Translational Animal Science, 2023, **7**, txad103
https://doi.org/10.1093/tas/txad103
Advance access publication 28 August 2023
Animal Health and Well Being

Evaluation of tissue depth, captive bolt penetration force and energy, and potential for bolt-thalamus contact in cadaver heads from physically castrated market barrows and immunocastrated boars

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Abstract

The main objective of this study was to describe tissue thicknesses of cadaver heads from physically castrated market barrows (PC MARKET BARROWS) and immunocastrated boars (IC BOARS) at the frontal penetrating captive bolt (PCB) placement. Other objectives were to describe differences in bolt force and energy requirements to penetrate and describe potential for bolt-thalamus contact. Forty-four heads were obtained from PC MARKET BARROWS (n = 22) and IC BOARS (n = 22) of similar age and size that were rendered insensible with CO_2. Mean HCW was 117.32 ± 3.52 kg. Snout to poll distance (cm) and maximum deflection distance (cm) were collected in duplicate. Heads were split at midline with a bandsaw and soft tissue and cranial thicknesses were measured with a digital caliper. Images of each cut surface were collected to evaluate the potential for thalamic damage. Tissue samples were retained from each half of each head and a universal tester was used to determine maximum force and energy of bolt penetration. There was no evidence to support a significant difference (P > 0.05) in tissue thicknesses between PC MARKET BARROWS and IC BOARS. Maximum deflection distance (maximum distance from a straight edge that was placed from the tip of the snout to the poll of the head) was not different (P = 0.10) between PC MARKET BARROWS (3.31 ± 0.10 cm) and IC BOARS (3.08 ± 0.10 cm). There was no evidence to support a difference (P = 0.77) in maximum force between PC MARKET BARROWS (7130.32 ± 483.23 N) and IC BOARS (6974.60 ± 463.70 N). There was also no evidence to support a difference (P = 0.62) in maximum energy between PC MARKET BARROWS (33.37 ± 2.77 J) and IC BOARS (32.04 ± 2.50 J). For PC MARKET BARROWS, there was a difference (P = 0.05) between the number of heads where the thalamus was located within the theoretical plane of bolt travel for market placement (21/21) versus mature placement (16/21). For IC BOARS, the number of heads where the thalamus was located within the plane of theoretical bolt path was not different between the two PCB placements (19/21 each). Overall, the data suggest that tissue profiles of PC MARKET BARROWS and IC BOARS do not differ at the frontal PCB placement site and the mechanical tools that are effective for PC MARKET BARROWS should also be effective for IC BOARS.

LAY SUMMARY

Euthanasia is a procedure that cannot be avoided on swine farms. One common method for euthanizing swine is penetrating captive bolt (PCB); this involves passing a metal bolt through the skull and into the brain, resulting in immediate unconsciousness. PCB use has been evaluated for market hogs and breeding sows and boars, but not for immunocastrated boars. It is unknown whether the skull thickness of immunocastrated boars is similar to that of market hogs, or if it increases and resembles that of mature breeding swine. Immunocastrated boars are becoming more common in US swine production because the painful procedure of castration is eliminated, the expression of sexual characteristics and associated meat quality defects are prevented. Evaluating the tissue profile at the common PCB application site on the front of the head allows for caretakers to select appropriate PCB devices for euthanasia of immunocastrated boars. We found that there was no evidence to support a difference between soft tissue thickness, cranial thickness, total tissue thickness, captive bolt penetration force and energy, and potential for bolt-thalamus contact between market hogs and immunocastrated boars. This finding suggests that PCB devices that are effective for market hogs should also be effective for immunocastrated boars.

Key words: captive bolt, euthanasia, immunocastration, stunning, swine, welfare

Introduction

Penetrating captive bolt (PCB) is an approved method of euthanasia for grow-finish and breeding swine (NPB and AASV, 2016; AVMA, 2020) and is commonly used to euthanize animals on-farm or upon arrival at a slaughter establishment, if needed. The use of a single PCB application to the front of the head has been validated for the euthanasia of market hogs (Woods, 2012) and these animals are rendered immediately insensible following this procedure. However, as pigs age, skull thickness increases, and PCB may become less effective

Received May 19, 2023 Accepted August 24, 2023.

at rendering an animal insensible (Woods, 2012; HSA, 2016; AVMA, 2020; EFSA, 2020; Anderson et al., 2022). This makes pigs one of the most difficult species to effectively stun using a PCB (HSA., 2016).

Immunocastrated (IC) boars have become more common in the United States over the past 20 years because a painful procedure (physical castration) is eliminated from a pig's life (Dunshea et al., 2013; McGlone, 2013). As farms work toward sustainability, eliminating harm from animals is an important focus of that goal (McGlone, 2013). Traditionally, pigs are physically castrated; this involves restraining the pig, making an incision on the scrotum, and using pressure to remove each testicle. This procedure usually occurs within the first 7 days of age (McGlone, 2013; Kress et al., 2019) and does not involve the use of pain mitigation, before, during, or after completion (McGlone, 2013). After the procedure, pigs have been observed presenting painful indicators including trembling, leg shaking, sliding, and tail jerking (reviewed by Ison et al., 2016). With immunocastration, two doses of a vaccine that immunized against GnRH are administered. This prevents the expression of sexual characteristics in the male, ultimately preventing the meat quality defect of boar-taint (Kress et al., 2019). The first dose of the vaccine is administered at approximately 12 weeks of age, and then the second dose is administered 4 to 6 weeks prior to slaughter (Kress et al., 2019). Improvest, a vaccine produced by Zoetis, should be administered at a dose of 2 mL no earlier than 9 weeks of age, and then repeated at a dose of 2 mL at least 4 weeks after the first dose. Male pigs should be slaughtered between 3 and 10 weeks after the second dose (Zoetis, 2020). Previous research shows that IC boars have a greater daily weight gain than physically castrated boars (Kress et al., 2019).

As boars mature, their skull thicknesses increase (NPB and AASV, 2016; Anderson et al., 2022). At the time of this study, it was not known whether the skull thickness of IC boars was similar to that of market barrows or if the skull thickness of IC boars more closely resembled that of a mature boar. Ensuring that all animals are rendered immediately insensible following a single PCB application is important for preventing pain and distress, and also ensures compliance with the Humane Methods of Slaughter Act (7 USC 1901) (United States House of Representatives Office of the Law Revision Counsel., 2023) and the regulations that enforce it (9 CFR 313) (United States Electronic Code of Federal Regulations, 2023). Compliance with this is critical for all animals on the property of a slaughter establishment, making it applicable to any animals that may need to be euthanized there as well.

There have been anecdotal reports that IC boars may respond differently to a PCB application than physically castrated market barrows (PC MARKET BARROWS), which creates concern and indicates the need for research to evaluate factors that may contribute to this. However, there is currently no peer-reviewed literature evaluating the PCB use for IC boars. It is unknown whether these observations are due to potential differences in skull thickness.

The primary objective of this study was to describe the tissue thicknesses of cadaver heads from PC MARKET BARROWS and IC BOARS at the common frontal PCB placement. Secondary objectives of the study included a) to describe the differences in force and energy requirements to penetrate the skull and b) to describe the potential for bolt-thalamus contact in PC MARKET BARROWS and IC BOARS. Our hypothesis was that differences in soft tissue thickness, cranial thickness, and total tissue thickness would be detected between heads from PC MARKET BARROWS and IC BOARS.

