INTERPRETATIVE SUMMARY: **Relationships between energy balance during early lactation, and cow performance, blood metabolites, and fertility: a meta-analysis of individual cow data**. *By Civiero et al.* Most dairy cows experience negative energy balance in early lactation. Data from 1,508 Holstein-Friesian dairy cow lactations were divided into quartiles (primiparous and multiparous cows separately) based on mean energy balance during 4 to 21 days in milk. Cows with improved energy balance in early lactation had a shorter interval to start of luteal activity, greater milk progesterone concentrations at start of luteal activity, and earlier first observed heats. However, early lactation energy balance did not affect conception rate to first service.

Relationships between energy balance during early lactation, and cow performance, blood metabolites, and fertility: a meta-analysis of individual cow data

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ABSTRACT

This study was designed to contribute to the understanding of the relationships between energy balance (EB) in early lactation (4 to 21 days in milk (DIM)) and fertility traits (interval to start of luteal activity (SLA), interval to first observed heat (FOH), and conception to first artificial insemination (AI)), and their associated relationships with cow performance and blood metabolites between 4 to 150 DIM. Individual cow data (488 primiparous and 1,020 multiparous lactations) from 27 experiments was analyzed. Data on cow performance, EB (on a metabolizable energy (ME) basis), and fertility traits were available for all cows, while milk progesterone data (to determine SLA) and periodic blood metabolite data were available for 1,042 and 1,055 lactations, respectively. Data from primiparous and multiparous cows were analyzed separately, with the datasets for the two parity groups divided into quartiles (Q1 – Q4) according to the average EB during 4 to 21 DIM (EB range for Q1 to Q4: primiparous, -120 to -49, -49 to -24, -24 to -3 and -3 to 92 MJ/d, respectively: multiparous, -191 to -79, -79 to -48, -48 to -22 and -22 to 93 MJ/d, respectively). Differences between EB quartiles for production and fertility traits were compared. In early lactation (4 to 21 DIM), moving from Q1 to Q4 mean DMI and ME intake increased while mean ECM decreased. During the same period, moving from Q1 to Q4 milk fat content, milk fat-to-protein ratio, and plasma non-esterified fatty acid and β-hydroxybutyrate concentrations decreased, while milk protein content and plasma glucose concentrations increased in both primiparous and multiparous cows. When examined over the entire experimental period (4 to 150 DIM), many of the trends in intakes and milk production remained, although the magnitude of the difference between quartiles was much reduced, while milk fat content did not differ between quartiles in primiparous cows. The percentage of cows with FOH before 42 DIM increased from Q1 to Q4 (from 46 to 72% in primiparous cows, and from 41 to 58% in multiparous cows). Interval from calving to SLA and to FOH decreased with increasing EB during 4 to 21 DIM, with these occurring 9.8 and 10.2 d earlier, respectively, in Q4 compared to Q1 (primiparous cows), and 7.4 and 5.9 d earlier, respectively, in Q4 compared to Q1 (multiparous cows). For each 10 MJ/d decrease in mean EB during 4 to 21 DIM, FOH was delayed by 1.2 and 0.8 d in primiparous and multiparous cows, respectively. However, neither d to first AI nor the percentage of cows that conceived to first AI, were affected by daily EB during 4 to 21 DIM in either primiparous or multiparous cows, and this is likely to reflect a return to a less metabolically stressed status at the time of AI. These results demonstrate that interval from calving to SLA and to FOH were reduced with increasing EB in early lactation, while early lactation EB had no effect on conception to the first service.

Key Words: dairy cattle, energy balance, fertility, blood metabolites.

INTRODUCTION

Over the last few decades the milk production potential of dairy cows in many countries has increased dramatically (Miglior et al., 2005). There is little evidence, however, that cows with greater milk yield have a better metabolic efficiency for milk production (**k_l**) than cows with lesser milk yield (Agnew et al., 1998). While part of the extra milk produced by greater yielding cows may be supported by their greater intakes (Ingvartsen and Andersen, 2000), intake capacity during early lactation has not kept pace with the increase in milk yields (Veerkamp et al., 2001). Instead, greater milk yields during early lactation have been driven largely by increased levels of body tissue mobilization, with this reflected in increasing extent and duration of negative energy balance (**NEB**; Agnew et al., 1998). During postpartum NEB, glucose is preferentially partitioned to the mammary gland, pancreatic insulin secretion in response to glucose is suppressed, peripheral tissues exhibit insulin resistance, and cows are susceptible to metabolic disorders (Leroy et al., 2008). In addition, energy balance (**EB**) can also influence fertility.

A number of studies have identified relationships between calculated EB and fertility traits. In one of the earliest studies (involving 13 dairy cows), Butler et al. (1981) concluded that EB during the first 20 d of lactation is important in determining the start of luteal activity (**SLA**). Similarly, in a study involving 134 dairy cows, de Vries et al. (1999) identified a relationship between the extent of energy deficit in early lactation and delay in first observed heat (**FOH**), while in a separate study involving 470 first lactation heifers, de Vries and Veerkamp (2000) observed that each 10 MJ (on net energy basis; **NE**) decrease in nadir EB was associated with a delay in ovulation of 1.25 d. Furthermore, cows with a smaller nadir EB and faster recovery to positive EB had fewer d open and a shorter calving interval (Patton et al., 2007; Carvalho et al., 2014). Using a large data set of almost 1,000 cows, Banos and Coffey (2009) observed genetic correlations between d to first estrus and a number of calculated EB traits.

In addition, other studies have demonstrated relationships between indirect indicators of EB and fertility traits. For example, increased BCS loss has been associated with a delay to first ovulation (Butler and Smith, 1989; Gobikrushanth et al., 2019), while Vercouteren et al. (2015) observed a negative relationship between cyclicity by 21 days in milk (**DIM**) and greater body weight (**BW**) loss. Others have examined the relationship between blood metabolites and fertility, with Dubuc et al. (2012) observing that lesser non-esterified fatty acid (**NEFA**) concentrations post calving were associated with earlier ovulation, while Macmillan et al. (2018) observed a greater incidence of ovulation by 35 d post-partum in cows with lesser NEFA and β-hydroxybutyrate (**BHB**) concentrations.

While relationships between ‘energy status’ and fertility traits have been established, few studies have been able to examine these in a holistic manner, including the inter-relationships with genetic index, energy intake, milk yield, milk composition, milk progesterone, body tissue and blood metabolites, especially using large datasets. In addition, as primiparous and multiparous cows differ in intakes, performance, endocrine and blood metabolite levels during the transition period (Macmillan et al., 2018), and as primiparous cows continue to grow during their first lactation, it might be expected that primiparous and multiparous dairy cows would exhibit different responses to NEB (Wathes et al., 2007). Thus, the primary objective of this study was to use a large individual cow dataset collected over a 20-yr period to examine the relationships between early lactation EB, and cow performance and blood metabolites, and the impact of early lactation EB on fertility outcomes such as: SLA, FOH, conception to first artificial insemination (**AI**), and time to conception, in both primiparous and multiparous cows.

MATERIALS AND METHODS

Experiments, treatments and cows

This study involved a meta-analysis of individual dairy cow data obtained from 27 individual studies which were conducted between 1996 to 2016 at the Agri-Food and Biosciences Institute (**AFBI**) in Hillsborough, Northern Ireland. The results of the majority of these studies have been published in peer reviewed scientific papers, conference proceedings, technical reports, and a PhD thesis (Appendix 1). A minimum prerequisite for the inclusion of any experiment in the analysis was that the experiment encompassed the 'early lactation period' (commencing within a few d of calving, and having a mean length of more than 90 d), included data to allow daily EB to be estimated (i.e. daily DMI, daily milk yield, regular milk composition, BW, and detailed information on the ingredients and chemical composition of the diets offered), and had detailed fertility data. The 27 experiments were variable in length, encompassing incomplete lactations, complete lactations, or multiple lactations. In the case of multi-lactation studies, each lactation was designated as a separate experiment within the analysis. While some full lactation and multi-lactation experiments involved periods of grazing in mid/late lactation, data included in the EB calculations were restricted to periods when cows were housed and when individual cow intakes were available. Within all experiments cows were transferred to a free stall cubicle house shortly after calving.

A total of 79 treatments were examined across the 27 studies, with the majority of treatments examining the impact of diet and/or management strategies on cow performance. Although a number of treatments involved 'alternative' cow genotypes, only Holstein cows were included within the analysis. Individual cows within experiments were excluded from the analysis if the housed period when individual feed intakes were measured was less than 42 d. Data recorded during the first three d of lactation were excluded from the analysis, while data was included in the analysis up until a maximum of 150 DIM (provided individual feed intake data was recorded during the entire period). In addition, cows with a lactation number > 6 were excluded from the analysis.

The final dataset comprised data from 1,508 individual lactations (derived from 1,009) individual cows, representing 488 and 1,020 lactations for primiparous and multiparous cows, respectively. Genetic indexes were sourced for the majority of the cows (936) from Animal Horticulture Development Board, UK, during December 2018. Cows with pedigree information had a mean Predicted Transmitting Ability (**PTA**) for milk yield of -37 (SD, 212.2) kg, a mean PTA for milk fat plus protein yield of 7.0 (SD, 7.32) kg, and a mean Profitable Lifetime Index (**PLI**) of £60 (SD, 150.5). The £PLI represents the additional profit a sire is expected to return from each of its milking daughters over her lifetime, compared with an average sire of £0 PLI, and comprises the following traits: production (34.4%), survival (15.1%), fertility (15.3%), udder health (13.7%), efficiency (11.8%), leg health (8.1%) and calving ability (1.6%). The fertility component of the index is comprised of 5 traits, as follows: calving interval, non-return rate, body condition score, milk yield around insemination, days from calving to first insemination, and number of inseminations needed to get a cow in calf (AHDB Dairy, 2020).

Diets offered

Diets offered were predominantly based on grass silage and concentrates. However, in a number of studies (n = 16) grass silage was partially replaced with corn silage (usually between 20 - 40% of the forage component of the diet). In addition, in one study, a small quantity of chopped wheat straw (0.3 kg/cow/d) was included in the diet. The mean forage: concentrate DM ratio across the 27 studies was 49: 51. In all studies the forage component of the diet was offered ad libitum (normally between 7 – 10% of the previous day's intake).

