Original Article

Associations between putative risk factors and poor colostrum yield in Holstein Friesian cattle

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Introduction

Calves need to ingest a volume equivalent to 10-15% of their bodyweight of high-quality (>50 g/L concentration of IgG) colostrum in the first 6-12 hours of life, to acquire passive immunity from their dams (McGuirk and Collins, 2004). If colostrum is not ingested in a timely fashion, colostrum quality is poor, or inadequate volumes of colostrum are produced by the dam, it is likely that neonatal calves may suffer from failure of passive transfer (FPT). Consequences of FPT include increased calfhood morbidity and mortality, poor growth rates, and poor reproductive and productive performance.

Colostrogenesis (the prepartum transfer of immunoglobulins from maternal circulation into mammary secretions) begins 5-6 weeks pre-calving. The volume of colostrum produced by the dam (colostrum yield) may be affected by: nutrition; environmental conditions; time interval from calving to milking; parity; dry period length; degree of calving difficulty; calf weight; calf sex; calf viability; cow BCS/body weight; milk production in previous lactation and dam health (Pritchett et al., 1991, Conneely et al., 2013, Gavin et al., 2018). Individual cow colostrum yield is highly variable. Mean yield of colostrum from approximately 700 pasture based dairy Jersey cows in Ireland was 6.7 kg (SD = 3.6 kg, range = 0.1 to 24 kg) (Conneely et al., 2013); while colostrum yield from one Jersey cow dairy farm in North America was 4.26 kg (range = 0 to 26.5 kg) (Gavin et al., 2018) and from Greek Holstein cows was 6.18 kg +- 3.77kg (Soufleri et al., 2019).

Colostrum yield may be influenced by environmental variables such as photoperiod, temperature and rainfall, the effect possibly lagging periods of exposure by an interval of weeks or months (Gavin et al., 2018). Temperature humidity index (THI) is a single value representing the combined effects of air temperature and humidity associated with thermal stress. Variation in THI has been associated with variation in milk yields (Herbut et al., 2019), and is moderately correlated with colostrum yields from multiparous cows, with a time lag of 1 to 2 months pre-calving (Gavin et al. 2018).

While risk factors for poor IgG concentration in colostrum have been extensively studied, there is little published literature on the phenomenon of low colostrum yields and associated risk factors (Conneely et al. 2013, Gavin et al. 2018). The aim of this observational study was to identify associated risk factors for poor colostrum yield in dairy cows to add to an insubstantial body of evidence. In particular, the objective was to quantify the effect of variation in those variables that the farmer can directly control: the length of the dry period, and nutritional inputs during the close-up transition period.

Materials and methods

Study site, animals and their management

Between November 2020 and October 2021 439 animals (n= 121 heifers, n=318 cows) from a single Scottish dairy farm were used as a convenience sample (under University of Glasgow ethics licence EA49/21) for an observational study. The herd, located in central Scotland, included approximately 550 Holstein Friesian milking cows housed throughout the year in free-stall barns. Cows were milked three times a day in a 24-a-side herringbone parlor with swing-over central milking units and produced on average 30 kg of milk/day (305-day milk yield 9200 kg/cow). The herd participated in a monthly testing program for milk yield and components (fat and protein) and in quarterly testing for somatic cell count (SCC), performed by the Cattle Information Service (CIS).

Cows were dried off abruptly approximately 60 days before expected calving date and were managed in two separate groups: far-off (up to 21 days before calving) and close-up (21 days to calving). Nulliparous animals were moved into a separate close-up pen about three weeks before calving. Dry cows and heifers were fed a total mixed ration (TMR) diet composed of a mix of silage, straw, and a blend of molasses and soybean meal. During the close-up period, the ration for all animals was supplemented with a vitamin-mineral premix (Translac, ForFarmers UK, IP30 9ND) to achieve a negative dietary cation-anion difference (DCAD, miliequivalents per kg of dietary dry matter, mEq/kg of DM). In October 2021, the DCAD diet was terminated and replaced with a calcium binder (Zeolite A). Close up cows' rations (fed for 21 days pre-calving) are detailed in Table 1 including the dates when changes were made to the rations.

