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ORIGINAL ARTICLE

Effects of a fine needle liver biopsy on the behavior and milk production of postpartum dairy cows

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ABSTRACT. The objective of this study was to investigate the effect of a fine needle liver biopsy (FNB) on the behavior and milk production of postpartum dairy cows during and after the procedure. In Experiment 1, cows at 10 ± 1.1 days in milk were assigned to receive either a FNB (n = 26), involving collection of a liver sample using an 18G spinal needle under local anesthesia, or a sham procedure (n = 26). Behavioral responses during the procedure, such as vocalizations, kicking, and abrupt movements, were recorded and analyzed by a trained observer. In Experiment 2, a separate group of cows (n = 48), also at 10 ± 1.1 days in milk, underwent a FNB, and their daily lying behavior, rumination, and milk yield were monitored using automated sensors over three consecutive days: the day before (day -1, baseline), the day of (day 0), and the day after the procedure (day +1). No significant differences were observed in the behavioral responses between cows that received FNB and those that underwent the sham procedure. Post-procedure, daily lying time, rumination time, and milk yield remained stable compared to baseline values. On day +1, an increase in the number of lying bouts and a decrease in bout duration were noted. The results of this study indicate that liver FNB has little immediate or short-term effect on behavior or milk production, suggesting that it is a minimally painful procedure, supporting its suitability for clinical and research applications.

Keywords: welfare, hepatic lipidosis, transition, dairy cattle, biopsy.

INTRODUCTION

Hepatic lipidosis, characterized by abnormal triacylglycerol accumulation in hepatocytes (Bobe *et al*., 2004), is a common metabolic disorder in dairy cows during the periparturient period that affects animals in both pasture-based (Spaans *et al*., 2022) and confinement systems (Jorritsma *et al*., 2001). Assessment of hepatic triacylglycerol content in cattle has traditionally relied on the conventional biopsy technique (Bobe *et al*., 2004; Mølgaard *et al*., 2012), which involves the use of large-gauge instruments (Beausoleil & Stafford, 2012; Mølgaard *et al*., 2012). Several studies have reported behavioral changes in cows undergoing this procedure, including reduced rumination time and increased restlessness, which may reflect acute pain (Beausoleil & Stafford, 2012; Mølgaard *et al*., 2012). Moreover, increased activity for up to 19 hours post-biopsy suggests the potential for longer-lasting discomfort following this procedure (Mølgaard *et al*., 2012). These findings highlight the need to explore alternative diagnostic approaches that minimize animal discomfort and support improved welfare outcomes.

Less invasive approaches for diagnosing hepatic lipidosis in dairy cattle include blood-based assessments of biomarkers associated with negative energy balance (Spaans *et al*., 2022) as well as measurements of liver enzymes, cholesterol, and bilirubin concentrations (Batista *et al*., 2022). However, these biochemical indicators are nonspecific and generally reliable only in cases of advanced hepatic damage (Pinedo & Melendez, 2022). On the other hand, transcutaneous ultrasonography represents a non-invasive diagnostic tool (Elshafey *et al*., 2023), but its sensitivity is limited, particularly when hepatic triacylglycerol concentrations are below 10% (Pinedo & Melendez, 2022). Accordingly, fine needle biopsy (FNB) has emerged as an alternative method for evaluating liver conditions in cattle (Melendez *et al*., 2018) and has also been applied in other species (Sellon *et al*., 2000; Wang *et al*., 2004). Compared to conventional liver biopsy, FNB is technically simpler, associated with a lower risk of hemorrhage (Rothuizen & Twedt, 2009), and more cost-effective (Pinedo & Melendez, 2022). Despite its advantages, the potential impact of FNB on cow behavior and milk production has not been evaluated.

The objectives of this study were 1) to evaluate the behavioral responses of postpartum dairy cows during a liver FNB procedure, and 2) to compare the lying and ruminating behavior, and milk production 24 h before the procedure (baseline day) and 24 and 48 h after the procedure. We hypothesized that liver FNB would elicit no significant changes in short-term behavior during or after the procedure, or in milk yield in dairy cows.

