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**Animal Health and Well Being**

# Quantification of cooling effects on basic tissue measurements and exposed cross-sectional brain area of cadaver heads from Holstein cows > 30 mo of age

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## Abstract

Penetrating captive bolt (PCB) is the primary method of preslaughter stunning for cattle and is also used for on-farm euthanasia. The objective of this study was to quantify the impact of cooling on the soft tissue thickness, cranial thickness, total tissue thickness, and cross-sectional brain area of cadaver heads collected from mature (> 30 mo of age) dairy cows following the application of a PCB stun in a frontal placement. Hide-on cadaver heads were obtained from culled dairy cows ($N = 37$) stunned in a frontal location using a handheld PCB device (Jarvis Model PAS—Type C 0.25R Caliber Captive Bolt, Long Bolt) at a commercial slaughter establishment. Following transport to the University of Wisconsin—River Falls, heads were split at midline along the bolt path by a bandsaw and then underwent FRESH, CHILL24, CHILL48, and CHILL72 refrigeration treatments. The FRESH treatment involved images collected immediately after splitting each head, the CHILL24 treatment involved images collected after 24 h of refrigeration, the CHIL48 treatment involved images collected after 48 h of refrigeration, and the CHILL72 treatment involved images collected after 72 h of refrigeration. Measurements of soft tissue thickness, cranial thickness, total tissue thickness, and cross-sectional brain area were recorded for each refrigeration treatment. Soft tissue thickness did not differ caudal to ($P = 0.3751$) or rostral to ($P = 0.2555$) the bolt path. Cranial thickness did not differ caudal to ($P = 0.9281$) or rostral to ($P = 0.9051$) the bolt path. Total tissue thickness did not differ caudal to ($P = 0.9225$; FRESH: 24.77 mm, CHILL24: 23.93 mm, CHILL48: 24.27 mm, CHILL72: 42.30, SE: 0.86) or rostral to ($P = 0.8931$; FRESH: 24.09 mm, CHILL24: 23.99, CHILL48: 24.26, CHILL72: 24.43 mm, SE: 0.79 mm) the bolt path. Cross-sectional brain area was not different ($P = 0.0971$) between refrigeration treatments (FRESH: 9,829.65 ± 163.87 mm$^2$, CHILL24: 10,012.00 ± 163.87 mm$^2$, CHILL48: 9,672.43 ± 163.87 mm$^2$, CHILL72: 10,235.00 ± 166.34 mm$^2$). This study demonstrated that FRESH tissue parameters can be determined from cattle cadaver heads refrigerated for 24, 48, or 72 h.

## Lay Summary

Euthanasia and preslaughter stunning are important to animal welfare. Penetrating captive bolt (PCB) is the primary method of preslaughter stunning for cattle and is also used for euthanasia. PCB causes severe brain damage by passing a metal bolt through the skull and into the brain of the animal. An immediate loss of consciousness should result from this process. There has been a growing interest in understanding various aspects of PCB stunning and euthanasia for cattle, including the impacts of bolt length and PCB placement. Much of the literature in this space includes the assessment of brain damage. The brain is a semifluid structure, which makes it difficult to assess brain damage after splitting cadaver heads with a bandsaw without refrigeration. It is unknown whether refrigerated cow heads can be used as a model for fresh heads, or if refrigeration significantly modifies the tissues. This study evaluated the impact of four refrigeration periods (0, 24, 48, and 72 h) on tissue parameters associated with the frontal PCB placement using cadaver heads from culled dairy cows. Refrigeration time did not change the tissue parameters, suggesting that a refrigeration period can be used to improve brain rigidity without mathematical adjustments to tissue parameters.

