Assessment of biomarkers for precision dairy calf rearing

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Executive summary

- During the transition period, most dairy cows enter a state of negative energy and protein balance due to the increased energy and protein demands of the end of pregnancy and beginning of lactation.
- Growth and health of dairy calves is essential for efficient heifer rearing and dairy beef production. Calves in the early rearing period are affected by maternal nutrition from late pregnancy effects and colostrum production.
- However, there is little known about predictors of poor calf development. Therefore, the present study aimed to establish predictors in the dam to enable early identification of calves with a higher likelihood of disease or reduced growth development.
- A total of 76 dairy cows were followed from 1 week prepartum to 3 weeks postpartum and measures such as body condition score (BCS), rumen fill score and serum metabolites were recorded at 1 week prepartum, 1 and 3 weeks postpartum. The calves of all 76 cows were then followed from 48h to 5 weeks of age and measures such as weight (to calculate average daily gain (ADG)), health indices and blood immune indicators were assessed.
- This report focuses on changes in dam BCS and measurements taken at 1 week prepartum as indicators for calf health and growth.
- There was limited effect of the dam measurements at 1 week prepartum on calf health from 48h to 5 weeks of age, indicated through serum IgG and calf health scores.
- However, a BCS loss of between -1 and -0.3 units over three weeks in dam BCS led to calves with a significantly slower ADG compared to those with a >-0.3 or no change. Furthermore, there was a tendency for calves from dams with a rumen fill score of 3 or less to have a slower ADG compared to those from dams with a RFS of 5. This data was supported by the same calves also having significantly fewer lymphocytes at 48h of age, suggesting a level of immunocompromisation. This which warrants further investigation.

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Introduction

The metabolic stress of dairy cows increases during the transition period. As such, most dairy cows enter a state of negative energy balance (NEB) and negative protein balance (NPB). In some cases, where nutrition is not adequately managed in the final stages of pregnancy, NEB and NPB will occur in the final two weeks prepartum where the energy and protein demands of pregnancy and the beginning of colostrum and milk production outweigh the intake. Indeed, most high yielding dairy cows will experience a loss of body condition during the first 80 days in milk due to a prioritisation of resources to the mammary gland to meet the requirements of milk production (de Vries et al., 1999). A substantial deficit during this period is known to lead to an increased risk of ketosis, a reduction in fertility, dry matter intake, milk yield and poorer health status. Notably, NEB pre- and postpartum results in a suppression of the immune response in dairy cows leading to production related disorders such as retained foetal membrane, mastitis and metritis (Goff, 2006).

Calves are dependent on their dam during pregnancy for energy provision and during the immediate post-natal period for passive immunity through colostrum consumption. Yet if the dam has an impaired metabolic or immune function then provision for the calf is likely to be impaired. Colostrum is the early milk produced by the dam within the first 3 days post-partum. Colostrum contains high concentrations of growth factors, cytokines and immunoglobulin G (IgG) (McGrath et al., 2016). Intake of IgG and the allowance of its transfer through the gut wall are essential for the newborn calf for passive immunity, which the calf is reliant on within the first 6 weeks of life. However, both IgG concentration in the colostrum and gut permeability reduce over the first 24 hours after birth. As such, calf serum IgG levels peak in the first 24 hours and subsequently gradually decline to minimum levels at around 4 weeks of age (Logan et al., 1973). Following the reduction in serum IgG derived from passive immunity, IgG serum levels increase again as calves begin to rely on the development of their own adaptive immune system. Thus, it is essential that calves receive an adequate supply, both in volume and quality of colostrum. Good quality colostrum is defined as having at least 50g/L of IgG and having a low bacterial load (Gibbons, 2014). However, colostrum quality is variable between animals due to breed and parity differences, but also pre-partum nutrition. Indeed, cows that experience weight loss during the dry period are four times more likely to produce colostrum with IgG concentrations of less than 50 g/L (Mulder et al., 2018). Furthermore, the effect of an inadequate nutritional supply to the dam in late pregnancy does not just affect the quality of colostrum and passive immunity to the calf. Neonatal calf immune and antioxidant capacity, as well as growth parameters, are impaired from dams deprived of nutritional energy (Gao et al., 2012). Thus, it is clear that the nutritional management of the dam is imperative for early development of the calf. Yet there is limited

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information as to the persistence of this effect on the calf, although calf development within the rearing period has implications on the production potential of the animal.

