



The Effects of Rib Biopsy on Performance Indices of Dairy Cattle



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Abstract

In dairy cattle, rib bone biopsy is used for the analysis of bone tissue. However, it is unclear how the serial rib biopsy collection impacts the lactation performance of dairy cows. The objective of this study was to examine the effect of serial rib biopsy collection on the performance indices of cows during lactation. Sixty-two multiparous Holstein cows were enrolled in the study, 29 of which were assigned randomly to the control group and 33 to biopsy group. Outer cortex rib biopsy samples were collected from the 11th and 12th ribs of both left and right sides of each cow. Rib biopsy was performed at early lactation, mid lactation, late lactation, and late pregnancy stages in biopsy group cows. Daily milk yield, concentrated feed intake and body weight data were collected by automated dairy management systems from both biopsy and control group cows. The results showed that variability in milk yield, concentrate feed intake and body weight was predominantly driven by between-cow variability. The within-cow variability in milk yield and feed was mostly related to lactation stage.

Body weight was not affected by the biopsy procedure or lactation stage. The analgesia protocol used was adequate in the prevention of post-operative pain and the monitoring regime adopted was successful in the early identification of post-operative complications. Overall, the serial rib biopsy procedure had no serious impact on the health of cows and samples collected were suitable for micro-computed tomography and histopathology evaluations.

Keywords: Rib Biopsy; Milk Yield; Bone Turnover; Automated Voluntary Milking

Abbreviations: CL: Confidence Limit; UCL: Upper Confidence Limit; LCL: Lower Confidence Limit; CI: Confidence Interval; E: Endosteal; P: Periosteal; SSI, surgical site infections.

Introduction

Biopsy of bone is commonly used by scientists or clinicians for the purpose of qualitative and quantitative evaluation of bone structure and remodeling, as well as for the diagnosis of bone disease [1-3]. In dairy cattle (*Bos taurus*), a rib bone biopsy technique has been described as the method of choice for the analysis of bone tissue [4-6]. The technique is relatively simple, relying on a bone trephine to cut a core of tissue from bone and has the advantage of allowing serial sampling from the same animal over time [4,6,7]. During the procedure, the surgeon can opt to obtain a sample from the outer external cortex alone if only cortical sampling is desired or perform a full thickness biopsy of the rib to include both external and internal cortices together with the intervening medullary trabecular bone.[5] Whilst the latter

procedure is inherently riskier than the external cortical biopsy, due to the close association of internal cortex and parietal pleura and thus the possibility of iatrogenic pneumothorax, no serious complications have been previously reported [8].

As a part of a prospective self-controlled study to investigate the changes in cortical bone with lactation in dairy cattle, we have adopted a unicortical approach to take samples from the 11th and 12th ribs of both left and right sides of each cow over a course of approximately one year. We adopted a very strict pre-emptive analgesia protocol and followed a validated pictorial guide in the monitoring of postoperative cows [9]. This was to ensure any residual discomfort was identified early on to allow for the administration of rescue analgesics in a bid to prevent the occurrence of breakthrough pain.

Given the stoic nature of many animals and particularly cattle in the face of acute and chronic pain, a question that needs to be answered relates to the effectiveness of the clinical monitoring measures often used in identifying less intense postoperative pain or otherwise where subclinical pain is present. This is a question of paramount importance for the purpose of improving the welfare outcome not only of any future experimental animals, but also for cattle that will be required to undergo a diagnostic or corrective surgical treatment sometime during their productive lives. The utilization of industry-standard robotic machinery as a means of monitoring production and animal health is gaining momentum in the dairy cattle industry. The wealth of such collected information creates an opportunity to use data science in ways that have not been possible before.

Whilst some work has started to emerge to utilize this data to improve farm performance and detect pain, more work is required on the latter given its welfare implication [10-12]. Whilst our primary goal was to study bone turnover over time in dairy cattle, we chose to use the opportunity to investigate the usefulness of automatically collected data in critically evaluating the impact of a surgical procedure on production and animal comfort. We report here the result of a modern statistical modelling method applied to data on concentrate intake, body weight and lactation performance of dairy cows that have undergone multiple rib biopsy procedures over the course of approximately one year.