**Key words:** captive bolt, cow, euthanasia, stunning, welfare

## Introduction

Penetrating captive bolt (PCB) is the primary method of preslaughter stunning used for cattle in North America. It is also an approved method of euthanasia for cattle (American Veterinary Medical Association [AVMA], 2016, 2020; Humane Slaughter Association [HSA], 2016; Canadian Food Inspection Agency [CFIA], 2019; American Association of Bovine Practitioners [AABP], 2023). PCB is also a method of stunning and euthanasia for other livestock species, including swine (AVMA, 2016, 2020; HSA, 2016; National Pork Board and American Association [NPB and AASV], 2016; CFIA, 2019), sheep (AVMA, 2016, 2020; HSA, 2016; CFIA, 2019), and goats (AVMA, 2016, 2020; HSA, 2016; CFIA, 2019). Recently, there has been an interest in better understanding various aspects of PCB use for cattle (Gilliam et al., 2012, 2016, 2018; Kline et al., 2019; Wagner et al., 2019; Casagrande et al., 2020; Robbins et al., 2021, 2023) and swine (Anderson et al., 2019, 2021a; Kramer et al., 2021). For cattle, varying bolt lengths (Kline et al., 2019;

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Wagner et al., 2019), physical landmarks for the frontal PCB placement (Gilliam et al., 2012, 2016, 2018), security stuns (Casagrande et al., 2020), and a second shot in the poll placement (Robbins et al., 2021, 2023) have been evaluated. For swine, alternative PCB placements have been evaluated for market hogs (Anderson et al., 2019) and sows and boars (Anderson et al., 2021a; Kramer et al., 2021).

Kline et al. (2019) described that assessing macroscopic brain damage following PCB stunning on heads from fed cattle that were not refrigerated was difficult. Specifically, differentiating damage from the PCB and tissue distortion that may occur during the splitting process may be difficult with heads that are not cooled prior to splitting (Kline et al., 2019; Anderson et al., 2021b). Anderson et al. (2021b) evaluated the impact of cooling on tissue parameters associated with a frontal PCB application for market hogs. At the time of the present study, there did not appear to be any published data to quantify the impact of cooling on the tissue parameters associated with cadaver heads from cattle following a PCB application. Wagner et al. (2019) and Casagrande et al. (2020) both utilized chilled heads to evaluate brain damage following PCB stuns applied as part of the slaughter process for fed cattle, but the tissue thicknesses associated with the bolt path were not assessed. Understanding whether tissue parameters change with the cooling of cadaver heads is important for future studies related to PCB stunning and euthanasia. Cooling cadaver heads prior to splitting allows for improved accuracy in brain damage assessments because there is less tissue distortion from the splitting process and the bolt path is visually distinguished (Anderson et al., 2021b).

The objective of this study was to quantify the impact of cooling on the soft tissue thickness, cranial thickness, total tissue thickness, and cross-sectional brain area of cadaver heads collected from mature (> 30 mo of age) commercially slaughtered dairy cows following the application of a penetrating captive bolt (PCB) stun in a frontal placement. Our hypothesis was that refrigeration treatments would not impact the soft tissue thickness, cranial thickness, total tissue thickness, or cross-sectional brain area measurements.

## Materials and Methods

### Animal Use Protocol

It was not necessary to submit an animal use protocol to the University of Wisconsin—River Falls Institutional Animal Care and Use Committee because live animals were not directly manipulated in this study. The cows from which the heads were obtained were slaughtered at a commercial slaughter establishment under inspection by the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) in accordance with the Humane Methods of Slaughter Act (7 USC 1901; United States House of Representatives Office of the Law Revision Council, 2023) and the regulations that enforce it (9 CFR 313; United States Electronic Code of Federal Regulations, 2023). This followed the precedent set by Anderson et al. (2019, 2021a, 2021b) and Hamilton et al. (2023) for an exemption from Institutional Animal Care and Use Committee approval.