Calf growth rate is important to ensure calving at two years of age. Ensuring heifers obtain a first calving as close to two years of age is optimal to reduce costs in the period of non-production (Boulton et al., 2015), whilst also preventing problems associated with calving immature animals. Furthermore, calving at two years of age has been demonstrated to increase total days in milk, milk yield and the percentage of life spent in milk over 5 years, as well as the likelihood of calving for a third time (Cooke et al., 2013). To promote calving at two years, heifers must reach an appropriate weight for first service, yet calves with a low average daily gain up to 180 days are less likely to reach an appropriate target weight (Bazeley et al., 2016). Thus, early calf development is essential in ensuring an optimal rearing period and subsequent lifetime performance. Therefore, it would be useful to be able to determine calves early on that are likely to need further management input. Early intervention will help them to achieve an optimal rearing period and as such improve the efficiency of heifer replacement rearing in dairy farming. Furthermore, calves not destined for the milking herd are normally sold to rearers for meat production, thus if a greater weight and a healthier calf can be achieved in the early rearing period before calves are sold, this is of benefit to future rearers.

The project aims to determine predictors of poor calf development to enable early identification of calves with a higher likelihood of disease or reduced growth development. Such calves would require further management input and so the aim of the project was to allow an improvement in the precision of calf management. Metabolic and immune biomarkers as well as general health characteristics were assessed as predictors, and were assessed in both the dam (1 week prepartum and up to three weeks postpartum) and the calf (up to 8 weeks of age).

Methods

Animals

The study took place at Trawsgoed dairy farm, Aberystwyth University between 20th March and 16th June, 2019. Primiparous and multiparous dairy cows (n=76) were recruited to a prospective study one week before calving. Cows within the trial consisted of Holstein Friesians (n=67), Ayrshire crosses (n=5) and Jersey crosses (n=4), with a mean parity of 2.19 ± 1.32. Cows remained a part of the dry cow or milking herd and followed the same management practices during the study. Briefly, cows were dried off at 60 days before calving, received dry cow antibiotics and teat sealants and were fed on a diet for dry cows up to calving. Following calving, cows were milked twice daily.

Following calving, all calves were recruited to the study and reared under standard farm practices. After calving, calves received 4L of colostrum within 12 hours of birth following iodine application to the navel. Once dry, a calf coat was placed on all calves. Calves were removed from their dam between 12 and 24h after calving and subsequently housed in straw pens with other calves of a similar age in groups of approximately 4. All calves were fed a total of 6L of combination of milk and milk replacer, provided over two meals up to 42 days. From 42 days onwards, calves were gradually weaned down to 2L per day of milk and milk replacer at weaning (75-80 days). A calf concentrate mix and chopped straw feed was provided to all calves from 5 days.

Cow data collection

At 1 week before the expected calving date and 1 and 3 weeks post calving, cows were weighed, body condition scored (on a 5 point scale; Edmonson *et al*., 1989) and the rumen fill scored noted (on a 5 point scale; Burfeind *et al*., 2010). Change in BCS was calculated between 1 week prepartum and 3 weeks postpartum. Behaviour data, (comprising of ruminating, feeding and resting), was collected for 5 days at each time point (2 days either side of the data collection date) from MooMonitor collars (Dairymaster, Tralee, Ireland). Daily milk yield was recorded up to 21 days in milk. Blood was collected from the tail vein at 1 week before calving and 1 and 3 weeks post calving. For blood cell analysis 5 mL of blood was collected into anticoagulant tubes (K3-ethylenediaminetetraacetic acid), inverted 10 times and stored at $4^{o}C$ before analysis. For metabolite, cytokine and IgG analysis 10 mL of blood was collected into tubes with no anticoagulant, centrifuged at 2000 x g for 15 minutes and the serum collected and stored at $-20^{o}C$ before analysis.

