Clinical trial protocol: The effects of a high or low plane of nutrition and an additional NSAID on wound healing and inflammation in 3-4-week-old calves disbudded via cautery

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Administrative information

This trial protocol was written in accordance to Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 Checklist (Chan et al., 2012). Funding for this study was received from the Ontario Ministry of Agriculture, Food, and Rural Affairs – University of Guelph Research Program. Grant proposals were written by CBW in collaboration with TJD, KDL, and TFD. Study sponsors and funders were not/will not be involved in study design, data collection, data management, analysis and interpretation of data, writing of the report, and decision of submission and publication.

Introduction

Various studies have found that immune function in calves is associated with their diet and nutrition (Pollock et al., 1994; Nonnecke et al., 2003; Foote et al., 2005; Ballou, 2012), but there has been less work done examining the roles of diet and nutrition on inflammation in calves. Two main studies have been conducted examining the length of time it takes for wounds to heal following disbudding procedures. The study by Huebner et al. (2012) found that wounds may be present for at least 3 weeks following disbudding, although in this study they did not follow calves until their wounds had fully healed. In the study by Adcock and Tucker (2018), they determined that it takes 9 weeks for wounds to fully heal following cautery disbudding, although in this study the calves were fed a diet of between 1.4-1.9L of milk replacer twice daily which is considered a low plane of nutrition and could confound the wound healing time. Therefore, the impact of diet and nutrition on the healing of lesions following cautery disbudding has not been studied yet. In transition cows, over-feeding results in increased biomarkers of inflammation (Vailati-Riboni et al., 2016), while a study by Kvidera et al. (2017) found that restricting feed in lactating cows results in a linear increase in serum haptoglobin and serum amyloid A, both markers of inflammation in cattle. More and more producers are feeding an increased nutritional plane to young dairy calves, but feed restriction is still very common (Vasseur et al., 2010). The Dairy Code of Practice requires that when feeding calves, “calves must receive a volume and quality of milk or milk replacer to maintain health, growth and vigour” (NFACC, 2009), this is left to interpretation by the producer as to how much milk or milk replacer is considered the proper quantity and/or quality. Under certain controlled conditions, feeding calves a total of 6L of milk or milk replacer per day could achieve these standards. However, there is evidence from other work showing that calves fed only 6L per day
show signs of chronic hunger (Rosenberger et al., 2017). As well, a study by Todd et al. (2017) found that an increased nutritional plane can improve both health and performance of calves. There is also evidence that increased nutritional plane early in life results in improved first lactation milk production in heifers (Gelsinger et al., 2016). Thus, work examining the role of early life nutrition on wound healing and inflammation following disbudding procedures would be useful to support the benefits of an increased nutritional plane in early life for calves.

Therefore, the primary objective of this study is to determine the impact of feeding a high plane of nutrition versus a low plane of nutrition on wound healing and inflammation in calves disbudded via a cautery iron. Secondary objectives of this study are to examine the effects of an additional NSAID post-disbudding on wound healing and inflammation, the effects early life parameters such as passive transfer status and health on wound healing later in life, and the effects of feeding a novel milk by-product based starter compared to a grain starter on wound healing and inflammation. It is hypothesized that calves fed a higher plane of nutrition and receiving an additional NSAID will have improved wound healing outcomes as well as decreased markers of inflammation post-disbudding. It is also hypothesized that calves with decreased health parameters early in life (sickness due to scours and/or pneumonia and/or failure of passive transfer) will have poorer wound healing compared to healthy calves.

Methods: Animals, interventions, assignment, and outcomes

This study will be conducted at the Elora Research Station – Dairy Facility with a target start date of October 1st, 2019 using heifer calves 18-25 days of age. Calves will be on a Calf Rail system (Förster Technik, Engen, Germany) and will be individually housed. As calves are moved to group housing at 78 days of age, staff will remove the sides of the empty pens, pressure wash and sanitize the sides of the pen, scrape out old bedding and place new bedding down before placing a new calf in the pen. The research team will visit the centre once a week to disbud heifers in the eligible age bracket as well as every day following the procedure for 7 days and then twice a week for 6 weeks, or until calves are fully weaned and moved to group housing (Figure 1). Calves will be randomly assigned to one of four treatment groups (30 calves per group based on an expected difference of 2 cm of wound diameter with a standard deviation of 4 cm). All heifer calves will be enrolled in trial. This project will be in collaboration with another research group conducting a nutrition trial at the same time using the same calves (AUP#3722).