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 (IACUC) because live animals were not directly manipulated in this study. The live pigs from which the cadaver 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 regulations in 9 CFR 313 (United States Electronic Code of Federal Regulations, 2023). This exemption from IACUC approval followed the precedent established by Anderson et al. (2019, 2021a, 2021b).

Description of Cadaver Heads

All data was collected on cadaver heads, to avoid the use of live animals in this research. This was done as it was not necessary to use live animals in order to quantify tissue thickness differences between PC MARKET BARROWS and IC BOARS. A total of 44 cadaver heads were obtained from PC MARKET BARROWS ($n = 22$) and IC BOARS ($n = 22$). 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 5, SD of 3.8, power of 0.8. The SD value was based on the SE value for total tissue thickness in Anderson et al. (2019). Source animals for each group were of similar age and genetic background. Each animal in the IC BOARS group was given a dose of Improvest (Gonadotropin Releasing Factor Analog-Diptheria Toxoid Conjugate, 0.2 mg/mL, Zoetis, Parsippany-Troy Hills, New Jersey) at week 33, and then a second dose at week 41. IC BOARS were slaughtered 130 d following placement in the finishing barn, and PC MARKET BARROWS were slaughtered 139 d following placement in the finishing barn. The pigs were rendered insensible with controlled atmosphere (CO_2), and heads were removed from their respective carcass following exsanguination. HCW (reported as mean ± SE) was collected for each animal (117.32 ± 3.52 kg).

After the heads were removed from their carcasses, the ears and jowls were removed from each head before they were frozen and shipped under refrigeration for head processing (approximate distance traveled: 1,931 km). Each head was individually bagged, and 6 to 7 heads were placed in a shipping cooler that consisted of a Styrofoam cooler within a cardboard box. Upon arrival at the University of Wisconsin—River Falls Meat Science Laboratory, the heads remained packaged in the same shipping coolers. The shipping coolers were stored in a walk-in cooler for ~69 h at 2 to 4 °C until the commencement of data collection. Heads remained frozen from the time of arrival to the time of the commencement of data collection. The use of frozen or chilled heads allows for the brain to stay intact when heads are split, as the tissue is more rigid, which allows for better identification of brain structures (Kline et al., 2019; Anderson et al., 2021a). At the time of head processing, the exposed internal cranial surface temperature (reported as mean ± SE) for all heads was 1.5 ± 0.2 °C.

Head Processing and Measurements

On the day of head processing, shipping coolers were removed from the walk-in cooler one at a time. The heads were then removed individually from their bags. Upon removal from the bags, the following external head measurements were collected in duplicate, by two observers, using a flexible measuring tape (Singer 218 60 in, The Singer Company Ltd., Boston, MA): snout to poll distance (cm) and maximum deflection distance (cm) (Figure 1). Snout to poll distance referred to the distance from the tip of the snout to the first point of contact between a taught measurement tape at the crest of the head. Maximum deflection distance referred to the maximum distance from a straight edge that was placed between the tip of the snout and the poll or the first point where the straight edge touched the head when placed from the tip of the snout. These measurements were adapted from Anderson et al. (2021b) and values were averaged prior to statistical analysis. The mean interobserver percent coefficient of variation for snout to poll distance was 1.9%. The mean interobserver percent coefficient of variation for maximum deflection distance was 6.0%. Head weight (kg) was also recorded for each head using a benchtop scale that was calibrated prior to use.

The location for the recommended frontal PCB placements for both market hogs and mature breeding swine was marked on each head using a permanent marker. The placement for market hogs was 2.54 cm above the top of the optic orbits at the midline (Woods et al., 2010; Anderson et al., 2019); the placement for mature hogs was 3.5 cm above the top of the optic orbits at the midline (Woods et al., 2010; Anderson et al., 2021b). All heads were split down the midline using a Hobart 6801 Vertical Meat Band Saw (Hobart, Troy, OH) equipped with a blade that was 0.06 mm thick, 360.68 cm long, with 4teeth/2.54 cm, and a hook angle of 3 °C (Product #: C78529545, Bunzl Processor Division, Riverside, MO). Following the cut a 150-mm digital caliper (HC Kenshin Electronic Digital Vernier Caliper, HC Kenshin, HuiChuang Technology, Fujian, China) was used by a single observer to measure soft tissue thickness (mm) and cranial thickness (mm) (Figure 2) at the frontal PCB placement for market hogs, located at the midline and 2.54 cm above the top of the optic orbits (adapted from Anderson et al., 2021b). Soft tissue thickness referred to the tissue on the surface of the skin from the market PCB placement—where the PCB would have been placed—to the exterior surface of the cranium along the theoretical bolt path. Cranial thickness referred to the thickness from the exterior surface of the cranium to the interior surface along the theoretical bolt path, as indicated by the line for the market PCB placement. Soft tissue and cranial thickness measurements were collected on both sides of the exposed cranium. Total tissue thickness (mm) for each side was determined by adding soft tissue and cranial thicknesses together for that head. Total tissue thickness referred to the total soft tissue and cranial thickness from the indicated placement line to the interior surface of the cranium, along the theoretical bolt path. The measurements taken from each side of the head were averaged prior to statistical analysis, such that there was one value for each measurement per head.

Digital images were taken 50.8 cm directly above and perpendicular to the exposed cut surface of each half of each head; these images included a 15-cm ruler for reference. All digital images also included a pin that was placed to indicate the location of the top of the optic orbits, this was done to indicate the reference point for PCB placements. In addition,

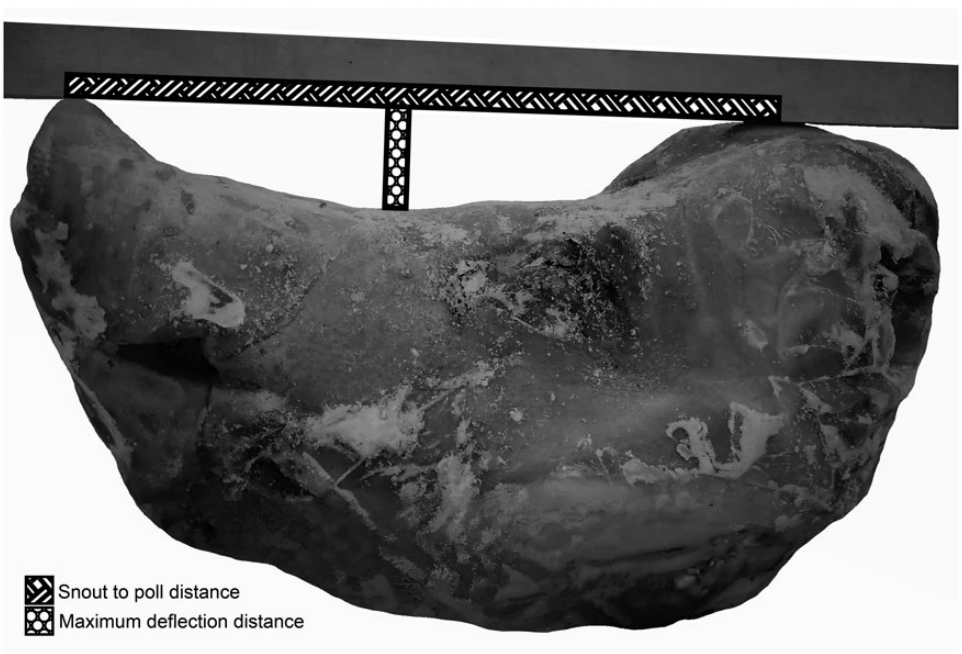

Figure 1. External head measurements. Snout to poll distance (cm)—the distance from the tip of the snout to the first point of contact between a taught measurement tape at the crest of the head. Maximum deflection distance (cm)—the maximum distance from a straight edge that was placed at the tip of the snout and the poll or first point where the straight edge touched the head when placed from the tip of the snout.

