Factors associated with daily weight gain in preweaned calves on dairy farms

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ABSTRACT

The preweaning period is vital in the development of calves on dairy farms and improving daily liveweight gain (DLWG) is important to both financial and carbon efficiency; minimising rearing costs and improving first lactation milk yields. In order to improve DLWG, veterinary advisors should provide advice that has both a large effect size as well as being consistently important on the majority of farms. Whilst a variety of factors have previously been identified as influencing the DLWG of preweaned calves, it can be challenging to determine their relative importance, which is essential for optimal on-farm management decisions. Regularised regression methods such as ridge or lasso regression provide a solution by penalising variable coefficients unless there is a proportional improvement in model performance. Elastic net regression incorporates both lasso and ridge penalties and was used in this research to provide a sparse model to accommodate strongly correlated predictors and provide robust coefficient estimates. Sixty randomly selected British dairy farms were enrolled to collect weigh tape data from preweaned calves at birth and weaning, resulting in data being available for 1,014 calves from 30 farms after filtering to remove poor quality data, with a mean DLWG of 0.79kg/d (range 0.49-1.06kg/d, SD 0.13). Farm management practices (e.g. colostrum, feeding, hygiene protocols), building dimensions, temperature/humidity and colostrum quality/bacteriology data were collected, resulting in 293 potential variables affecting farm level DLWG. Bootstrapped elastic net regression models identified 17 variables as having both a large effect size and high stability. Increasing the maximum preweaned age within the first housing group (0.001kg/d per 1d increase, 90% bootstrap confidence interval (BCI): 0.000-0.002), increased mean environmental temperature within the first month of life (0.012kg/d per 1°C increase, 90% BCI: 0.002-0.037) and increased mean volume of milk feeding (0.012kg/d per 1L increase, 90% BCI: 0.001-0.024) were associated with increased DLWG. An increase in the number of days between the cleaning out of calving pen (-0.001kg/d per 1d increase, 90% BCI: -0.001-0.000) and group housing pens (-0.001kg/d per 1d increase, 90% BCI: -0.002-0.000) were both associated with decreased DLWG. Through bootstrapped elastic net regression, a small number of stable variables have been identified as most likely to have the largest effect size on DLWG in preweaned calves. Many of these variables represent practical aspects of management with a focus around stocking demographics, milk/colostrum feeding, environmental hygiene and environmental temperature; these variables should now be tested in a randomised controlled trial to elucidate causality.

INTRODUCTION

The preweaning period is critical to the development and future performance of both dairy and dairy-cross beef calves on dairy farms in Great Britain (GB). A key outcome routinely measured across the preweaning period is daily liveweight gain (DLWG), which has been shown to be a crucial factor in the efficient rearing of productive dairy heifers (Chester-Jones et al., 2017). Studies in GB have highlighted the importance of achieving a calving age of 23-25 months in minimizing rearing costs for dairy heifers (Boulton et al., 2017), and have suggested that increased bodyweight at 30d was associated with a reduced age at first calving (Brickell et al., 2009a). Alongside improvements in efficiency of heifer rearing, preweaning DLWG has also been shown to significantly impact first lactation milk yield, with each 0.1kg of DLWG being associated with a 85-113kg increase in milk yield during first lactation (Soberon et al., 2012). Although this finding has not always been replicated in similar studies (Davis Rincker et al., 2011; Kiezebrink et al., 2015) a meta-analysis concluded that whilst other aspects of management are potentially more important than preweaning DLWG, an increase in DLWG above 0.5kg/d can enhance first lactation performance (Gelsinger et al., 2016). Although in one UK study only 28% of dairy farms routinely record heifer growth rates (Boulton et al., 2015), significant variations between farms have been reported (Bazeley et al., 2016). Concerns have been raised over the environmental impact of both dairy and beef production, finding a high degree of heterogeneity in the environmental impact between producers (Poore and Nemecek, 2018), suggesting efficiency at farm level may play an important role in greenhouse gas emissions as well as productivity.

A variety of factors have been reported to affect DLWG including colostrum supplementation, milk feeding protocol and composition (Brickell et al., 2009), total milk solids being fed, housing management (Johnson et al., 2018) and environmental temperature

(Shivley et al., 2018). Whilst there is often an economic cost associated with alterations in preweaning calf management, the improved efficiency of rearing, reduced calving to conception interval and increased first lactation yields associated with increased DLWG produce an economic saving to off-set higher preweaning costs (Boulton et al., 2017; Johnson et al., 2018).

Whilst there are many potential factors influencing the DLWG of preweaned calves, their relative importance remains unknown, and this is essential for optimal on-farm management decisions (Lima et al., 2020). Datasets containing large numbers of explanatory variables are more likely to contain potentially spurious correlations, which can confound conventional modelling approaches, resulting in inflated coefficients and over fitting, particularly when the number of predictors (p) exceeds that of the observations (n) (Hastie et al., 2015; Kuhn and Johnson, 2013). Conventional regression models are likely to perform poorly in relatively small samples, firstly in the selection of truly causal variables, but also in the estimation of regression coefficients; particularly important when considering conditional selection bias (Dormann et al., 2013). One solution is the use of regularized regression (Zou and Hastie, 2005), where model coefficients are penalized unless there is a proportional improvement in model performance (Kuhn and Johnson, 2013). This penalization can take the form of reducing coefficients to near zero in the case of ridge regression (Hoerl and Kennard, 2006), or reducing the coefficient to zero in the case of lasso regression (Tibshirani, 1996). Regularisation techniques have also been shown to effectively deal with both confounding and collinearity which have historically been a concern with conventional model approaches and provide more accurate measures of both accuracy and uncertainty (Greenland, 2007). The elastic net algorithm makes use of both lasso and ridge regression, often outperforming both, whilst also producing a sparse model that can accommodate strongly correlated predictors (Zou and Hastie, 2005) often found when exploring farm management practices.

Bootstrapping of regression models allows a robust estimate of coefficients (Breiman, 1996), alongside an estimate of variable stability; an estimate of the likely reproducibility of a variable effect in the target population. Used in conjunction with regularized regression, this approach has been utilized in animal health as a method of identifying a relatively small set of variables with a large and consistent influence on key outcomes (Lima et al., 2020).

There are a wide variety of feeding, management and housing practices involved in achieving improved DLWG rates in preweaned calves and whilst there have been several studies examining individual areas of calf feeding, environment and management, research examining these factors in a holistic manner is currently lacking. The aim of this study was to evaluate a wide variety of management variables using bootstrapped regularized regression to identify factors most likely to affect preweaning DLWG in calves on GB dairy farms.