A wide range of concentrate types, feeding levels, feeding strategies and feeding methodologies were adopted within and between studies, according to the objectives of each individual study. The concentrate supplements consisted principally of cereal grains (e.g. barley, wheat, maize), protein supplements (e.g. soybean meal, canola meal), and fibrous by-products from the food industry (e.g. corn gluten meal, sugar-beet pulp, citrus pulp). Additional energy sources (e.g. Megalac® and molasses) were included in some concentrates, while most concentrates contained a mineral/vitamin supplement. The concentrate component of the diets was offered either mixed with the forages (partial mixed ration), separate from the forages (via in-parlor of out-of-parlor feeders), or via a combination of these practices.

Breeding management and fertility records

Heifers entering the AFBI herd have a target age at first calving of 24 months (actual, 24.5 months). Cows in the herd calve from early September through to late December (‘Autumn’ calving), and from early January through to mid-April (‘Spring’ calving). Heat detection commences after calving. Heat detection is based on visual observations, although tail paint was used in a number of studies as an aid to heat detection. There are three defined periods of heat detection during the d, at approximately 10.00 h, 14.00 h and 20.00 h, although all heats observed throughout the d are recorded. Cows with uterine infection (normally based on stock person observations) are normally treated within 3 to 4 wk of calving. During the early years of the dataset, cows failing to show signs of estrus within 8 wk of calving were examined by a veterinarian, and cows with ovarian dysfunction treated as appropriate. During more recent years, veterinary interventions were delayed until approximately wk 10 to 12 of lactation. An exception to the above was cows identified with cystic ovaries, which were treated as soon as the problem was identified.

Throughout the study period all cows in the herd were bred by AI. A minimum 42-d voluntary waiting period was adopted with all cows. Within the autumn and spring calving components of the herd, breeding commenced early in December and early April, respectively. Cows were typically inseminated once per d by trained AFBI staff (assisted on occasions by a local breeding company). Cows observed in estrus after 10.00 h were inseminated the following morning. Pregnancy status was determined by a veterinarian using trans-rectal ultrasonography (scanner) at least 32 d after insemination. Fertility records included cows treated for uterine infection, cows treated with hormones (progesterone, prostaglandin, estradiol benzoate, or gonadotrophin releasing hormone; **GnRH**), observed heats, inseminations, pregnancy diagnosis, and subsequent calving details.

Animal measurements

A number of animal measurement protocols changed over the 20-yr period during which the 27 experiments were undertaken, while others remained largely unchanged. The feed intake of each individual cow was recorded daily using feed-boxes mounted on weigh cells, access to which was controlled by a Calan Gate feeding system (American Calan Inc., Northwood, NH, USA) linked to an electronic cow identification system. All diets were offered ad libitum. In all experiments cows were milked twice daily, with milk yields recorded automatically at each milking, and a total daily milk yield for each cow determined for each 24-h period. In early experiments (n = 7) milk samples were taken in proportion to yield during six consecutive milkings (either weekly or fortnightly), and a single bulked sample analyzed for each wk or fortnight. However, in later experiments (n = 20) samples were taken during two consecutive milkings (normally on a weekly basis) and each individual sample analyzed, and a weighted composition for the 24-h sampling period subsequently determined. Samples in all experiments were analyzed for fat, protein, and lactose concentrations using mid-infrared milk analysis. Fat-to-protein ratio (**FPR**) in milk was calculated as milk fat content (g/kg) divided by milk protein contents (g/kg). The equation (Eq. 1) given by Tyrrell and Reid (1965) was used to calculate the gross energy (**GE**) content of the milk, where fat, protein and lactose content are presented as g/kg:

$$GE, MJ/kg = [0.0384\ x\ fat] + [0.0223\ x\ protein] + [0.0199\ x\ lactose] - 0.108 \quad [1]$$

Energy corrected milk yield (kg/d) was calculated assuming the GE content of 1 kg 'standard milk' to be 3.1 MJ/kg (i.e. for milk containing 4.0% fat, 3.2% crude protein, and 4.8% lactose, as described by Muñoz et al. (2015), according to Eq. 2:

$$ECM, kg/d = \frac{milk\ yield\ (kg/d)\ x\ GE\ (MJ/kg)}{3.1} \quad [2]$$

Milk energy output (MJ/cow per d) was calculated by multiplying the GE content of milk (Eq. 1) by the daily milk yield. Feed efficiency was calculated by dividing ECM yield (kg/d) by the total DMI (kg/d).

In early studies (n = 4) BW was recorded weekly, immediately after pm milking. However, in later studies BW was recorded twice daily (immediately after each milking) using an automated weighbridge, and an average BW calculated for each wk. Body condition scores (BCS) were recorded weekly or fortnightly through each lactation, with BCS assessed on a 1 to 5 scale (Edmonson et al., 1989). Blood samples were collected (from the tail vein) in 26 of the 27 studies, normally between 8.00 – 10.00 h, while the frequency of blood sampling varied according to experiments (normally one sample every 14 – 28 d, until approximately wk 12 of lactation, with less frequent sampling thereafter). Blood serum was subsequently analyzed for BHB and NEFA concentrations, while plasma was analyzed for glucose concentrations.

Milk samples for progesterone determination were taken twice weekly (Monday and Thursday) from each cow in 21 studies for approximately 50 DIM. Milk progesterone concentrations were determined using an enzyme-linked immune-sorbent assay kit (Ridgeway Science Ltd, Gloucestershire, UK), based on the method of Sauer et al., (1986), as described in details by McCoy et al., (2006). Interval to the **SLA** was defined as the interval from calving to the first of at least two consecutive increases in milk progesterone concentrations of >3.0 ng/mL (Darwash et al., 1997). Peak progesterone concentration during first luteal cycle was recorded.

Determination of energy contents of the feedstuffs

In all experiments samples of grass and corn silages offered were collected daily and oven dry matter (**ODM**) determined, with fresh samples normally analyzed weekly for nitrogen (**N**), GE and fermentation products. Silage ODM contents were subsequently corrected for volatile losses during drying, with all intakes presented on a volatile corrected DM basis. Samples of dried silage were composited for each 2 to 4 wk period and subsequently analyzed for fiber and ash concentrations.

In early experiments (n = 8) the digestible OM in total DM content (**DOMD**, %) of silages was determined by offering the silage to sheep confined in 'digestibility crates' at maintenance level (normally 4 sheep per silage). The metabolizable energy (**ME**) content of these silages was then estimated by multiplying the DOMD by 0.16 (assuming that one percentage point of DOMD equates to 0.16 MJ/kg DM of ME (AFRC, 1993). The calculated ME values were then corrected to 'production level of feeding' by multiplying by 0.97 (MAFF, 1975; 1984). In later experiments (n = 20), the ME value of the forages offered were derived using NIRS as described by Park et al. (1998). In two experiments where neither sheep digestibility data nor NIRS predictions were available, silage DOMD was initially predicted from nutrient composition (DM, Ash, CP, and NDF) and fermentation characteristics of the silages (lactic acid: total VFA ratio) as described by Yan and Agnew (2004: Eq. 14b), and silage ME content estimated by multiplying the DOMD by 0.16. The mean ME content of the silages offered was 11.3±0.58 for grass silages and 11.2±0.35 for corn silages, while the ME content of wheat straw was assumed to be 6.0 MJ/kg DM (FeedByte®, SRUC, Edinburgh, UK).

Concentrates offered were normally sampled weekly, and composite samples analyzed for each 2 to 4 wk period. The ME content of each concentrate was calculated using the ME content of each individual ingredient, based on values reported in UK feed composition tables (FeedByte®). The mean calculated ME content of the concentrate offered was 12.9 (SD, 0.25) MJ/kg DM. Total ME intakes were determined as the sum of the DM intake of each diet component multiplied by the ME content of that component. Further details of analytical methods used to determine the chemical composition of the feedstuffs and fermentation quality of the silages are presented within the individual studies listed in Appendix 1.

Calculations of estimated energy balances (EB)

Individual cow EB values were initially calculated on a daily basis. Daily EB calculations utilized daily DMI and daily milk yield values. For data that was not available on a daily basis (i.e. BW and milk composition data), measured values were applied to each d during the 3 d period pre and post the d of measurement (in the case of weekly measurements) or to the 7 d period pre, and to the 6 d period post the d of measurement (in the case of fortnightly measurements). The mean ME content of all individual silage samples taken from each silo was applied to all d during which that silage was offered.

The daily EB (MJ of ME/d) of each individual cow was calculated using equations contained within ‘Feed into Milk’ (**FIM**), the current UK dairy cow rationing system, as the difference between the cow’s total ME requirements (maintenance, milk production, and activity) and total ME intake (Agnew et al., 2004). The sum of ME requirements for maintenance (including activity: standing, vertical movement and body position changes) and milk production ($ME_{maint+milk}$: MJ/kg of $BW^{0.75}$) was determined using Eq. 4.

$$ME_{maint+milk} = \frac{log_e\left[\left[\frac{[5.06 - Milk\ E.per\ kg\ of\ BW^{0.75}]}{[5.06 + 0.453]}\right]\right]}{-0.1326}$$

[4]

Pregnancy requirements were excluded from the EB calculations in the present study since data used within this analysis was until a maximum of 150 DIM, a 42 d voluntary waiting period is adopted within the AFBI herd, and energy cost of pregnancy is only accounted for from wk 14 of gestation in FIM. Energy requirements for 'walking' were included within the EB calculations as described by Agnew et al. (2004: shown in Eq. 5), using the term $(0.0013 \times BW)/k_m$, with the efficiency of utilization of ME for walking assumed to be the same as that for maintenance ($\boldsymbol{k_m}$; AFRC, 1993). This assumes a distance walked of 500 m, which was considered appropriate for housed cows on the AFBI farm.

Finally, daily EB (MJ/d) was calculated by using the following equation:

$$EB, MJ\ of\ ME/d = \left([ME_{maint+milk} \times BW^{0.75}] + \left[\frac{[0.0013 \times BW]}{k_m}\right] - 10\right) - MEi$$

[5]

The term ME_i is the ME intake (MJ/cow per d). Mean weekly EB values were subsequently calculated for each wk post-calving (up to a maximum of wk 20), with calving date considered as d 1 of wk 1 of lactation. Actual values for BCS were collated on a weekly basis using calving dates as reference points.