Calf data collection

At 48h after calving, all calves were weighed, the rectal temperature taken and health indicators determined according to the University of Wisconsin calf health scores chart (faecal scores omitted; https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf). Measurements were repeated at 2 and 4 weeks of age. Calf average daily gain (ADG) was calculated from 48h to 4 weeks of age. Blood was collected from the jugular vein at 48h and 5 weeks of age, in the same manner as described for cow blood samples.

Blood analysis

Blood was analysed via a haematology analyser (Mythic 18 Vet Haematology Analyser; Woodley Equipment Company Ltd, Horwich, UK) within 6 hours of collection to establish immune cell content. The level of white blood cells was assessed in the blood samples from dams and calves and the percentage of the morphotypes (lymphocytes, monocytes and granulocytes).

Serum metabolites (NEFA and BHB) in dams were analysed by RANDOX Daytona^{+} (Randox Laboratories Ltd, Crumlin, UK). For serum IgG analysis in calves, samples were diluted 1:400,000 and analysed by a bovine IgG ELISA kit, as described by the manufacturer (GenWay Biotech Inc, San Diego, USA).

Statistical analysis

All statistical analysis was completed on GenStat 19th Edition (VSNI, Hemel Hempstead, UK). Significance was declared when the probability of no difference between means fell below 0.05. Dams were grouped based on each tested parameter (see results section for groupings) and the effect on calf ADG, total health scores, immune cells and IgG assessed via an unbalanced design ANOVA with Bonferoni post-hoc test. When assessing data that included the prepartum dam parameters, four dams and their calves were removed from the analysis due to the prepartum data being missing.

Results / Discussion

Due to the number of results generated in this study, key results only will be discussed here. These were the effect of change in dam BCS on calf health and growth, and the effect of pre-calving indicators in the dam on calf health and growth.

Minimal loss in Dam BCS led to greater average daily weight gain in calves but had no impact on calf health.

Dams were grouped based on their change in BCS between a week prepartum and 3 weeks postpartum. No dams had excessive BCS gain. Calves from dams who had a mild loss of BCS (less than 0.3 BCS) or no change were found to have a greater ADG up to 4 weeks of age compared to the calves from dams with moderate BCS change (

Table 1; P<0.05). Cows with mild loss in BCS had lower milk yields between 7 and 21 DIM than those with greater BCS loss, and this may be associated with the reduced drain on the energy and protein resources of the animal for milk (

Table 1; P<0.001). Postpartum dairy cows mobilise fat reserves to support high milk yields and a dramatic increase in energy demands at the udder (Ruda et al., 2019). Previous research has demonstrated that dams deprived of nutritional energy in the prepartum period will produce calves that have a lower birthweight (Gao et al., 2012). However, this is the first study to demonstrate that this can persist throughout the early rearing period. Although these data do not determine long term effects on heifer performance, a low ADG in the early rearing period identifies calves with poorer performance and persistence in the herd as lactating cows (Handcock et al., 2020). However, it was demonstrated in the present study that there was no effect of a change in BCS on the health of the calves, as assessed through the Wisconsin calf health scores at 48h, 2 and 4 weeks after birth (

Table 2; P>0.05). Furthermore, this was supported by there being no difference between calf white blood cells and immune parameters such as IgG (data not displayed).

Table 1: The effect of change in dam BCS between 1 week prepartum and 21 days postpartum on mean calf ADG (kg) between 48h and 4 weeks of age and on dam mean milk yield (kg) between 7 and 21 days in milk. Dams (n=72) were split into three groups, severe BCS loss (≤-1; n=22) moderate BCS loss (>-1 and ≤-0.3; n=28) and mild to no BCS loss (>-0.3; n=22). Means within a row that do not share a superscript letter indicates a significant difference (Calf ADG P<0.05; Dam milk yield P<0.001).