Materials and methods

Animals

Cattle used for the purpose of this study were held at the dairy facility of the Dookie campus of the University of Melbourne. This facility is equipped with the Lely Astronaut robotic milking system (Lely, Maassluis, Netherlands) that allows automated voluntary milking, and automates other management processes including grazing and concentrate feeding. The Lely software database maintains a detailed record of health and management data for individual cows. All animal procedures used in this study were approved by the Animal Ethics Committee of the Faculty of Veterinary and Agricultural Sciences, the University of Melbourne (AEC 1814625; Project ID No. 10205) and were conducted in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes (2013).

Sixty-two multiparous Holstein cows were enrolled in the trial from November 2019 to June 2021. First biopsy cows were randomly recruited based on their availability at early lactation and control cows were later recruited randomly to match the same criteria of biopsy cows. Thirty-three were assigned to the bone biopsy group and twenty-nine to the control group. The biopsy and control cows had similar parity (median = 2, range = 2-5) and days in milk (median = 60, range = 34-70) at enrolment. Rib biopsy collection was performed at four different timepoints starting from early lactation (4-8 weeks postpartum), mid lactation (18-22

weeks postpartum), late lactation (35-39 weeks postpartum) and late pregnancy stage (dry period) in biopsy group cows. The first and third biopsies were performed on the left and right 11th ribs, respectively and the second and fourth biopsies on the left and right 12th ribs respectively. Therefore, each rib was biopsied only once. No biopsy collection procedure was performed on control cows.

Biopsy Procedure

Rib bone biopsy samples were collected following a method previously described by Little [4]. Pre-emptive analgesia was provided by use of meloxicam (Metacam 20 mg/100ml, Boehringer Ingelheim, Australia) administered subcutaneously at a 25 mg/kg dose at least 20 minutes prior to skin incisions. The cows were restrained in a crush for the surgical procedure. Surgical site preparation was carried out by clipping the hair over a wide area surrounding the biopsy sites followed by routine aseptic cleaning of the skin using a chlorhexidine gluconate (4% w/v) solution and a chlorhexidine gluconate (0.5% w/v)-ethanol (70% w/v) spray (Microshield, Australia). An intercostal nerve block using a total of 6 ml lidocaine 2% (w/v; Ilium, Australia) was performed on the most proximal aspect of each rib to be biopsied as well as one adjacent rib on either side after the initial cleaning of the skin.

This was followed by a line block between the first and second round of scrubs which was performed by injecting lidocaine 2% (w/v) into all tissue layers on the proposed incision line from skin to periosteum as well as the immediate cranial and caudal regions. After the onset of local anesthesia, skin was incised over the biopsy site to a maximum of 8 cm (typically 6 cm). The incision was continued into the areolar tissues and muscles followed by a subperiosteal dissection of the rib to expose the outer cortex. Next, a 16 mm Galt trephine (Medicon, Germany) was used to remove a cylinder of bone from the external cortex of the rib taking care to avoid the internal cortex and pleura. Some medullary trabecular bone was also removed with this cortical sample [Figure 1]. Following a brief lavage of the surgical site to remove any loose debris, the skin incision was closed using 2/0 Optilene (B. Braun, Australia) in a cruciate and a simple interrupted pattern. If required, bleeding vessels were ligated using 3/0 polydioxanones (Ethicon, United States). An insecticidal and repellent (Troy, Australia) treatment was also utilized on and around the incision line. No prophylactic antibiotics were used in this study.

Post-operative monitoring

Cattle were confined to an accessible feed pad for 24 hours post-operatively to allow for the early stage of monitoring to be completed. This was done 3 times daily to identify any signs of local reactions, pain or distress using a variety of local, systemic, and behavioural clues as per the monitoring sheet presented in Figure 2. On the second day after surgery, cattle were returned to the herd and monitored once a day until day 14 when sutures were removed.

Milk yield, feed intake and weight

Daily milk yield, body weight and concentrate feed intake data were collected by the Lely robotic milking system for both biopsy and control cows. The dates of data collection were the same

for both biopsy and control groups. The feed intake data did not include pasture consumed. The data was collected 7 days pre- and 7 days post-surgery. The arithmetic mean of 7 days observations was used for analysis.