All dry animals were housed in straw yards. Calves were born in the multiple close-up pens and removed from their dams within 2 hours. Colostrum was then harvested at first milking time (04:00, 12:00, and 20:00 h). Animals had their first milking colostrum yield measured by farm staff using a graduated bucket. Volumes were estimated to the nearest half L and recorded (together with animal identification number) on a white board in the milking parlour, before the information was transferred to a spreadsheet (Microsoft Excel version 10) for further analysis.

Further data on each individual cow was collected from DairyCOMP305 (Valley Agricultural Software, Tulare, CA, USA). For each animal, the data included: lactation number; predicted transmitting ability for milk 'PTA milk' (L); birth date; dry-off date; calving date; calving ease; calf sex; mean somatic cell count (based on the last 3 tests) (cells/ml); dry matter intakes (kg/cow/day); percentages of starch and protein in the pre-calving diet. In addition, meteorological data were obtained from Metoffice (Devon, UK) for the nearest weather station (6.8 miles from the study farm): daily mean temperature (°C); daily rainfall (mm) and ultraviolet light index (one UV index equivalent to 25 mW per square metre). For temperature data, the temperature range was also calculated (°C).

A sample size calculation was completed using Epitools online calculation tool (Ausvet, Canberra, Australia) to estimate a single mean, assuming a population standard deviation of 2 L, with desired precision of 0.2 L and confidence level of 0.95; this indicated that 385 animals would be required.

Statistical analysis

Statistical analyses used Stata (StataCorp LLC, Texas, USA) and R (R core-Team 2018). Exploratory analysis was conducted for each variable by tabulation and plotting histograms, scatterplots and boxplots. Continuous variables were tested for normality using the Shapiro-Wilk test with consideration of the histograms.

Manipulation of variables

Cattle of 5 lactations and over were collapsed into a single category because only 22 animals were in lactations 6-8. Calving ease was classified as 1 (no assistance), and 2 or more (assistance of any kind); since only 4 animals were categorised as calving ease score 3 (assistance using a calving aid) and no enrolled animals underwent caesarean section (calving ease score 4). For the purposes of this analysis (and due to the non-normal distribution of these variables) the number of days dry and previous lactation length were divided into quartiles. Dry period length prior to calving was also recategorized as <60 days and ≥60 days for logistic regression modelling. Colostrum yield was not normally distributed (Shapiro-wilk test <0.01) and was transformed by the square root function for linear regression and inverse probability weighting (IPW) modelling. To reflect the commonly recommended quality threshold, the data were also dichotomised into 4 L or more and less than 4 L to reflect the recommended minimum 10% requirement for the calves born on the study farm (approximately 40 kg birth weight). It is acknowledged that dichotomisation of outcome variables is commonly used in clinical practice, but can be regarded as problematic (Altman and Royston, 2006). It is included here, in addition to an analysis using continuous data, because it has become standard clinical practice.

Univariable analysis

Each continuous and categorical risk factor variable was examined in turn using univariable logistic regression models; and significance for inclusion in further linear and logistic multivariable modelling was declared at p=0.2 and for IPW models at p=0.05.

Multivariable generalised linear and logistic regression analysis

Two outcome variables were considered: the continuous outcome of square root of colostrum yield and the dichotomous outcome of colostrum yield ≥4 L and <4 L. For both linear and logistic regression modelling, confounding between explanatory variables was noted where regression coefficients varied by ≥20%. All biologically plausible interaction terms were explored (declaring significance at $p \leq 0.05$) including lactation and calving ease, calf sex and calving ease, as well as temperature and UV indices, season, and calving month. Interactions between all dietary measures were also explored. Variables were then excluded from the multivariable models using a process of backwards stepwise elimination. Multivariable models were compared conservatively using the likelihood ratio test (significance declared at $p \leq 0.05$) and Akaike Information Criteria (AIC), with a choice of model with the smallest AIC.