Statistical analysis

Within each analyses primiparous and multiparous cows were analyzed separately, while the dataset (or part of the data set) for the two parity groups was divided into quartiles (Q1 – Q4) according to the average EB during 4 to 21 DIM. The primary analysis (on which the EB quartiles described were derived) excluded all cows where FOH followed hormonal intervention (233 cows excluded). This analysis compared cow genetic index, cow performance (intakes, milk production and composition, body tissue and blood metabolites), interval (in d) between calving and FOH, and the percentage of cows with FOH pre-d 42, within each EB quartile. The second analysis excluded cows where first AI followed hormone treatment (279 cows excluded) and compared interval (in d) between calving and first AI, and conception to first AI, within each EB quartile. The third analysis involved cows with milk progesterone data available, but excluded cows where SLA followed hormone treatment (37 cows excluded). This analysis compared interval from calving to SLA, peak progesterone concentration at SLA, and the percentage of cows with SLA pre-d 42, within each EB quartile. The final analysis involved the complete dataset and compared the percentage of cows treated for uterine infection, percentage of cows where the FOH followed hormone intervention, and the percentage of cows where first AI followed hormone intervention, within each EB quartile. Individual cows were not fully nested within a study since individual cows were often used in more than one study. All continuous data were analyzed by REML variance component analysis and differences between treatments tested using Fishers unprotected least significance difference test. The model for continuous data included ‘experiment’ (1 – 27) and cow as the random effect, and ‘EB quartile’ (1 – 4) as the fixed effect. For continuous data for the period 4 to 150 DIM, days-on-experiment was also included in the model as a fixed effect, if significant. Binomial data were analyzed via Generalized Linear Model regression analysis using the binomial distribution with a logit link function, and differences between treatments tested using Chi square probability test. The model for binomial data included 'experiment' (1 – 27) and cow as the random effect, and EB quartile (1 – 4) as the fixed effect.

Least mean squares evaluating the interaction between EB quartile (1, 2, 3 and 4) and DIM, were determined for BCS, BW and EB (using weekly data), and general trends produced for the experimental period. The model included experiment and cow within study as random effects.

Data from 401 primiparous cows and 742 multiparous cows were included in a survival analysis to examine the effect of EB quartile (mean of 4 to 21 DIM) on interval from calving to first observed estrus (until 80 DIM). Cows which had a FOH following hormone intervention were excluded from the analysis, as were cows removed from the dataset prior to d 80. Cows which had a FOH after 80 DIM were assumed censored (10, 7, 4 and 4 for primiparous cows in Q1 – Q4; 13, 12, 7 and 11 for multiparous cows in Q1 – Q4). Similarly, data from 462 primiparous cows and 923 multiparous cows were included in a survival analysis to examine the effect of EB quartile (mean of 4 to 21 DIM) on 'non pregnancy' (up to 200 DIM). Cows which had a first AI following hormone intervention were excluded from the analysis, as were cows removed from the dataset prior to d 200. Cows which had a first AI after 200 DIM were assumed censored (29, 17, 17 and 23 for primiparous cows in Q1 – Q4, respectively; 64, 55, 55 and 39 for multiparous cows in Q1 – Q4, respectively). Within each of these survival analyses, the effect of EB quartile on cows without an observed heat and cows not pregnant (survival) was compared using four tests: Log-rank, Wilcoxon (Breslow), Tarone-Ware and Wilcoxon (Peto-Prentice). Kaplan-Meier survival functions were estimated for each EB quartile within each of primiparous and multiparous cows. All statistical analyses were performed using GenStat, Version 20.1 (VSN International Limited, 2019). For all models, statistical significance was declared at $P \leq 0.05$ and trends at $P > 0.05$ to $P < 0.10$.

RESULTS

With the exception of PTA for fertility, which increased between Q1 and Q4 ($P = 0.025$), none of the genetic values presented for primiparous cows differed ($P > 0.10$) between EB quartiles (Table 1). With multiparous cows, Q1 cows had a lesser PLI than Q4 cows ($P = 0.048$), while none of the other genetic values presented differed between quartiles ($P > 0.10$).

With primiparous cows (Table 2) neither concentrate percentage in the diet nor BW differed ($P > 0.05$) between quartiles in either early lactation (4 to 21 DIM), or over the entire experimental period (4 to 150 DIM). During both periods DMI and ME intake increased from Q1 to Q4 ($P < 0.001$), while milk protein content increased in early lactation ($P = 0.008$) and over the entire lactation ($P = 0.002$). In contrast, milk yield, ECM yield and ECM/DMI (Table 2) decreased from Q1 through to Q4 in both periods ($P < 0.001$). Milk fat content decreased from Q1 to Q4 in early lactation ($P < 0.001$), but not over the entire experimental period ($P > 0.05$), while milk fat-to-protein ratio decreased from Q1 to Q4 during both early lactation ($P < 0.001$) and over the entire period ($P = 0.005$). Body condition score did not differ between quartiles during early lactation (4 to 21 DIM), but increased from Q1 to Q4 over the entire experimental period ($P < 0.001$). During early lactation plasma NEFA and BHB concentrations decreased from Q1 to Q4 ($\mathrm{P} < 0.001$), while plasma glucose concentrations increased ($P < 0.001$), with similar effects observed over the entire experimental period ($\mathrm{P} = 0.008$, $\mathrm{P} = 0.004$ and $\mathrm{P} < 0.001$, respectively).

Multiparous cows in each of EB quartiles 1 – 4 had a mean lactation number of 3.3, 2.9, 2.9 and 2.8, respectively ($P = 0.021$: Table 3). Concentrate proportion in the diet did not differ ($P > 0.10$) between quartiles in either period. Total DMI and total ME intake increased ($P < 0.001$) from Q1 through to Q4 in early lactation, but not over the entire experimental period ($P > 0.05$). Each of milk yield, ECM, ECM/DMI and BW decreased ($P < 0.001$) from Q1 to Q4 during both periods. Milk fat content and FPR decreased from Q1 to Q4 in early lactation ($P < 0.001$), and over the entire experimental period (P = 0.002 and P < 0.001, respectively), while milk protein content followed the reverse trend in both periods (P < 0.001). While BCS decreased from Q1 to Q4 in early lactation (P < 0.001) BCS did not differ between quartiles over the entire experimental period (P > 0.05). During both experimental periods plasma NEFA and BHB concentrations decreased from Q1 to Q4 (P < 0.001), while plasma glucose concentrations increased (P < 0.001).

Neither the percentage of primiparous cows treated for uterine infection, nor the percentage of primiparous cows where FOH followed hormone intervention, differed between quartiles (P > 0.10) (Table 4). While d to FOH decreased from Q1 to Q4 (P = 0.049), the percentage of cows with FOH pre d 42 followed the reverse trend, increasing from Q1 to Q4 (P = 0.019). None of the percentage of cows where first AI followed hormone intervention, d to first AI, conception to first AI, or cows pregnant during the first 21 or 42 of the breeding season, differed between quartiles in primiparous cows (P > 0.05). However, there was a tendency for an increased percentage of cows in Q3 and Q4 to be pregnancy during the first 84 d of the breeding season (P = 0.072). For the sub-set of primiparous cows for which progesterone data was available (Table 4), the interval from calving to SLA decreased from Q1 to Q4 (P < 0.001), while peak progesterone concentration at SLA and the percentage of cows with SLA pre d 42 increased from Q1 to Q4 (P < 0.001 and P = 0.009, respectively).

Within the multiparous cow dataset, the percentage cows treated for uterine infection and the percentage of cows where FOH followed hormone intervention, did not differ between EB quartiles (P > 0.10) (Table 5). However, d to FOH was greater with cows in Q1 than cows in Q4 (P = 0.012), while the percentage of cows with FOH pre d 42 increased from Q1 to Q4 (P = 0.038). None of the percentage of multiparous cows where first AI followed hormone treatment, d to first AI, conception to first AI, and cows pregnant during the first 21 or 42 d of the breeding season differed between quartiles (P > 0.05). However, while the percentage of cows pregnant during the first 84 d of the breeding season tended to differ between quartiles (*P* = 0.087), there was no consistent trend between Q1 – Q4. Days from calving to SLA decreased (by 5.8 d) from Q1 to Q4 (*P* = 0.003), while peak progesterone concentration increased by 3.7 ng/mL between Q1 and Q4 (*P* = 0.026). The percentage of multiparous cows with SLA pre d 42 did not differ between quartiles (*P* > 0.10).

Time trends for BCS, BW and EB within each of EB quartiles 1 - 4 are presented in Figure 1 for primiparous and multiparous cows. With the exception of BCS for multiparous cows (*P* = 0.101; Figure 1B) and BW for primiparous cows (*P* = 0.398; Figure 1C) all other parameters differed between quartiles. In addition, there was a significant effect of DIM on all parameters (*P* < 0.001), and a significant interaction between EB quartile and DIM (*P* < 0.001).

Survival curves (FOH before d 80 DIM) for each of the EB quartiles within primiparous and multiparous cows, produced using the Kaplan-Meier survival function, are presented in Figures 2A and 2B, respectively. The estimated time to 25%, 50% and 75% of primiparous cows having a FOH was 33, 50 (95% CI: 44 – 53) and 62 d (Q1), 24, 40 (95% CI: 35 – 45) and 57 d (Q2), 22, 39 (95% CI: 32 – 45) and 56 d (Q3), 21, 34 (95% CI: 30 - 37) and 46 d (Q4). Similarly, the estimated time to 25%, 50% and 75% of multiparous cows having a FOH was 32, 47 (95% CI: 42 – 53) and 61 d (Q1), 26, 43 (95% CI: 38 – 47) and 61 d (Q2), 24, 41 (95% CI: 36 – 45) and 57 d (Q3), 20, 36 (95% CI: 33 - 40) and 54 d (Q4). Differences in survival between EB quartiles, tested using the Log-rank, Wilcoxon (Breslow), Tarone-Ware, Wilcoxon (Peto-Prentice) were found to be significant in both primiparous (All, P < 0.001) and multiparous (*P* < 0.039, *P* < 0.002, *P* < 0.007 and *P* < 0.002, respectively) cows.

Survival curves (not-pregnant cows before d 200 DIM) for each of the EB quartiles within primiparous and multiparous cows, produced using the Kaplan-Meier survival function, are presented in Figures 3A and 3B, respectively. The estimated time to 25%, 50% and 75% of primiparous cows becoming pregnant were 81, 112 (95% CI: 98 – 137) and 187 d (Q1), 82, 119 (95% CI: 107 – 137) and 168 d (Q2), 72, 96 (95% CI: 86 – 108) and 139 d (Q3), 78, 111 (95% CI: 96 - 118) and 162 d (Q4). Similarly, the estimated time to 25%, 50% and 75% of multiparous cows becoming pregnant were 82, 117 (95% CI: 109 – 129) and 177 d (Q1), 75, 108 (95% CI: 99 – 118) and 177 d (Q2), 76, 116 (95% CI: 105 – 125) and 185 d (Q3), 76, 104 (95% CI: 98 - 111) and 151 d (Q4). Differences in survival between EB quartiles, tested using the Log-rank, Wilcoxon (Breslow), Tarone-Ware, Wilcoxon (Peto-Prentice) were as follows in primiparous cows ($P < 0.143$, $P < 0.062$, $P < 0.087$ and $P < 0.064$, respectively) and for multiparous cows ($P = 0.191$, $P = 0.301$, $P = 0.240$, $P = 0.295$, respectively).