MATERIALS AND METHODS

This study was conducted at the Austral Agricultural Experimental Station of the Universidad Austral de Chile in Valdivia, Chile (39°46'42.0" S, 73°13'45.0" W). The experimental farm maintains a total of 160 Holstein–Friesian cows, managed as a typical grazing herd under a two-seasonal calving system, with approximately 90 cows calving in spring and 70 calving in autumn, with 60-40% being the multiparous-primiparous rate. The Animal Care and Use Committee of the Universidad Austral de Chile approved all procedures (Protocol ID: No. 459/2022 and No. 572/2025).

Animals and handling

This study included 100 clinically healthy multiparous Holstein cows allocated across two experiments (Experiment 1: n = 52, year 2023; Experiment 2: n = 48, year 2022). On the day of the procedure, the cows had a mean (± SD) body condition score of 3.25 ± 0.56, and an average parity of 4.16 ± 1.88. All animals were managed as a single herd under a rotational grazing system, with access to pasture composed of mixed grasses and legumes (16.9% DM; 10.00% ash; 21.9% CP; 51.28% NDF; 33.44% ADF; 2.86 Mcal/kg ME; 117.62 g/kg WSC). Water troughs were located within 5 m of the electric fencing. Cows were milked twice daily (05:00 and 14:00) in a milking parlor and received a concentrate ration based on individual milk production (87% DM, 14% CP, 22% NDF, 11% ADF, 55% WSC, and 4% EE). Herd management practices, including pasture rotation, were conducted according to routine farm protocols with decisions made by the farm manager.

Fine needle liver biopsy procedure

To minimize the effects on the animals of the handling in the cattle chute used to performed the procedure (which was equipped with a head-locking mechanism to ensure animal and operator safety), cows were also partially restrained on the day before the procedure, for a time duration that was similar to the time that they will be restrained on the day of the procedure. During fine-needle biopsy (FNB), cows were partially restrained in a chute. The biopsy site, located at the right 11th intercostal space, was identified using ultrasonography with a 2.5 MHz transducer following a trichotomy. The area was then aseptically prepared using 70% ethanol and 10% povidone-iodine. Local anesthesia was administered via subcutaneous injection of 1 mL of bupivacaine hydrochloride (Richmond Vet Pharma®). A skin incision of less than 1 cm was made using a sterile scalpel to facilitate needle entry. An 18G × 3.5" spinal needle (NIPRO®) was inserted through the intercostal muscles and peritoneum (approximately 2.5" of needle length). Upon reaching the liver, the stylet was removed to permit complete needle insertion. The needle was then partially withdrawn, slightly redirected to obtain tissue from a different area, and fully reinserted thrice to collect the liver sample. A topical antiseptic spray was applied to the site following needle removal. The procedure was performed in small groups (one to five cows per day) and lasted an average of 3 min and 54 s (range: 2:43 to 5:41 min) per cow, measured from anesthetic infiltration to antiseptic application. Two experienced veterinarians performed all procedures.

Experiment 1

To evaluate the effect of a FNB on behavior during the procedure, 52 cows were allocated into two groups (n = 26 cows per group): 1) the FNB group, in which a liver tissue sample was collected, and 2) the sham control group, in which cows were handled in the same manner as those in the FNB group, except for skin incision and puncture. Cows in both groups were matched based on parity (FNB: 3.4 ± 1.7; mean ± SD; Control: 2.5 ± 1.6; $P = 0.18$) but differed in body condition score (FNB: 3.0 ± 0.9; Control: 3.25 ± 0.2; $P < 0.05$). All procedures were conducted on day 10 postpartum (± 1 d) for both groups, which took place between 8:00 and 9:00 AM, after the morning milking.

Experiment 2

A different group of 48 cows was used for Experiment 2, where all cows underwent an FNB at 10 ± 1.1 d postpartum. Cows were assessed over three consecutive days: the day before the FNB (day -1, used as the baseline value), the day of the procedure (day 0), and the day following the procedure (day +1), to measure lying behavior, rumination, and milk production. Each day, cows were brought into a pen after morning milking and subsequently moved into a head-locking chute to partially immobilize them for a duration comparable to the FNB procedure. Following the procedure, cows were allowed to return to the feed yard or paddock for up to two hours before being returned to pasture.