	Dam BCS change				
	Severe	Moderate	Mild to none	SED	P value
Calf ADG (kg/d)	0.59[ab]	0.47[a]	0.63[b]	0.058	0.014
Milk yield (kg/d)	33.17[a]	34.93[a]	23.6[b]	1.755	<0.001

Table 2: The change in dam BCS between 1 week prepartum and 21 days postpartum has no effect on mean Wisconsin calf health scores, (total value based on temperature, cough, nasal and eye scores) at 48h, 2 and 4 weeks of age. Dams (n=72) were split into three groups, severe BCS loss (≤-1; n=22) moderate BCS loss (>-1 and ≤-0.3; n=28) and mild to no BCS loss (>-0.3; n=22).

	Dam BCS change				
	Severe	Moderate	Mild to none	SED	P value
48 h	1.91	2	1.82	0.235	0.732
2 weeks	3.55	2.71	2.73	0.453	0.125
4 weeks	2.75	2.41	2.42	0.446	0.692

Dam RFS at 7 days prepartum tends to be associated with a greater average daily weight gain in calves and has some effect on calf health

Dams were grouped based on their RFS (5-point scale) at one week prepartum. Calves from dams that had a RFS of 5, tended to have a greater ADG between 48h and 4 weeks of age compared to calves from dams with a RFS of 3 or less (

Table 3; P=0.072). Suggesting that those dams that were eating more prepartum had a greater likelihood of producing a calf with improved growth rates. To assess if the assessment of RFS was linked to the dams that experienced a change in BCS, the effect of RFS on milk yield and change in BCS between one week prepartum and 21 days postpartum was assessed. It was demonstrated that there was no effect of RFS on either dam average milk yield (7-21 DIM) or change in BCS (

Table 3), therefore the calves identified in the RFS analysis were likely independent to those identified in

Table 1. However, on assessment of calf health parameters, it was found that calves from dams with a RFS of 5, tended to have a greater total calf health score at 2 week of age (

Table 4; p=0.058) and greater blood lymphocyte levels 48h after birth (

Table 4; p=0.038). However, there was no effect of RFS on calf health scores at 48h or 4 weeks of age (

Table 4), the overall WBC level or % of the mononuclear cells or granulocytes at 48h or 5weeks of age (data not displayed).

Table 3: The effect of dam RFS at 1 week prepartum on mean calf ADG (kg) between 48h and 4 weeks of age; dam mean milk yield (kg) between 7 and 21 days in milk and dam change in BCS between 7 days prepartum and 21days postpartum. Dams (n=71) were split into three groups, a RFS of 3 or below

	Dam RFS			SED	P value
	≤3	4	5		
Calf health scores					
48 h	1.8	1.82	2.13	0.245	0.302
2 weeks	2.47	2.79	3.61[ŧ]	0.472	0.058
4 weeks	2.93	2.2	2.7	0.457	0.231
Calf lymphocyte (% of WBC)					
48h	3.98[a]	5.1[ab]	6.14[b]	0.869	0.038
5weeks	5.86	6.39	6.3	0.522	0.596

(n=15); 4 (n=33) or 5 (n=23). Means within a row that do not share a superscript letter indicates a statistical trend (p<0.1).

	Dam RFS ≤3	4	5	SED	P value
Calf ADG (kg/d)	0.46[a]	0.54[ab]	0.61[b]	0.062	0.072
Milk yield (kg/d)	31.42	31.29	30.12	2.43	0.834
Dam BCS change 7D prepartum to 21D postpartum	-0.73	-0.56	-0.66	0.157	0.567

Table 4: The effect of dam RFS at 1 week prepartum on mean Wisconsin calf health scores (total value based on temperature, cough, nasal and eye scores) at 48h, 2 and 4 weeks of age and calf lymphocyte level (% of WBC) at 48h and 5 weeks of age. Dams (n=71) were split into three groups, a RFS of 3 or below (n=15); 4 (n=33) or 5 (n=23). Means within a row that do not share a superscript letter indicates a significant difference (p<0.05). ǂ indicates a statistical trend (p<0.1).

	Dam RFS ≤3	4	5	SED	P value
Calf health scores					
48 h	1.8	1.82	2.13	0.245	0.302
2 weeks	2.47	2.79	3.61ǂ	0.472	0.058
4 weeks	2.93	2.2	2.7	0.457	0.231
Calf lymphocyte (% of WBC)					
48h	3.98[a]	5.1[ab]	6.14[b]	0.869	0.038
5weeks	5.86	6.39	6.3	0.522	0.596

Dam health status and blood WBC levels do not have an effect on calf growth or health parameters.