DISCUSSION

Primiparous and multiparous cows were examined separately within this study as the former have significant energy requirements for growth, and are more sensitive to NEB than multiparous cows (Wathes et al., 2007; Macmillan et al., 2018). During the period between 4 to 21 DIM, 78% of primiparous cows and 87% of multiparous cows had a mean EB that was negative. While there is a significant body of research evidence that ‘energy status’ in early lactation can have adverse effects on fertility traits, many studies have involved relatively small numbers of cows, while other studies have involved indirect measures of EB such as BCS and BW (Buckley et al., 2003; Middleton et al., 2019). In contrast, the current study allows the relationships between calculated EB (determined as the difference between ME intake minus energy requirements, according to equations within Feed into Milk (Agnew et al., 2004)) and fertility parameters to be examined using a large dataset. Furthermore, the availability of information on cow genotype and milk progesterone, together with detailed information on DMI, milk production, milk composition, body weight, BCS, and blood metabolites during both early lactation, and over the first 150 d of lactation, provide a more holistic picture within which to interpret the EB trends and the fertility outcomes observed.

Relationships between DMI, milk production and energy balance

During the early lactation period (4 to 21 DIM) EB within both parity groups was determined to a large extent by the relationship between DMI and ECM yield, cows with a greater DMI and a lesser ECM having an increased EB. While differences in DMI and ECM continued to drive EB in primiparous cows over the entire experimental period (4 to 150 DIM), differences in EB profiles between quartiles in multiparous cows were driven largely by ECM and not intakes. Broadly similar relationships between ECM and EB have been observed by Patton et al. (2007).

The lesser intakes and greater milk yields observed with increasing NEB did not appear to be driven by diet, as concentrate proportion in the diet (an important driver of intake: Lawrence et al., 2015), did not differ between quartiles in either primiparous or multiparous cows. Similarly, these differences are unlikely to have been due to the relatively small difference in lactation number between quartiles with the multiparous cows, while PTA for milk also did not differ between quartiles. While pre-partum management can influence performance post calving, with high BCS cows known to have lesser DMI following calving (Roche et al., 2009; Weber et al., 2013), differences in BCS between EB quartiles were small in both primiparous and multiparous cows. Furthermore, Roche et al. (2009) have suggested that a reduction in intake post-calving only becomes an issue for cows with a BCS greater than 3.5 (5-point scale), considerably greater than mean BCS in the current study.

The increased concentrations of NEFA and BHB in blood in the lower quartile cows may also have inhibited intake during the early lactation. According to the ‘hepatic oxidation theory’ (Allen et al., 2009), for animals in a lipolytic state with shortage of glucose precursors, increasing NEFA concentration, and consequently higher hepatic oxidation, can impact the satiety center decreasing feed intake. Furthermore, greater concentrations of BHB (a ketone body) can also suppress feed intake by hepatic oxidation (Allen and Piantoni, 2013).

Relationships between milk composition, blood metabolites and energy balance

The milk composition and blood metabolite data fully support the EB trends observed. Consistent across both primiparous and multiparous cows, those with a greater NEB during d 4 to 21 DIM had a greater milk fat content. That PTA for milk fat did not differ between quartiles suggests that this was not a 'genetic effect'. Rather, greater milk fat contents in early lactation, as observed previously by Gobikrushanth et al. (2019), is associated with cows mobilizing body tissue reserves, and the incorporation of longer chain length fatty acids into milk fat (Bauman and Griinari, 2001). This is supported by the blood metabolite data for this period, and is reflected in greater NEFA concentrations (arising from the mobilization of body fat reserves to produce glycerol for energy) and greater BHB concentrations (arising from the incomplete oxidation of NEFA to ketones) (Allen and Piantoni, 2013). Blood glucose concentrations followed the reverse trend, increasing with increasing EB, as observed by Gross et al. (2011). The decrease in milk protein content with decreasing EB is also as expected (Beever et al., 2001), and is likely due to a reduced supply of amino acids for milk protein synthesis due to insufficient energy supply for microbial protein synthesis, and for protein synthesis in mammary gland (Nousiainen et al., 2004). The differing trends in milk fat and milk protein contents were reflected in a decreasing FPR ratio with increasing EB. Fat-to-protein ratio is often highlighted as an indicator of energy status (Gross and Bruckmaier, 2019), with a FPR >1.5 claimed to be indicative of cows with sever metabolic stress (Heuer et al., 1999). While differences in mean BCS between quartiles in early lactation (4 – 21 DIM) appear small, the weekly trends presented in Figure 1 highlight distinct differences between primiparous and multiparous cows at this time. For example, while primiparous cows within all EB quartiles had a similar BCS at calving, the range in BCS for multiparous cows was much greater, cows with the highest BCS being in the lowest EB quartile, and vice versa for cows with the lowest BCS. Thus multiparous cows with greater BCS at calving were more prone to body tissue loss.

While the magnitude of many of the differences between EB quartiles observed in early lactation decreased when observe over the entire experimental period, most differences did remain. This is hardly surprising given that differences in weekly EB, BCS and BW profiles between EB quartiles remained throughout the duration of the study. Nevertheless, the large differences in milk fat content observed in early lactation had largely disappeared when examined over the entire experimental period (although still significant in multiparous cows).

Effect of energy balance on incidence of uterine infection and hormone treatments

The link between energy status and uterine infection has also been established, with Vercouteren et al. (2015) observing that cows that lost less BW had a lesser incidence of metritis, while Galvão et al. (2010) found the incidence of uterine diseases (both clinical and subclinical) to be positively correlated with increasing NEB. Thus the absence of an effect of EB quartile on the percentage of cows treated for uterine infection in the current study was surprising. It is possible that this is due to ‘treatment’ for infection being on the basis of visual observation followed by clinical examination, rather than a structured check of all cows. In addition, the percentage of cows observed with uterine infection in the current study was relatively low (13%), compared to 25% observed by Galvão et al. (2010) on commercial farms, perhaps reflecting a higher standard of management within a research environment than on commercial farms.

That the percentage of cows treated with hormones was not affected by EB quartile is perhaps not surprising as interventions did not take place before 70 - 80 DIM in the majority of studies, a time when actual EB differences between quartiles were much smaller.

Nevertheless, these cows were not included in the analyses of FOH due to the impact of human intervention, rather than natural onset of estrus (Lucy et al., 2004).

Relationships between energy balance, start of luteal activity and first observed heat

Data on SLA was available from a sub-set of cows, with SLA occurring an average of 12.7 d earlier than FOH. This is not unexpected as progesterone priming influences how estradiol stimulates the hypothalamus, and consequently estrus expression (Sauls et al., 2017). Also, poor energy status during early lactation may decrease estradiol production in the pre-ovulatory follicle, and reduce the sensitivity of the hypothalamus to estradiol resulting in 'silent ovulations' (Ranasinghe et al., 2010). The effects of EB in early lactation on both SLA and FOH were fully aligned, both of these events occurring earlier with improved early lactation energy status. In addition, the percentage of cows showing both SLA and FOH before 42 DIM increased, or tended to increase, with increasing EB in both primiparous cows and multiparous cows, respectively. The overall effect of the latter is clearly highlighted in Figure 2A and 2B. A number of authors (Windig et al., 2008; Patton et al., 2007) have observed that increasing NEB during early lactation is highly correlated with the increase in interval to first ovulation. This effect was quantified by De Vries and Veerkamp (2000), who observed that each 10 MJ decrease in nadir EB (NE_L/d) in primiparous cows corresponded to a delay in ovulation of 1.25 d. Similarly, within the current study each 10 MJ decrease in daily EB (ME basis) (approx. 6.4 MJ/d on a NE_L basis) in early lactation increased the delay to FOH by 1.2 and 0.8 d in primiparous and multiparous cows, respectively, suggesting that the former were more sensitive to NEB. This may reflect the fact that primiparous cows also have a significant competing demand for energy for growth (Wathes et al., 2007; Macmillan et al., 2018).

Cows with improved EB and earlier SLA also had greater milk progesterone concentrations at SLA, supporting the observations of Spicer et al. (1993) of a positive correlation between

EB and progesterone concentration during the first estrous cycle. While Windig et al. (2008) observed that cows with a greater milk production had lesser peak progesterone concentrations, as observed in the current study, Moore et al. (2014) noted that greater circulating progesterone concentrations are primarily due to greater corpus luteum synthetic capacity (rather than differences in progesterone clearance rates).

The relationships observed between EB and each of SLA and FOH, are aligned with many of the trends in the production data, and findings of earlier studies. For example, as d to SLA and FOH decreased, total DMI increased while milk yield decreased, in agreement with the findings of Kadokawa et al. (2006), Patton et al. (2007) and Macmillan et al. (2018). Similarly, as in the current study, previous research has identified relationships between the early resumption of reproductive activity and greater milk protein content (Patton et al., 2007), lesser milk fat content (Kadokawa et al., 2006), and consequently a lesser FPR.

A number of studies have observed strong relationships between BCS loss and SLA. For example, Gobikrushanth et al. (2019) found SLA to be delayed in cows that lost more than 0.75 BCS unit (scale 1-5) before 35 DIM, while Barletta et al. (2017) found that greater loss of BCS during the transition period was a key factor in delaying the initiation of ovarian activity after calving. Furthermore, in a study involving 19 Northern Ireland dairy farms (McCoy et al., 2006) SLA was delayed in cows with a lesser BCS during the first 100 d of lactation, while Buckley et al. (2003) observed a reduced likelihood of submission for breeding in cows with greater BW loss in early lactation and a lesser nadir BCS. While BCS profiles in Figure 1 suggest that BCS loss was relatively modest with cows in all EB quartiles, even in Q1, the EB profiles suggest that these cows were mobilizing substantial quantities of body tissue reserves. Thus it is likely that these cows were mobilizing significant amounts of abdominal adipose tissue, something which is more likely to occur with cows in relatively low BCS, as in the current study.