Behavior and milk production data collection

In Experiment 1, the behavior of each cow was continuously recorded using two video cameras during the FNB or sham procedure. Videos were captured from two perspectives: frontal and right lateral. A trained observer evaluated the videos and recorded the frequency of various behaviors exhibited during the procedure. Owing to the visual distinguishability of the differences in the instruments used for the FNB and sham procedures in the lateral video record, a blind observer could not be used. Intraobserver reliability was assessed using the Concordance Correlation Coefficient for each behavior (Table 1).

Table 1. Behavioral responses of dairy cows were recorded during liver fine-needle biopsy (FNB) or sham procedure, along with the Concordance Correlation Coefficient (CCC) for intraobserver reliability.

Behavior	Definition	CCC
Head shaking	The cow moves its head rapidly, both side to side and up and down. A series of head movements in rapid succession is considered as one event.	0.98
Vocalization	The cow makes sounds or moos with its mouth open or closed.	1.00
Turn the head to the right flank	The cow moves her head from the middle of her basal mid-plane to her right side. Once she returns to her mid-plane and turns her head again, it was counted as a new event.	0.99
Kicks	The cow hits the inner walls of the chute with the hind limb. Lifting the hind limb up and forward and then pushing it sideways or backwards.	0.99
Abrupt movements	The cow moves her body abruptly and performs escape or evasion behavior, including lunging forward or backward, hitting the inner walls of the chute, then returning to the original position.	0.99

The definition of different behaviors were adapted from: Mølgaard et al. (2012), and Beausoleil and Stafford (2012).

In Experiment 2, variations in daily rumination and lying behavior were continuously monitored from 00:00 on the day before the FNB (day -1) to 23:59 on the day following the procedure (day +1) using automated monitoring devices. Rumination time was assessed using the Hi-Tag Rumination Collar System (SCR Engineers Ltd., Netanya, Israel), which records rumination duration at two-hour intervals and transmits data via radio frequency to dedicated software for storage and analysis. Lying behavior was determined using data loggers (HOBO Pendant G Acceleration Data Logger; Onset Computer Corp., Bourne, MA, USA) attached to one of the hind limbs of the cow over the tibia-tarsal joint, using flexible self-adhesive bandages. These devices registered cow posture (lying or standing) at one-minute intervals. From these data, the daily lying time, number of lying bouts, and average bout duration were calculated, following the methodology described by Ledgerwood *et al*. (2010). Additionally, individual milk yield was recorded at each milking using a flow sensor (MPC580, DeLaval, Tumba, Sweden), with data exported and processed using DelPro® Software (DeLaval, Tumba, Sweden).

Statistical analysis

All statistical analyses were conducted using the 4.4.2 version of RStudio (R Foundation for Statistical Computing, Vienna, Austria; www.R-project.org), based on datasets generated in Microsoft Excel for each variable. A significance level of $P < 0.05$ was used to determine statistical differences. Only animals with complete datasets were included in this analysis. Due to the limited number of postpartum cows on the farm, all eligible animals from this group were enrolled to maximize the sample size and ensure adequate representation.

To compare behavioral responses between the Control and FNB groups in Experiment 1, the Mann–Whitney U test was used, as the behavioral variables did not meet the assumptions of normality, as assessed through the Shapiro-Wilk test.

In Experiment 2, data analysis was limited by a reduced sample size, resulting from data loss associated with device malfunctions (n = 10 cows) and power outages (n = 7 cows). The final sample sizes were as follows: lying time (n = 39), lying bouts (n = 38), bout duration (n = 38), rumination time (n = 41), and milk production (n = 31). The distribution of each variable was assessed using the Shapiro–Wilk test. Repeated measures ANOVA was conducted for variables with a normal distribution, with the individual cow considered a random effect and the day of observation (days -1, 0, and +1) as a fixed effect. Post hoc pairwise comparisons were performed using the Bonferroni test to compare values on days 0 and +1 to the baseline (day -1). Assumptions of absence of outliers and sphericity were evaluated, the latter using Mauchly's test. The Kruskal-Wallis test was applied for lying bouts and bout duration, which did not meet the normality assumption. The Wilcoxon test was performed as a post hoc analysis of nonparametric variables.