Administrative Details																	
Date:																	
Time:																	
Animal ID:																	
Procedure: bone biopsy from the rib																	
Anaesthesia administered: 2% lignocaine (intercostal nerve block + line block)																	
Analgesia administered: meloxicam (0.5 mg/kg SC once preoperatively)																	
Signs																	
<i>Do not leave fields blank as this will be read as the sign not having been monitored.</i>																	
Days	0	1			2	3	4	5	6	7	8	9	10	11	12	13	14
		A	B	C													
Local reactions																	
Visible swelling																	
Redness (hyperaemia and congestion)																	
Haemorrhage																	
Discharge																	
Wound healing score (1-4)																	
Evidence of eggs or maggots																	
Milk Production																	
Drop in Milk Production																	
Any sign of distress/pain																	
Change in feeding																	
Pacing																	
Vocalisation																	
Facial expression and body posture																	
Comments																	
<i>Provide detail of any signs, body weight or condition changes, or other observations of note.</i>																	
Investigator																	

Figure 1: The rib biopsy procedure.

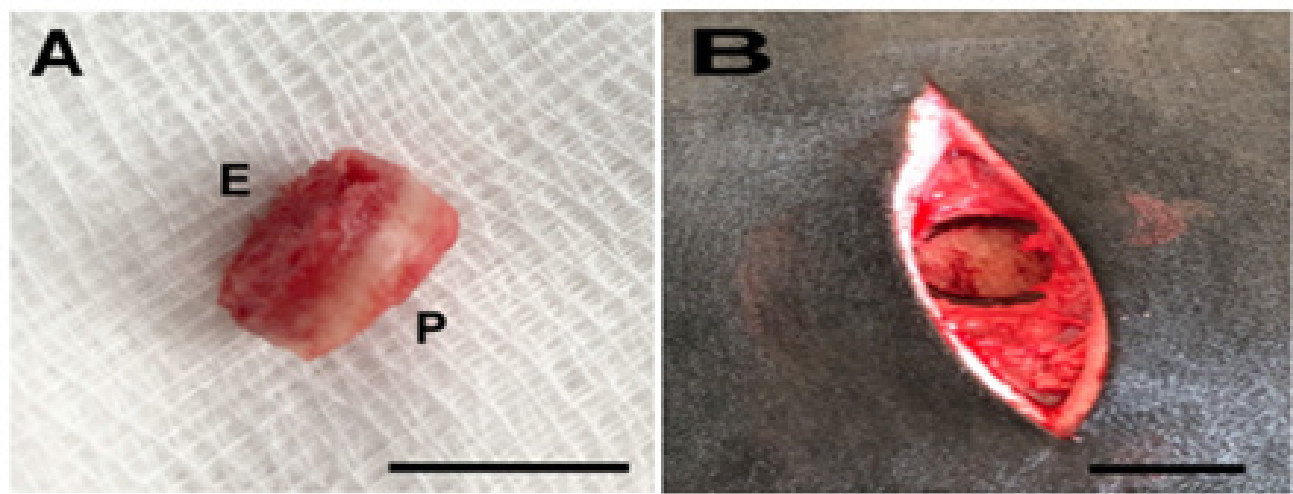


Figure 2: Monitoring Criteria Sheet.

Statistical analysis

Statistical analysis was performed in R.[13] Milk yield, concentrate intake and body weight were defined as response variables in mixed linear models using 'lme4' package [14]. Predictor variables were the stages of lactation, time (before and after biopsy procedure), treatment (control and biopsy) with subject level random effects. The model was implemented as shown in (Eq.1).

Eq.1. Model equation used for linear mixed models to evaluate response variable (milk yield[liters], concentrate feed intake [kg] and liveweight [kg] with predicted variables (stages of lactation [early, mid, late], time [before & after] and treatment [biopsy & control]. Y: response variable, (1+ stage|ID): random intercept/slope(stage), ϵ : residual error. The * indicates interacting terms that include all combinations of variables stage, time, and treatment.

The goodness-of-fit was checked by residual analysis and scatter plots. Marginal and conditional coefficients of determination (R^2) [15] were used to describe the variability explained by the models, as implemented in 'muMin' package. [16] Marginal R-squared (mR^2) provides the variance explained by fixed effects (stage, time, and treatment) and conditional R-squared (cR^2) provides the variance explained by the entire model including both fixed and random effects. The package 'em means' was used for pairwise comparisons among lactation stages and for estimating marginal means [17].