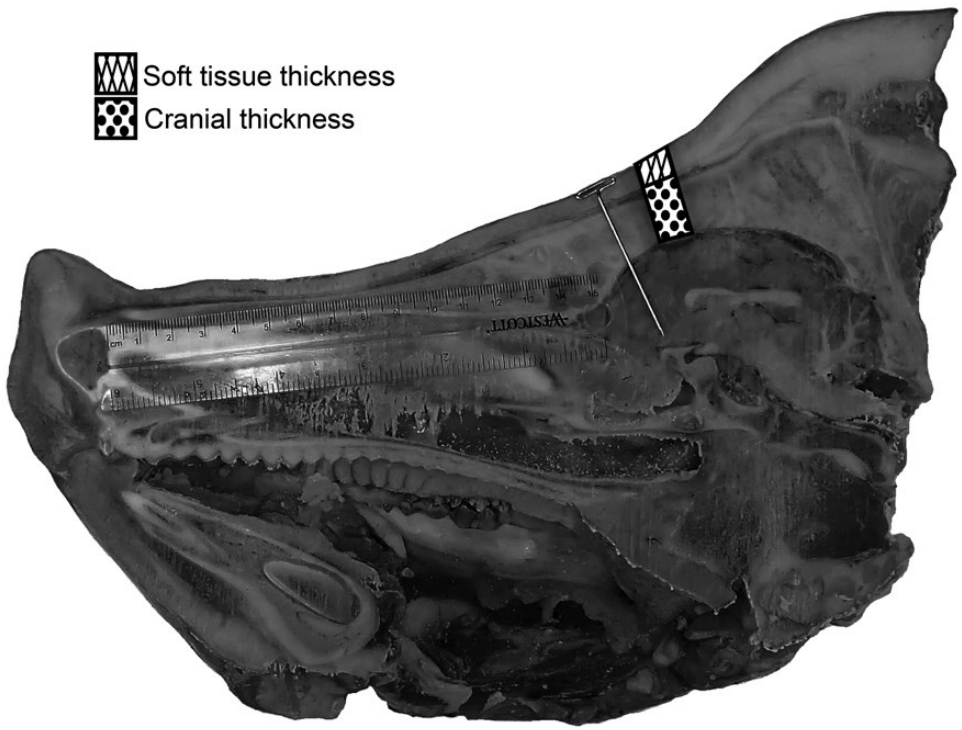

Figure 2. Tissue thickness measurements. Soft tissue thickness (mm)—the thickness from the tissue on the surface of the skin from the market PCB placement, where the PCB would have been placed, to the exterior surface of the cranium along the theoretical bolt path. Cranial thickness (mm)—the thickness from the exterior surface of the cranium to the interior surface along the theoretical bolt path.

images were taken with a painted wooden dowel indicating the theoretical bolt path at the PCB placements for market hogs and mature breeding swine. The three digital images that were taken for each half of each head were 1) pin only, 2) pin with rod indicating market PCB placement, 3) pin with rod indicating PCB mature placement. Thermal images (Model E8 FLIR Systems, Boston, MA) were collected from each half of each head once split to record maximum exposed intracranial temperature. These images were also taken 50.8 cm directly above and perpendicular to the exposed cut surface of each half of each head, but did not include a ruler, pin, or rod.

Force Testing Measurements

Following tissue thickness measurements and imaging procedures, a 15 × 6 cm rectangular sample of cranial and soft tissue was removed using a Hobart 6801 Vertical Meat Band Saw (Hobart, Troy, OH) equipped with a blade that was 0.06 mm thick, 360.68 cm long, with 4 teeth/2.54 cm, and a hook angle of 3 °C (Product #: C78529545, Bunzl Processor Division, Riverside, MO). These samples were cut such that the line indicating the market PCB placement was centered, rostral to caudal, and that the tissue covering the brain was still intact. Samples were packaged individually in unsealed vacuum bags and then into large storage totes (Sterilite 1466-27 Gallon Industrial Tote, Sterilite Corp., Townsend, MA). The samples were held in a walk-in cooler for 48 h at 2 to 4 °C and then frozen for 53 d at −10 °C. Samples were placed into a cooler to thaw for 25 h at 2 to 4 °C before testing.

A custom-built apparatus was fabricated from steel with two 15.2 cm long parallel sides, separated by a 12.8-cm opening (Figure 3). The space available for the tissue thickness was 15.2 cm × 10.8 cm, due to the thickness of the steel. A cylindrical metal piece that was 6.3 cm in diameter and 5 cm tall was welded to the bottom of the holding apparatus so that it could be placed over the portion of the machine where the bolt extends into during testing; this piece had a 3.8-cm diameter hole for the bolt to travel through. Several 1- to 2-cm thick metal shims were made to assist in sample positioning within the apparatus. One metal shim was added on the side of the sample nearest midline, and then additional metal shims were added to the other side as needed until the sample was secured within the apparatus.

A new bolt from a commercially-available PCB (Order no.: 1120006, Jarvis Corp., Middletown, CT) was modified, such that 16 cm starting at the muzzle end of the bolt was retained and ~5.5 cm nearest the combustion chamber was discarded, and fit into the upper jaw of a QTest/150 "Universal Tester" (Serial # M216990070699, MTS Systems Corporation, Eden Prairie, MN). Once each tissue sample was placed and fit tightly within the holding apparatus, the Universal Tester forced the bolt into and through the tissue sample at a rate of 7.62 cm/min. The machine was manually stopped when it became obvious that the bolt had penetrated through the entirety of the soft and cranial tissue and into the brain. This was indicated by a peak in force shown on TestWorks4 software (MTS Systems Corporation, Eden Prairie, MN). Figure 4 shows the holding apparatus with a sample, on the Universal Tester. Maximum force values were collected from a tissue sample from each side of each head on both the PC MARKET BARROWS and IC BOARS. A value of maximum force was produced by the Universal Tester and recorded. Following measurement of penetration force, a data processing utility made using LabVIEW 2018 SP1 (National Instruments, Austin, TX) integrated the area under the Force vs. Displacement Curve measured by the

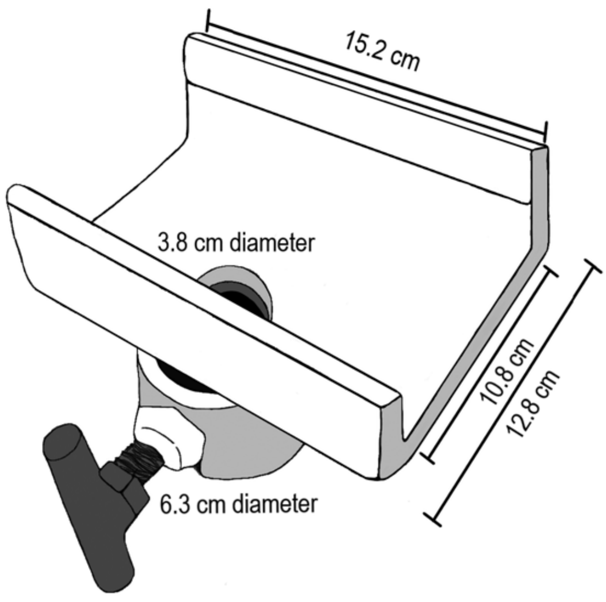

Figure 3. Depiction of tissue sample holding apparatus for force and energy measurements.