MATERIALS AND METHODS

One hundred and twenty dairy farms were selected at random from a list of suppliers to a large GB supermarket. Selected farms were sent an initial information letter, followed up with a telephone call to recruit farms until 60 were recruited. One hundred and seven farms were contacted, with 47 declining to participate, resulting in 60 participating farms. Recruited farms were visited between 17th December 2018 and 14th February 2019 at which time a detailed calf management questionnaire was conducted, alongside the measurement of calf housing dimensions and environmental parameters. Ethical approval was given by the University of Nottingham ethics committee (2119 170911)

Management questionnaire

The management questionnaire contained 374 questions, including sections on general farm background, farmer demographics, calving area management, current calf morbidity and mortality, colostrum management, milk feeding, weaning and building management. A full copy of the questionnaire is available in *Supplementary materials i*.

Building measurements

Building parameters were recorded for all buildings in which the calves spent ≥24h during the preweaning period, including calving pens. The mean number of cows or calves typically within the pen and building were recorded as a farmer estimate, as was the number of pens within the building and the number of days spent in each environment. The shape of the pens and building were recorded as was the roof design (i.e. dual-pitched, mono-pitched, hutch etc) and ridge outlet design (open, cranked, covered etc).

Bedding depth, roof coverage (% of housed area covered by a roof) and the number of mechanical ventilation fans present was recorded numerically. The availability of water, sharing of water both within and between pens and the presence of water over bedding was recorded. The presence of cobwebs, bedding wetness/contamination, and ammonia levels were scored subjectively on a 0-10 scale (10 being “high”) and nesting score on a 1-3 scale (1: calves’ legs fully visible in recumbency, 2: calves’ legs partially visible in recumbency, 3: calves’ legs not visible in recumbency) (Lago et al., 2006).

Building dimensions were recorded using a laser measurer (LDM-100, CEM, Kolkata, India) including the widths and lengths of all pens and buildings, the height to eaves and ridges of buildings, with details being collected on wall material (concrete, space boarding, mesh etc), proportion of wall being solid/ventilated, and measurements of bedded/non-bedded areas. The airspace per pen and airspace per shed was calculated from recorded dimensions, as was typical stocking density (m^2 available per animal calculated using farmer estimated mean stocking numbers). Building dimensions were calculated both as individual measurements for each feeding period, and as mean values amalgamated across the entire preweaning period (e.g. a calf being housed for 7d in a building whilst being fed colostrum with a ridge height of 2m followed by being housed in a building with a ridge height of 4m for 63d whilst being fed milk replacer (MR), would have a feed1 ridge height of 2m, a feed2 ridge height of 4m and a mean ridge height of 3.8m).

Measurements of environmental conditions were taken on the day of visit including minimum/maximum airspeed at both human (1.5m) and calf (0.5m) level, environmental inside/outside temperature, relative humidity, minimum/maximum light intensity (lux) using a handheld monitor (Enviro-meter, Fisher scientific, Leicestershire), as well as temperature of all four walls, bedding and outside ground temperature via a laser thermometer (IR-801, ATP, Leicestershire, UK).

Data loggers (EL-CC-2, Lascar, Wiltshire, UK) were installed in all separate calf buildings on each farm at 1.5m height, up to a maximum of 3 buildings, depending on the number of buildings used. A data logger was installed outside the main calf shed at 1.5m height on each farm. Loggers were set to record temperature and humidity every 10 minutes and were posted back to the authors via a prepaid envelope in July 2019.

Feeding measurements

On farms that were feeding milk replacer (MR), the youngstock manager was asked to measure the quantity of MR powder as typically prepared for feeding, which was then weighed using digital weigh scales (CL series, OHAUS, Nänikon, Switzerland) so the true weight could be recorded in addition to the desired weight. Farmers were informed of variations in true/desired milk replacer concentration on completion of the trial. Details of the MR ingredients and constituents were also recorded. Water temperature used to mix MR powder was measured using a laser thermometer (IR-801, ATP, Leicestershire, UK). Milk replacer constituent data were not collected on farms not feeding milk replacer, so milk replacer constituent variables were recorded as “NA” for these farms.

Colostrum and immune transfer

Farmers were trained in refractometer use during the farm visit, and a Brix refractometer (Model RHB-325G with 0-32% range, YHequipment, Shenzhen, China) was provided to all participating farmers. Farmers were instructed to record the Brix % (Quigley et al., 2013) of colostrum samples as least weekly. Colostrum bacteriology sampling kits were provided to farmers, consisting of 6 sample pots containing glycerol with instructions to sample the next 6 colostrum samples available (within one month) at the point of calf feeding (i.e. from the teat/bottle/tube depending on colostrum feeding method) which were frozen immediately on-farm as described in previous colostrum studies (Mcaloon et al., 2016), and asked to post the samples within one month of the first sample being taken. Ice packs were provided for samples which were sent directly to the laboratory where temperature checks were performed on arrival. Total bacterial counts were performed after 72h incubation at 30^{o}C and coliform counts were performed after 72h incubation at 37^{o}C.

Farmers were asked to consider blood sampling a sample of calves for passive transfer of immunity with their routine veterinarian, but this was voluntary and to be performed only if there was deemed to be a valid clinical justification by the local veterinarian.

Farmer recording

Calf weight at birth and weaning were collected by farmers using a weigh tape which was provided to all farmers (MSD animal health, Milton Keynes, UK). All farmers were trained in the correct use of the weigh tapes by the lead researcher including a demonstration of use during the farm visit. Farmers were asked to record the breed, sex, time period between birth and colostrum feeding and whether calving assistance was required. Farmers were contacted every 2-4 weeks via text, email or telephone throughout the trial to encourage compliance.

Descriptive analysis

All data analysis was conducted in R (R Core Team, 2020). Birth and weaning weights and dates were used to calculate an average DLWG for each calf. Calf level variables were converted to farm level variables, i.e. percentage births receiving calving assistance, mean time from birth to colostrum feeding.

Blood sample and colostrum Brix results were used to calculate a percentage failure of passive transfer and low colostrum quality rate respectively for each farm. “Failure” of passive transfer was determined when defined as total protein <5.2g/dl ; Brix % <7.8% ; ZST <14.6g/L (Zakian et al., 2018), and colostrum was determined as being a “failure” in terms of quality when Brix % <22% (Quigley et al., 2013). Similarly, the percentage of colostrum samples failing in terms of total bacteria count (TBC) and coliform count (CC) was calculated for each farm, with a "failure" being when ≥100,000 for TBC and ≥10,000 for CC as previously suggested by McGuirk and Collins (2004).

Data from the day of logger installation and the last 2 weeks of recording were removed to ensure temperature readings during installation or posting were not included in any subsequent analysis. Data from the loggers were analysed both individually and amalgamated into farm level "inside" and "outside" mean, median, max and min measurements. Mean, median, min, max and SD of both temperature and humidity were calculated for all calves for the first 7, 30 and 60 days of life for inside building and outside building temperatures respectively.