### Description of Cadaver Heads

Thirty-seven heads ($N$ = 37), with the hide on, were obtained from mature (> 30 mo of age) Holstein dairy cows (average hot carcass weight = 279.43 ± 43.28 kg, mean ± SD) that were commercially slaughtered at a regional processing facility under federal inspection. All source animals were stunned with a PCB (Jarvis Model PAS-Type C 0.25R Caliber Captive Bolt, Long Bolt; Order #: 4144062, Jarvis Corp., Middletown, computed tomography) with Jarvis Black HMF (heavy metal free) 0.25R Caliber, 5 GR (Order #: 1176036). A power calculation to determine sample size was conducted using the POWER procedure of SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC) with the following parameters: detectable difference of 0.8, SD of 1.2, and power of 0.8. The detectable difference was derived from soft tissue thickness measurements caudal to the bolt path of market hogs at 0 and 24 h of refrigeration, and the SD value was back-calculated from the SE values for the same measurements (Anderson et al., 2021b). All heads were removed from their respective carcasses by plant personnel via knife incision between the atlas and axis. All heads were placed in large storage totes with secured lids (Sterilite 1466 - 27 Gallon Industrial Tote, Sterilite Corp., Townsend, MA) with two heads per tote, and transported (distance traveled: 201 km; duration: ~ 2 h) to the University of Wisconsin—River Falls Meat Science Laboratory and Animal Welfare Lab.

### Head Processing

Upon arrival at UWRF, dentition was used to confirm that each head was sourced from a cow over 30 mo of age. Heads were considered to be from cows over 30 mo of age when at least one of the second set of permanent incisors had erupted (i.e., passed through the gumline; USDA FSIS, 2021). Stun placement was also assessed for each head. The target stun placement was at the intersection of two imaginary lines, each drawn from the lateral canthus of the eye to the center of the base of the opposite horn or poll (AVMA, 2016, 2020; AABP, 2023). This target placement was identified for each head and a transparency sheet with a bullseye diagram was placed such that the center of the bullseye was located at the target stun placement. Then, the actual stun placement was marked on the transparency sheet with permanent marker. All dentition and stun placement assessments were performed by AAR and KDV. Each head was split along the bolt path following the PCB application with a Hobart 6801 Vertical Meat Band Saw (Hobart, Troy, OH) equipped with a 0.06 mm thick, 360.68 cm long blade with 4 teeth/2.54 cm and a 3-degree hook angle (Product #: C78529545, Bunzl Processor Division, Riverside, MO), as described by Anderson et al. (2021a) and Hamilton et al. (2023). All heads were split prior to refrigeration treatments.

After each cut, digital and thermal images were captured for the left and right sides of each head with the cameras positioned 50.8 cm directly above and perpendicular to the exposed cut surface (Anderson et al., 2021a; Hamilton et al., 2023). Digital images were taken with a digital camera (Olympus Tough TG-6 Waterproof Camera, OM Digital Solutions Corp., Tokyo, Japan) and included a 15 cm ruler for reference. Thermal images were collected using a FLIR E8 thermal camera (FLIR Systems, Boston, MA); for object temperature above 0 °C and ambient temperatures between 10 and 35 °C, the temperature emission accuracy for this camera was ± 0.2 °C or ± 2% of reading.

### Refrigeration Treatments and Thermal Image Assessment

The FRESH treatment referred to the head weights and images collected immediately following each cut. It should be noted that the FRESH images were collected at least 2 h after exsanguination and following transport to UWRF; this was the soonest that images could be collected postmortem. After these images were collected, both sides of each head were placed into a clean storage tote, with two heads per tote. Each storage tote was stored with the lid slightly offset in a walk-in cooler held at 2 to 4 °C for a total of 72 h. Heads were removed from the cooler and the imaging process was repeated at 24 h (CHILL24), 48 h (CHILL48), and 72 h (CHILL72) after the FRESH images were collected. All heads were placed into clean storage totes, as previously described, and stored in the walk-in cooler between the CHILL24, CHILL48, and CHILL72 timepoints. Using thermal images, the maximum intracranial temperature of each head at each timepoint (FRESH, CHILL24, CHILL48, and CHILL72) was determined using the FLIR Tools software (FLIR Systems), as described by Anderson et al. (2021b).

