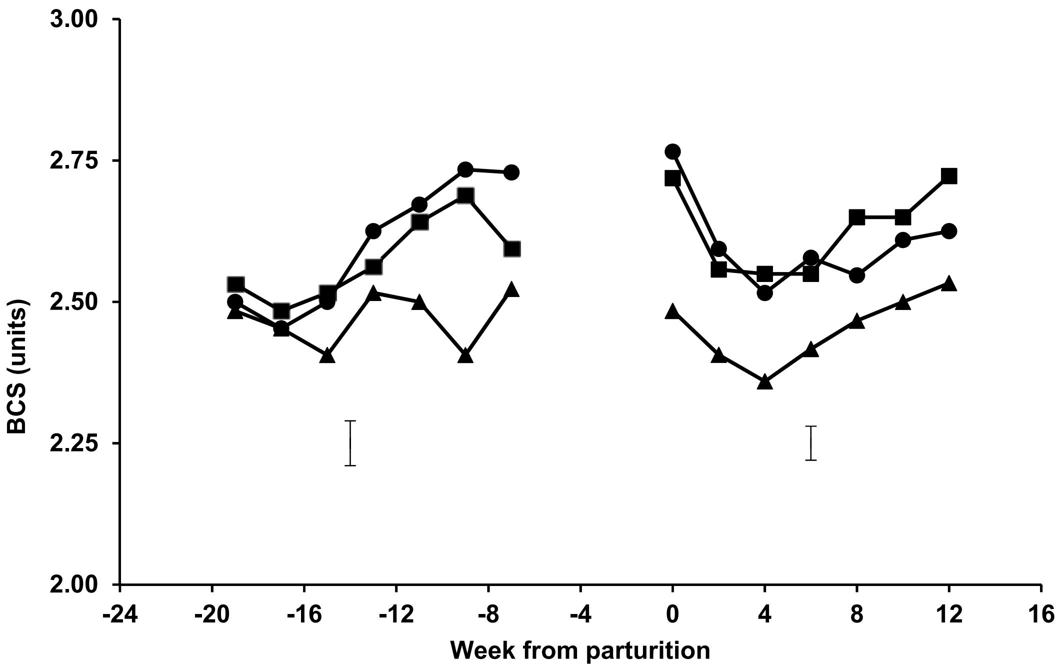

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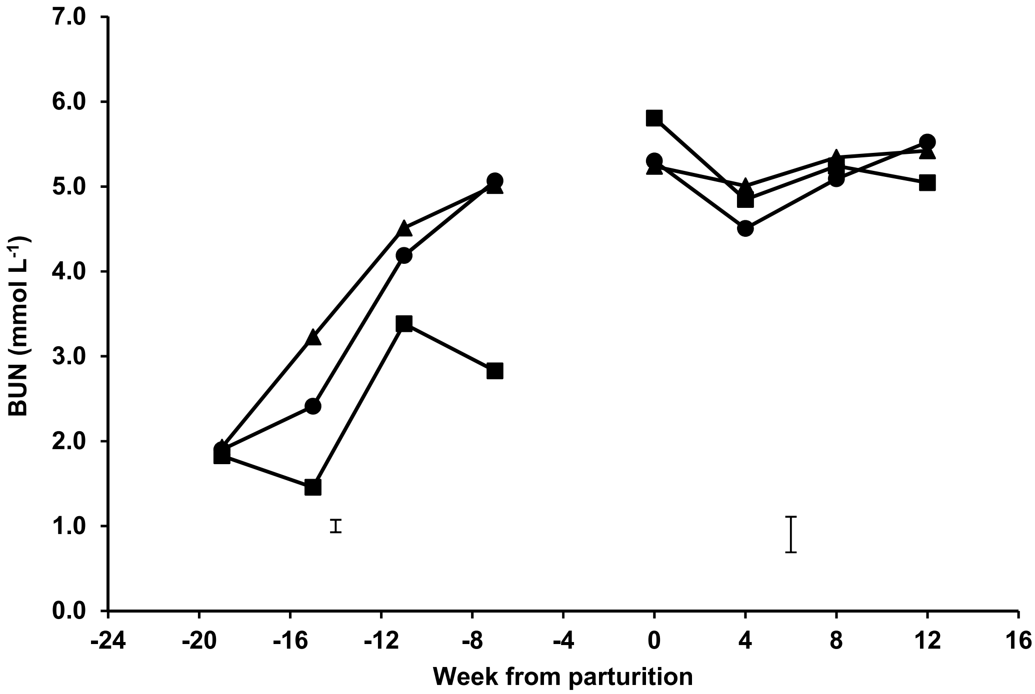