Statistical analysis

Some subjective variables (farmer views on disease levels, willingness for financial investment etc) were excluded and categorical variables were removed when <2 levels were present (a full list of variables is presented in *Supplementary materials ii*). All numeric variables were centred and scaled (divided by one standard deviation) using "center" and "scale" inside the preProcess function within the caret package (Kuhn. et al., 2018). Farm management variables with missing data were converted to categorical variables including an "Unknown" category for missing data. Missing data for the percentage of colostrum samples failing on CC or TBC count (n=1) and temperature/humidity inside (n=3) and outside (n=5) buildings were imputed using the rfImpute function from the randomforest package (Liaw, 2018).

Elastic net models employ a balance of lasso (L1) and ridge (L2) penalties and in the current study took the form (Friedman et al., 2010):

$$SSE_{enet} = \frac{1}{2n} \sum_{i=1}^{n} \left(y_i - \hat{y}_i\right)^2 + \lambda \ [\sum_{j=1}^{p} (\frac{1}{2} \ (1 - \alpha) \ \beta_j^2 \ + \ \alpha \, \beta j \ ]$$

where SSEenet represented the elastic net loss function to be minimised, *i* represented each observation and *n* the number of observations. y*i* and ŷ*i* represented the observed and predicted outcome for the *i*th observation respectively, *j* denoted a predictor variable with *p* the number of predictor variables in total. |β| represented absolute values of the regression coefficients. Hyperparameters that represent the penalty (λ_E) and the relative proportion of penalisation on either the sum of the square of the coefficients or the unsquared coefficients (α) were optimised by 10 x 5-fold cross validation to minimise MAE.

A preliminary elastic net model was created using the glmnet (Friedman et al., 2010) and caret (Kuhn. et al., 2018) packages to inform grid parameters for α and λ. A full elastic net model was then created, again using glmnet and caret.

Based on the α and λ values in of the full model, a dense and sufficiently wide grid of α and λ values was created for future bootstrapped models. One thousand bootstrap samples were taken to enable estimations of bootstrap intervals (Efron and Tibshirani, 1994), and an elastic net model was built for each sample using the glmnet package (Friedman et al., 2010). Five-fold cross validation was repeated 10 times for each model. The dense grid of α and λ values was used for tuning, and optimal models were selected for each bootstrap sample based on minimising the mean absolute error (MAE). Final model coefficient values for all variables were saved for each iteration of the bootstrap sample process, and the percentage of models which contained each variable was subsequently calculated as a measure of variable stability. Coefficients were converted back to original units by dividing the coefficient by the standard deviation (SD) used to pre-process the variable from the original dataset. Models were interpreted utilising both stability and coefficient distributions as follows. A 90% bootstrap confidence interval (BCI) was calculated for each variable and a bootstrap p-value defined as one minus the proportion of coefficient estimates on the majority side of zero (proportion below zero if the mean coefficient was above zero, and proportion above zero if mean coefficient was above zero). Variables with a bootstrap stability >50% and a bootstrap p-value <0.025 were deemed to be both stable and have an effect size with a high probability of being > or < 0 and were therefore selected to comprise a final model.

RESULTS

Descriptive statistics

Farms were predominantly in the South West and Midlands of England with 46, 11 and 3 in England, Scotland and Wales respectively. Herd size was a mean of 312, ranging from 72 to 2100 milking cows, with a mean milk yield of 9323 L/305d, ranging from 6500-12000 L/305d. Fifty farms milked cows using a conventional milking parlour, with 10 being robotically milked.

A total of 6,973 weights of 4,552 unique calves were recorded from 37 farms, with twenty-three farms not returning any calf weight data despite repeated contacts. Seven of the 37 farms had poor quality data, with data being recorded in an incorrect format which meant it was not possible to calculate DLWG and were excluded, resulting in 30 farms in the final dataset. Four of these 30 farms did not record date of weaning and these were estimated using mean age of weaning as recorded during the farm management questionnaire. As a cross-check, models were repeated without these four farms included to ensure no significant alterations in model outcomes. Individual calves from the final 30 farms with weights at selling rather than weaning were removed (n=318), and subsequently calves without a birth or weaning date (n=55) were excluded from the analysis.

After processing and filtering, the final dataset of calf weights included 1,014 calves that had both a birth and weaning weight from 30 farms. The mean DLWG for each farm was calculated as an outcome variable at farm level. Six hundred and eighty-nine colostrum brix recordings, 249 colostrum bacteriology and 280 calf blood sample results were returned from 26, 39 and 18 farms respectively. One hundred and three data loggers were returned from 40 farms which resulted in 2,459,108 recordings for temperature and humidity.

Of the 30 farms included in the final dataset, including data from 1,014 calves, the mean DLWG was 0.79kg/d (range 0.49-1.06kg/d, SD 0.13, Figure 1). Calves were fed a mean of 5.62L/d of either whole milk or milk replacer during the preweaning period (2.93-9.24L/d, SD 1.37) and were stocked at a mean rate of 2.59m^2/calf (1.14-5.00m^2/calf, SD 0.93). Mean temperatures within the first month of life were very similar between both inside and outside shed measurements, with a mean temperature of 10.35 C (6.36-14.90, SD 1.71) and 9.85 (6.46-13.28, SD 1.68) for inside and outside shed temperatures respectively. Calving pens were cleaned out a mean of every 32d (3.5-105d, SD 27.67) and group calf pens were cleaned out a mean of every 55d (5-180d, SD 38.22). The maximum length of time after birth calves would go without colostrum had a mean of 8.8h (2-24h, SD 4.3) and the mean proportion of colostrum samples failing in terms of TBC and CC was 34.9% (0-100%, SD 32.4) and 10.7% (0-83.3%, SD 22.6) respectively.

Temperature and humidity analysis illustrated minimal differences in mean daily temperature between inside and outside temperatures (Figure 2). Hourly temperature analysis demonstrated that calf buildings were generally slightly warmer overnight than the outside temperature and were slightly colder during the day, however these differences were relatively small, generally in the region of 2°C.

Statistical analysis

Two hundred and ninety-three variables were available for predicting DLWG at farm level which would be included in the final analysis. Preliminary elastic net models indicated optimal α and λ values at 0.55 and 0.039 respectively. A dense grid including α values of 0, 0.2, 0.4, 0.6, 0.8, 1 and λ values of 0.001, 0.002, 0.003, 0.004, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.1 was used for bootstrapping. A median of 0.4 and 0.001 was selected throughout the 1,000 bootstrap sample repeats for α and λ respectively.