Directed acyclic graphs (DAGs)

Directed acyclic graphs (DAGs) were constructed for the dataset (Textor et al. 2016). The DAG in our study was constructed with the DAGitty software (University of Utrecht, http://dagitty.net). DAGs offer systematic representations of causal relationships and have become an established framework for the analysis of causal inference in epidemiology, often being used to determine covariate adjustment sets for minimizing confounding bias. We constructed a DAG with number of days dry pre-calving as the main exposure variable of interest. All recorded variables were included in the model – first drawn as unconnected nodes, and then with directional lines (arrows with direction indicating causality) between nodes if there was a good *a priori* reason to believe that one node might influence another. Linking two nodes with an arrow does not mean that there is a relationship, but that it is conceivable that there might be. For example, season is known to affect temperature, which in turn might drive colostrum yield, while season might have a direct effect on colostrum yield, and colostrum yield could never influence season or temperature. Arrows are drawn from one node to another as testable, plausible hypotheses of causality. Figure 1 shows the DAG diagram with colostrum yield (blue symbol with "I") as the outcome variable of interest. Dry period length and cows' ration components (DM, E, starch, protein and DCAD) were identified as exposure variables whose effects were the object of this study (yellow symbols), since unlike other variables that might have direct effects on colostrum yield, diet composition and dry-period duration can be manipulated by farmers. According to this DAG, the potential confounders (and hence the minimum set of variables for which adjustment is required in inverse probability weighting (IPW)) for the dry-period length were parity and previous lactation length (pink symbols), with pink arrows indicating biasing paths. The blue symbols represent other ancestors of the outcome variable for which adjustment would be unnecessary (mediators and competing variables).

Inverse probability weighting and doubly-robust regression

Inverse probability weighting (IPW) is a causal inference approach that can be used to adjust for confounding. It involves weighting each observation by its inverse probability of exposure, so that the confounding is removed and the marginal effect of exposure on the outcome can be estimated. After construction of the DAG to identify the minimum adjustment set of variables, the *ipw* package in R (van der Wal and Geskus, 2011) was used to fit marginal structural models by inverse probability weighting. The *survey* package in R (Lumley, 2004, 2020) was used to calculate doubly robust effect estimates.

Lagged time series analysis

Time series analyses (between November 2020 and October 2021) were completed in Stata using the *tsset* (to manage the time series settings of the dataset), *corrgram* (to produce correlograms) and *xcorr* (to measure cross-correlation) functions. A cross-correlation function is a representation of the linear correlation between independent variables and the outcome at different lags plotted against the current lag. Monthly time lags were used to examine the relationship between UV and temperature (up to 3 months previously) and colostrum yield.

Results

Description of data

Data were collected from 439 cattle. Somatic cell count data were missing from 13 animals because they either calved after the somatic cell count test (3 monthly) or died before they were tested. Only 318 observations were available for the variables of previous lactation length and number of days dry, since heifers were excluded.

Fourteen cows produced no colostrum at all. The majority of these animals (9/14, 64.29%) were first and second lactation, and produced female calves (8/14, 57.14%).

Overall mean colostrum yield was 4.59 L (95%CI=4.29-4.83, SD= 2.94, range=0-15 L). Figures 2 and 3 show the distribution of measured colostrum yields and boxplots of measured colostrum yield by lactation, respectively. Figure 4 shows the mean first milking colostrum yields of primiparous and multiparous cows.

Univariable analysis

Tables 2 and 3 detail the descriptive statistics and the results of the univariable regression models for both continuous and categorical predictor variables. An increasing temperature range (°C) was negatively associated ($p<0.01$) with colostrum yield and an increasing dry period length was associated with better colostrum yield (($p<0.01$). Older cows produced more colostrum than lactation 1 and 2 animals ($p<0.01$). None of the ration variables were significantly associated with colostrum yield ($p>0.05$).

Multivariable generalised linear models

The results of the final multivariable linear regression model are shown in Table 4. A higher temperature range was associated with lower colostrum yield ($p<0.01$) and a longer dry period length was associated with higher colostrum yield ($p<0.01$). Lactation 1 animals were not included, as heifers had no dry period length recorded, but compared with lactation 2 animals, lactation 3 animals produced more colostrum (p=0.04). No significant confounding or interaction terms ($p<0.05$) were measured between these variables for colostrum yield.