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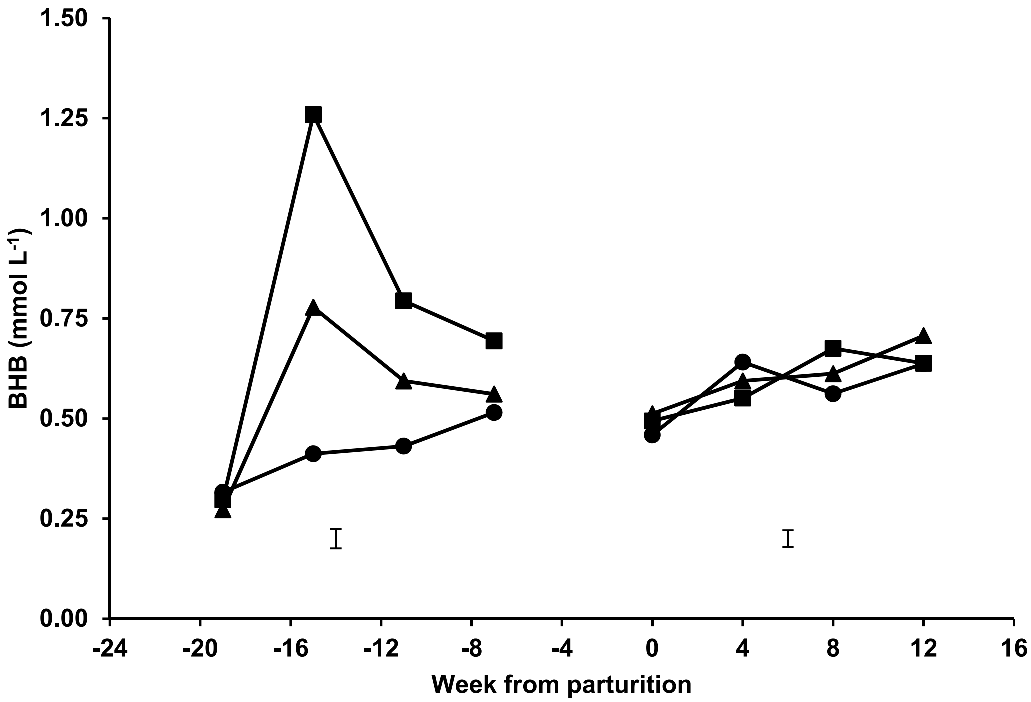

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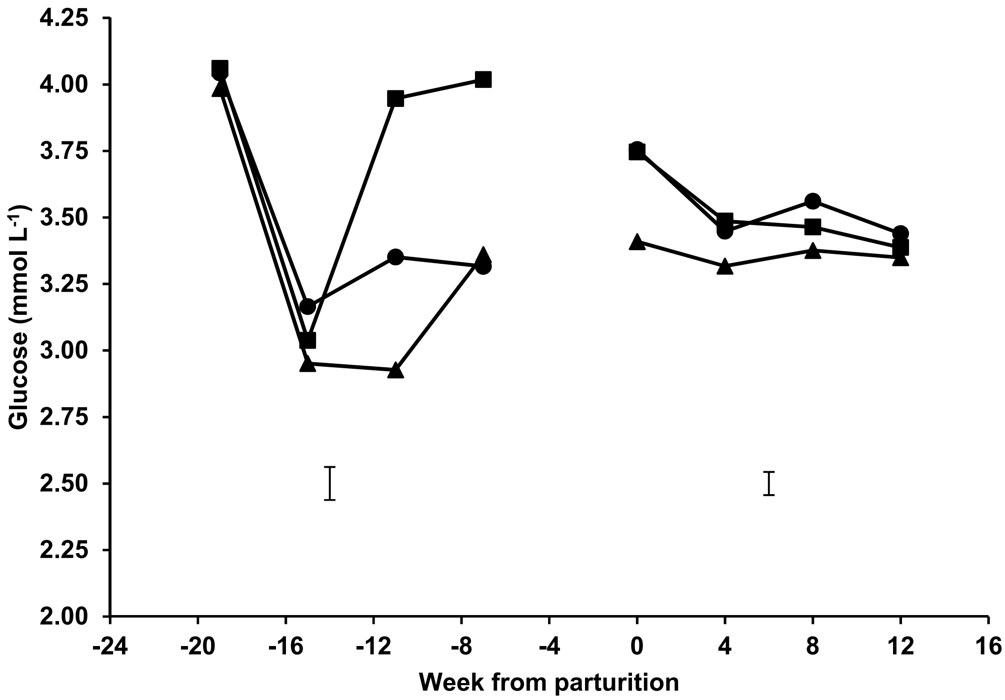