Intervention groups

1) HIGH: offered 3L of milk 5 times daily, injection of placebo at +3 days relative to disbudding

2) HIGH + NSAID: offered 3L of milk 5 times daily, injection of meloxicam at +3 days relative to disbudding

3) LOW: offered 3L of milk twice daily, injection of placebo at +3 days relative to disbudding

4) LOW + NSAID: offered 3L of milk twice daily, injection of meloxicam at +3 days relative to disbudding
All calves will receive a lidocaine cornual nerve block and a subcutaneous (SC) injection of meloxicam 15 minutes prior to cautery disbudding.

**Intervention allocation and blinding**

This will be a 2X2 factorial design study to assess the effects of plane of nutrition as well as an additional NSAID on wound healing and inflammation in young calves following cautery disbudding. Calves will be assigned randomly to one of four treatment groups from birth; only heifer calves will be eligible for trial. A random pattern of the treatment groups will be created using a random number generator and repeated until 120 calves have been enrolled. Treatments will be assigned based on the treatment assigned to the next available pen; the heifer calf placed in that pen will then be enrolled onto the corresponding treatment. This randomization of allocation of treatments will control for the effect of location within a room. The researcher performing the algometry measurements, wound photographing and measuring, health scoring, and disbudding will be blinded to the treatments which the calves are assigned to.

**Intervention administration**

Calves will be assigned to their plane of nutrition when they are old enough to be trained on the Calf Rail (6th feeding) and offered the same amount of milk until they are weaned. Starter diet will be offered to calves from day 5 of life. The second injection of meloxicam or placebo will be administered to calves 3 days post-disbudding and will be given by the collaborating graduate student who will not be blinded to the treatment groups. Lidocaine cornual nerve blocks and subcutaneous meloxicam injections will be administered 15 minutes prior to cautery procedure. Hair around the horn buds will be clipped with electric clippers 1 hour before baseline algometry measures are taken. Researchers will observe calves for a minimum of 4 hours on the day of disbudding following the procedure.

**Outcomes**

1. **Wound healing** Photos will be taken of the wounds following the system described by Adcock and Tucker (2018). Wounds will be scored on a scale from 1-7 based on the stage of healing and the tissue present. Photos and measurements will be taken directly after disbudding, +3-, +7-, +10-, +14-, +17-, +21-, +24-, +28-, +31-, +35-, +38-, and +42 days relative to disbudding, or twice a week until calves are moved out of calf rooms and into group housing. Wound healing will be assessed based on how long in days it takes for a calf’s wounds to fully heal. An external outcome assessor who is blind to all treatment groups will assess the photos and score them based on their stage of healing. This is the primary outcome which the sample size is based off of.

2. **Wound assessment** The diameter and depth of each wound will be measured directly after disbudding, +3-, +7-, +10-, +14-, +17-, +21-, +24-, +28-, +31-, +35-, +38-, and +42 days relative to disbudding, or twice a week until calves are moved out of calf rooms. These measurements will be taken by one assessor and data from each wound (left and right) will be assessed independently of each other.
3. **Pressure sensitivity** A pressure force algometer will be used as described by Winder et al. (2017), and readings will be taken at -1 hour, 0 minutes, +4 hours, +3-, +7-, +10-, +14-, +17-, and +21 days relative to disbudding. Algometry will be measured in kgf based on the amount of force applied to the areas around the horn bud until the calf shows signs of distress. One trained researcher will take all algometry readings for the duration of the trial. Average algometry score for all four locations around both horn buds at each time point will be calculated to assess overall sensitivity of each calf.

4. **Haptoglobin** Haptoglobin will be measured in mg/mL. 10 mL of blood will be taken from the jugular vein via venipuncture at -1-hour, +1 day, +4 hours, +2-, +3-, +4-, +5-, +6-, +7 days relative to disbudding.