Of the 293 available variables, 18 variables were selected in at least 50% of the models as shown in Table 1. Variables with a stability >50% and bootstrap p-value <0.025 were included in the final model, resulting in 17 variables being selected. Model coefficients, illustrated in Figure 3, are depicted as the effect of a 1SD change in each independent variable on DLWG (kg/d). The stability and bootstrap p-value of all variables with stability >50% are illustrated in Figure 4.

An older maximum age within first preweaned housing group (the maximum age a calf might reach in the first housing group) was associated with increased DLWG, with a stability of 93.4%, and a coefficient of 0.001 (90% BCI: 0.000-0.002kg/d) indicating a mean increase of 10g/d for every 10d age for the oldest calf within the first preweaned group. There was also an effect of increased mean volume of milk/MR fed on DLWG, with a stability of 78.9% and a coefficient of 0.012 (90% BCI: 0.001-0.024kg/d), indicating an increase in feed volume of 1L was associated with an increase in DLWG of around 12g/d. An increased mean daily inside environmental temperature within the first week, month or two months of life was also associated with improved DLWG, with stabilities of 59.5%, 82.8% and 62.5% respectively, with temperature in the first month of life (°C) having a coefficient of 0.012 (90% BCI: 0.002-

0.037kg/d), suggesting a 5°C increase in environmental temperature would increase DLWG by 60g/d. Increases in the maximum time to colostrum feeding was associated with a decrease DLWG, with a stability of 78.1% and coefficient of -0.004 (90% BCI: -0.010 - 0.000kg/d), suggesting a 6h delay in max colostrum feeding time would decrease DLWG by 24g/d over the preweaning period.

Longer periods between the cleaning out of group housing and calving pens were associated with decreased DLWG, with 73.0% and 71.8% stability respectively, with coefficients of -0.001 (90% BCI -0.002 – 0.000kg/d) and -0.001 (90% BCI -0.001 – 0.000kg/d), suggesting that a 1wk delay in cleaning out of either the calving pen or group housing would be associated with a 7g/d reduction in DLWG, and a simultaneous 1wk delay in both calving pen and group housing would be associated with a 14g/d reduction. Increased space allowance per calf was associated with an increased DLWG, with a stability of 63.9% and coefficient of 0.010 (90% BCI 0.001-0.025kg/d), indicating an increase in DLWG of 10g/d for each additional m^2 of space per calf.

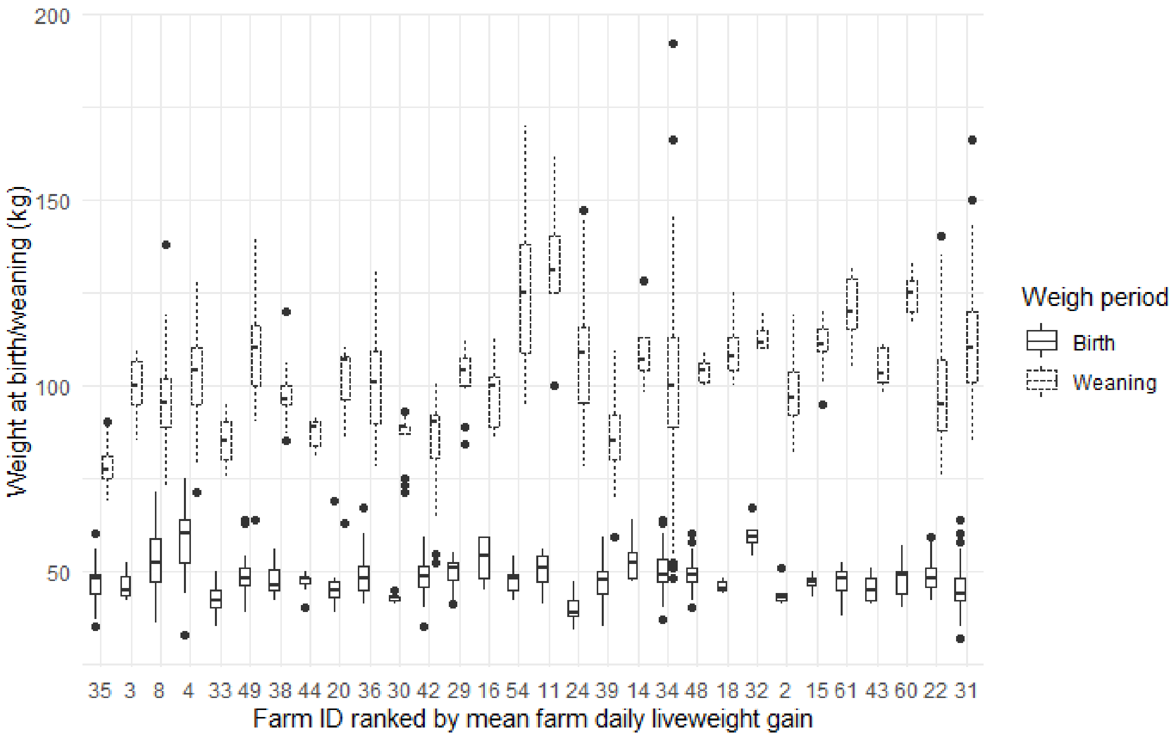

Figure 1

Birth and weaning weights (kg) estimated by weigh tape on each farm ranked by mean farm daily liveweight gain (kg/d)