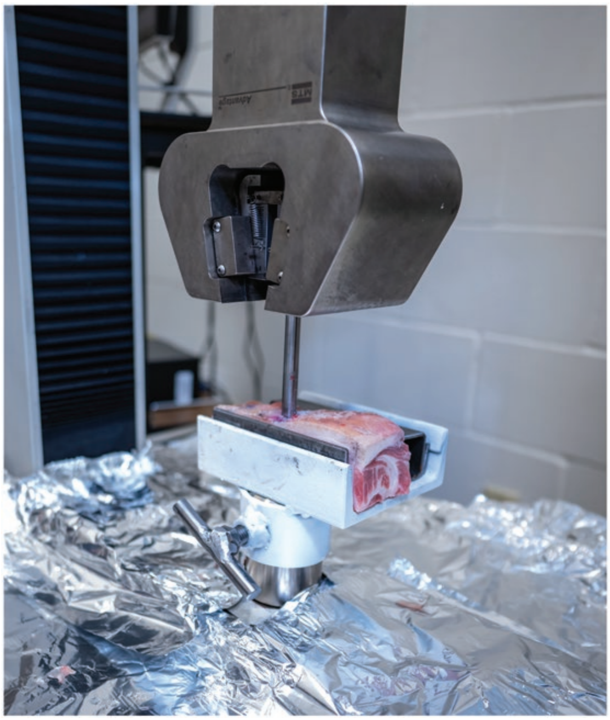

Figure 4. Tissue sample mounted in holding apparatus attached to the MTS Universal Testing machine during a test.

Universal Tester; this integration produced penetration energy values (J).

Theoretical Bolt Path Assessment

Digital images were assessed by a single observer to determine whether the theoretical bolt path for the market PCB placement and the mature PCB placement was located within the plane of the thalamus. For each head, an image that included the wooden dowel at the market frontal PCB placement was assessed, and an image that included the wooden dowel at the mature frontal PCB placement was assessed. The location of the thalamus was determined based on information presented by Anderson et al. (2021b). The theoretical bolt path for either placement was considered to have been in the plane of the thalamus when any part of the theoretical bolt path would have reached the any part of the thalamus, assuming a theoretical bolt path of unlimited length. This was recorded on a yes/no basis: yes meant the theoretical bolt travel path would have made contact with the thalamus, had the bolt been long enough to reach that region of the brain; no meant the theoretical bolt path would not have made contact with any part of the thalamus, had the bolt been long enough to reach that region of the brain.

Statistical Analyses

Continuous data outcomes (soft tissue thickness, cranial thickness, total tissue thickness, maximum deflection distance, HCW, head weight, head temperature, bolt penetration force, and bolt penetration energy) for treatment effects (IC BOARS, PC MARKET BARROWS) were assessed for normality with the histogram statement within the UNIVARIATE procedure followed by analysis with models constructed in the MIXED procedure of SAS Enterprise Guide 7.1 (Statistical Analysis System Institute, Inc., Cary, NC). Models included treatment effects only. The random effect of shipping cooler was included in the initial models and automatically removed because it was not statistically significant ($P > 0.05$). Mean separation was determined using Student's *t*-tests. Differences between means were recognized as significant when $P \leq 0.05$.

Discrete data outcomes (thalamic location within the hypothetical plane of bolt travel) were analyzed for differences in occurrence between treatment groups with Fisher's exact tests constructed in the FREQ procedure of SAS Enterprise Guide 7.1 (Statistical Analysis System Institute, Inc., Cary, NC). Differences in occurrence of discrete outcomes between treatment groups were recognized as significant when $P \leq 0.05$.

Within the sample populations of IC BOARS and PC MARKET BARROWS, the relationships of maximum deflection distance, maximum bolt penetration force, and maximum bolt penetration energy with soft tissue thickness, cranial thickness, total tissue thickness, HCW, and head weight were assessed for correlations using models constructed in the REGRESSION procedure of SAS Enterprise Guide 7.1 (Statistical Analysis System Institute, Inc., Cary, NC). Relationships between regression variables were recognized as significant when $P \leq 0.05$.

Results and Discussion

Tissue thickness measurements for PC MARKET BARROWS and IC BOARS can be found in Table 1. For all tissue thickness measurements, results are reported as means ± SE. Differences in tissue thicknesses were not observed ($P > 0.05$) between PC MARKET BARROWS and IC BOARS. There was no evidence to support a difference ($P = 0.7036$) in soft tissue thickness between PC MARKET BARROWS (8.91 ± 0.28 mm) and IC BOARS (9.06 ± 0.28 mm). For cranial thickness, there was no evidence to support a significant difference ($P = 0.8678$) between PC MARKET BARROWS (23.11 ± 0.83 mm) and IC BOARS (22.91 ± 0.83 mm). There was no evidence to support a difference ($P = 0.9730$) in total tissue thickness between PC MARKET BARROWS (32.02 ± 0.97 mm) and IC BOARS (31.98 ± 0.97 mm). These results indicate that the tissue profile of IC BOARS is similar to that of PC MARKET BARROWS and that PCB devices recommended to be effective for the euthanasia of PC MARKET BARROWS are likely to be effective for IC BOARS, based on the tissue the bolt must pass through in order to reach the brain. The soft tissue thickness, cranial thickness, and total tissue thickness results reported here for both PC MARKET BARROWS and IC BOARS are similar to the soft tissue thickness, cranial thickness, and total tissue thickness values at the same frontal PCB placement for market hogs found by Anderson et al. (2019) for market hogs with an estimated BW of 136 kg (8.3, 23.4, and 31.7 mm, respectively). These findings are further corroborated by similar results from Anderson et al. (2021a) for market hogs with an estimated BW of 136 kg (soft tissue thickness = 6.7 mm, cranial thickness = 16.8 mm, and total tissue thickness = 22.9 mm).

On the contrary, IC BOARS did not have cranial thicknesses, and subsequent total tissue thicknesses, that resembled those of mature boars weighing more than 200 kg BW. Anderson et al. (2021b) reported the mean cranial thickness at the frontal PCB placement for mature was 34.8 mm and the mean total tissue thickness at the frontal PCB placement for mature was 41.2 mm. It should be noted that the soft the amount of soft tissue thickness at the frontal PCB placement site does not appear to thicken as pigs age; Anderson et al. (2021b) reported a mean soft tissue thickness of 6.4 mm for mature boars at the frontal PCB placement.