Inverse probability weighting and doubly robust regression

As determined by examination of the DAG, the minimum adjustment set of variables for the exposure variable of dry period length was previous lactation length and parity. Primiparous cows were removed from the analysis since they had no previous lactation and no dry period length. Because no confounders were identified for nutrition variables in the DAG, they were not included in the IPW analysis. Marginal effect models for the effect of dry period length on colostrum yield using the continuous outcome of square root of colostrum yield (adjusting for previous lactation length and parity) revealed that the effect of dry period length in multiparous cows was 0.054 L increase of colostrum yield for each additional day dry (coefficient= 0.0029, SE=0.0008, 95%CI=0.001-0.004, p=0.01)

Multivariable logistic regression models

The results of the final multivariable logistic regression model are shown in Table 5. For the logistic model, cows with a dry period length of 60 days or more had greater odds of producing ≥4 L of colostrum than animals with dry period length <60 days (OR=2.36, 95%CI=1.35-4.11, $p<0.01$). A greater temperature range was associated with lower colostrum yield (OR=0.89, 95%CI=0.83-0.95). For every unit increase in UV index, the odds of colostrum yield ≥4L increased (OR=1.61, 95%CI=1.19-2.18). Temperature ranges and UV indices were associated with season; with spring and summer months having a mean temperature range of 9.95°C, versus 6.64°C for autumn and winter months, however season was not significantly associated with colostrum yield. Similarly, mean UV indices for spring/summer months were 1.78 units compared with 0.32 units for autumn/winter months, however season was not significantly associated with colostrum yield.

Time series analysis

Cross correlation coefficients for the autocorrelations between time (monthly lags) and colostrum yield were calculated and are shown in Table 5. UV and temperature parameters were more closely cross correlated with colostrum yield in primiparous animals. Higher temperature ranges in the month before calving were negatively associated with colostrum yield in multiparous cows (cross correlation coefficient (CCF) 0.25), and in primiparous cows the relationship was in the same direction but of slightly weaker magnitude (CCF 0.24). Higher UV indices the month before calving were positively associated with colostrum yield, particularly in primiparous cows (CCF 0.47). Higher mean temperatures in the month and 2 months before calving were positively associated with colostrum yield in multiparous (CCF 0.30) and primiparous (CCF 0.42) cattle respectively.

Discussion

The aims of this observational study were to identify risk factors for poor colostrum yield in dairy cows and particularly to quantify the effect of variation in those variables that the farmer can directly control: the length of the dry period, and the nutritional inputs during the close-up transition period. Significant associations were observed between some putative risk factors and colostrum yield, particularly temperature range, number of days dry before calving and UV index.

Nutritional variation had little effect on colostrum yield in this study, which was somewhat surprising since it has been shown in ovine work that nutrition pre-calving can affect colostrum volume (Swanson et al. 2008). However a similar study in cattle produced inconclusive results (Mann et al. 2016): IgG concentration in colostrum was affected by dry period nutrition, but the same was not shown for yield of colostrum in dry cows fed 100, 120 and 150% of their energy requirements. In sheep, greater volumes of colostrum were produced by animals fed 100% of requirements from day 50 of gestation to lambing compared to underfed animals (Swanson et al., 2008). Mellor et al., (1986) also demonstrated that underfeeding pregnant ewes reduced udder development and colostrum yield, as underfed ewes suffered a delayed decrease in circulating concentration of progesterone resulting in a delay in lactogenesis and poor colostrum production. Work in beef cows demonstrated higher yields in housed cows than outwintered (related to nutrition) independent of IgG concentration (Logan, 1977). A small study of 20 multiparous dairy cows fed low starch diets showed that 27% failed to produce at least 5 kg of colostrum (Litherland et al., 2011). In our study, within the range of nutrients offered, it seems that nutrition was not a limiting factor on colostrum yield. While there was no significant association between 'close up' (3 weeks pre-calving) rations and colostrum yield in the study population, body condition score data was not examined. Excessive body condition score loss between dry off and calving leading to negative energy balance may be related to poor colostrum yield and is a risk factor which warrants further investigation. Nutritional inputs were measured by a commercial laboratory and rations were prepared by an independent nutritional consultant, but actual point of feeding values and animal intakes were not directly measured or verified by the authors, which may have caused minor dilution bias, possibly leading to underestimation of possible effect sizes.

An increasing number of days dry was associated with increased colostrum yield, even when confounding factors (parity and previous lactation length) were adjusted for. This may be due to cows with longer dry periods being afforded more time to metabolically prepare for calving after cessation of milk production. Dry period length (pre-calving) has been implicated in colostrum yield in other work - compared with cows with a 75-day dry period; cows with a 45-day dry period had a 1.88 times greater odds of low colostrum production (Gavin et al., 2018). In the current study, the effect of dry period length in multiparous cows was a 0.054 L increase of colostrum yield for each additional day dry. Similarly, in the logistic regression models a dry period length of ≥60 days resulted in a higher colostrum yield (≥4L $p<0.01$). The effect of previous lactation length has also been measured and was found to be significant but small (Gavin et al., 2018); however, we observed no significant effect of previous lactation length at the multivariable level.