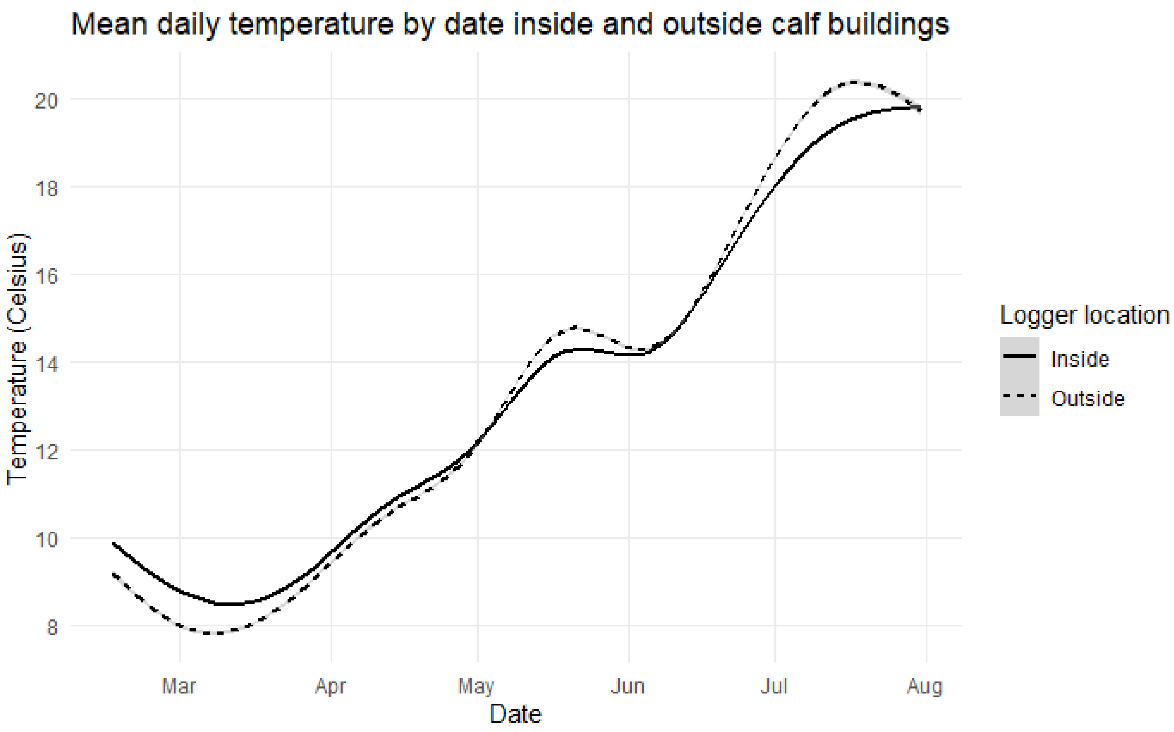

Figure 2

Mean daily temperature (Celsius) by date for data loggers placed inside and outside calf buildings respectively.

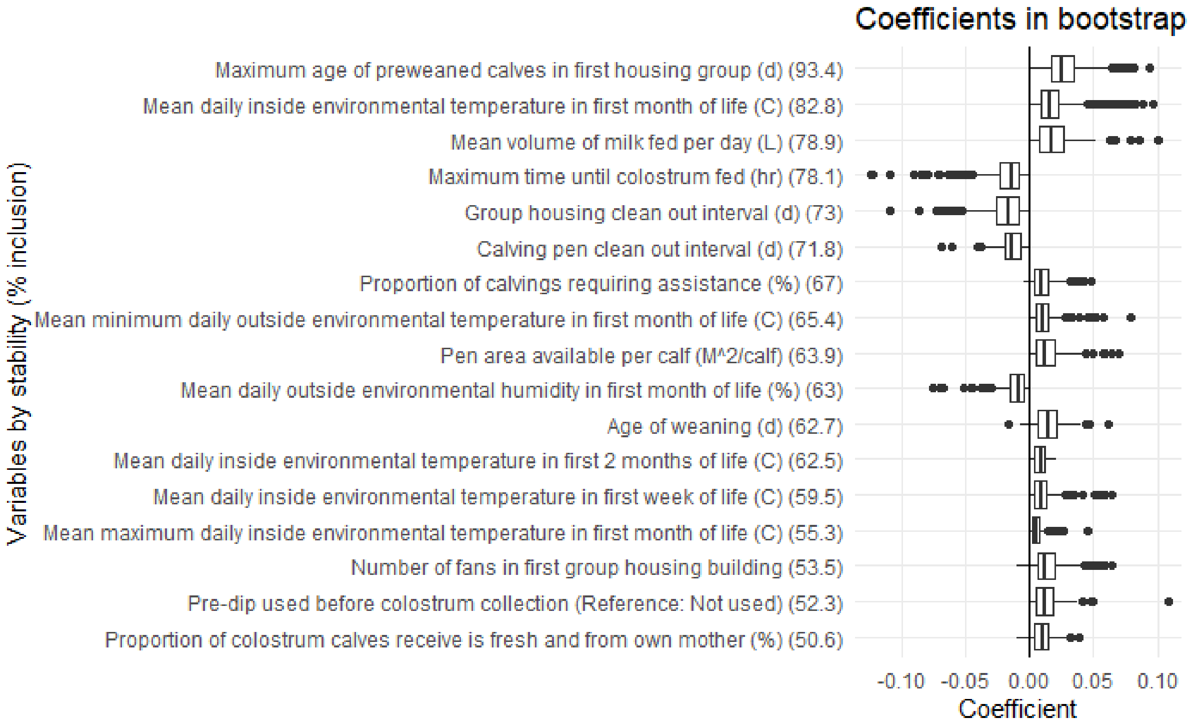

Figure 3

Coefficient distributions and variable stability for variables selected in at least 50% of models across 1000 bootstrapped samples. Coefficient estimates for numeric variables represent the change in daily liveweight gain (kg/d) associated with a change of 1SD in each variable.

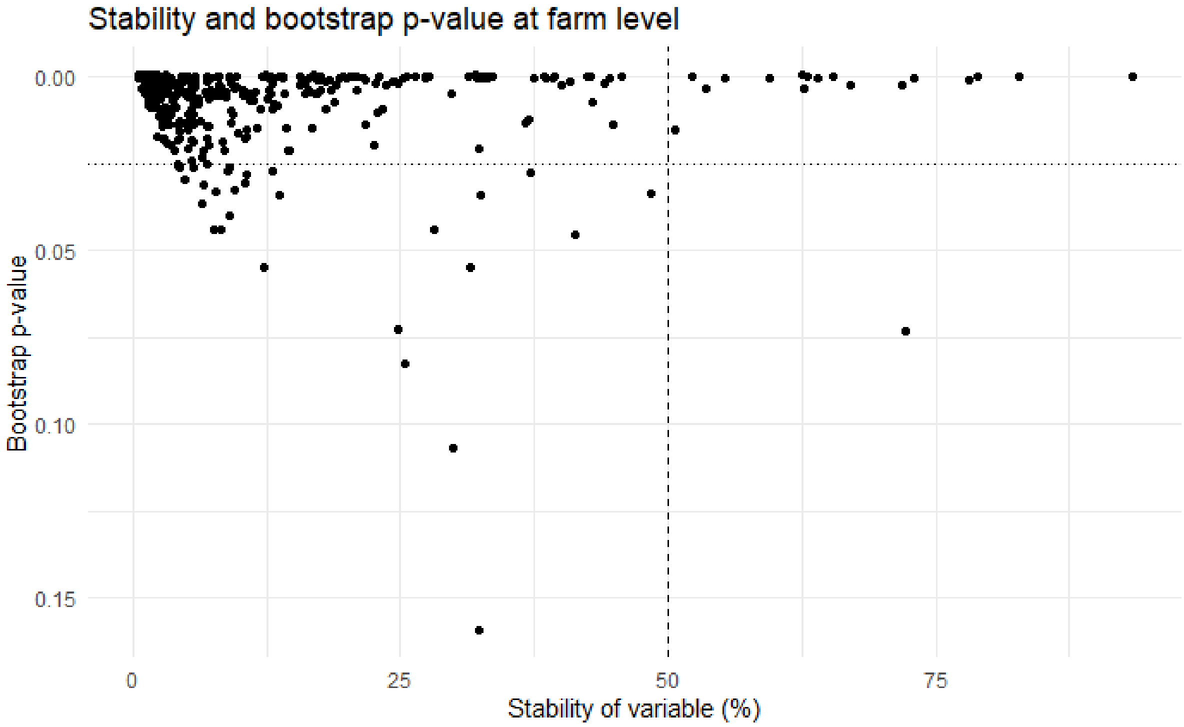

Figure 4

Bootstrap p-value by stability of variables (%). Points jittered to avoid overlapping. Variables selected for final model were above 50% stability (dashed line) with a bootstrap p-value of <0.025 (dotted line).