Atkins: Figure 6

Table 1 Ingredient and chemical composition of the dry cow and lactation TMR diet offered to heifers following a period of out-wintering on fodder beet with grass silage, perennial ryegrass with grass silage, or housed for the winter with grass silage and concentrates

	Dry cow	**Lactation**
Ingredient		
Chopped wheat straw	498	---
Maize silage	178	413
Lucerne silage	106	89
Sweet starch[†]	---	127
Soy hulls	---	60
Pot ale syrup	120	60
Molasses	1.8	6.4
Rapeseed meal	21	77
Wheat distillers dark grains	21	77
Soy bean meal	8.9	32
Palm kernel	5.9	21
Rumen protected fat[‡]	---	16
Urea	7.1	5.3
Dry cow minerals[§]	13	---
Lactating cow minerals[¶]	---	5.3
Limestone flour	8.9	5.3
Magnesium chloride	4.4	---
Aspergillus oryzae extract[††]	---	4.0
Vitamins[‡‡]	4.4	---
Buffer[§§]	---	2.2
Anti-mycotoxin agent[¶¶]	---	0.9
Chemical composition		
DM (g kg^{-1})	426	460
CP (g kg^{-1} DM	137	180
NDF (gkg^{-1} DM)	574	391
Ash (g kg^{-1} DM)	95	76
Ether extract (g kg^{-1} DM)	15	38

[†]KW Alternative Feeds, Andover, UK; g/kg 360 cake products, 140 breakfast cereals, 140 cocoa hulls, 140 wheat feed, 70 sugar confectionery, 140 flour.

[‡]Megalac, Volac International Ltd, Royston, UK

[§]**Contained the following macro minerals (g/kg):** 50 P, 250 Mg, 70 Na and trace elements (mg/kg); 6000 Zn, 5000 Mn, 2500 mg Cu, 350 I, 70 Co, 35 Se.

[¶]**Contained the following macro minerals (g/kg):** 210 Ca, 100 Mg, 50 Na, 30 P and trace elements (mg/kg) 6000 Zn, 5000 Mn, 2500 Cu, 400 I, 70 Co, 40 Se.

[††]Provimi Amaferm, Cargill PLC, Surrey, UK

[‡‡]Provimi LiFT, Cargill PLC, Surrey, UK

[§§]Acidbuf, Celtic Sea Minerals, Cork, Ireland

[¶¶]Toxisorb, Biotal, Worcestershire, UK

Table 2 Chemical analysis of the forages and concentrate fed to heifers out-wintered on fodder beet with grass silage (F), perennial ryegrass with grass silage (G), or housed for the winter with grass silage and concentrates (H). Values are the mean of winter feed type bulked by month (n=3, ±SD)†

	Fodder beet		Perennial ryegrass		Concentrate		Grass silage	
DM (g kg^{-1})	143	(±5.8)	140	(±11.9)	842	(±2.16)	401	(±98.8)
ME (MJ kg^{-1} DM)	---		11.8		---		11.0	
CP (g kg^{-1} DM)	117	(±2.2)	213	(±9.5)	178	(±11.6)	136	(±12.0)
NDF (g kg^{-1} DM)	221	(±10.4)	517	(±15.2)	277	(±22.7)	453	(±53.7)
WSC (g kg^{-1} DM)‡	415	(±9.5)	135	(±8.7)	80	(±5.4)	38	(±14.6)
Ca (g kg^{-1} DM)	3.3	(±0.48)	3.4	(±0.47)	4.3	(±0.47)	4.8	(±0.14)
K (g kg^{-1} DM)	34	(±1.6)	37	(±4.5)	13	(±1.4)	41	(±2.8)
Mg (g kg^{-1} DM)	2.6	(±0.28)	1.7	(±0.16)	2.6	(±0.003)	2.1	(±0.16)
Na (g kg^{-1} DM)	3.2	(±0.42)	2.1	(±0.33)	1.2	(±0.19)	0.6	(±0.09)
P (g kg^{-1} DM)	3.0	(±0.05)	4.4	(±0.17)	5.1	(±0.14)	2.8	(±0.10)
S (g kg^{-1} DM)	0.5	(±0.02)	4.9	(±0.05)	4.8	(±0.07)	4.6	(±0.45)
Co (mg kg^{-1} DM)	0.05	(±0.009)	0.10	(±0.035)	0.11	(±0.005)	0.04	(±0.014)
Cu (mg kg^{-1} DM)	7.2	(±0.36)	6.9	(±1.16)	7.1	(±0.47)	3.8	(±0.57)
Fe (mg kg^{-1} DM)	182	(±30.0)	427	(±119.3)	327	(±63.0)	131	(±22.0)
Mn (mg kg^{-1} DM)	43	(±3.6)	38	(±6.8)	53	(±2.1)	196	(±9.0)
Mo (mg kg^{-1} DM)	0.68	(±0.222)	0.56	(±0.019)	< 0.001		0.02	(±0.036)
Se (mg kg^{-1} DM)	0.09	(±0.003)	0.15	(±0.012)	0.16	(±0.040)	0.08	(±0.005)
Zn (mg kg^{-1} DM)	42.5	(±3.01)	23.9	(±0.74)	37.1	(±2.96)	21.8	(±0.95)