We assessed the statistical power ($1 - \beta$; post hoc) of all analyses performed in the study using the program G*Power for Windows, validated by Faul *et al*. (2007).

RESULTS

Experiment 1

We found no differences in the behaviors measured during the procedure between the Control and FNB groups ($P > 0.05$; Table 2). Behaviors were observed in most animals, including turning their head toward the right flank (in 65.4% of cows in both groups), kicking (in 76.8% of cows in the control and 84.6% in the FNB), and abrupt movements (in 73.1% of cows in the control and 92.3% in the FNB), although at low frequency. While these behaviors were prevalent in most animals, few cows exhibited them with a high frequency; therefore, the standard deviation for every behavior was higher than the mean (Figure 1).

Table 2. Frequency of behaviors exhibited by dairy cows during liver fine-needle biopsy (FNB biopsy) or sham procedure (control group).

Behavior	Control	FNB	*P*-value
	Median (Q1 – Q3)	Median (Q1 – Q3)	
Vocalization	0 (0 – 0)	0 (0 – 0)	0.57
Head shaking	0 (0 – 1.75)	0.5 (0 – 2.75)	0.41
Turn the head to the right flank	1 (0 – 2)	1 (0 – 2)	0.73
Kicks	2 (1 – 5)	4 (2 – 7.75)	0.17
Abrupt movements	1.5 (0.25 – 2.75)	3 (2 – 4)	0.07

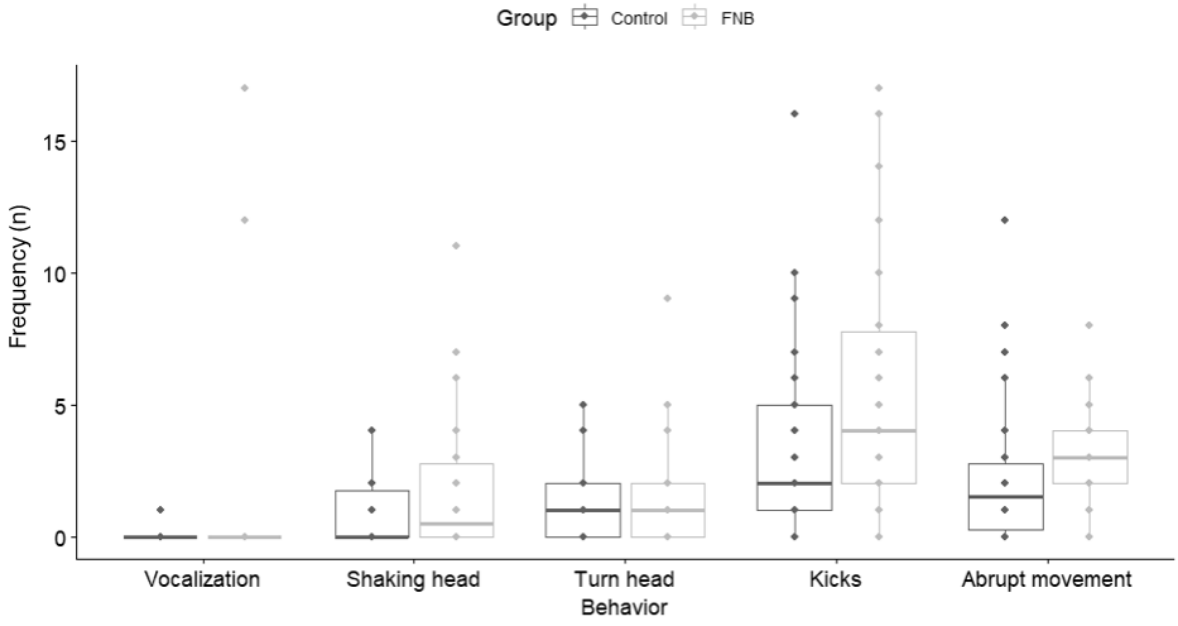

Figure 1. Boxplot for behaviors evaluated during liver fine needle biopsy (FNB) procedure (median of number of times that the behavior was performed).