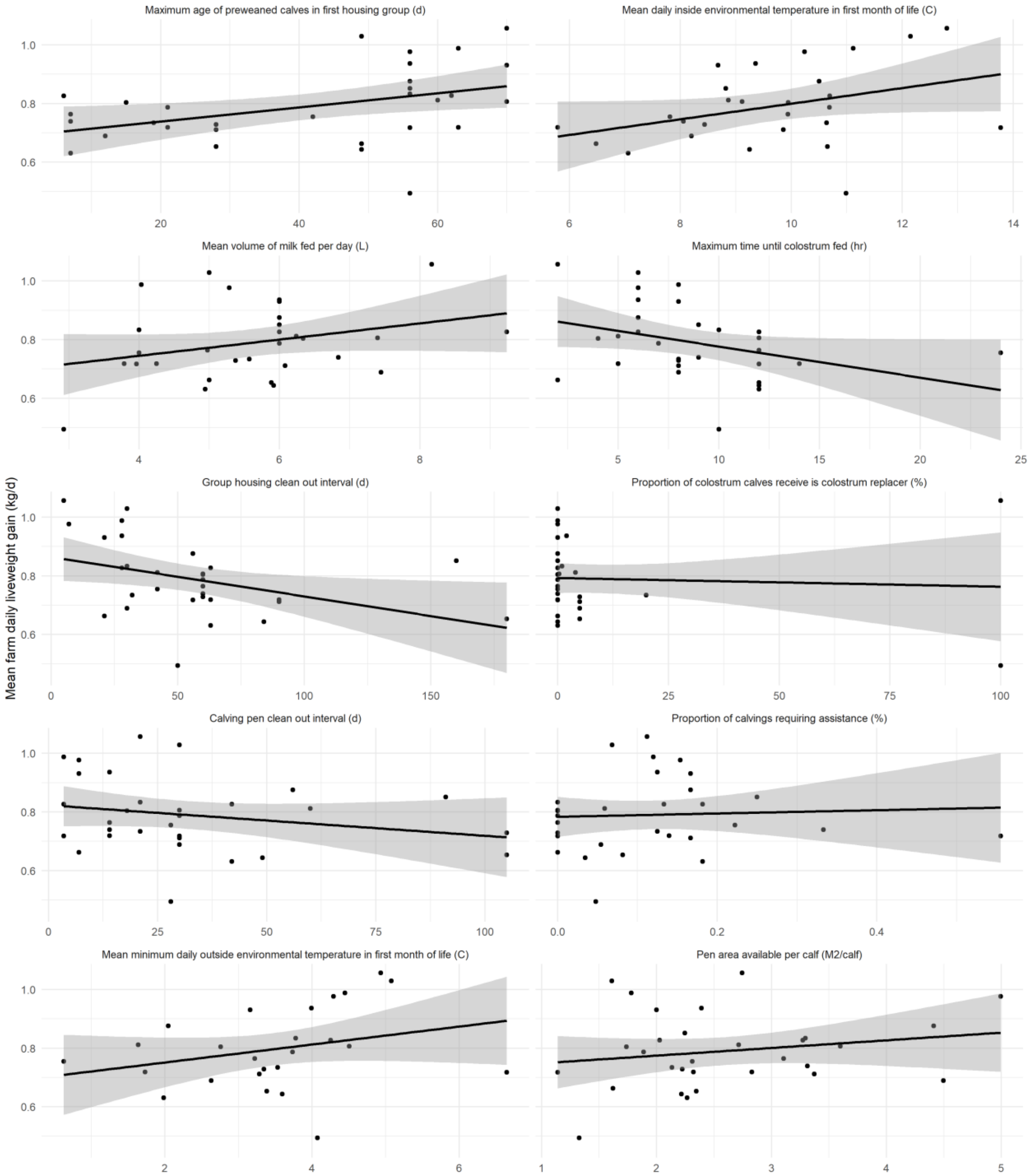

Figure 5

Descriptive presentation of the 10 highest stability variables identified from bootstrapped regularised regression model against mean farm daily liveweight gain (kg/d)

Variable (unit)	*Stability (%)*	*Mean coefficient*	*90% Bootstrap confidence interval*	*Bootstrap P-value*
Maximum age of preweaned calves in first housing group (d)	93.4	0.001	(0.000 - 0.002)	<0.001
Mean daily inside environmental temperature in first month of life (C)	82.8	0.012	(0.002 - 0.037)	<0.001
Mean volume of milk fed per day (L)	78.9	0.012	(0.001 - 0.024)	<0.001
Maximum time until colostrum fed (h)	78.1	-0.004	(-0.01 - 0.000)	0.001
Group housing clean out interval (d)	73	-0.001	(-0.002 - 0.000)	<0.001
Proportion of colostrum calves receive is colostrum replacer (%)	72.2	-0.001	(-0.003 - 0.000)	0.073
Calving pen clean out interval (d)	71.8	-0.001	(-0.001 - 0.000)	<0.001
Proportion of calvings requiring assistance (%)	67	0.001	(0.000 - 0.003)	0.002
Mean minimum daily outside environmental temperature in first month of life (C)	65.4	0.009	(0.001 - 0.020)	<0.001
Pen area available per calf (M2/calf)	63.9	0.010	(0.001 - 0.025)	<0.001
Mean daily outside environmental humidity in first month of life (%)	63	-0.004	(-0.010 - 0.000)	<0.001
Age of weaning (d)	62.7	0.002	(0.000 - 0.004)	0.004
Mean daily inside environmental temperature in first 2 months of life (C)	62.5	0.005	(0.001 - 0.009)	<0.001
Mean daily inside environmental temperature in first week of life (C)	59.5	0.007	(0.000 - 0.019)	<0.001
Mean maximum daily inside environmental temperature in first month of life (C)	55.3	0.001	(0.000 - 0.003)	<0.001
Number of fans in first group housing building (n)	53.5	0.025	(0.002 - 0.062)	0.004
Pre-dip used before colostrum collection (Reference: Not used)	52.3	0.012	(0.001 - 0.030)	<0.001
Proportion of colostrum calves receive is fresh and from own mother (%)	50.6	0.000	(0.000 - 0.001)	0.015

Table 1

Variable stability, coefficient estimates, 90% bootstrap confidence intervals and bootstrap p-value for variables present in the final model of calf preweaning daily liveweight gain (kg/d). Coefficient estimates for numerical variables relate to the effect of a 1-unit change in daily liveweight gain.