Age effects on colostrum yield are inconsistently reported. Lower colostral weight was recorded in first lactation heifers in some studies (Kruse, 1970; Conneely et al., 2013), in contrast to other work, which reported no significant association between parity and colostrum volume (Kehoe et al., 2011). In the current study, there was a significant positive association between cow age and colostrum yield only in the univariable analysis. Compared with the youngest animals at calving, older cows had greater odds (OR 1.38-2.27) of colostrum yield less than 2.7 kg in Jersey animals (Gavin et al., 2018). This differs from the trend observed in the univariable regression results from the current work, in which older cows (lactation >2) produced more colostrum than first and second lactation animals ($p<0.01$). In the Gavin study (2018), significantly more multiparous than primiparous animals produced no colostrum, particularly in the winter months (December), however the profile of the 14 animals that produced no colostrum in the current work was skewed towards younger animals (9/14, 64.29% of cases). Heifer lactation yields are often lower than those of older animals, so it is generally expected that colostrum yield should also be lower (Logan 1978; Horan et al., 2005), although the two are not always related. A small-scale Swiss study evaluated the effects of colostrum yield on subsequent lactation yield and found first-colostrum yield (mean= 19.4 kg) and cumulative milk production of 100, 200, and 305 lactation days were not significantly correlated in multiparous or primiparous cows (Kessler et al., 2014).

Irish work reported a coefficient of genetic variation of 22.3% (Conneely et al., 2013) for colostrum yield, whereas in other work additive genetic variance was found for all colostrum traits except yield (Soufleri et al., 2019). Despite no effect of sire or dam PTA (predicted transmitting ability) observed in the current work, genetic analysis showed extreme colostrum yield (low vs. high) followed some sire lines (Gavin et al., 2018). This breed effect on colostrum production has also been reported in a study comparing Holstein, Jersey, Ayrshire and Guernsey animals (range in colostrum yield 3.1lb to 61.8lb, equivalent to 1.41kg to 28.03kg) (Parrish, 1950), but there have been many changes in sire selection for dairy herds since that work was published.

An increasing daily temperature range was negatively associated with colostrum yield ($p<0.01$). The number of days with a maximum temperature (for 21 days before calving) greater than 23°C has been previously shown to be negatively correlated with colostrum yield (Cabral et al., 2016), however absolute maximum daily temperatures were not associated with low colostrum yield in the current study. Heat stress in cattle causes numerous physiological and behavioural disturbances (Herbut et al., 2019) resulting in decreased dry matter intake and reduced efficiency of milk yield (West et al., 2003). Other studies have demonstrated that while colostrum composition is affected by heat stress in animals, colostrum yield is not (Nardone et al., 1997, Karimi et al., 2015). A high temperature range may have resulted in cows affording valuable metabolic resources to thermoregulation which may have affected colostrum production.

North American research has associated colostrum deficiency with season, with some dairy herds reporting poor colostrum yields in the autumn and winter months (Gavin et al., 2018). Specifically, Gavin et al., (2018) observed a decline in production of 0.17 kg/cow per week between June and December, with a more pronounced effect of decreased colostrum yield in multiparous rather than primiparous cows. However, neither month calved, nor season had a significant effect on colostrum yield in the current work.

Time series analysis revealed moderate correlations between temperature measures (time lags of 1 to 3 months) and colostrum yield, but this was not consistent in primiparous and multiparous animals. Despite the animals on the study farm being housed year-round, there were positive associations observed between increasing UV index and colostrum production both on the day of calving (in the logistic models) and in the time series (lag) analysis (particularly in primiparous animals). To the authors' knowledge, the effect of UV exposure on colostrum yield in dairy cows has not previously been studied, however photoperiod has a known effect on milk production through associations with the hormones melatonin and prolactin, both of which are involved in colostrogenesis and lactogenesis (Auchtung et al., 2015). Dry cows exposed to a short-day photoperiod were observed to produce approximately 3.1 kg/day more milk during the next lactation than dry cows exposed to a long-day photoperiod; while lactating cows exposed to a longer day photoperiod had higher prolactin concentrations and produced more milk (Dahl and Petitclerc, 2003). Photoperiod has not been shown to affect IgG concentrations in the colostrum of Holstein cows (Morin et al., 2010), nor has the effect of photoperiod been consistently associated with colostrum yield despite its association with colostrogenic hormones (Auchtung et al., 2005).