### Tissue Measurements

Following the methods described by Anderson et al. (2021a, 2021b), measurements of soft tissue thickness (mm), cranial thickness (mm), and cross-sectional brain area ($mm^2$) (Figure 1) were determined from digital images of both sides of each head at the FRESH, CHILL24, CHILL48, and CHILL72 timepoints using an online irregular area calculator (SketchandCalc, iCalc, Inc., Palm Coast, FL). All tissue depth and cross-sectional brain area measurements were performed by KNA or EMH.

Tissue depth measurements were adapted from Anderson et al. (2019, 2021a, 2021b) and Hamilton et al. (2023). Soft tissue thickness (mm) referred to the tissue from the PCB application site to the exterior surface of the cranium along the bolt path and cranial thickness (mm) referred to the exterior surface of the cranium to the interior surface of the cranium along the bolt path. Total tissue thickness (mm) was the total distance along the bolt path from the PCB application to the interior surface of the cranium and was determined by the summation of soft tissue thickness and cranial thickness. The tissue depth measurements of all heads were determined by averaging the measurements from the left and right sides of each head for the caudal and rostral aspects of the bolt path (Anderson et al., 2021b). Cross-sectional brain area ($mm^2$) referred to the cross-sectional area of the exposed brain within the plane of bolt travel (Anderson et al., 2019, 2021a, 2021b) and was determined for the left and right side of each head; these measurements were averaged prior to statistical analysis.

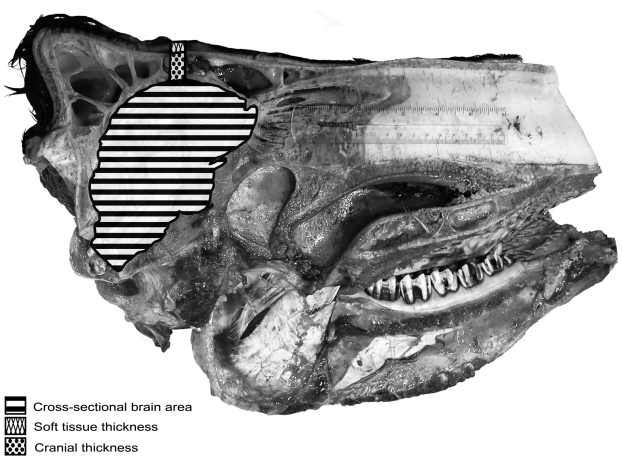


**Figure 1.** Tissue thickness and brain area measurements. Soft tissue thickness—the tissue from the application site on the surface of the skin to the exterior surface of the cranium. Cranial thickness—the tissue from the exterior surface of the cranium to the interior surface of the cranium along the bolt path. Cross-sectional brain area—the cross-sectional surface area of the exposed brain within the plane of bolt travel. Soft tissue thickness and cranial thickness were measured at the caudal and rostral aspects of the bolt path on the right and left sides of each split head. Values were averaged prior to statistical analysis.

### Statistical Analyses

All continuous data outcomes (head weight, maximum head temperature, soft tissue thickness caudal to the bolt path, soft tissue thickness rostral to the bolt path, cranial thickness caudal to the bolt path, cranial thickness rostral to the bolt path, total tissue thickness caudal to the bolt path, total tissue thickness rostral to the bolt path, and cross-sectional brain area) for treatment effects (FRESH, CHILL24, CHILL48, and CHILL72) were assessed for normality via the histogram statement within the UNIVARIATE procedure of SAS Enterprise Guide 7.1 (Statistical Analysis Systems Institute, Inc. These outcomes were then analyzed for refrigeration treatment effects (FRESH, CHILL24, CHILL48, and CHILL72) with models constructed in the MIXED procedure of SAS Enterprise Guide 7.1 (Statistical Analysis System Institute, Inc.) with the Satterthwaite method for calculation of degrees of freedom. A repeated statement with head identification number as the subject was included in all models. Models included the main effect of treatment (FRESH, CHILL24, CHILL48, and CHILL72) only. Mean separation was determined using Student's *T*-tests, protected by the Bonferroni-Holm adjustment for multiple comparisons. Differences between means were recognized as significant when $P < 0.05$.