Results

Each biopsy procedure took approximately 9 to 15 minutes to complete. There were no signs of discomfort or pain indicated

by vocalization or body posture, and the cows remained calm throughout surgery; however, local anesthetic supplementation was required in two cases. The total number of biopsies collected was 119 out of the intended 132. Three cows were not sampled at all timepoints due to their death (1) or being sold off (2) due to low milk production. Sampling of seven cows was not completed because they were not pregnant or dried off at late timepoint sampling. Out of 118 biopsies collected, post-operative complications were seen in 5 cows (4.2%). These complications involved the formation of surgical site abscesses or seepage. These cows were identified through our daily monitoring regimen and production data was not indicative of any noticeable changes that could point to an issue; however, the small number of these animals precluded the use of a meaningful statistical analysis.

These were managed satisfactorily with a simple abscess incision and drainage placement together with antibiotic therapy (4 cows) or an antibiotic alone (1 cow). Bacterial culture results indicated a light and moderate growth of *Aerococcus viridans* and *Trueperella pyogenes* respectively in two instances. The species of bacteria in samples were identified using standard laboratory procedure by a commercial veterinary diagnostic laboratory. All other cows showed no sign of local reactions or pain using the facial grimace and body posture evaluation method [9]. As expected, milk yield was highest in early lactation and dropped over time reaching its lowest levels in late lactation in both biopsy and control cows [Figure 3]. Daily milk yield data showed that whilst there were fluctuations in milk production following surgery, there were no detectable differences between the overall trends of biopsied cows and those observed in the control cows [Figure 3].

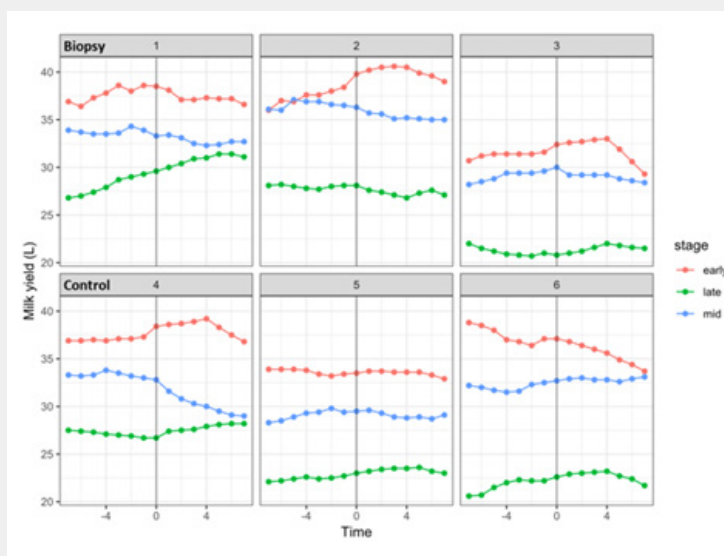


Figure 3: Line plot of daily milk yield of biopsy and control lactating Holstein cows before and after rib biopsy procedures during early, mid, and late lactation.

Our modelling demonstrated that lactation stages, biopsy procedure and time (7 days pre- and post-surgery) collectively explained 39% of variation observed in milk yield (mR²: 0.39, cR²: 0.96), 14% in concentrate feed intake (mR²: 0.14, cR²: 0.87) and 2% in body weight (mR²: 0.02, cR²: 0.93) of cows (Table 1). The variability in milk yield was mostly related to lactation stage and time of the year. Milk yield gradually decreased in mid lactation (β :

-7.04, 95% CI: -8.95 to -5.35) to the lowest level at late lactation (β : -13.00, 95% CI: -15.79 to -10.56) compared to early lactation (reference level). In line with this, concentrate feed intake was lower in late lactation (β : -1.34, 95% CI: -2.23 to -0.46) compared to early lactation. The body weight was higher in mid (β : 22.18, 95% CI: 9.65 to 34.65) and late lactation (β : 25.15, 95% CI: 11.30 to 39.11) compared to early lactation [Table 1].

Table 1: Mixed linear model describing the effects of biopsy procedure and stages of lactation on average milk yield (litres), average feed intake (kg) and average body weight (kg) of biopsy and control groups.