Since this was an observational study on a commercial dairy farm, with farmer recorded colostrum yields which were only measured to the nearest half L, there might have been some degree of dilution bias, with the result that effects of potential risk factors might be underestimated. Colostrum volume (litres) rather than colostrum weight (kg) was measured on farm as this is the measure of colostrum yield with which producers are most familiar, and industry recommendations specify the volume of colostrum to be fed to newborn calves rather than the weight. In addition, the density of colostrum is approximately 1.048 $g.mL^{-1}$ (McGrath et al., 2016) such that 1 litre of colostrum approximates 1kg of colostrum.

Conclusions

The current work showed that the risk factors of UV index, dry period length and temperature range were associated with colostrum yield. This study adds to a body of evidence which investigates the risk factors for poor colostrum yield. However, more work is necessary to definitively identify risk factors and to develop strategies for farmers to mitigate the risk to neonatal calves of low colostrum production, which could have serious implications for dairy farming enterprise.

Conflict of interest statement

This data was collected in the course of normal clinical work and we did not receive funding for this project. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper. Data was analysed under University of Glasgow ethics licence EA49/21.

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Table 1. Dated diet specifications for close up ration (fed for approximately 3 weeks prior to calving) for 439 dairy cows on a Scottish farm enrolled between November 2020 and October 2021

	Date										
	2020			2021							
	09/10	26/11	01/12	08/01	21/01	23/03	16/04	25/06	05/07	06/09	19/10
DMI (kg/cow)	8.3	9.9	12.0	11.2	12.1	12.7	12.8	12.9	12.9	13.2	12.9
DMI forage	6.9	8.7	10.2	9.5	10.4	10.8	10.4	10.5	10.3	10.7	8.7
Starch (%)	37	21	40	43	15	16	39	40	40	40	40
Crude protein (%)	14	13.7	14	16	13.0	11.4	13.6	13.3	14.9	14.1	16.6
Energy (MJ/cow/d)	74	90.6	102	103	114	114	118	111	114	125	112
DCAD (mEq/kg DM)	-287	-223	-161	-8	-145	-113	-100	-152	-83	30	Ca- binder

Footnote: Ca- binder (Zeolite) was introduced to lower urine pH, DCAD =dietary cation anion difference.

Table 2: Descriptive stats and univariable analyses for continuous predictor variables using colostrum yield as a continuous (square root of colostrum yield) and colostrum yield as a categorical (≥4 or <4 litres) outcome

Variable	n	mean	SD	minimum	maximum	95%CI	p-value (continuous)	p-value (categorical)
Colostrum yield (L)	439	4.59	2.94	0	15.00	4.29-4.83	outcome	outcome
Month dried off	318	5.95	3.55	1.00	12.00	5.56-6.34	0.11	0.97
Month calved	439	6.07	3.38	1.00	12.00	5.76-6.39	0.73	0.34
PTA milk (kg)	439	355.70	9.32	-96.00	831.00	337.39-374.01	0.01	0.03
PTA milk sire (kg)	439	467.21	331.71	-179.00	996.00	436.10-498.33	0.08	0.03
Days dry before calving	318	62.03	35.94	26.00	255.00	58.06-65.99	<0.01	<0.01
Previous lactation length (days)	318	353.85	57.80	250.00	510.00	347.47-360.23	<0.01	<0.01
Somatic cell count (mean/ml)	426	175.82	447.09	6.00	5083.00	133.24-218.40	0.65	0.34
Temperature (mean daily ◦C)	439	9.09	5.58	-10.60	19.50	8.57-9.62	0.54	0.28
Temperature (maximum daily ◦C)	439	13.28	6.17	-3.00	27.30	12.71-13.86	0.89	0.56
Temperature (minimum daily ◦C)	439	4.90	5.72	-18.2	15.90	4.37-5.44	0.18	0.14
Daily temperature range (◦C)	439	8.38	4.12	0.90	19.30	7.99-8.77	0.04	0.20
UV index (units)	439	1.09	1.00	<0.01	4.01	0.99-1.18	0.33	0.07
Rainfall (mm)	439	2.33	4.41	0	31.4	1.92-2.74	0.47	0.65
Stocking density (%)	238	105.40	24.68	40.74	162.96	101.64-107.36	0.41	0.19