5. **Serum total protein** 10 mL of blood will be taken from the jugular vein via venipuncture when calves are between 2-9 days old. Serum total protein will be measured in g/dL using a cut point of 5.5 g/dL where above or equal to this value is considered successful passive transfer and below this value is considered failure of passive transfer.

6. **Feeding behaviour and milk consumption** The automated milk feeder (AMF) will record information on each calf’s average consumption per feeding session, average length of feeding session and the average drinking speed per feeding session. These will all be analyzed as separate outcomes.

7. **Standing and laying behaviour** HOBO data loggers will be attached to the inner rear leg of each calf using cohesive bandage when calves are 11 days old, these will be replaced weekly to download the data and switch rear legs. Data will be collected in 1 min increments and each day will be analyzed separately, with total time spent laying (min/d), number of laying bouts (bouts/d), and average laying bout length (min/d) offered for analysis. Data will be assessed from 1 week prior to disbudding to 1-week post-disbudding.

8. **Health score** Health scores will be taken on each calf twice a week from birth until they leave the calf rooms (around 11 weeks old). This will be measured using the University of Wisconsin Calf Health Scorer app (McGuirk) which is used to assign a score of 0-3 to each calf for various health attributes. Calves will be scored on temperature, fecal consistency, cough, navel, joints, eyes, ears, and nose. Scores for each attribute will be added together to determine a final cumulative score for each individual calf on each day health scores are assessed. Any abnormal scores that may indicate sickness will be reported to the staff for further assessment and treatment, this includes a score of 3 for fecal, temperature, and navel and a score of 2 or above for eyes, ears, joints, cough, and nose.

**Methods: Data collection, management, and analysis**

CBW will train the research team and one assessor will take all algometry readings and perform every disbudding procedure. Baseline values will be taken for feed and milk consumption, pressure sensitivity (-1 hour), and haptoglobin (-1 hour). Algometry readings, wound diameter,
wound depth, as well as any additional notes for each day will be recorded on paper by the researchers and will be stored and kept in a locked lab at the end of each collection day. Health score data will be recorded on the University of Wisconsin Calf Health Scorer app on an iPad which will also be kept in a locked lab at the end of each collection day. Data will then be transferred to an excel file and checked for errors prior to cleaning. At +7 days post-disbudding, HOBO loggers will be removed from calves and data will be saved to a research computer as well as to an online database (OneDrive) and a USB stick until it can be analyzed. Photos of the wounds will be uploaded to a research computer as well as an online database (OneDrive) and will be scored using the 7-point scale developed by Adcock and Tucker (2018) by one external outcome assessor blind to all treatment groups. Blood samples will be kept on ice during the collection days. Serum samples for haptoglobin and serum total protein will be spun down in a centrifuge by the end of each trial day and kept at -20C. Haptoglobin samples will be run as a single batch using the Roche Cobas 6000 c501 and biochemistry analyzer with methemoglobin stock reagent using formulas and operating conditions developed by J.G. Skinner laboratory, Veterinary Investigation Centre (Aberdeen, Scotland). Serum total protein will be measured using a refractometer.

**Statistical analysis**

Data will be exported into STATA15 (Stata/IC Version 15.1 for Mac, StatCorp, College Station, TX) and descriptive statistics reviewed for normality and variation. Univariate association of treatment group on outcome will be assessed for binary outcomes with logistic regression, continuous outcomes with linear regression, and count data with both Poisson and negative binomial models, with the negative binomial model used if the overdispersion term is significant. Zero-inflated models will be built if there are a large number of zeros in the data. Baseline data will be used as a fixed effect and disbudding date as a random effect. For outcomes with repeated measures, date will be included as a random effect, time as a fixed effect, and a treatment by time interaction will be examined.

**Ethics and dissemination**

Ethical approval has been obtained prior to the start of trial (AUP#4268). Changes to the protocol will be reported as protocol deviations in the relevant manuscript. All calves will be receiving industry standards for best practice for disbudding as they will all receive both local anesthesia and analgesia. Results will be analyzed during the winter of 2021 and should be submitted for publishing by fall 2021.

**References**


Figure 1. Timeline of sample collection relative to disbudding procedure.