The relationships between blood metabolites and SLA and FOH in the current study agree with earlier findings. For example, Dubuc et al. (2012) and Bossaert et al. (2008) observed a relationship between lesser NEFA concentrations and earlier FOH, while Kawashima et al. (2012) observed a similar effect with greater blood glucose concentration, and lesser blood NEFA concentration. Similarly, Macmillan et al. (2018) observed that cows that had ovulated before 35 DIM had a greater glucose, and lesser NEFA and BHB concentrations compared with cows that ovulated after 35 DIM. It has been suggested that cows with greater serum NEFA, BHB and lesser glucose concentrations have a greater risk of prolonged postpartum anovulation and consequently lesser reproductive efficiency, and as such might benefit from targeted preventive therapy (Wathes, 2012; Vercouteren et al., 2015).

Greater yielding dairy cows make ‘metabolic decisions’ about the utilization of scarce resources such as energy, and in early lactation nutrients are preferentially directed to milk production rather than to initiate pregnancy (Friggens, 2003). The delay in FOH and SLA between Q1 and Q4 in primiparous cows (9.8 and 10.2 d delay, respectively) and multiparous cows (7.4 and 5.9 d delay, respectively) reflect the difference in mean EB profiles (range from -67 to +14 MJ/d in primiparous cows, and -104 to -6 MJ/d in multiparous cows), and is likely due to the impact of energy on activity of the hypothalamic-pituitary-ovarian axis (**HPO**) (Wathes, 2012). The effects of energetic stress on the function of the HPO axis have been examined primarily at the hypothalamus and anterior pituitary, and the loss of pulsatile LH secretion has been shown to result from prolonged inadequate intake of dietary energy (Beam and Butler, 1999; Bisinotto et al., 2012). The underlying mechanism by which NEB reduces LH release is likely to involve the supply of energy to neurons, and hormonal modulation of hypothalamic and pituitary cells (Schneider, 2004). For example, glucose and insulin are the substances that are most likely to exert an impact on HPO, and to influence GnRH secretion, consequently reducing LH pulse and causing a delay in the resumption of reproductive activity

(Leroy et al., 2008). In addition, glucose is the preferred energy substrate for neuron metabolism, and lesser concentrations of glucose can inhibit the GnRH pulse generator (Schneider, 2004). In the current study plasma glucose increased as d to FOH and SLA decreased (Q1 to Q4: from 3.20 to 3.47 mmol/l in primiparous cows, and from 2.89 to 3.08 mmol/l in multiparous cows).

Energy balance and fertility outcomes

There were no differences between quartiles in the number of cows treated with hormones prior to first AI, with these treated cows excluded from the subsequent analysis. Nevertheless, for those cows that were cycling normally, interval from calving to first AI was still determined in part by management decisions. For example, a minimum voluntary waiting period of 42 d was adopted for all cows, while for many cows a longer ‘delay’ occurred to align with the breeding season start dates (namely early December and early April for autumn and spring calving ‘herds’, respectively). This helps explain why, despite differences between quartiles in interval to FOH, there were no differences in interval to first AI between quartiles (which occurred at a mean of 70.4 and 70.0 d in primiparous and multiparous cows, respectively).

A key finding of this study was that mean conception rate to first AI, the percentage of cows pregnant by 21, 42 and 84 d after start breeding season, and the percentage of non-pregnant cows over the first 150 d of lactation were unaffected by early lactation EB. In contrast, Patton et al. (2007) and Gümen et al. (2005)found that cows with a severe NEB in early lactation had a reduced conception rate at time of breeding. Similarly, a number of studies have established relationships between changes in BCS in early lactation, and pregnancy outcomes. For example, Middleton et al. (2019) found that cows that maintained or gained BCS during the first 30 DIM had increased conception at first AI than those that lost BCS. Similarly, Barletta et al. (2017) observed a greater conception rate (47%) in cows that gained (+0.35 units) BCS in early lactation compared to those that either maintained BCS (33% conception rate) or lost (-0.38 units) BCS (18% conception rate). Carvalho et al. (2014) observed poorer quality embryos in cows that had lost BCS in early lactation.

Nevertheless, in the current study there was a tendency for primiparous cows in Q4 to have an increased pregnancy rate at 84 d after the start of the breeding season, while data in Figure 3B suggests a greater long term pregnancy rate in Q4 multiparous cows. The absence of a clear effect in the current study are likely due to the delay in interval to first AI, and the fact that cows had moved to a less severe metabolic state at the time of AI (on average, 71 DIM). This was highlighted when mean data for the entire experimental period was examined, with all cows having a much-improved EB during this period, with this reflected in the much smaller differences between EB quartiles in milk fat content, milk fat-to-protein ratio and blood metabolites. With regards the latter, in a large scale study Chapinal et al. (2012) observed no relationship between early lactation NEFA and BHB concentration, and subsequent pregnancy rate to first AI. In contrast, Ospina et al. (2010) found a 16% decrease in risk of pregnancy for cows with high (≥ 0.72 mmol/L) NEFA concentrations, with this level only slightly greater than that observed in Q1 cows in early lactation in the current study (0.63 and 0.70 mmol/L for primiparous and multiparous, respectively). The latter is important as it is known that increased NEFA concentrations can adversely affect oocyte quality (Leroy et al. 2005 and 2008). In addition, adequate blood glucose levels are necessary for proper functioning of, and preparation of the ovary, oviducts and uterus (Wathes et al., 2011: Garverick et al., 2013).

Within the current dataset PTA for fertility in primiparous cows increased by 2.2 units between Q1 and Q4, with each 1 unit increase expected to reduce calving interval by approximately 0.6 d and to improve non-return rate by 0.25% (AHDB Dairy, 2020). While this may have made a small contribution to the earlier FOH observed, this was not reflected in a difference in fertility outcomes. Nevertheless, given that PTA for fertility for primiparous cows within the overall dataset ranged from -14 to +11.9, and that there was very considerable overlap in PTA values between quartiles, the relative absence of a genetic-phenotypic relationship is unsurprising. PTA for fertility did not differ between quartiles in multiparous cows, in agreement with the absence of an effect on fertility outcomes observed.

A number of possible reasons why clear relationships between EB and fertility outcomes were not observed in this study have been discussed. However, the potential limitations of numbers of cows involved in the analysis must also be considered. Although numbers were substantially greater than in many other studies, the number of cows within each EB quartile was 122 and 255 cows for primiparous and multiparous cows, respectively.

CONCLUSION

Dairy cows with more severe NEB during early lactation (4 to 21 DIM) had a lesser DMI and greater ECM yields, while more severe NEB was also reflected in greater milk fat content, and increased concentrations of NEFA and BHB in serum. In addition, increasing NEB in early lactation was associated with a delay in FOH and postpartum SLA. For each 10 MJ/d increase in mean NEB (ME basis) during 4-21 DIM, FOH was delayed in by 1.2 and 0.8 d in primiparous and multiparous cows, respectively. However, early lactation EB had no effect on conception to first service.

ACKNOWLEDGEMENTS

This work was supported by the Department of Agriculture and Rural Development for Northern Ireland (DAERA) as part of the DAFM NutriGen project. Maurício Civiero was in receipt of a scholarship from Programa de Bolsas Universitárias de Santa Catarina (UNIEDU/ FUMDES) and thanks Centro Universitário Vale do Iguaçu (UNIGUAÇU) for providing time to complete this work. Thanks also to Dr Mark Little (Trouw Nutrition UK) for valuable comments on the manuscript.

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APPENDIX 1

Publications (and experiment ID code, in bold) describing the experiments from which individual animal data was obtained (all experiments undertaken at the Agri-Food and BioSciences Institute, UK, between 1996 and 2016).

Ferris, C. P., F. J. Gordon, D. C. Patterson, and C. S. Mayne, and M. A. McCoy. 2003. A short-term comparison of the performance of four grassland-based systems of milk production for autumn-calving dairy cows. Grass Forage Sci. 58:192–209. **AFBI experiment codes C18 and C24.**

Ferris, C.P., D. C. Patterson, M. A. McCoy, and D. J. Kilpatrick. 2010. Effect of offering dairy cows diets differing in phosphorus concentration over four successive lactations: 1. Food intake, milk production, tissue changes and blood metabolites. Animal 4:545–559. **AFBI experiment codes C36, C40, C42 and C48.**

Ferris, C.P., Doody, D.G., Laughlin, R., Watson, C.J. and Watson, S. 2013. Cow performance within contrasting milk production systems over three successive lactations. In: Proc. British Grassland Soc. 11th Research Conference: Science and Practice for Grass-Based Systems. 2 - 3 September 2013, Dumfries, Scotland. Paper 6.3. **AFBI experiment codes C54, D91 and D100.**

Ferris, C.P., Gordon, F.J., Patterson, D.C. and Murphy, J. 2002. A three year comparison of four contrasting grassland based systems of milk production. In: Profitable grass and forage: meeting the needs of the farmer and society. Proc. of the British Grassland Soc. Winter Meeting, 27-28 November 2002. Stafford, UK. **AFBI experiment codes C27 and C30.**

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Gilmore, H.S. 2010. An investigation of factors affecting reproductive function in the high genetic merit Holstein-Friesian dairy cow. PhD Thesis, School of Biological Sci., Queen's University Belfast. **AFBI experiment code D84.**

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Johnston, D. J., Theodoridou, K., Gordon, A. W., Yan T., McRoberts, W.C. and Ferris C. P. 2019. Field bean inclusion in the diet of early-lactation dairy cows: Effect on performance and nutrient utilization. J. Dairy Sci, 102: 10887 - 10902. **AFBI experiment code D127.**

Keady, T. W. J., and C. S. Mayne. 2002. The effect of two levels of nutrient intake on milk production of two dairy cow genotypes. Proc. British Soc. Animal Sci. Page: 12. **AFBI experiment code D40(Yr 2).**

Keady, T. W. J., and C. S. Mayne. 2003. An evaluation of the effect of concentrate proportion of the diet during previous and present lactations on animal performance of two breeds of lactating dairy cows. Proc. British Soc. Animal Sci. Page: 24. **AFBI experiment code D40(Yr3).**

Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. G. Wylie, K. L. Ingvarsten, A. Hameleers, M. A. McCoy, C. S. Mayne, C. P. Ferris. 2011. Effect of pre calving and post calving dietary energy level on performance and blood metabolite concentrations of dairy cows throughout lactation. J. Dairy Sci. 94:808–823. **AFBI experiment code D64.**

Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. G. Wylie, and C. S. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. J. Dairy Sci. 92: 1001–1012. **AFBI experiment code D71.**

Law, R. A., McGettrick, S. A., and Ferris, C.P. 2012. Effect of three different concentrate build-up strategies in early lactation on production performance, health and fertility of high yielding dairy cows. End of project report for AgriSearch, D-54-11. Acces. Sep. 03, 2020 https://www.agrisearch.org/dairy/completed-dairy/feeding-dairy-completed/125-effect-of-three-different-concentrate-build-up-strategies-in-early-lactation-on-production-performance-health-and-fertility-of-high-yielding-dairy-cows. **AFBI experiment code D97.**