Footnote: PTA= predicted transmitting ability genetic parameter

Month dried off and month calved measurements are 1-12 where 1=January and 12=December

Table 3 Descriptive statistics from univariable analyses for categorical predictor variables using colostrum yield as a continuous (square root of colostrum yield) and categorical (≥ or <4 litres) outcome

Variable	Category	N	Proportion (%)	p-value (continuous)	p-value (categorical)
Calf gender	female	169	169/439 (38.49)	ref	ref
	male	270	270/439 (61.50)	<0.01	<0.01
Calving ease	no assistance	403	403/439 (91.80)	ref	ref
	assistance	36	36/439(8.20)	0.08	0.10
Clinical mastitis	no	374	374/439 (85.19)	ref	ref
	yes	65	65/439 (14.81)	0.38	0.47
Number of days dry	<60 days	219	219/318 (68.87)	ref	ref
	≥60 days	99	99/318 (31.13)	<0.01	<0.01
Lactation number	1	121	121/439 (27.56)	ref	ref
	2	96	96/439 (21.87)	0.1	0.05
	3	84	84/439 (19.13)	<0.01	<0.01
	4	72	72/439(16.40)	<0.01	<0.01
	5+	66	66/439(15.03)	<0.01	<0.01

Table 4: Final multivariable linear regression model showing risk factors associated with colostrum yield from 318 cows on a Scottish dairy farm using the continuous outcome of square root of colostrum yield

Variable	Category	Coefficient	SE	95%CI	p-value
Daily temperature range	-	-0.02	0.01	-0.04- -0.002	<0.01
Number of days dry	-	0.004	0.001	0.002-0.006	<0.01
Lactation category	2	ref	ref	ref	ref
	3	0.23	0.11	0.01-0.44	0.04
	4	0.15	0.11	-0.08-0.37	0.19
	5+	0.21	0.12	-0.03-0.45	0.08

Table 5: Final multivariable logistic regression model showing risk factors associated with colostrum yield from 439 cows on a Scottish dairy farm using colostrum yield categorised into greater than or equal to 4 litres or less than 4 litres as the outcome of interest. An OR >1 indicates a higher odds of achieving ≥4 litres colostrum yield.

Variable	Category	OR	SE	95%CI	p-value
Daily temperature range		0.89	0.03	0.83- -0.95	<0.01
Number of days dry	<60 days	ref			
	60 days or more	2.36	0.67	1.35-4.11	<0.01
UV (units)		1.61	0.25	1.19-2.18	<0.01

Table 6. Time-series cross-correlation coefficients for colostrum yield with temperature variables and uv index from a cross-correlation function analysis for the time series from November 2020 to October 2021. Each time lag represents one month.

Independent variable	Colostrum production			
	Lag	Primiparous	Multiparous	Overall
UV (units)	-1	0.47	0.05	0.33
	-2	0.55	-0.07	0.16
	-3	0.40	-0.33	-0.17
Mean temperature (◦C)	-1	0.02	0.30	0.38
	-2	0.42	0.15	0.37
	-3	0.39	-0.18	0.02
Maximum temperature (◦C)	-1	0.06	0.21	0.34
	-2	0.41	0.14	0.36
	-3	0.44	-0.14	0.07
Temperature difference (◦C)	-1	0.24	-0.25	0.03
	-2	0.22	0.05	0.16
	-3	0.50	0.09	0.26

Figure captions

Fig 1: Directed acrylic graph showing causal pathways, conditional independence and confounding variables for colostrum yield.

Fig 2. Frequency distribution of first milking colostrum yield (L) from 439 (318 multiparous and 12 primiparous) Holstein Friesian dairy cows from a Scottish dairy farm recorded between October 2020 and November 2021.

Fig 3. Box plot showing first milking colostrum yield (L) by lactation yield for 439 439 (318 multiparous and 12 primiparous) Holstein Friesian dairy cows from a Scottish dairy farm recorded between October 2020 and November 2021.

Fig 4. Mean first milking colostrum yield (L) by calendar month for 121 primiparous and 318 multiparous Holstein Friesian animals from a Scottish dairy farm recorded between October 2020 and November 2021.