Effects of a topically applied anaesthetic on the behaviour, pain sensitivity and weight gain of dairy calves following thermocautery disbudding with a local anaesthetic

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Supplementary Information 1. Assessment of activity of calves following disbudding.

A total of 30 eligible calves per farm were randomly allocated to have activity monitors (Heyrex, Wellington, NZ) attached using a calf collar. The monitors were attached with two cable ties to a calf collar (Shoof, Hamilton, NZ) to allow them to sit snuggly against the larynx. The monitors are designed for dogs and house an array of sensors designed to code and record motion in different axes relative to the wearer. The records of coded movements create digital signatures that are characteristic of specific behaviour patterns such as walking, running, and sleeping. The output can then be downloaded into an Excel (version 10) spreadsheet (Microsoft USA), with the data recorded as the change in acceleration force, ΔG, per 15 minutes. Heyrex activity data were collected for a continuous 24-hour period from three farms.

Statistical Analysis

The outcome variable, total ΔG, was analysed using linear regression, with farm, treatment group and time recorded as potential risk factors.

Results

There were 88 calves that had Heyrex information collected over the entire 24-hour period post-disbudding.

No single treatment group was significantly different to another treatment group. However, calves that were disbudded under sedation were significantly less active than calves disbudded fully conscious, restrained in a crate ($p = 0.02$).

When different time periods were assessed, it was noted that the difference in activity in sedated and non-sedated calves was almost entirely explained by the first 3 hours after disbudding. There was no residual effect of sedation on activity after the first 3 hours.

There was a difference in activity between farms, with calves from Farm 2 having a 39% lower ΔG output compared to calves from Farm 1 ($p < 0.001$). Model diagnostics identified two animals that were influential on the outcomes; both of these were from Farm 1, one calf was in the Crate-TA group, and the other was in the Sedation-TA group. Both of these calves were significantly more active than the other calves. Considered rationale for these outlying activity results included possible failure of cornual nerve block, unexpected reaction to the product, or high innate behavioural activity. Once the two calves were removed from the analysis as a result of being influential observations, the calves in the Sedated-TA group were significantly less active than calves in the Crate-control group. However, as a causal conclusion could not be ascertained, they remained in the model.

Supplementary Information 2. Subjective assessment of pain response following disbudding.

In addition to the algometer measurements, the person applying the algometer also subjectively graded the calf's pain response as low, moderate, high or extreme. A low response was classified as an animal that had either no response after reasonable pressure or was slow to respond and waited for pressure to be applied before responding with a mild movement of the head. A moderate response was a faster response than a low responder but still allowed pressure to be applied before responding. The response was stronger and more purposeful. A high response was a calf that had a very fast response to the stimulation and pressure could only just be applied before the calf would forcefully react with some aggression and a very purposeful movement of the head. The extreme response was a very fast response and could hardly be touched before responding. The response was extremely forceful and aggressive and very difficult for the assistant to restrain.

Statistical Analysis

The subjective scoring of the pain response was re-categorised into two categories; high or extreme and moderate or low response. This outcome was modelled using a generalised linear mixed effects model, using a similar process as for the algometer readings, assessing the odds of calves showing a high or extreme compared to a moderate or low response.

Results

There were 2,354 subjective scores recorded, with 793 (34%) high or extreme pain responses elicited by calves.

For the model comparing pain sensitivity between sedated and non-sedated calves there was a significant interaction between time and treatment. All measurements from 0–1 hours post disbudding were removed from the subsequent analysis.

The mixed logistic regression analysis for the subjective pain responses is presented in Supplementary Table 1. Sedated calves had a significantly reduced odds of having a high or extreme subjective pain score compared to non-sedated calves ($p < 0.001$). Again, there was no statistically significant difference between meloxicam or topical anaesthetic in either sedated or crate disbudded calves.

Supplementary Table1. Results from the generalised linear mixed effects model on the effect of treatment on the subjective pain response (low-medium) and (high-extreme) following disbudding, with time 0-1 hours removed (1,828 measurements from 124 calves).