Stages	Milk Yield				Concentrate Feed Intake				Body Weight			
	β	LCL [§]	UCL [¶]	p-value [†]	β	LCL	UCL	p-value [†]	β	LCL	UCL	p-value [†]
Early lactation	0 [‡]	—	—	—	0 [‡]	—	—	—	0 [‡]	—	—	—
Mid lactation	-7.04	-8.95	-5.35	<0.001	-0.28	-1.14	0.56	0.521	22.18	9.65	34.65	0.001
Late lactation	-13	-15.79	-10.56	<0.001	-1.34	-2.23	-0.46	0.004	25.15	11.3	39.11	0.001
Time before (Before)	1.31	0.45	2.17	0.004	1.13	0.7	1.55	<0.001	18.54	9.5	27.58	0
Treatment (Control)	-9.06	-12.36	-5.93	0	0.96	-0.18	2.06	0.09	-1.84	-28.5	24.41	0.893
Late lactation: Before	-2.01	-3.27	-0.75	0.002	-0.91	-1.53	-0.3	0.004	-16.11	-28.8	-3.44	0.015
Mid lactation: Before	-0.99	-2.22	0.23	0.12	-0.72	-1.32	-0.12	0.021	-21.53	-34	-9.06	0.001
Late lactation: Control	3.66	-0.14	7.96	0.024	-1.25	-2.49	0.02	0.052	-24.45	-44.9	-3.82	0.023
Mid lactation: Control	3.25	0.67	6.11	0.004	-0.84	-1.96	0.32	0.15	2.25	-17.4	21.97	0.823
Time before: Control	-1.65	-2.91	-0.39	0.012	-1	-1.61	-0.39	0.002	-9.48	-22.2	3.19	0.151
Late lactation: Before: Control	2.42	0.59	4.25	0.012	1.32	0.43	2.21	0.005	21.59	3.06	40.13	0.026
Mid lactation: Before: Control	1.54	-0.24	3.33	0.097	0.61	-0.26	1.48	0.177	5.01	-14.2	24.21	0.616
Intercept	38.46	35.91	41.32	<0.001	9.92	9.05	10.8	<0.001	597.8	577	619.5	<0.001
R-squared marginal	0.39				0.14				0.02			
R-squared conditional	0.96				0.87				0.93			

[†] p-values are for the test hypothesis of coefficient value 0 and have been generated by approximate degrees of freedom

[‡] = reference level

[§]LCL = 95% Lower confidence limit

[¶]UCL = 95% Upper confidence limits

Table 2 summarizes the estimated marginal means of milk yield, concentrate feed intake and body weight calculated from the mixed linear models. The difference in marginal means is presented in Table 3 of the 3 stages of lactation analyzed, most changes were observed during the early lactation stage. The predicted mean of milk yield at the early lactation stage for biopsy cows was slightly decreased from 39.8 liters (95% CL: 37.2 to 42.4) to 38.5 liters (95% CL: 35.9 to 41) following biopsies whilst the predicted mean for early lactating control cows mildly increased

during the same time frame (29.1 liters, 95% CL: 26.4 to 31.7 liters before the time of surgery in the biopsy group and 29.4 liters, 95% CL: 26.7 to 32.1 liters afterwards). No significant changes were observed in milk yield following biopsies in mid and late lactation stages. Interestingly, the overall milk yield values were higher in early (mean: 38.5, 95% CL: 35.9 to 41), mid (mean: 31.4, 95% CL: 29.4 to 33.4) and late lactation (mean: 25.5, 95% CL: 23.3 to 27.6) post-surgery compared to control cows [Tables 2 and 3].

Table 2: Estimated marginal means of average milk yield, concentrate feed intake and body weight obtained from fitted mixed model (Table 1)

Stage	Time	Treatment	Milk (litres)			Feed (Kg)			Body Weight (Kg)		
			Marginal mean	LCL†	UCL‡	Marginal mean	LCL†	UCL‡	Marginal mean	LCL†	UCL‡
Early	Before	Biopsy	39.8	37.2	42.4	11.1	10.2	11.94	616	594	638
Early	After	Biopsy	38.5	35.9	41	9.92	9.03	10.81	598	576	620
Early	Before	Control	29.1	26.4	31.7	11	10.1	11.94	605	583	627
Early	After	Control	29.4	26.7	32.1	10.9	9.95	11.81	596	574	618
Mid	Before	Biopsy	31.7	29.8	33.7	10.1	9.51	10.59	617	596	638
Mid	After	Biopsy	31.4	29.4	33.4	9.64	9.1	10.18	620	599	641
Mid	Before	Control	25.8	23.8	27.9	9.78	9.22	10.35	613	590	636
Mid	After	Control	25.6	23.6	27.6	9.77	9.2	10.33	620	597	643
Late	Before	Biopsy	24.8	22.6	26.9	8.79	7.98	9.6	625	604	647
Late	After	Biopsy	25.5	23.3	27.6	8.58	7.77	9.39	623	601	645
Late	Before	Control	20.1	17.9	22.3	8.83	7.98	9.68	611	588	634
Late	After	Control	20.1	17.8	22.3	8.29	7.44	9.14	597	574	620

†LCL = 95% Lower confidence limit

‡UCL = 95% Upper confidence limit

Table 3: Pairwise comparisons of predicted means of milk yield, body weight and concentrate feed intake at different stages of lactation.