Health status of dams was tested for the effect on calf ADG between 48h and 4weeks of age; calf health status at 48h, 2 and 4 weeks of age and calf immune blood parameters at 48h and 5 weeks of age (data not show). Dams that were noted as unhealthy (n=26) due to a diagnosis of Johne's disease or a production related disease (e.g. mastitis, endometritis) up to 21 days postpartum, did not produce calves with reduced growth or suboptimal health or immune parameters, compared to healthy cows (n=50). It was thought that unhealthy cows would have a greater reduction in BCS between a week prepartum and 21 days postpartum as well as a reduced RFS to indicate feed intake, and as such an effect on calf growth. However, there was only an effect of dam health status at three weeks postpartum, with a reduction in RFS ($p=0.023$) and rumination activity ($p=0.021$) and an increase in time spent resting ($p=0.023$) in unhealthy animals compared to healthy animals (data not shown). As such, dam health is not a good indicator for calf growth or health, but RFS, rumination and resting activity data at three weeks postpartum, may be used as an indicator for dams that are unhealthy.

Furthermore, dams were grouped based on their WBC level one-week prepartum (low: $<6 \times 10^3/\mu l$; medium: ≥ 6 and $<8 \times 10^3/\mu l$ or high $\geq 8 \times 10^3/\mu l$) and the effect of WBC level assessed on calf parameters. As expected, based on the health status data, dam WBC level at one week prepartum did not have an effect on calf ADG, health status or immune parameters in the blood (data not show).

Greater dam NEFA and BHB concentration at 7 days prepartum tends to be associated with a greater average daily weight gain in calves but only BHB has an effect on calf white blood cell levels

Dams were grouped based on serum NEFA concentration (mMol) at one-week prepartum. There was a statistical tendency that calves from dams that had greater serum NEFA concentration (≥ 0.8 mMol) at one week prepartum, had a greater ADG between 48h and 4 weeks of age ($p<0.1$), although there was no effect on calf health scores or blood immune parameters (Table 5).

Such a result is surprising as serum NEFA concentrations reflect the mobilisation of fat reserves and therefore, greater concentrations are associated with dams that are in more severe negative energy balance (Ospina et al., 2013). Thus, based on the previous results in this study, which demonstrated that calves from dams with severe BCS change (one week prepartum to three weeks postpartum) had a reduced ADG, it was expected that a greater NEFA concentration would also lead to a reduced ADG. Therefore, 48h calf body weight was assessed to understand whether calves in the higher NEFA concentration group had lower birthweights. However, it was determined that birthweights of calves from dams with a NEFA concentration of ≥0.8 mMol, were no different to those of the calves from dams with low serum NEFA concentrations (<0.4 mMol). It must be noted that the data only demonstrates a trend and that the number of dams in the higher serum NEFA concentration group is fairly low (n=14) compared to the other groups (n=21 and n=36).

Table 5: The effect of dam serum NEFA (mMol) at 1 week prepartum on mean calf ADG (kg) between 48h and 4 weeks of age and calf health scores at 48h, 2 and 4 weeks of age. Dams (n=71) were split into three groups, NEFA mMol of <0.4 (n=21); ≥0.4 - <0.8 (n=36) or ≥0.8 (n=14). Means within a row that do not share a superscript letter indicates a statistical trend (p<0.1).