Non-significant ($P > 0.05$) linear regression results for PC MARKET BARROWS can be observed in Table 2. There were no significant linear relationships for PC MARKET BARROWS between total tissue thickness and maximum deflection distance ($R^2 = 0.1538$, $P = 0.0711$). There were no significant linear relationships for PC MARKET BARROWS between total tissue thickness and HCW ($R^2 = 0.1043$, $P = 0.1426$) and maximum deflection distance ($R^2 = 0.0030$, $P = 0.8087$). There were no significant relationships in PC MARKET BARROWS between cranial tissue thickness and HCW ($R^2 = 0.0475$, $P = 0.3297$). Non-significant ($P > 0.05$) linear regression results for IC BOARS can be observed in Table 3. There were no significant linear relationships for IC BOARS identified between total tissue thickness and maximum deflection distance ($R^2 = 0.0055$, $P = 0.7437$) and HCW ($R^2 = 0.0211$, $P = 0.5778$). There were no significant linear relationships for IC BOARS identified between cranial tissue

Table 1. Soft tissue, cranial thickness, total tissue thickness, maximum deflection distance, and head characteristics from cadaver heads of physically castrated market barrows (PC MARKET BARROWS) and immunocastrated boars (IC BOARS)

Dependent variable	Treatment						
	PC MARKET BARROWS			IC BOARS			
	LS Means	*n*	SE	LS Means	*n*	SE	*P*-value
Hot carcass weight, kg	116.46	22	3.57	117.99	17	3.49	0.6949
Head weight, kg	5.87	22	0.12	6.15	22	0.12	0.1089
Head temperature, °C	1.5	22	0.25	1.4	22	0.21	0.6193
Soft tissue thickness, mm	8.91	22	0.28	9.06	22	0.28	0.7036
Cranial thickness, mm	23.11	22	0.83	22.91	22	0.83	0.8678
Total tissue thickness, mm	32.02	22	0.97	31.98	22	0.97	0.9730
Maximum deflection distance, cm	3.31	22	0.10	3.08	22	0.10	0.1036

Table 2. Linear regression relationships between head characteristics and tissue thicknesses, penetration energy, and maximum force for physically castrated market barrows (PC MARKET BARROWS)

Variables (*Y*; *X*)	R^2	*P*-value
Energy, J; maximum deflection distance, cm	0.0396	0.3870
Energy, J; HCW, kg	0.0938	0.1769
Energy, J; head weight, kg	0.0590	0.2886
Energy, J; total tissue thickness, mm	0.0663	0.2598
Energy, J; cranial thickness, mm	0.0637	0.2695
Force, N; maximum deflection distance, cm	0.0086	0.6819
Force, N; HCW, kg	0.1465	0.0787
Force, N; head weight, kg	0.0011	0.8857
Force, N; total tissue thickness, mm	0.0129	0.6150
Force, N; cranial thickness, mm	0.0026	0.8203
Total tissue thickness, mm; maximum deflection distance, cm	0.1538	0.0711
Soft tissue thickness, mm; maximum deflection distance, cm	0.0030	0.8087
Total tissue thickness, mm; HCW, kg	0.1043	0.1426
Cranial thickness, mm; HCW, kg	0.0475	0.3297

Table 3. Linear regression relationships between head characteristics and tissue thicknesses, penetration energy, and maximum force for immunocastrated boars (IC BOARS)

Variables (*Y*; *X*)	R^2	*P*-value
Energy, J; maximum deflection distance, cm	0.0305	0.5023
Energy, J; HCW, kg	0.0834	0.3168
Energy, J; head weight, kg	0.0798	0.2719
Energy, J; total tissue thickness, mm	0.0489	0.3939
Energy, J; cranial thickness cranial, mm	0.0104	0.6964
Force, N; maximum deflection distance, cm	0.0181	0.5944
Force, N; HCW, kg	0.1287	0.1891
Force, N; head weight, kg	0.0202	0.5733
Force, N; total tissue thickness, mm	0.0003	0.9415
Force, N; cranial thickness, mm	0.0223	0.5543
Total tissue thickness, mm; maximum deflection distance, cm	0.0055	0.7437
Cranial thickness, mm; maximum deflection distance, cm	0.0046	0.7653
Soft tissue thickness, mm; maximum deflection distance, cm	0.0024	0.8300
Total tissue thickness, mm; HCW, kg	0.0211	0.5778
Cranial thickness, mm; HCW, kg	0.0514	0.3815
Soft tissue thickness, mm; HCW, kg	0.0242	0.5512

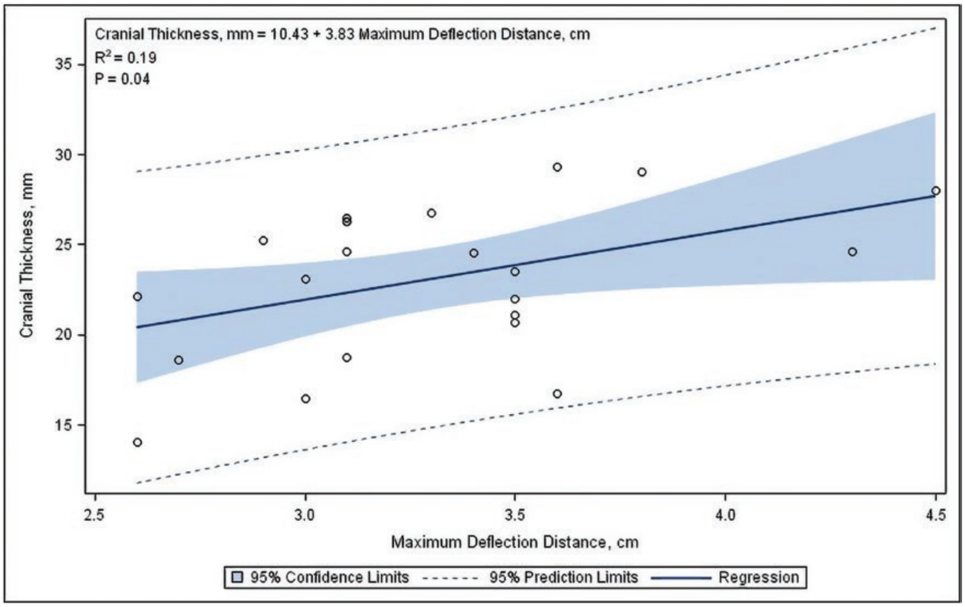

Figure 5. Relationship of cranial thickness (mm) and maximum deflection distance (cm) on the frontal surface of cadaver heads from PC MARKET BARROWS (*n* = 22).

thickness and maximum deflection distance ($R^2 = 0.0046$, $P = 0.7653$) and HCW ($R^2 = 0.0514$, $P = 0.3815$). There were no significant linear relationships for IC BOARS identified between soft tissue thickness and maximum deflection distance ($R^2 = 0.0024$, $P = 0.8300$) and HCW ($R^2 = 0.0242$, $P = 0.5512$).

External head measurements for PC MARKET BARROWS and IC BOARS can be observed in Table 1. There was no evidence to support a significant difference ($P = 0.1036$) between maximum deflection distance in PC MARKET BARROWS (3.31 ± 0.10 cm) and IC BOARS (3.08 ± 0.10 cm). There was no evidence to support a significant difference ($P = 0.6949$) in HCW between PC MARKET BARROWS (116.46 ± 3.57 kg) and IC BOARS (117.99 ± 3.49 kg). There was no evidence to support a significant difference ($P = 0.1089$) in head weight between PC MARKET BARROWS (5.87 ± 0.12 kg) and IC BOARS (6.15 ± 0.12 kg).