Statistical power and effect size analysis showed that the behaviors shaking head and kicks had moderate power (71 and 46%, respectively) and moderate size effect (Cohen's d: 0.63 and 0.44, respectively); and vocalization, turning head to the right flank and abrupt movements had low power (32, 11 and 32%, respectively) and low effect size (0.34, 0.12 and 0.25, respectively).

Experiment 2

No significant differences in daily lying time were detected ($P > 0.05$) on the day of the FNB (day 0: 6.84 ± 1.7 h/day) or the following day (day +1: 7.65 ± 1.4 h/day) when compared to baseline (day -1: 7.21 ± 1.83 h/day; Figure 2A). However, cows in the FNB group exhibited more lying bouts on day +1 compared to the baseline (adjusted $P = 0.005$; Figure 2B), accompanied by a shorter average lying bout duration (adjusted $P = 0.021$; Figure 2C). Rumination time was not affected by FNB ($P = 0.668$), with mean (± SD) values of 7.72 ± 1.2, 7.56 ± 1.3, and 7.71 ± 1.1 h/day for days -1, 0, and +1, respectively (Figure 2D). Similarly, no effect was observed on daily milk production ($P = 0.996$), with mean (± SD) yields of 29.4 ± 4.74, 29.3 ± 4.57, and 29.4 ± 5.11 L/day on days -1, 0, and +1, respectively (Figure 3).

The statistical power detected for the variables corresponding to Experiment 2 was as follows: 99% for lying time, 57% for lying bouts and bout duration, 53% for rumination time, and 5% for milk production. The size effect was determined by ANOVA. In lying bouts and bout duration cases, *F*-value was set to 0.4.