Law, R.A., McGettrick, S. and Ferris, C.P. 2011. Effect of concentrate build-up strategy in early lactation on production performance, health and fertility of high-yielding dairy cows. In: Advances in Animal Biosciences-Food Security-Challenges and Opportunities for Animal Science. Proc. British Soc. Animal Sci and the Assoc. of Vet. Teaching and Research Work. April 2011. p 005. **AFBI experiment code D90.**

Purcell, P. J., R. A. Law, A. W. Gordon, S. A. McGettrick, and C. P. Ferris. 2016. Effect of concentrate feeding method on the performance of dairy cows in early to mid-lactation. J. Dairy Sci. 99:2811–2824. **AFBI experiment code D102.**

Purcell, P.J., Law, R.A. and Ferris, C.P. 2015. Effect of concentrate feed rate within a feed-to-yield system on the performance of dairy cows in early to mid-lactation. In: Advances in Animal Science – Science with Impact. Proc. British Soc. Animal Sci. April 2015, Vol 6 Part 2. Cambridge University Press. Page 192. **AFBI experiment code D107.**

Vance, E. R., C. P. Ferris, C. T. Elliot, H. M. Hartley, and D. J. Kilpatrick. 2013. Comparison of the performance of Holstein-Friesian and Jersey x Holstein-Friesian crossbred dairy cows within three contrasting grassland-based systems of milk production. Livest. Sci. 151:66–79. **AFBI experiment code C49 and C51.**

Vance, E. R., C. P. Ferris, C. T. Elliot, S. A. McGettrick, and D. J. Kilpatrick. 2012. Food intake, milk production, and tissue changes of Holstein-Friesian and Jersey × Holstein-

Friesian dairy cows within a medium-input grazing system and a high-input total confinement system. J. Dairy Sci. 95: 1527–1544. **AFBI experiment code C54(V).**

Young, F.J., Hameleers, A., Patterson, D.C., and Mayne, C.S., 2006. Effect of additional concentrate supplementation for dairy cows at three time points in lactation. In: Proc. British Soc. Animal Sci. p 123. **AFBI experiment code D56.**

Table 1. Predicted transmitting ability (PTA) for primiparous and multiparous cows within each EB quartile, with quartiles based on mean daily energy balance during 4 to 21 DIM

	Quartiles (mean daily energy balance during 4 to 21 DIM)					
	Q1	Q2	Q3	Q4		
Primiparous cows	(-120 to -49 MJ/d)	(-49 to -24 MJ/d)	(-24 to -3 MJ/d)	(-3 to 92 MJ/d)	SED	*P*-value
PTA milk (kg)	18.5	-9.2	-15.8	-43.9	26.93	0.208
PTA milk fat (kg)	6.4	5.3	4.3	3.7	0.98	0.065
PTA milk protein (kg)	6.0	5.2	4.7	4.2	0.71	0.117
PTA milk fat (%)	0.07	0.07	0.06	0.06	0.012	0.842
PTA milk protein (%)	0.06	0.07	0.06	0.07	0.006	0.545
PTA fertility	-1.6^{a}	-0.7^{ab}	-0.6^{ab}	0.6^{b}	0.66	0.025
Profitable Lifetime Index (£)	86.6	83.9	70.7	79.9	16.30	0.777
	Q1	Q2	Q3	Q4		
Multiparous cows	(-191 to -79 MJ/d)	(-79 to -48 MJ/d)	(-48 to -22 MJ/d)	(-22 to 93 MJ/d)	SED	*P*-value
PTA milk (kg)	-64.9	-55.8	-43.4	-39.2	11.23	0.140
PTA milk fat (kg)	2.4	2.5	3.0	3.2	0.32	0.085
PTA milk protein (kg)	3.2	3.4	3.6	3.8	0.27	0.227
PTA milk fat (%)	0.06	0.06	0.06	0.06	0.002	0.973
PTA milk protein (%)	0.07	0.07	0.07	0.07	0.001	0.434
PTA fertility	-0.5	-0.7	-0.5	-0.5	0.17	0.270
Profitable Lifetime Index (£)	40.6^{a}	45.2^{ab}	55.8^{b}	58.7^{b}	6.83	0.048

^{a,b} Values within a row with different superscript lowercase letters differ at $P < 0.05$.

Table 2. Concentrate proportion in the diet, performance indicators and biological responses of primiparous cows during 4 to 21 DIM, and during 4 to 150 DIM, with quartiles based on mean daily energy balance during 4 to 21 DIM[1]

	Quartiles (mean daily energy balance during 4 to 21 DIM)[1]					
Item[2]	Q1 (-120 to -49 MJ/d)	Q2 (-49 to -24 MJ/d)	Q3 (-24 to -3 MJ/d)	Q4 (-3 to 92 MJ/d)	SED	*P*-value
Days 4 to 21 in milk						
Concentrate proportion in diet	0.50	0.53	0.54	0.52	0.015	0.123
Total DMI (kg/d)	11.5^{a}	12.7^{b}	13.2^{c}	14.3^{d}	0.22	<0.001
Total ME intake (MJ/d)	140^{a}	154^{b}	161^{c}	175^{d}	2.71	<0.001
Milk yield (kg/d)	25.2^{d}	24.0^{c}	22.2^{b}	21.1^{a}	0.44	<0.001
ECM (kg/d)	28.4^{d}	26.0^{c}	23.4^{b}	22.0^{a}	0.48	<0.001
ECM/DMI	2.52^{d}	2.01^{c}	1.79^{b}	1.55^{a}	0.020	<0.001
EB (MJ/d)	-67^{a}	-35^{b}	-15^{c}	14^{d}	0.86	<0.001
Milk fat (g/kg)	49.4^{c}	45.6^{b}	43.4^{a}	42.4^{a}	0.80	<0.001
Milk protein (g/kg)	34.0^{a}	34.8^{b}	35.2^{b}	35.3^{b}	0.37	0.008
FPR	1.46^{c}	1.32^{b}	1.24^{a}	1.21^{a}	0.024	<0.001
BW (kg)	521	514	507	511	5.72	0.123
BCS	2.74	2.69	2.73	2.70	0.036	0.484
Plasma NEFA (mmol/mL)	0.63^{c}	0.53^{b}	0.51^{b}	0.43^{a}	0.041	<0.001
Plasma BHB (mmol/l)	0.75^{b}	0.61^{a}	0.54^{a}	0.52^{a}	0.055	<0.001
Plasma glucose (mmol/l)	3.20^{a}	3.33^{b}	3.43^{c}	3.47^{c}	0.053	<0.001
Days 4 to 150 in milk						
Concentrate proportion in diet	0.50	0.52	0.52	0.52	0.015	0.242
Total DMI (kg/d)	15.4^{a}	16^{b}	16.2^{b}	16.7^{c}	0.24	<0.001
Total ME intake (MJ/d)	187^{a}	195^{b}	197^{b}	204^{c}	3.12	<0.001
Milk yield (kg/d)	28.7^{c}	27.8^{c}	26.2^{b}	24.9^{a}	0.54	<0.001
ECM (kg/d)	29.5^{d}	28.3^{c}	26.6^{b}	25.6^{a}	0.50	<0.001
ECM/DMI	1.95^{d}	1.79^{c}	1.65^{b}	1.53^{a}	0.019	<0.001
EB (MJ/d)	-22^{a}	-7^{b}	3^{c}	17^{d}	2.39	<0.001
Milk fat (g/kg)	41.6	41.0	40.7	41.4	0.59	0.435

Milk protein (g/kg)	32.5^{a}	33.0^{ab}	33.2^{bc}	33.7^{c}	0.29	0.002
FPR	1.28^{b}	1.25^{a}	1.23^{a}	1.23^{a}	0.017	0.005
BW (kg)	512	514	516	520	5.5	0.535
BCS	2.51^{a}	2.52^{a}	2.59^{b}	2.62^{b}	0.027	<0.001
Plasma NEFA (mmol/mL)	0.44^{b}	0.41^{b}	0.37^{a}	0.36^{a}	0.023	0.008
Plasma BHB (mmol/l)	0.66^{b}	0.60^{a}	0.59^{a}	0.58^{a}	0.024	0.004
Plasma glucose (mmol/l)	3.26^{a}	3.37^{b}	3.45^{c}	3.49^{c}	0.026	<0.001

[a,b,c,d] Values within a row with different superscript lowercase letters differ at $P < 0.05$.

[1] Excludes cows where first observed heat followed hormone intervention.

[2] ECM= energy corrected milk; ECM/DMI= gross feed efficiency; EB= energy balance; FPR= milk fat-to-protein-ratio; NEFA= non-esterified fatty acids; BHB= β-hydroxybutyrate.

Table 3. Concentrate proportion in the diet, performance indicators and biological responses of multiparous cows during 4 to 21 DIM, and during 4 to 150 DIM, with quartiles based on mean daily energy balance during 4 to 21 DIM.