DISCUSSION

Of the 293 variables available, 17 were found to have both high stability and an effect size with a low bootstrap p-value when predicting mean farm DLWG. Many of these variables represent similar practical aspects of management and largely focus around stocking demographics, milk/colostrum feeding, environmental hygiene and environmental temperature.

Increases in several environmental temperature variables at a variety of age ranges appeared to be associated with improved DLWG. The lower critical temperature is 20°C for calves up to 3 weeks old (National Research Council, 2001) however the mean UK temperature in 2019 was only 9.4°C, with winter 2018/19 temperatures averaging only 5.2°C (Met Office, 2019). As neonatal calves are likely to be far below their lower critical temperature for much of the year in GB, it is perhaps unsurprising that lower environmental temperatures were associated with reductions in DLWG in the current study, as calorific requirements are likely to increase in cold temperatures (National Research Council, 2001). Calves in GB are generally housed in naturally ventilated buildings, and temperatures inside similar calf buildings have previously been found to be similar to outside environmental temperatures across Europe (Seedorf et al., 1998). These findings were replicated in the current study, with mean daily temperatures recorded inside calf buildings being almost identical to those outside on each farm as shown in Figure 2. Since the design of the building appears to have minimal effect on environmental temperatures, it may be that active heating elements could have a positive effect on calf DLWG. Methods to limit weather exposure and therefore reduce heat loss through convection should also be further explored. Negative effects of colder environmental temperatures on calf mortality at national level have recently been reported in GB (Hyde et al., 2020), and the effects of interventions that improve calves’ thermal environment on both mortality and DLWG could be directly explored in future studies. It is worth noting that the positive effect of increasing temperature on DLWG is likely to be country-specific, and caution should be taken extrapolating these results to countries with differing weather conditions to GB. Despite the apparent importance of environmental temperature, calf jacket usage did not have a consistent beneficial effect on DLWG, similar to previous findings (Earley et al., 2004; Scoley et al., 2019).

Increased milk volume was found to improve DLWG during the preweaning period. Results from a previous meta-analysis suggest increased milk replacer rates were associated with increased DLWG during the preweaning period (Hu et al., 2020), and whilst concerns have been raised that feeding calves a higher allowance of milk increased total feed costs, research has demonstrated a decreased total cost per kg of liveweight gain with increased milk feeding (Hawkins et al., 2019). Despite research suggesting calves will voluntarily drink up to 13.3L (95% CI 12.4-14.2L) of milk by 26d of age (Curtis et al., 2018), recent UK surveys suggest calves are routinely being fed 4-8L, with considerable variation in feeding practices due to a lack of scientific consensus in effective protocols to promote growth and future performance (Palczynski et al., 2020). Calves fed only 6L a day have been reported to show chronic signs of hunger (Rosenberger et al., 2017), however, only 8 out of the 30 farms in the final dataset reported feeding greater than 6L/calf on average. Whilst there are likely to be objective gains in terms of productivity when increasing milk feeding, the benefits to calf welfare of increased milk volume allowance should also be considered.

The association of an increased maximum age of preweaned calves in the first housing group (the maximum age a calf might reach in the first housing group) with increased DLWG suggests that calves remaining within one housing group throughout weaning may benefit from higher DLWG than those being housed in several housing groups within the preweaning period. This may be in part due to calves' tendency to learn from their companions, potentially increasing early intakes. There may also be an effect of minimising group changes in preweaned calves, as calves housed for longer within the first housing group would consequently experience fewer social group changes in early life than those spending a shorter time in the first housing group. A review into the effects of group housing found that social housing improves intakes and weight gains (Costa et al., 2016), which has been reinforced by more recent studies reporting increased growth rates when calves are group housed rather than housed individually (Curtis et al., 2018; Johnson et al., 2019). Whilst there are concerns that group housing might be disadvantageous from a disease transmission perspective, any health risks associated with group housing can likely be mitigated with appropriate management (Costa et al., 2016). In addition to the association between an increased maximum age of preweaned calves in the first housing group, an association between increased weaning age and increased DLWG was also found and is likely to be reflective of exponential growth rates during the preweaning period.

An increased interval between the complete cleaning out of both group housing and calving pens was associated with a reduced DLWG. Previous research has suggested that neonatal calf diarrhea is negatively associated with DLWG (Windeyer et al., 2014). These results may support findings from the current study that environmental hygiene of both the calving pen and group housing environment are consistently important for improving DLWG, potentially through reduced disease levels.

A longer maximum time until colostrum feeding, a decreased percentage of calves receiving fresh colostrum from their own mother and failure to use a pre-dip prior to colostrum collection were all consistently associated with reduced DLWG. This suggests that colostrum feeding timing, substance and hygiene during collection may all play an important role in

DLWG. Failure of passive transfer has previously been negatively associated with DLWG (Windeyer et al., 2014). Timing, substance and importance of maintaining low bacterial levels within colostrum in reducing the risk of failure of passive immunity transfer (FPT) has been reported in previous studies (Gelsinger et al., 2015; Godden, 2008). As FPT is known to increase the risk of disease (Raboisson et al., 2016), the effects of colostrum management on DLWG may be indirect effects due to reduced rates of FPT reducing the incidence of neonatal calf disease and therefore mitigating the negative effects of disease on DLWG (Windeyer et al., 2014). This was challenging to investigate in the current study, as a relatively low number farms returned FPT data, with 12/30 farms having unknown levels of FPT.

An increased frequency of veterinary visits was also associated with a higher DLWG. The stability of this variable however was only 43.9% with a mean coefficient only -0.001 kg/d per additional 1d between veterinary visits (90% BCI -0.003 – 0.000, bootstrap p-value 0.045), suggesting that whilst other variables may initially result in a larger effect size on a greater number of farms than an increased rate of veterinary visits, an increased frequency of veterinary visits may play a role in improving the productivity of preweaned calves. Previous studies examining factors associated with mortality have suggested that the absence of active/routine calf health involvement from a veterinarian was also associated with a higher rate of calf mortality (Renaud et al., 2018).