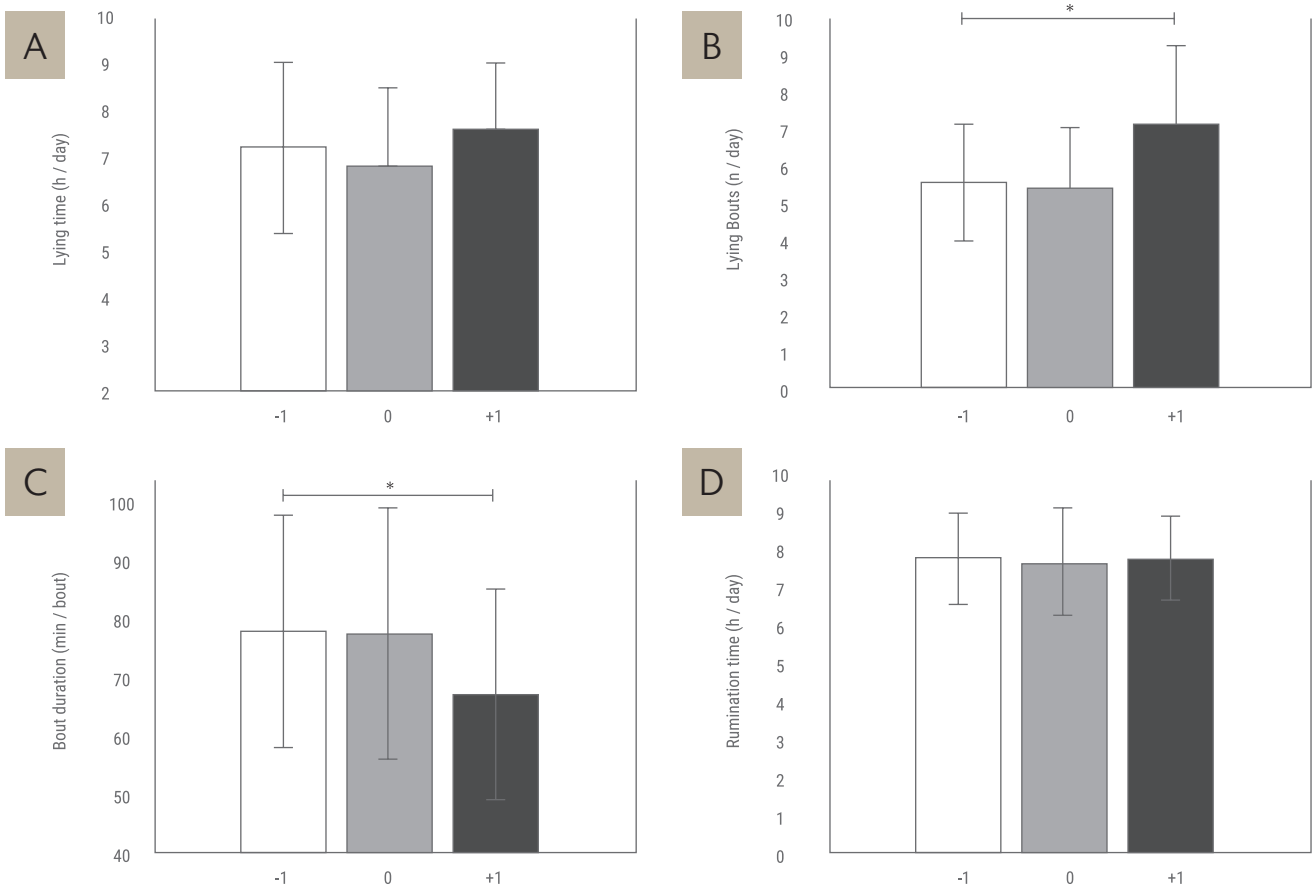

Figure 2. Lying time (A; h/d; n = 39), number of lying bouts (B; n/d; n = 38), lying bout duration (C; min/bout; n = 38), and daily rumination time (D; h/d; n = 41) (mean ± SD) over a three-days of observation period in dairy cows that underwent fine needle biopsy (FNB) at 10 days in milk (day 0). * $P < 0.05$.

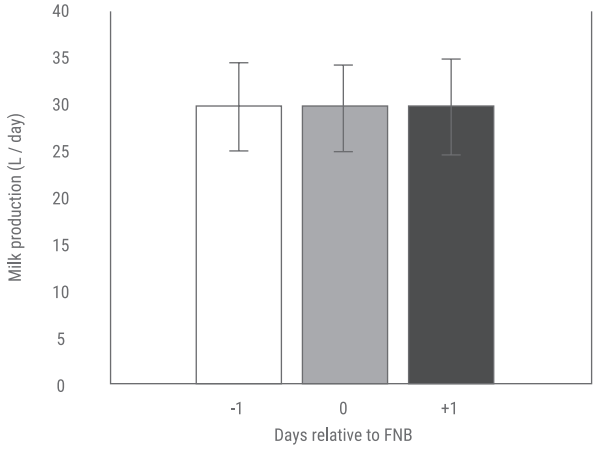

Figure 3. Milk production (L/day; n = 31; mean ± SD) over the three-days of observation in dairy cows that received a fine needle biopsy (FNB) at 10 days in milk (day 0).

DISCUSSION

This study investigated the behavioral responses and milk production of postpartum dairy cows during and after liver FNB. The stated hypothesis was partially rejected, as no significant differences in measured outcomes were detected during the procedure itself compared to the sham procedure. However, it is possible that the lack of statistical significance in the more frequent kicking and abrupt movements in the FNB group was due to the lack of statistical power. Similarly, no differences were detected in most of the measured outcomes on the day after the FNB procedure. However, cows in the FNB group had a small but significant increase in the number of lying bouts and a shorter average lying bout duration on day +1 compared to the baseline. Our interpretation of the collective results is that we cannot entirely exclude the possibility that cows may experience some level of pain or discomfort from liver FNB, considering that cows may experience pain related to FNB in a manner that could not be assessed in this study. However, if any, the FNB procedure has little immediate or short-term effect on behavior or milk production.