## Results and Discussion

Weights, temperatures, tissue thickness measurements, and cross-sectional brain areas collected from this study can be observed in Table 1 and are reported as mean ± SE.

There was no evidence ($P = 0.8890$) to support a difference in head weight between the FRESH (7.37 ± 0.12 kg), CHILL24 (7.25 ± 0.17 kg), CHILL48 (7.22 ± 0.17 kg), or CHILL72 (7.20 ± 0.17 kg) treatments. This is consistent with the findings of Anderson et al. (2021b), who reported that a 24 h refrigeration period did not impact the head weight of cadaver heads from market hogs.

The greatest ($P < 0.05$) maximum head temperature was observed in FRESH heads (31.72 ± 0.18 °C). Maximum head temperature decreased ($P < 0.05$) to 9.14 ± 0.18 °C after 24 h of refrigeration (CHILL24) and further decreased ($P < 0.05$) to 7.80 ± 0.18 °C after an additional 24 h of refrigeration (CHILL48). There was no evidence to support a difference ($P > 0.05$) in head temperature between 48 (CHILL48) and 72 h of refrigeration (CHILL72, 7.77 ± 0.20 °C). Anderson et al. (2021b) observed a 27.9 °C decrease in mean head temperature following a 24 h refrigeration period for cadaver heads from market hogs; in the present study, the mean head

**Table 1.** Effects of cooling on head weight, temperature, and tissue parameters of cadaver heads from mature (> 30 mo of age) Holstein dairy cows

| Dependent Variable | Treatment[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FRESH | | | CHILL24 | | | CHILL48 | | | CHILL72 | | |
| | *n* | LS Means | SE | *n* | LS Means | SE | *n* | LS Means | SE | *n* | LS Means | SE |
| Head weight, kg | 37 | 7.37 | 0.17 | 37 | 7.25 | 0.17 | 37 | 7.22 | 0.17 | 37 | 7.20 | 0.17 |
| Max head temp, ° C | 37 | 31.72[a] | 0.18 | 37 | 9.14[b] | 0.18 | 36 | 7.80[c] | 0.18 | 32 | 7.77[c] | 0.20 |
| Soft tissue thickness caudal, mm | 37 | 6.34 | 0.23 | 37 | 6.05 | 0.23 | 37 | 6.69 | 0.23 | 37 | 6.45 | 0.23 |
| Soft tissue thickness rostral, mm | 37 | 6.03 | 0.23 | 37 | 6.18 | 0.23 | 37 | 6.57 | 0.23 | 37 | 6.16 | 0.23 |
| Cranial thickness caudal, mm | 37 | 18.39 | 0.86 | 37 | 17.88 | 0.86 | 37 | 17.58 | 0.86 | 37 | 17.85 | 0.86 |
| Cranial thickness rostral, mm | 37 | 18.08 | 0.78 | 37 | 17.73 | 0.78 | 37 | 17.69 | 0.78 | 37 | 17.25 | 0.78 |
| Total tissue thickness caudal, mm | 37 | 24.77 | 0.86 | 37 | 23.93 | 0.86 | 37 | 24.27 | 0.86 | 37 | 24.30 | 0.86 |
| Total tissue thickness rostral, mm | 37 | 24.09 | 0.79 | 37 | 23.99 | 0.79 | 37 | 24.26 | 0.79 | 37 | 23.43 | 0.79 |
| Brain area, $mm^2$ | 34 | 9,829.65 | 163.87 | 34 | 10,012.00 | 163.87 | 34 | 9,672.43 | 163.87 | 33 | 10,235 | 166.34 |