	Quartiles (mean daily energy balance during 4 to 21 DIM)[1]					
Item[2]	Q1 (-191 to -79 MJ/d)	Q2 (-79 to -48 MJ/d)	Q3 (-48 to -22 MJ/d)	Q4 (-22 to 93 MJ/d)	SED	*P*-value
Lactation number (upper and lower confidence limit)	3.3 (3.00 – 3.59)	2.9 (2.66 – 3.20)	2.9 (2.62 – 3.15)	2.8 (2.50 – 3.03)	-	0.021
Days 4 to 21 in milk						
Concentrate proportion in diet	0.52	0.52	0.50	0.50	0.009	0.785
Total DMI (kg/d)	15.5^{a}	16.8^{b}	16.8^{b}	18.0^{c}	0.24	<0.001
Total ME intake (MJ/d)	188^{a}	204^{b}	205^{b}	219^{c}	3.01	<0.001
Milk yield (kg/d)	37.2^{d}	35.4^{c}	32.2^{b}	29.5^{a}	0.50	<0.001
ECM (kg/d)	41.9^{d}	38.3^{c}	34.7^{b}	30.9^{a}	0.54	<0.001
ECM/DMI	2.73^{d}	2.28^{c}	2.10^{b}	1.75^{a}	0.017	<0.001
EB (MJ/d)	-103^{a}	-61^{b}	-34^{c}	-5^{d}	0.91	<0.001
Milk fat (g/kg)	49.6^{c}	46.0^{b}	45.3^{b}	42.6^{a}	0.58	<0.001
Milk protein (g/kg)	34.8^{a}	35.3^{b}	35.7^{bc}	36.0^{c}	0.26	<0.001
FPR	1.43^{c}	1.30^{b}	1.27^{b}	1.19^{a}	0.017	<0.001
BW (kg)	609^{d}	598^{c}	579^{b}	565^{a}	5.6	<0.001
BCS	2.58^{b}	2.53^{b}	2.45^{a}	2.45^{a}	0.029	<0.001
Plasma NEFA (mmol/mL)	0.70^{d}	0.58^{c}	0.52^{b}	0.42^{a}	0.028	<0.001
Plasma BHB (mmol/l)	0.82^{c}	0.74^{b}	0.69^{b}	0.61^{a}	0.038	<0.001
Plasma glucose (mmol/l)	2.89^{a}	2.97^{b}	3.00^{b}	3.08^{c}	0.036	<0.001
Days 4 to 150 in milk						
Concentrate proportion in diet	0.51	0.51	0.51	0.51	0.009	0.818
Total DMI (kg/d)	19.8	20.1	19.9	20.3	0.21	0.086
Total ME intake (MJ/d)	241	245	242	247	2.70	0.053
Milk yield (kg/d)	38.7^{d}	37.1^{c}	35.1^{b}	29.5^{a}	0.50	<0.001
ECM (kg/d)	39.9^{d}	37.7^{c}	35.7^{b}	33.7^{a}	0.47	<0.001

ECM/DMI	2.03^{d}	1.89^{c}	1.80^{b}	1.68^{a}	0.022	<0.001
EB (MJ/d)	-35^{a}	-17^{b}	-7^{c}	11^{d}	2.23	<0.001
Milk fat (g/kg)	42.6^{b}	41.5^{a}	41.4^{a}	41.2^{a}	0.39	0.002
Milk protein (g/kg)	32.6^{a}	33.0^{b}	33.3^{b}	33.8^{c}	0.17	<0.001
FPR	1.31^{c}	1.25^{b}	1.24^{ab}	1.22^{a}	0.011	<0.001
BW (kg)	602^{b}	595^{b}	585^{a}	578^{a}	4.8	<0.001
BCS	2.37	2.39	2.39	2.40	0.020	0.654
Plasma NEFA (mmol/mL)	0.45^{d}	0.37^{c}	0.34^{b}	0.28^{a}	0.013	<0.001
Plasma BHB (mmol/l)	0.69^{c}	0.64^{b}	0.63^{ab}	0.60^{a}	0.016	<0.001
Plasma glucose (mmol/l)	3.13^{a}	3.18^{b}	3.19^{b}	3.25^{c}	0.017	<0.001

[a,b,c,d] Values within a row with different superscript lowercase letters differ at $P < 0.05$.

[1] Excludes cows where first observed heat followed hormone intervention.

[2] ECM= energy corrected milk; ECM/DMI= gross feed efficiency; EB= energy balance; FPR= milk fat-to-protein-ratio; NEFA= non-esterified fatty acids; BHB= β-hydroxybutyrate.

Table 4. Mean fertility performance of primiparous cows within EB quartiles based on mean daily energy balance during 4 to 21 DIM (for binomial data, upper and lower confidence limit in parenthesis)

	Quartiles (mean daily energy balance during 4 to 21 DIM)					
Item[1]	Q1 (-120 to -49 MJ/d)	Q2 (-49 to -24 MJ/d)	Q3 (-24 to -3 MJ/d)	Q4 (-3 to 92 MJ/d)	SED	*P*-value
Percentage of cows treated for uterine infection (entire dataset)[2]	14 (7 - 27)	11 (5 - 23)	18 (9 - 33)	10 (4 -21)	-	0.283
Percentage of cows where FOH followed hormone intervention (entire dataset)[2]	17 (9 - 30)	13 (7 - 24)	9 (4 - 17)	9 (4 - 17)	-	0.273
Days to FOH[3]	47.7^{b}	42.6^{ab}	41.4^{ab}	37.9^{a}	2.38	0.049
Percentage of cows with FOH pre d 42[3]	46 (31 – 62)	58 (43 – 72)	54 (38 – 68)	72 (57 – 84)	-	0.019
Percentage of cows where first AI followed hormone intervention (entire dataset)[2]	20 (10 - 34)	20 (11 - 34)	12 (6 - 23)	14 (7 - 26)	-	0.360
Days to first AI[3]	72.4	71.1	68.9	69.3	3.31	0.735
Conception to first AI (percentage)[4]	32 (24 - 43	30 (21 - 39)	32 (23 - 41)	31 (22 - 40)	-	0.974
Cows pregnant during						
First 21 d of breeding season (percentage)	33 (24 - 43)	24 (16 - 33)	28 (20 - 38)	26 (18 -36)	-	0.507
First 42 d of breeding season (percentage)	48 (39 - 58)	40 (31 - 49)	52 (42 - 60)	46 (37 - 56)	-	0.355
First 84 d of breeding season (percentage)	70 (61 - 78)	66 (56 - 74)	79 (70 - 86)	78 (70 - 86)	-	0.072
For sub-set of cows with progesterone data available[5]						
Interval from calving to SLA	34.6^{c}	28.8^{b}	31.6^{bc}	24.4^{a}	1.59	<0.001
Peak progesterone concentration at SLA (ng/mL)	26.7^{a}	33.6^{bc}	30.5^{ab}	34.2^{c}	1.95	<0.001
Percentage of cows with SLA pre d 42	69 (51 - 83)	83 (69 - 92)	74 (57 - 85)	91 (79 - 96)	-	0.009

[a,b,c] Values within a row with different superscript differ at $P < 0.05$.

[1] FOH= first observed heat; SLA= start of luteal activity; AI= artificial insemination.

[2] Based on entire data set: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 within the entire data set were -120 to -50, -50 to -24, -24 to -3 and -3 to 92 MJ/d, respectively

[3] Excludes cows where first observed heat followed hormone intervention.

[4] Excludes cows were first AI followed hormone intervention: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 were -111 to -50, -50 to -24, -24 to -4 and -4 to 92 MJ/d, respectively.

[5] Excludes cows where SLA followed hormone intervention: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 within the data sub-set were -120 to -50, -50 to -25, -25 to -3 and -3 to 69 MJ/d, respectively.

Table 5. Mean fertility performance of multiparous cows within EB quartiles based on mean daily energy balance during 4 to 21 DIM (for binomial data, upper and lower confidence limit in parenthesis

	Quartiles (mean daily energy balance during 4 to 21 DIM)					
Item[1]	Q1 (-191 to -79 MJ/d)	Q2 (-79 to -48 MJ/d)	Q3 (-48 to -22 MJ/d)	Q4 (-22 to 93 MJ/d)	SED	*P*-value
Percentage of cows treated for uterine infection (entire dataset)[2]	15 (9 - 22)	11 (6 - 18)	14 (9 - 22)	13 (8 -21)	-	0.741
Percentage of cows where FOH followed hormone intervention (entire dataset)[2]	17 (10 - 27)	15 (9 - 24)	14 (8 - 22)	17 (10 - 27)	-	0.766
Days to FOH[3]	49.0^{b}	44.9^{ab}	42.9^{a}	41.6^{a}	2.07	0.012
Percentage of cows with FOH pre d 42[3]	41 (29 - 53)	49 (38 - 61)	55 (43 - 66)	58 (46 - 69)	-	0.038
Percentage of cows where first AI followed hormone intervention (entire dataset)[2]	19 (12 - 29)	21 (14 - 31)	16 (9 - 24)	19 (12 - 29)	-	0.445
Days to first AI[4]	72.7	69.9	68.9	68.6	3.04	0.557
Conception to first AI (percentage)[4]	28 (22 - 35)	29 (24 - 36)	26 (21 - 33)	33 (26 - 40)	-	0.672
Cows pregnant during						
First 21 d of breeding season (percentage)	22 (16 - 29)	20 (15 - 27)	25 (19 - 33)	24 (18 -32)	-	0.629
First 42 d of breeding season (percentage)	43 (35 - 50)	45 (38 - 53)	39 (32 - 47)	45 (37 - 53)	-	0.554
First 84 d of breeding season (percentage)	73 (65 - 80)	75 (68 - 82)	68 (50 - 75)	78 (70 - 84)	-	0.087
For sub-set of cows with progesterone data available[5]						
Interval from calving to SLA	35.7^{b}	29.4^{a}	31.0^{a}	29.8^{a}	1.29	0.003
Peak progesterone concentration at SLA (ng/mL)	24.4^{a}	25.9^{ab}	27.4^{b}	28.1^{b}	2.14	0.026

Percentage of cows with SLA pre d 42	70 (59 - 80)	83 (75 - 90)	78 (68 - 86)	79 (69 - 86)	-	0.103

[a,b] Values within a row with different superscript differ at $P < 0.05$.

[1] FOH= first observed heat; SLA= start of luteal activity; AI= artificial insemination.

[2] Based on entire data set: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 within the entire data set were -191 – -80, -80 – -47, -47 – -22 and -22 – 93 MJ/d, respectively.

[3] Excludes cows where first observed heat followed hormone intervention.

[4] Excludes cows were first AI followed hormone intervention: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 were -191 – -79, -79 – -48, -48 – -21 and -21 – 93 MJ/d, respectively.

[5] Excludes cows where SLA followed hormone intervention: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 within the data sub-set were -185 – -71, -71 – -43, -43 – -16 and -16 – 93 MJ/d, respectively.