For heads from PC MARKET BARROWS, a positive linear relationship was identified for maximum deflection distance and cranial thickness (Figure 5) via simple linear regression. This regression was calculated to predict cranial thickness based upon maximum deflection distance. For each centimeter of maximum deflection distance, the expected cranial thickness increased 3.83 ± 1.74 mm (intercept = 10.43 ± 5.82, $R^2 = 0.1947$, $P = 0.0398$). For PC MARKET BARROWS, significant linear relationships between maximum deflection distance and soft tissue thickness (R^2 = 0.0030, P = 0.8087) or total tissue thickness (R^2 = 0.1538, P = 0.0711) were not observed. For IC BOARS, significant linear relationships between maximum deflection distance and soft tissue thickness (R^2 = 0.0024, P = 0.8300), cranial thickness (R^2 = 0.0046, P = 0.7653), and total tissue thickness (R^2 = 0.0055, P = 0.7437) were not observed. These findings indicate that, for the sample of cadaver heads included in our study, maximum deflection distance had predictive value for cranial thickness of PC MARKET BARROWS but was not a significant predictor of soft tissue thickness or total tissue thickness for PC MARKET BARROWS and was not a significant predictor of tissue thicknesses for IC BOARS. In mature sows (BW > 200 kg), maximum deflection distance was observed to be a predictive factor of cranial thickness and total tissue thickness (Anderson et al., 2021b). Anderson et al. (2021b) observed maximum deflection distances that ranged from <2 cm to >6 cm, along with cranial thicknesses that ranged from <40 mm to >60 mm and total tissue thicknesses that ranged just over 40 mm to nearly 70 mm. In the present study, we observed maximum deflection distances that ranged from 2.3 to 4.5 cm, along with cranial thicknesses that ranged from 14.09 to 30.24 mm and total tissue thicknesses that ranged from 20.91 to 41.13 mm. Woods (2012) investigated the impact of the shape of the frontal plate, "plank" or "dish" profiles and reported challenges in achieving death in some animals with a "dish" face, but no animals with a "plank" face; it should be noted that all animals that did not achieve death following a single PCB application to the front of the head were mature breeding swine, estimated to weight more than 200 kg. As such, the frontal profile of the head and the underlying tissue thickness may be related in mature breeding swine, but more investigation is required to better understand the relationship between the frontal profile of the head and tissue thicknesses for market weight animals. We observed that maximum deflection distance may be related to cranial thickness for PC MARKET BARROWS, but more replication would be valuable to understanding the relationship between maximum deflection distance and other tissue thicknesses. It should be noted that the relationship between the frontal profile of the head and tissue thicknesses has not formally been evaluated in mature boars.

In addition, for heads from PC MARKET BARROWS, a positive linear relationship was identified for HCW and soft tissue thickness (Figure 6) via simple linear regression. This regression was calculated to predict soft tissue thickness based upon HCW; for each kilogram of HCW, the expected soft tissue thickness increased by 0.05 ± 0.02 mm (intercept = 3.38 ± 2.04, R^2 = 0.2717, P = 0.0129). For PC MARKET BARROWS, significant linear relationships between HCW and cranial thickness (R^2 = 0.0475, P = 0.3297) or HCW and total tissue thickness (R^2 = 0.1043, P = 0.8087) were not observed. For IC BOARS, significant linear relationships between HCW and soft tissue thickness (R^2 = 0.0242, P = 0.5512), cranial thickness (R^2 = 0.0514, P = 0.3815), and total tissue thickness (R^2 = 0.0211, P = 0.5778) were not observed. These findings indicate that, for the sample of cadaver heads included in our study, HCW had predictive value for soft tissue thickness of PC MARKET BARROWS but was not a significant predictor of cranial thickness or total tissue thickness for PC MARKET BARROWS and was not a significant predictor of tissue thicknesses for IC BOARS.

Positive linear relationships were identified for head weight and soft tissue thickness (Figure 7), cranial thickness (Figure 8), and total tissue thickness (Figure 9) for heads from PC MARKET BARROWS via simple linear regressions. These regressions were calculated to predict tissue thicknesses based upon head weight. For each kilogram of head weight, the expected soft tissue thickness increased by 1.07 ± 0.44 mm (Intercept = 2.64 ± 2.63, R^2 = 0.2234, P = 0.0263). For each kilogram of head weight, the expected cranial tissue thickness increased by 4.40 ± 1.33 mm (intercept = -2.72 ± 7.84, R^2 = 0.3535, P = 0.0035). For each kilogram of head weight, the expected total tissue thickness increased by 5.47 ± 1.50 mm (intercept = -0.08 ± 8.82, R^2 = 0.4003, P = 0.0016). Positive linear relationships were also identified for head weight and soft tissue thickness (Figure 10), cranial thickness (Figure 11),

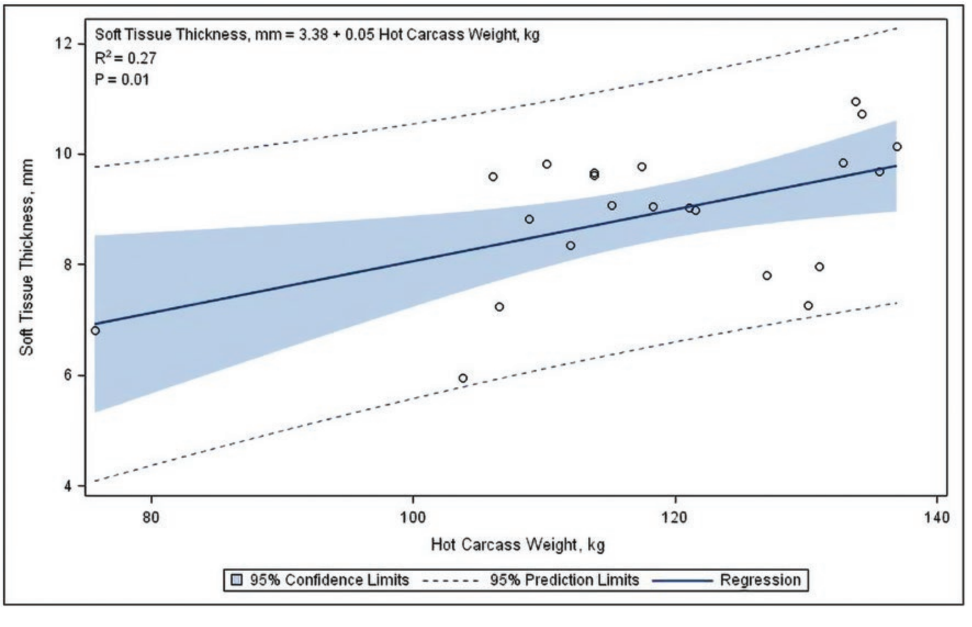

Figure 6. Relationship of soft tissue thickness (mm) and hot carcass weight (kg) of cadaver heads from PC MARKET BARROWS (n = 22).

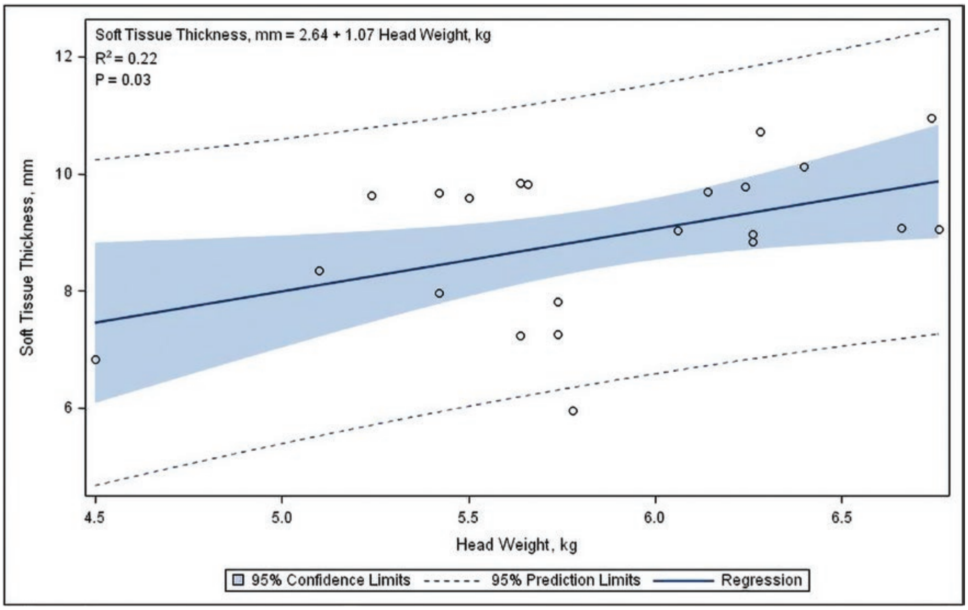

Figure 7. Relationship of soft tissue thickness (mm) and head weight (kg) of cadaver heads from PC MARKET BARROWS (*n* = 22).