Behavior-based pain assessment has become increasingly common in animal research, particularly for evaluating the effects of painful procedures in cattle (Adcock & Tucker, 2018; Gleerup *et al*., 2015). Such assessments have been applied to procedures such as disbudding or dehorning (Stafford & Mellor, 2011), identification marking (Adcock & Tucker, 2018), and various tissue biopsies (Miller-Cushon *et al*., 2019; Mølgaard *et al*., 2012). While most studies have focused on post-procedural responses, our study offers novel insights into behavioral reactions during diagnostic procedures. We found that the behavioral responses during the liver FNB were similar to those observed during the sham procedure. This contrasts with conventional liver biopsy, which has been associated with pain-related behaviors in dairy cows, such as increased shaking and vocalizations, compared to control animals (Mølgaard *et al*., 2012). Similarly, splenic FNB in dogs has been shown to induce signs of discomfort during the procedure, including vocalizations and directed attention toward the puncture site (Launay *et al*., 2023). Among other factors, the needle gauge influences pain perception (Adcock & Tucker, 2018). For instance, Launay *et al*. (2023) reported reduced signs of discomfort when using a smaller-gauge spinal needle for splenic fine needle puncture in dogs. Based on these findings, it is plausible that liver FNB performed with 18G spinal needles may cause less pain than liver biopsy procedures, which typically use larger 16G needles or trocars.

Our study found that lying time remained consistent throughout the three-day observation period. However, a statistically significant difference was observed on day +1 when compared to baseline values, with an increase in the number of lying bouts and a decrease in their duration. Given the slight numerical variation, this result may have been influenced by the large deviation observed. Lying behavior in cattle can be affected by various procedures. For example, Miller-Cushon *et al*. (2019) reported that cows undergoing mammary biopsy exhibited increased lying time compared to controls. Conversely, dairy cows subjected to conventional liver biopsy displayed more restlessness post-procedure than during the control evaluation (Mølgaard *et al*., 2012). These findings suggest that, while painful procedures can alter lying behavior in dairy cattle, the specific effects appear to depend on the nature of the procedure.

We observed no differences in daily rumination time according to the baseline. Rumination is a highly motivated behavior that reflects an adequate state of health (Stafford & Mellor, 2011) and is commonly affected in the presence of disease, stress or painful procedures (Adcock & Tucker, 2018; Beauchemin, 2018; Sylvester *et al*., 2004). For example, a decrease in rumination time has been reported following procedures, such as liver biopsy (Mølgaard *et al*., 2012), disbudding (Sylvester *et al*., 2004), and castration (Pieler *et al*., 2013). The findings of the aforementioned studies suggest that such procedures induce pain, which may lead to temporary disruptions in rumination behavior.

Daily milk production remained consistent throughout the three observation days. Although changes in milk production may not always indicate impaired animal well-being, reductions can occur in response to illness or stress (Tucker *et al*., 2021). For instance, Newby *et al*. (2014) observed a decrease in milk yield on the day of surgery following a fistula procedure. Similarly, procedures such as right omentopexy or toggle suturing in cows with displaced abomasum have been shown to reduce milk yield by 15 to 30 liters over a 5-month period, compared to control cows (Melendez *et al*., 2017). These findings suggest that less invasive procedures, such as liver FNB, do not significantly affect milk production in dairy cows.

The limitations of this study are related to the small sample size used, which resulted in low statistical power in most analyses of the evaluated variables. Thus, we strongly recommend that future studies employ larger sample sizes, which will enable us to detect any differences among the procedures or days of observation, if they exist.

CONCLUSIONS

Our study demonstrated that in postpartum dairy cows, liver FNB induces a behavioral response similar to that of a sham procedure. Additionally, although we found a difference in lying bouts and bout duration on the day after the procedure, daily rumination, lying time, and milk production remained unaffected after FNB when compared to baseline values. These findings suggest that FNB is a procedure that elicits no painful responses, making it suitable for both clinical use in field conditions and research applications.

STATEMENTS & DECLARATIONS

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Statement of animal rights

The methods described in the present study were approved under permission numbers 459-2022 and 572-2025 by the Animal Care Committee at Universidad Austral de Chile.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Autor Contributions

E. Pavez-Muñoz and P. Sepúlveda-Varas conceived and designed the experiments. R. Chihuailaf and C. Ríos secured the project funding. E. Pavez-Muñoz conducted the fieldwork, performed the data analysis, and wrote the manuscript. A. Ruiz-Salazar and N. Fryderup assisted with fieldwork. P. Sepúlveda-Varas, R. Chihuailaf, J. P. Keim, M. M. Fry, and C. Ríos contributed to the manuscript revision. All authors reviewed and provided feedback on earlier versions of the manuscript and approved its final version.

Data Availability

The data set generated and/or analyzed during the current study is available from the corresponding author upon reasonable request.

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