Contrast			Milk Yield				Concentrate Feed Intake				Body Weight			
Stage	Time	Treatment	Estimate	LCL‡	UCL§	p-value†	Estimate	LCL‡	UCL§	p-value†	Estimate	LCL‡	UCL§	p-value†
Early	After	Biopsy	-1.31	-2.19	-0.43	0	-1.13	-1.56	-0.7	<.0001	-18.54	-27.79	-9.29	0
Early	Before	biopsy												
Early	Before	biopsy	10.71	8.06	13.36	<.0001	0.04	-1.09	1.17	0.95	11.32	-15.97	38.61	0.41
Early	Before	biopsy												
Early	After	Biopsy	0.34	-0.6	1.28	0.48	-0.13	-0.58	0.33	0.58	-9.06	-18.15	0.03	0.05
Early	Before	biopsy												
Mid	After	Biopsy	-0.32	-1.21	0.57	0.48	-0.41	-0.84	0.02	0.06	3	-5.8	11.79	0.5
Mid	Before	Biopsy												
Mid	Before	Biopsy	5.92	3.77	8.07	<.0001	0.27	-0.48	1.01	0.48	4.06	-21.63	29.76	0.76
Mid	Before	Control												
Mid	After	Control	-0.21	-1.15	0.73	0.66	-0.02	-0.47	0.44	0.95	7.47	-4.4	19.34	0.22
Mid	Before	Control												
Late	After	Biopsy	0.7	-0.24	1.64	0.14	-0.21	-0.67	0.24	0.35	-2.43	-11.52	6.66	0.6
Late	Before	Biopsy												
Late	Before	Biopsy	4.63	1.88	7.38	0	-0.03	-1.18	1.11	0.95	14.18	-14.22	42.58	0.32
Late	Before	Control												
Late	After	Control	-0.07	-1.06	0.92	0.89	-0.53	-1.01	-0.06	0.03	-14.54	-24.97	-4.1	0.01
Late	Before	Control												

† p-values are for the test hypothesis of coefficient value 0

‡ p-values and contrast are not adjusted for multiple comparisons

‡LCL = 95% Lower confidence limit

§UCL = 95% Upper confidence limit

Like the milk yield data, the predicted mean of daily concentrate feed intake of early lactating biopsy cows decreased from 11.05 kg/day (95% CL: 10.15 to 11.94) pre-operatively to 9.92 kg/day (95% CL: 9.03 to 10.81) in the post-operative period whilst in control cows, values remained rather constant (11.01 kg/day [CL:10.08-11.94] vs 10.88 [CL: 9.95-11.81]). A similar, however, less noticeable decline was also noted in biopsy cows in mid lactation but not in late lactation when this relationship reversed with control cows showing a decrease in feed intake despite no significant change in cows in the biopsy groups [Table 2].

Changes observed in body weight followed the same pattern with values from cows in the biopsy and control group decreasing following the early lactation biopsies. The body weight remained similar in both groups after the mid lactation biopsies, followed by a relatively stable trend after late lactation biopsies despite weight loss in control cows during the same period [Table 2].

Discussion

The purpose of this study was to determine the physiological effects of a bone biopsy procedure at various stages of lactation in dairy cows using automatic data collected on milk yield, concentrate feed intake and body weight. Overall, the serial rib biopsy procedure had no serious or lasting impact on the health of the cows enrolled in this study. Although changes in milk yield and concentrate feed intake could be detected following early and occasionally mid lactation biopsy, the effect was generally negligible at least from the financial standpoint. The analgesia protocol used appeared to be adequate in the prevention of post-operative breakthrough pain and the monitoring regime adopted was successful in the early identification of post-operative surgical site infections (SSI). The rib biopsy samples collected were suitable for micro-computed tomography and histopathology evaluations.