[1]FRESH no refrigeration treatment; CHILL24 24 h of refrigeration at 2 to 4 °C; CHILL48 48 h of refrigeration at 2 to 4 °C; CHILL72 72 h of refrigeration at 2 to 4 °C.
[a-c]Superscripts that differ within a row identify significant differences between means within dependent variables across refrigeration treatments ($P \leq 0.05$).

temperature decreased 22.58 °C following a 24 h refrigeration period. The lesser decrease in head temperature observed in the current study may have been the result of different storage methods during the refrigeration period. Anderson et al. (2021b) wrapped each head with polyvinylchloride film to hold the two sides of the head together during the 24 h refrigeration period; wrapped heads were then stored on a metal shelf in the walk-in cooler. Heads in the present study were stored in large storage totes, with two heads per tote, which may have provided some level of insulation and prevented the same rate of temperature decline as observed by Anderson et al. (2021b). Anderson et al (2021a) reported that the mean temperature of cadaver heads from mature sows and boars was 7.70 °C following a refrigeration period of approximately 62 h, which is similar to the head temperatures observed at 48 and 72 h of refrigeration in our study. The heads in the Anderson et al. (2021a) study were stored in a similar manner to those in the current study. It is also possible that morphological differences, such as differences in adipose deposition and composition may have contributed to the lesser degree of cooling observed in the present study compared to Anderson et al. (2021b). Wood et al. (2008) described that both the muscle and adipose tissue of pork contain higher proportions of unsaturated fat than beef.

There was no evidence to support a difference in soft tissue thickness caudal ($P = 0.3751$) or rostral ($P = 0.2555$) to the bolt path between refrigeration treatments. Soft tissue thickness caudal to the bolt path was 6.34 ± 0.23 mm for FRESH heads, 6.05 ± 0.23 mm for CHILL24 heads, 6.69 ± 0.13 mm for CHILL48 heads, and 6.45 ± 0.22 mm for CHILL72 heads. Soft tissue thickness rostral to the bolt path was 6.03 ± 0.23 mm for FRESH heads, 6.18 ± 0.23 mm for CHILL24 heads, 6.57 ± 0.22 mm for CHILL48 heads, and 6.16 ± 0.23 mm for CHILL72 heads. Anderson et al. (2021b) reported a decrease in the soft tissue thickness, both caudal and rostral to the bolt path, of cadaver heads from market hogs following a 24 h refrigeration period and found that a correction factor of 1.12 was necessary to determine the soft tissue thickness of unrefrigerated cadaver heads from heads that had been chilled for 24 h. It is possible that the differences between that study and the present one are due to species differences between cattle and swine. It is also possible that the differences between the two studies result from how heads were stored during the refrigeration period: Anderson et al. (2021b) wrapped each swine head with polyvinylchloride film prior to refrigeration, which could have compressed the soft tissue.

There was no evidence to support a difference in cranial thickness caudal ($P = 0.9281$) or rostral ($P = 0.9051$) to the bolt path between refrigeration treatments. Cranial thickness caudal to the bolt path was 18.39 ± 0.86 mm for FRESH heads, 17.88 ± 0.86 mm for CHILL24 heads, 17.58 ± 0.86 for CHILL48 heads, and 17.85 ± 0.86 for CHILL48 HEADS. Cranial thickness rostral to the bolt path was 18.08 ± 0.78 mm for FRESH heads, 17.23 ± 0.78 for CHILL24 heads, 17.69 ± 0.78 mm for CHILL48 heads, and 17.25 ± 0.78 mm for CHILL48 heads. There was no evidence to support a difference in total tissue thickness caudal ($P = 0.9225$) or rostral ($P = 0.8931$) to the bolt path. Total tissue thickness caudal to the bolt path was 24.77 ± 0.86 mm for FRESH heads, 23.93 ± 0.86 mm for CHILL24 heads, 24.27 ± 0.86 mm for CHILL48 heads, and 24.30 ± 0.86 mm for CHILL48 heads. Total tissue thickness rostral to the bolt path was 24.09 ± 0.79 mm for FRESH heads, 23.99 ± 0.79 for CHILL24 heads, 24.26 ± 0.79 for CHILL48 heads, and 24.43 ± 0.79 mm for CHILL72 heads. This has been demonstrated in previous research (Anderson et al., 2021b), where the cranial thickness and total tissue thickness of market hog heads did not change following a 24 h refrigeration period.