There were occasionally variables that despite having low stability still appeared to have a large effect size such as increasing the age (d) at which dry feed is provided, with a coefficient of -0.003 (90% BCI -0.007-0.000) and bootstrap p-value of 0.002 despite a relatively low stability of 40.0%. Similarly increasing the age (d) at which free water access is provided had a coefficient of -0.003 (90% BCI -0.008-0.000) and bootstrap p-value of

0.013. This suggests that whilst these variables are not necessarily important on the majority of farms, there are certain situations where they might have a larger effect size than other variables, with a 7d delay in either the provision of dry food or free water access after birth being associated with a 21g/d decrease in DLWG across the preweaning period, and a simultaneous delay in both dry food and free water access being associated with a 42g/d decrease in DLWG. The optimal stability threshold chosen is a product of mimising error rates, with increases in stability threshold reducing the risk of false positives (Meinshausen and Bühlmann, 2010). In this study, a stability threshold of 50% was applied in order to optimise potential false positive and negative rates (Figure 4). A full table of variable coefficients is available in *Supplementary materials iii*.

There were numerous calf management factors identified in this study with the potential for major improvements (relatively large effect size) to DLWG on the minority of farms (low stability). Conversely there were some factors that might have a minor impact (relatively small effect size) on the majority of farms (high stability). It would be impractical however for veterinary advisors to suggest implementation of a large number of management interventions aimed at improving DLWG, when many of these would have limited impact. The identification of key factors likely to have the largest impact on the DLWG (effect size) of preweaned calves on the highest proportion of farms (stability) is consequently extremely important, enabling veterinary advisors to suggest the small number of interventions likely to have maximum gain in the majority of cases. Whilst the methods utilised in this research have identified several stable variables with relatively large effect sizes, it is possible a number of variables with an effect on DLWG might have remained undetected due to sample size constraints. The use of the elastic net algorithm means a conventional sample size calculation is unlikely to be appropriate, however by taking the standard deviation from the current research (0.13kg/d) and 15 farms per group, conventional sample size calculations indicate an 80% chance of detecting a relatively large difference of 0.14kg/d in daily liveweight gain, at a significance level of 0.05. Whilst this method of sample size calculation would not be appropriate when using elastic net, it suggests that variables with relatively small effect sizes might only be detected if a larger sample size was available. Methods such as sparse principal component analysis were also considered as a suitable approach to high dimensionality datasets (Zou et al., 2006), but the elastic net algorithm was chosen for improved interpretability and capability of p>>n datasets.

Whilst relatively small datasets can be prone to overfitting and be sensitive to outlying values, the use of both bootstrap sampling and model selection via cross-validation will greatly reduce this risk. As individual observations will only be present in ~63.2% of bootstrap samples (Borra and Di Ciaccio, 2010), and only 80% of observations will be randomly selected for each of the 10 repeats of 5-fold cross validation, the effect of any potential outlying variables is greatly reduced, resulting in robust estimates of coefficients. As shown in Figure 5, the risk of excessive leverage from individual observations appears to be low from key variables. The low stability of farm ID (supplementary data 3) suggests management variables were more important than the farm itself.

A potential limitation to the estimation of DLWG in this research was the use of weigh tapes rather than calibrated weigh scales. The use of weigh tapes has been shown to provide reliable estimates of BW in young calves (Heinrichs et al., 1992), and although there have been concerns about the accuracy of tape measures (Dingwell et al., 2006; Heinrichs et al., 2007) further validation studies have suggested this method to be sufficiently accurate (Bond et al., 2015), with the use of weigh tapes being suggested as practical when scales are unavailable or impractical (Atkinson et al., 2017). The use of a birth and weaning weight measurements should ameliorate user variability to a degree since users consistently overestimating/underestimating birthweights would be likely to consistently overestimate/underestimate weaning weights, resulting in relatively accurate DLWG estimates at farm level. From analysis of individual farm estimates of birth and weaning weight (Figure 1) it appears that estimates of birthweights are relatively consistent. Whilst there is likely to be a degree of random variation introduced by weigh tapes, such random error will tend to make model parameter estimates conservative (wider standard errors) rather than introduce bias (Hutcheon et al., 2010). There is the potential that farm management practices varied from responses given in the management questionnaire, however the authors feel that farmers were generally honest and accurate with their responses, even when these responses might not represent best practice. There may also be limitations in the collection of environmental temperature, as data loggers were placed at 1.5m height, and thus may not accurately represent the calves' local environment when in recumbency. Whilst logger placement at calf level may be desirable, it is logistically more challenging due to the propensity of calves to vigorously explore novel items in their environment, potentially damaging the loggers. Preliminary trials suggested that differences between 0.5m and 1.5m height within calf housing had relatively minor differences in absolute temperature, and followed similar trends in temperature changes, without the risk of damage to the data loggers. Visit dates were spread between December to February, meaning calves on some farms might be exposed to unequal environmental conditions depending on their visit date, however, this variation would be captured by the temperature and humidity variables.

The generalisability of this study is unknown since there may be some differences between farms randomly selected from a supermarket group and the general dairy farm population in GB, often having minimum management standards and volume requirements. Participation in the study was voluntary which may have introduced a degree of bias by selecting more proactive farmers, although the majority of farms contacted agreed to participate in the study

(55% of 110 farms contacted), with a similar proportion of participating farms returning high quality data (50%). Whilst there is the potential for farms within the final dataset to vary from the general population, we believe they are likely to be a largely representative sample of GB dairy farms.

An important additional limitation of this research is that associations identified during the observational study should not be interpreted as causal, and further studies investigating the effects of these interventions as a randomised controlled study should form the basis of future research.

CONCLUSIONS

Increasing the maximum preweaned age within the first housing group, increasing both milk feeding volume and environmental temperature, ensuring regular cleaning out of both group housing pens calving pens and ensuring rapid feeding of colostrum were found to be associated with improvements in DLWG at farm level.

DLWG in the preweaning period has been shown to be extremely important in the efficient rearing of both dairy and dairy cross beef calves. There are numerous variables likely to affect DLWG in preweaned calves, and it is essential that veterinary advisors are able to target a small number of stable variables likely to have the largest effect size. By performing elastic net regression in conjunction with bootstrap sampling techniques, a small number of stable variables have been identified that are most likely to have the largest effect size on DLWG in preweaned calves. Further research should be conducted to test the impact of interventions to improve these consistently important management factors on the DLWG of preweaned calves on GB dairy farms.

ACKNOWLEDGEMENTS

This research was carried out as part of a wider calf health research project funded by the Agricultural and Horticulture Development Board (AHDB; Warwickshire, UK), the AgriFood Charities Partnership (Bedford, UK), and the University of Nottingham School of Veterinary Medicine and Science (Leicestershire, UK).

Thanks to MSD (Milton Keynes, UK) for providing weigh tapes for use in the trial, and to Dr. Claire Windeyer (University of Calgary Faculty of Veterinary Medicine) for providing invaluable feedback on the calf management questionnaire.

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