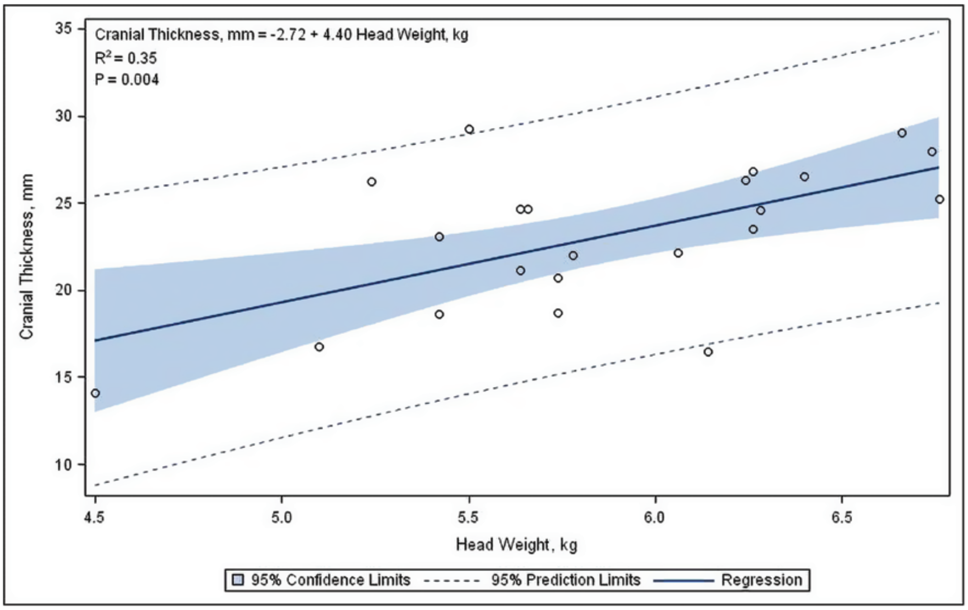

Figure 8. Relationship of cranial thickness (mm) and head weight (kg) of cadaver heads of PC MARKET BARROWS (*n* = 22).

and total tissue thickness (Figure 12) for heads from IC BOARS via simple linear regressions. These regressions were calculated to predict tissue thicknesses based upon head weight. For each kilogram of head weight, the expected soft tissue thickness increased 1.06 ± 0.46 mm (intercept = 2.52 ± 2.85, R^2 = 0.2102, P = 0.0319). For each kilogram of head weight, the expected cranial tissue thickness increased 4.44 ± 0.99 mm (intercept = -4.39 ± 6.14, R^2 = 0.4995, P = 0.0002). For each kilogram of head weight, the expected total tissue thickness increased 5.50 ± 1.06 mm (Intercept = -1.88 ± 6.54, R^2 = 0.5744, $P < 0.0001$). As head weight increased, soft, cranial, and total tissue thicknesses increased for both PC MARKET BARROWS and IC BOARS. This is also demonstrated in previous studies (Anderson et al., 2021b) where head weight was found to be a predictor of cranial and total tissue thickness. In this study, head weight was also a predictor of soft tissue thickness, which has not been observed in previous studies.

Potential for Thalamic Damage

Whether the thalamus was located within the theoretical bolt path can be observed in Table 4. For the PCB placement for market hogs (2.54 cm above the top of the optic orbits), there was no evidence to support a difference (P = 0.2878) between the number of heads from PC MARKET BARROWS (21 of 21; 100.0%) and IC BOARS (19 of 21; 90.5%) where the thalamus was located within the plane of theoretical bolt travel. For the PCB placement for mature swine (3.5 cm above the top of the optic orbits), there was no evidence to support a difference (P = 0.4099) between the number of

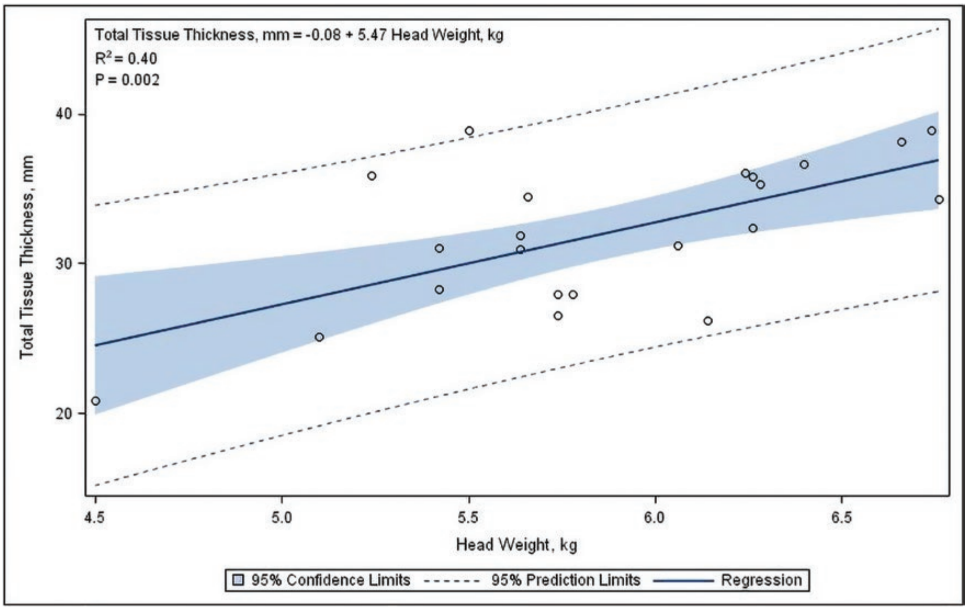

Figure 9. Relationship of total tissue thickness (mm) and head weight (kg) of cadaver heads of PC MARKET BARROWS (n = 22).