†DM was analysed weekly during the out-wintering period (n=13)

‡Water soluble carbohydrate

Table 3 Mean pre- and post-partum performance and blood metabolites in heifers which had been out-wintered on fodder beet with grass silage (F), perennial ryegrass with grass silage (G), or housed for the winter with grass silage and concentrates (H)

	F	G	H	s.e.m	*P*-value
Pre-partum					
Grass silage DMI (kg d^{-1} cow^{-1})[†]	3.6	4.5	8.5	-	-
Total DM intake (kg d^{-1} cow^{-1})[†]	10.6	10.6	9.5	-	-
Initial LW (kg)	475	476	479	14.3	0.979
LW at housing (kg)	583	558	575	13.2	0.418
LW change (kg d^{-1} cow^{-1})[‡]	1.24[a§]	0.95[b]	1.11[a]	0.050	0.001
Initial BCS	2.53	2.48	2.50	0.088	0.930
BCS at housing	2.61[ab]	2.42[b]	2.72[a]	0.073	0.026
BCS change[‡]	0.08[ab]	-0.06[b]	0.22[a]	0.050	0.002
Blood metabolites[‡]					
BUN (mmol L^{-1})	2.37[b]	3.67[a]	3.39[a]	0.102	< 0.001
Glucose (mmol L^{-1})	3.77[a]	3.31[c]	3.47[b]	0.064	< 0.001
3-OHB (mmol L^{-1})	0.76[a]	0.55[c]	0.42[b]	0.032	< 0.001
Post-partum					
Calf birth-weight (kg)	37.6	39.4	40.7	1.38	0.281
DMI (kg d^{-1} cow^{-1})	18.1	18.9	18.3	0.44	0.389
Parturition BW (kg)	560	543	565	11.7	0.390
Final BW (kg)	550	534	555	8.6	0.213
LW gain (kg d^{-1} cow^{-1})	0.11	0.10	0.07	0.087	0.951
Parturition BCS	2.72[a]	2.48[b]	2.77[a]	0.064	0.007
Final BCS	2.72	2.57	2.62	0.047	0.116
BCS change	-0.01	0.08	-0.18	0.073	0.052
Blood metabolites					
BUN (mmol L^{-1})	5.24	5.25	5.11	0.138	0.715
Glucose (mmol L^{-1})	3.52	3.36	3.55	0.062	0.085
BHB (mmol L^{-1})	0.59	0.61	0.58	0.026	0.694

[†]Estimate based on crop DM yield and residual DM yield
[‡]Out-wintering period only
[§]Means within a row with a different superscript differ ($P < 0.05$)

Table 4. Mean milk yield and milk composition during the first 12 weeks of lactation and first lactation fertility of primiparous heifers which had been out-wintered on fodder beet with grass silage (F), perennial ryegrass with grass silage (G), or housed for the winter with grass silage and concentrates (H)

	F	**G**	**H**	**s.e.m[†]**	***P*-value**
Milk (kg d^{-1})	30.1	31.3	30.7	0.44	0.120
Fat (g kg^{-1})	$35.4^{b\dagger}$	37.1^{ab}	37.9^{a}	0.62	0.027
Fat (kg d^{-1})	1.05^{b}	1.16^{a}	1.16^{a}	0.024	0.006
Protein (g kg^{-1})	32.1^{a}	31.2^{b}	31.6^{ab}	0.22	0.026
Protein (kg d^{-1})	0.95	0.97	0.96	0.012	0.357
Fat + protein (kg d^{-1})	2.00^{b}	2.13^{a}	2.11^{a}	0.032	0.017
Milk SCC (log_{10} mL^{-1})	1.77^{a}	1.52^{b}	1.66^{a}	0.038	0.014

[†]Significant effect of time for all parameters ($P < 0.001$) except for milk SCC ($P < 0.01$). There were no treatment x time interactions ($P > 0.05$)
[‡]Means within a row with a different superscript differ ($P < 0.05$).