The study showed that the variability in milk yield, concentrate feed intake and body weight was predominantly driven by between-cow variability. The within-cow variability in milk yield and concentrate feed intake was mostly related to lactation stage, and whilst a drop in milk yield and concentrate intake was observed following biopsies in the test group at early lactation, the change diminished over time and disappeared in late lactation. These effects appear to be proportional to production levels; assuming that these effects are relatively constant at each stage, they are most apparent when milk yield is larger. This finding is somewhat expected as early lactating cows in modern farming systems are at their peak production and in a negative energy balance. They are therefore very sensitive to stress and environmental changes. Stress can interfere with the oxytocin pathway hindering milk letdown and limiting milk quantity in both humans and cows [18-21]. Handling and surgery are well known stressors in animals and can explain some of the changes seen [22-24]. Additionally, cows were isolated before surgery

and often had to line up for up to five hours in cattle races that precluded feeding and potentially introduced more stress. The intensive monitoring regime following biopsies also meant that cattle did not have access to concentrate feed for 24 hours and that they could not volunteer to be milked.

Another possibility is related to differences in pasture intake between the control and biopsy groups which can well explain the changes seen in milk yield and concentrate feed intake postoperatively. Because pasture intake was not measured in this study, this remains undetermined. Taken together, it is highly likely that the changes observed were not directly related to the biopsy procedure or any associated pain, especially as cows in the late lactation stage did not demonstrate such changes, despite undergoing the exact same procedures. In keeping with this conclusion, a previous study conducted on lactating cows did not find any long-term effects on milk yield and composition, dry matter intake or mammary gland health when cattle were subjected to repeated mammary gland biopsies which although different to bone biopsies, can be considered as invasive if not more [25]. Similarly, a previous study reported no change in dry matter intake and average daily weight gain in steers subjected to a single biopsy of the 12th rib [7].

During late lactation, feed intake in cows in the control group dropped, which was accompanied by a subtle weight loss. This effect was not observed in cows in the biopsy group. A possible explanation for this observation lies in the fact that lower milk production at this stage would translate into less nutrient demand thus less feed intake and a potential for weight loss. The stress associated with artificial insemination and/or pregnancies that generally occurred around this time could also be a contributing factor. Although the control cows were randomly assigned to match the biopsy group, another possible explanation for this difference could lie in the normal variation of production efficiency, rendering cows in the biopsy group as generally better producers. Regardless, using current data, we are not able to provide any conclusive explanation for this observation. While our analysis and post-operative monitoring of cows did not indicate any breakthrough pain, considering the current findings, we think there is still value in trying to use the collected data by automated dairy systems to identify cows at risk of developing various physiological issues.

Inclusion of other physiological parameters such as heart rate, respiratory rate, body temperature, rumination time and frequency, etc. in the dataset could further improve the chances of identifying subclinical pain via a data orientated approach. Work in the past, for example, was promising when a similar statistical approach was used to identify lame cattle [10]. Other investigators have used machine learning and image-based methods for such analysis and successful detection of experimental noxious events and cow behaviour. [26,12] Given the enormous capabilities of machine learning methods and neural networks, and the stress-

free nature of data collection by robotic systems, it is inevitable that these methods will be widely integrated in the future further reducing the need for manual monitoring of farm animals and improving animal welfare.

There are very few studies that report the SSI risk in cows. The risk of SSI has been reported to be between 1.3 % to 25.9 % following caesarean section in cow [27-30]. In small animals, the incidence of SSI complications has been reported to be between 0.8% and 18.1% depending on the type of surgery, [31] and in horses, the rate is said to be higher at around 9-20% [32]. The incidence of post-operative SSI during the current study period was 4.2% which is substantially lower than previously reported given the conditions free range animals involved in this study were exposed to and the relatively wet climate of the region in which Dookie Agricultural College is situated (mean 46.5 mm rainfall per month; mean 4.9 days of rain > 1 mm per month using the 30-year average until 2020) [33].

Conclusion

Our current study suggests that data collected by automated dairy management systems is of high value in identifying physiological disturbances when a data-oriented approach is utilized. Future smart farm management systems are likely to take advantage of this principle to identify animals at risk early and thus improve welfare outcomes. Our analysis also indicates that the previously described rib biopsy technique has no expected negative impact in lactating cows and will allow comparisons over an extended period if desired. Farmers, researchers, and veterinarians can use this information while planning their analgesic and monitoring regimens.

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