There was no evidence to support a difference ($P = 0.0971$) in cross-sectional brain area between the FRESH (9,829.65 ± 163.87 $mm^2$), CHILL24 (10,012.00 ± 163.87 $mm^2$), CHILL48 (9,672.43 ± 163.87 $mm^2$), or CHILL72 (10,235.00 ± 166.34 $mm^2$) heads. Anderson et al. (2021b) reported that a 24 h refrigeration did not impact the cross-sectional brain area of cadaver heads from market hogs. A limitation of this study is that tissue thickness and brain area measurements could not be obtained until heads had arrived at UWRF; as a result, the soonest after exsanguination that FRESH images were taken was 2 h. It is unknown whether the 2 h transportation from the slaughter establishment to UWRF, or the time between exsanguination and splitting, impacted tissue thickness or brain area measurements.

Gilliam et al. (2012, 2016) reported challenges associated with the use of cadaver heads from cattle that were frozen and then thawed prior to use. Traumatic brain injury (TBI) could not be reliably assessed for heads that were frozen and then thawed 36 h prior to PCB application (Gilliam et al, 2012). The TBI assessment metric described by Gilliam et al. (2012) had been used successfully for horseheads following gunshot (Millar and Mills, 2000). This TBI scoring system has since been successfully used for brains from swine that had recently received a PCB application (Woods, 2012; Kramer et al., 2021). Gilliam et al. (2016) suggested that brain tissue may have shifted back into the bolt path as a result of freezing and thawing the heads, leading to challenges in identifying the bolt path via computed tomography scans of the heads.

Due to the semifluid structure of the brain, Kline et al. (2019) recommended a 12 to 24 h cooling time prior to splitting cadaver heads in future studies because of challenges associated with assessment of brain damage when heads are not cooled. Following this recommendation, various head cooling times have been employed prior to splitting cadaver heads to assess brain damage following a PCB application and challenges with brain damage assessment have not been reported (Wagner et al., 2019; Casagrande et al., 2020; Anderson et al., 2021a). Wagner et al. (2019) collected cattle heads from a slaughter establishment and cooled the heads for 24 h at 0 °C prior to splitting. Similarly, Casagrande et al. (2020) collected cattle heads from a slaughter establishment and cooled them at approximately 48 h at 2 °C prior to splitting. Anderson et al. (2021a) collected sow and boar heads from a slaughter establishment and cooled the heads for approximately 62 h at 2 to 4 °C prior to splitting. In the present study, heads were split prior to refrigeration to facilitate the comparison of FRESH, CHILL24, CHILL48, and CHILL72 tissue thickness measurements.

The results of the present study suggest that tissue depth and brain area measurements were not impacted by a cooling period of up to 72 h.

## Implications

This study was intended to determine whether a refrigeration period of 0, 24, 48, or 72 h impacted tissue parameters of cadaver heads from cattle following a PCB application. Our results did not identify differences in soft tissue thickness, cranial thickness, total tissue thickness, or cross-sectional brain area between refrigeration treatments. Understanding the effect, or lack thereof, of cooling on the tissue dimensions of cattle heads is important and valuable for future investigations of PCB stunning and euthanasia that use cadaver heads as a model.

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## Conflict of interest statement

The authors have no conflicts of interest to disclose.

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