Variable	Estimate	Std Error	Odds Ratio	Lower 95% CI	Upper 95% CI	P value
Intercept	-2.28	0.61				
Treatment						
Crate-control						Reference
Crate-meloxicam	-0.36	0.62	0.7	0.2	2.37	0.56
Crate-TA	-0.23	0.64	0.79	0.23	2.8	0.72
Sedation-control	-1.76	0.66	0.17	0.05	0.62	0.01
Sedation-meloxicam	-1.96	0.66	0.14	0.04	0.51	0.003
Sedation-TA [a]	-2.3	0.65	0.10	0.03	0.36	<0.001
Farm						
Farm 1						Reference
Farm 2	0.32	0.51	1.38	0.51	3.71	0.53
Farm 3	-1.5	0.48	0.22	0.09	0.57	0.002
Time (hours)						
12–24						Reference
2–5 [b]	-0.69	0.17	0.50	0.36	0.71	<0.001
6–11	0.76	0.15	2.14	1.6	2.86	<0.001
Location						
Left top						Reference
Left side	0.75	0.17	2.11	1.51	2.96	<0.001
Right top	0.18	0.17	1.19	0.85	1.67	0.3
Right side	0.38	0.17	1.46	1.04	2.05	0.03

[a]. Interpretation: In comparison to calves disbudded following a cornual nerve block and restrained in a crate, calves disbudded following a cornual nerve block and under sedation with Tri-Solfen product applied on the horn bud wound after disbudding were 90% less likely to show a high or extreme subjective pain response when pressure was applied to the disbudding wound (95%CI=64%-97% reduced odds).

[b]. Interpretation: In comparison to the calves tested between 12-24 hours, calves tested between 2-5 hours had were 50% times less likely to show a low-medium subjective pain response when pressure was applied to the disbudding wound (95%CI=29-64% reduced odds).

Supplementary Table 2. Results of the final linear regression model for the effect of treatment group on average daily gain (kg/day) between 0 and 7 days following disbudding in 310 calves from three farms in the Waikato region of New Zealand

Variable	Estimate	Std. Error	Lower 95% CI	Upper 95% CI	P value
Intercept	0.62	0.075	-	-	<0.0001
Farm					
Farm 1					Reference
Farm 2	-0.042	0.085	-0.208	0.124	0.623
Farm 3	-0.082	0.067	-0.213	0.048	0.217
Treatment					
Crate-control					Reference
Crate-meloxicam	0.047	0.065	-0.08	0.174	0.472
Crate-TA	0.011	0.065	-0.116	0.138	0.866
Sedation-control	0.125	0.065	-0.002	0.252	0.055
Sedation-meloxicam [a]	0.144	0.066	0.015	0.274	0.03
Sedation-TA	0.089	0.066	-0.041	0.218	0.181

[a]. Interpretation: Calves that were disbudded under sedation, with a cornual nerve block applied and Meloxicam provided 10 minutes prior to the procedure in comparison to calves disbudded following a cornual nerve block, fully conscious and restrained in a crate grew 144g/day (95%CI 15g-274g) more in the 7 days following the disbudding procedure.

Supplementary Table 3. Results of the final linear regression model for the effect of treatment group on average daily gain (kg/day) between 0 and 28 days following disbudding in 315 calves from three farms in the Waikato region of New Zealand.

Variable	Estimate	Std Error	Lower 95% CI	Upper 95% CI	P value
Intercept	0.639	0.033	-	-	<0.0001
Farm					
Farm 1					Reference
Farm 2	0.085	0.039	0.009	0.162	0.029
Farm 3	0.056	0.027	0.003	0.109	0.038
Treatment					
Crate-control					Reference
Crate-meloxicam	0.018	0.036	-0.052	0.089	0.611
Crate-TA	-0.025	0.036	-0.094	0.045	0.489
Sedation-control	0.049	0.035	-0.02	0.119	0.164
Sedation-meloxicam	0.04	0.037	-0.032	0.112	0.279
Sedation-TA	0.06	0.036	-0.01	0.13	0.094