	Dam serum NEFA (mMol)				
	<0.4	≥0.4 and <0.8	≥0.8	SED	P value
Calf ADG (kg/d)	0.48^{a}	0.54^{ab}	0.64^{b}	0.065	0.082
Calf health scores					
48 h	2	1.92	1.85	0.258	0.860
2 weeks	3.29	2.86	2.69	0.498	0.495
4 weeks	2.76	2.46	2	0.475	0.362

Dams were grouped based on serum BHB concentration (mMol) at one-week prepartum. Similarly to serum NEFA concentrations, calves from dams that had greater serum BHB concentration (≥500 µMol) at one week prepartum, had a greater ADG between 48h and 4 weeks of age (

Table *6*; p<0.05), compared to those from dams with a lower concentration (≥300 - <400 µMol). B-hydroxybutyrate (BHB) is a ketone body and produced as part of lipid metabolism thus, as with NEFA, greater concentrations are found in serum when cows are in NEB and mobilising fat reserves. As for serum NEFA, the effect of serum BHB prepartum was assessed on calf body weight at 48h to ensure that the increased ADG was not due to a reduced body weight, however, there was no effect. However, calves from dams with serum BHB concentrations of ≥400 - <500 µMol had a reduced % of granulocytes compared to those from dams with lower concentration of BHB (<300 µMol) (

Table *6*; P<0.05), which was also supported by a tendency for less WBC (

Table *6*; P=0.054). However, these results did not subsequently result in an effect on a reduction in calf health scores (

Table *6*), suggesting that although interesting from an immunological perspective, the overall health of the calves was not impacted.

Table 6: The effect of dam serum BHB (µMol) at 1 week prepartum on mean calf ADG (kg) between 48h and 4 weeks of age; calf health scores at 48h, 2 and 4 weeks of age and blood immune cells at 48h of age. Dams (n=71) were split into three groups, BHB µMol of <300 (n=13); ≥300 - <400 (n=23);

400 - <500 (n=25) or ≥500 (n=10). Means within a row that do not share a superscript letter indicates a significant difference (P<0.05).

	Dam serum BHB (µMol)				SED	P value
	<300	≥300 - <400	≥400 - <500	≥500		
Calf ADG (kg/d)	0.48[a]	0.49[ab]	0.57[abc]	0.69[ac]	0.071	0.036
Calf health scores						
48 h	2.15	2.04	1.88	1.5	0.283	0.215
2 weeks	2.92	3.26	2.84	2.9	0.581	0.823
4 weeks	2.25	2.77	2.61	2.25	0.577	0.729
Calf immune cells (48h after calving)						
WBC (x 10^3/µl)	12.52	9.86	9	10.38	1.316	0.054
Granulocytes (% of WBC)	4.17[a]	2.78[ab]	2.53[b]	3.06[ab]	0.549	0.018
Lymphocytes (% of WBC)	6.2	5.09	4.34	4.6	1.009	0.366
Monocytes (% of WBC)	2.15	1.87	1.83	2.2	0.26	0.391

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Summary

The present study demonstrates that there are easy to use on farm measures which can be used to collect information from dams on the potential performance of calves, although the most useful for early detection is potentially the use of rumen fill score. Overall, limited effects were demonstrated of the measures assessed on calf health. Importantly, no effect was established on IgG serum concentration in calves at 48h or 5 weeks of age, which implies that there was no effect of nutritional status of the dam in this data set on colostrum IgG uptake in calves. As expected, measures which are linked to dam nutrition are important for predicting the ADG of calves in the early rearing period. Specifically, a moderate change in body condition score (loss of >-1 but ≤-0.3 on a 5 point scale) of dams between 1 week prepartum and 21 days postpartum, resulted in calves with a slower ADG up to 4 weeks of age compared to those with mild to no BCS change. However, the use of this measure requires farmers to wait until 3 weeks postpartum to be able to define calves that are likely to require further support in their development. Therefore, although the present data only demonstrated a statistical tendency, dam rumen fill score at 1 week prepartum may be a better measure for farmers to use as an early identification tool. Calves from dams with a RFS or 3 or below (on a 5 point scale) at one week pre-partum, tended to be more likely to have a slower ADG than those from dams with a RFS of 5. It must be noted that there were no dams with a RFS of 1 and so if the data set included more severe RFS and a greater number of animals, there may have been a greater effect seen. Furthermore, although there were no overall effects of dam RFS on calf health, there was a reduction in lymphocytes as a % of total white blood cells, in calves from dams with a RFS of 3 or less. This suggests that there was a level of immunocompromisation in these animals and further research into the extent of the effect on these animals is warranted.

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