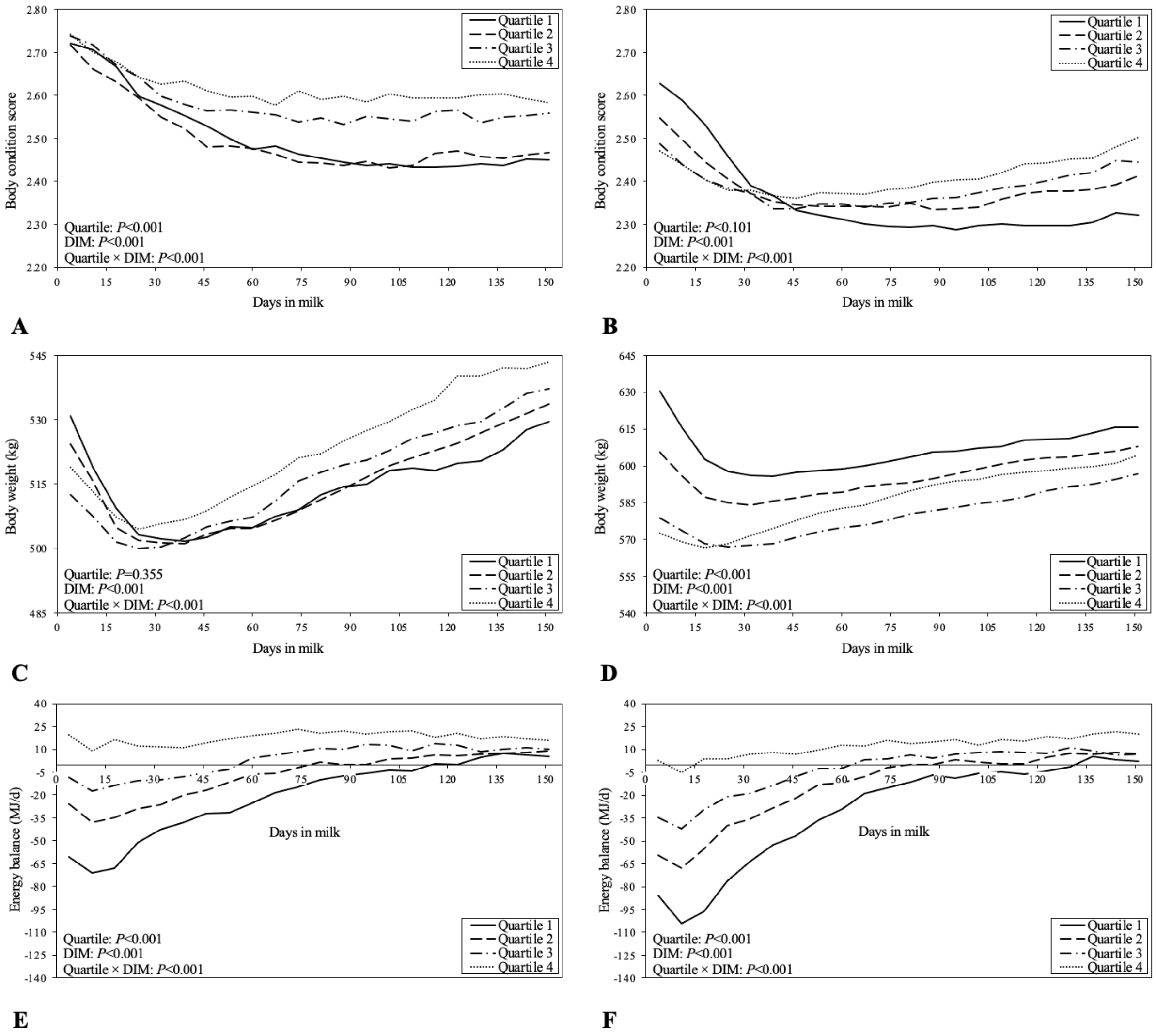

Figure 1. Least squares means (weekly basis, from 4 to 150 DIM), for body condition score (Figures 1A and 1B, primiparous and multiparous cows respectively), body weight (Figures 1C and 1D, primiparous and multiparous cows respectively), and daily energy balance (Figures 1E and 1F, primiparous and multiparous cows respectively) for cows within EB quartiles (Q1, Q2, Q3, and Q4) during early lactat

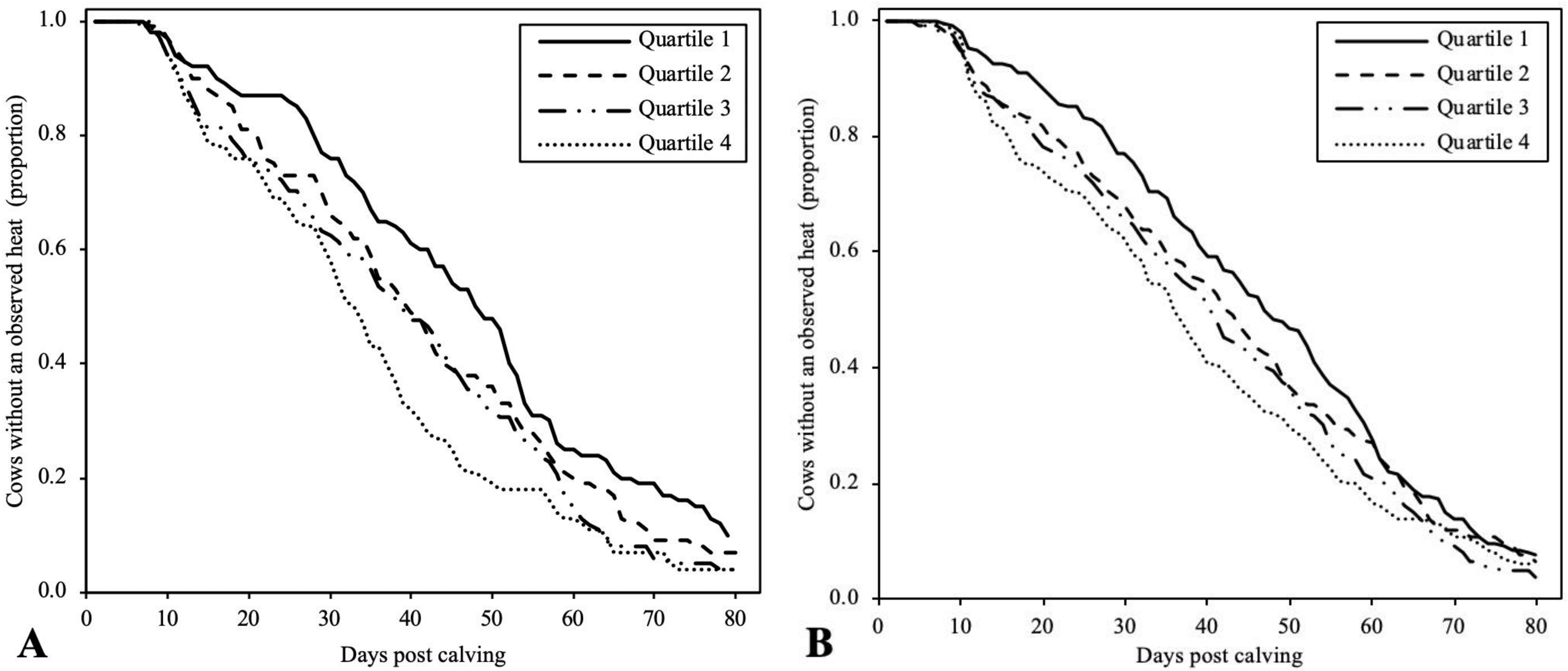

Figure 2. Survival curves showing the effect of EB quartile (based on mean daily EB during 4 to 21 DIM) on the proportion of (A) primiparous (*P* < 0.001: Q1 – 4, -120 to -49, -49 to -24, -24 to -3 and -3 to 92 MJ/d, respectively) and (B) multiparous cows (*P* < 0.002: Q1 – 4, -191 to -79, -79 to -48, -48 to -21 and -21 to 93 MJ/d, respectively) without an observed heat during the first 80 DIM.

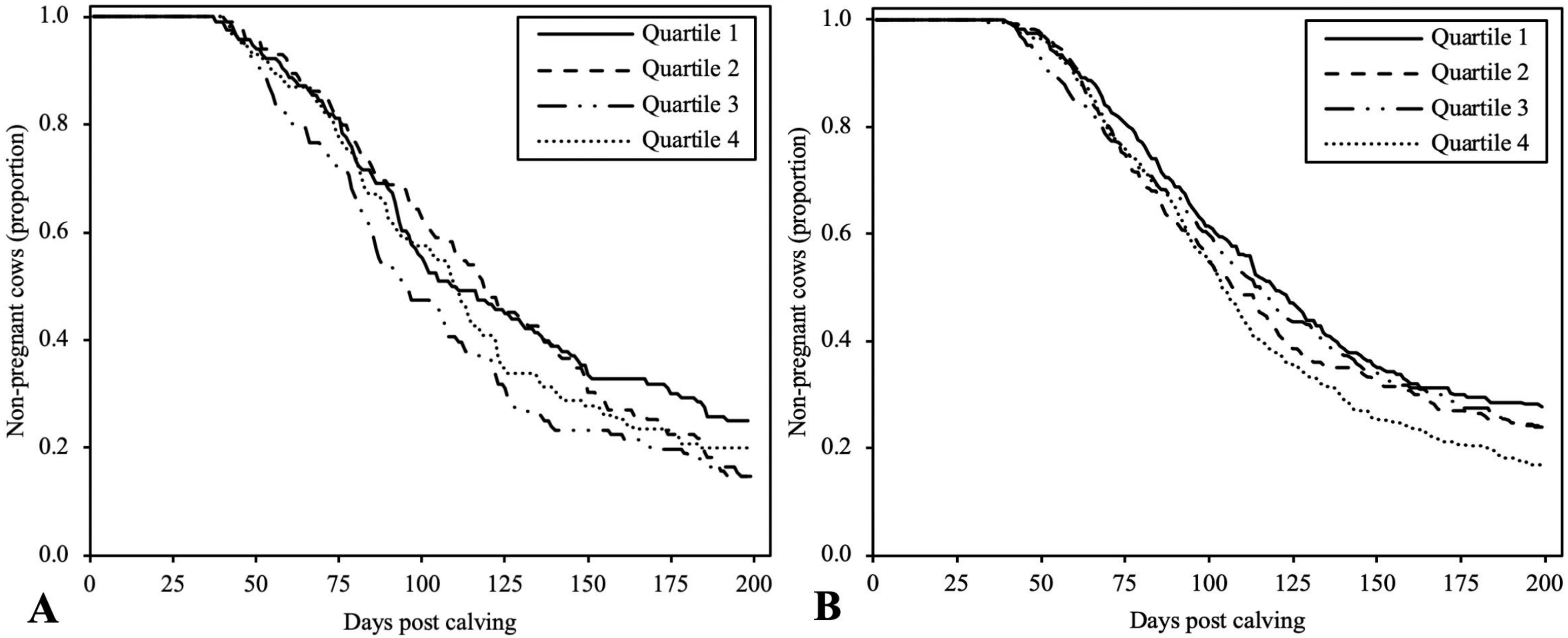

Figure 3. Survival curves showing the effect of EB quartile (based on mean daily EB during d 4 to 21 DIM) on the proportion of (A) primiparous ($P > 0.05$)]: Q1 – 4, -120 to -50, -50 to -24, -24 to -3 and -3 to 92 MJ/d, respectively) and (B) multiparous cows ($P > 0.05$): Q1 – 4, -191 to -80, -80 to -47, -47 to -22 and -22 to 93 MJ/d, respectively) that were not pregnant during the first 200 DIM.