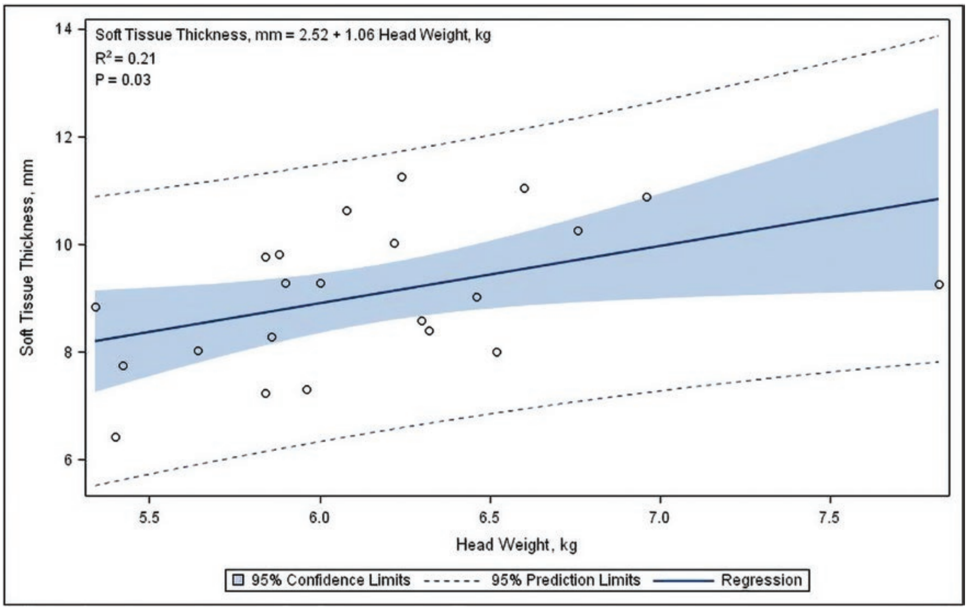

Figure 10. Relationship of soft tissue thickness (mm) and head weight (kg) of cadaver heads of IC BOARS (n = 22).

heads from PC MARKET BARROWS (16 of 21; 76.2%) and IC BOARS (19 of 21; 90.5%) where the thalamus was located within the plane of theoretical bolt travel. For PC MARKET BARROWS, there was a difference (P = 0.0478) between the number of heads where the thalamus was located within the theoretical plane of bolt travel for the market placement (21 of 21; 100.0%) and the mature placement (16 of 21; 76.2%). For IC BOARS, there was no evidence to support a difference (P = 1.0000) between the number of heads where the thalamus was located within the theoretical plane of bolt travel for the market placement (19 of 21; 90.5%) and the mature placement (19 of 21; 90.5%). While the thalamus, specifically, was not located within the theoretical plane of bolt travel for all heads, the brain, in general, was located within the theoretical plane of bolt travel for all heads in both treatments (PC MARKET BARROWS and IC BOARS) and PCB placements (market placement and mature placement). The existing literature has not evaluated the potential for thalamic damage a PCB application to the mature frontal PCB placement for sows and boars weighing more than 200 kg. Anderson et al. (2021b) observed damage to the diencephalon for 50% (13 of 26) of sow heads and 50% (6 of 12) boar heads. In addition, Anderson et al. (2021b) found that the brain was located within the plane of bolt travel associated with the frontal PCB placement for mature swine in 100% (42 of 42) heads from sows and 100% (17 of 17) of heads from boars. Kramer et al. (2021) evaluated brain damage following a frontal PCB application for sows and

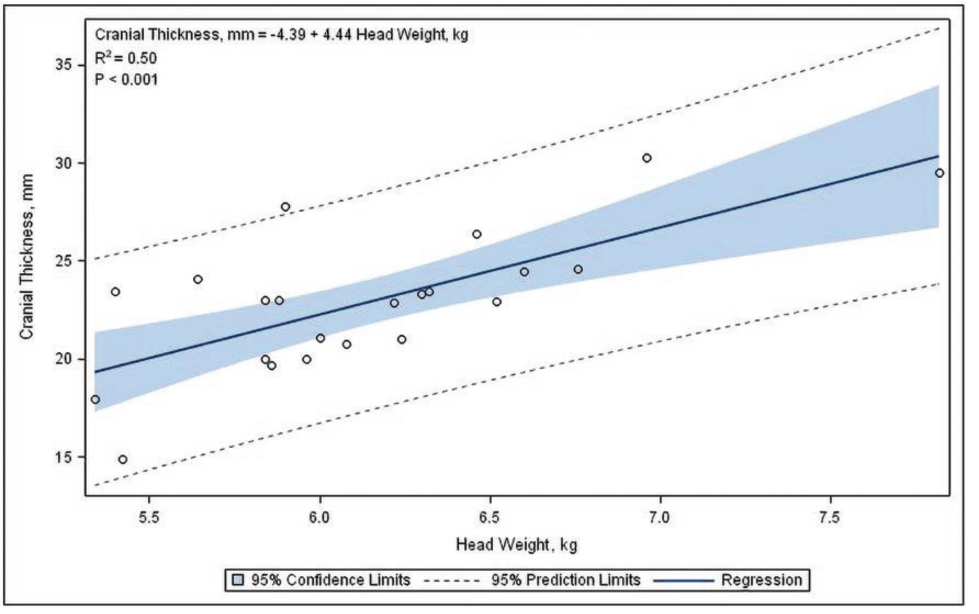

Figure 11. Relationship of cranial thickness (mm) and head weight (kg) of cadaver heads of IC BOARS (*n* = 22).

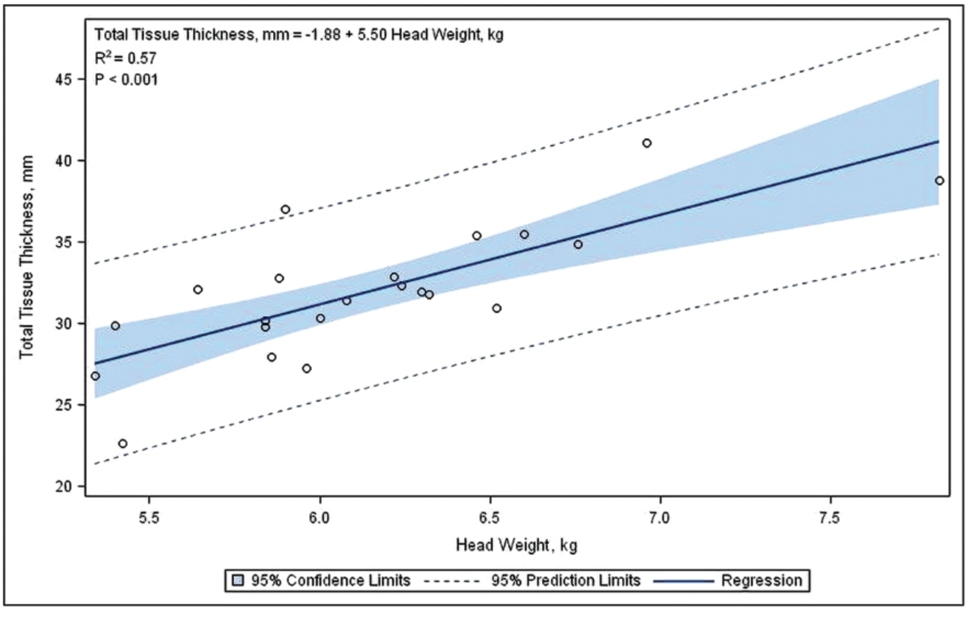

Figure 12. Relationship of total tissue thickness (mm) and head weight (kg) of cadaver heads of IC BOARS (*n* = 22).

Table 4. Percentage of cadaver heads from physically castrated market barrows (PC MARKET BARROWS) and immunocastrated boars (IC BOARS) in which the thalamus was located within the theoretical plane of bolt travel

	PC MARKET BARROWS	IC BOARS
Market frontal PCB placement[1]	21/21 (100.0%)[a,x]	19/21 (90.5%)[a,z]
Mature frontal PCB placement[2]	16/21 (76.2%)[b,y]	19/21 (90.5%)[b,z]

[1]Market frontal PCB placement: 2.54 cm above the top of the optic orbits at the midline.
[2]Mature frontal PCB placement: 3.5 cm above the top of the optic orbits at the midline.
[a,b]Superscript letters that differ within a row identify significant differences between sex for a PCB placement. (a:a, *P* = 0.2878; b:b, *P* = 0.4099).
[x,z]Superscripts that differ within a column identify significant differences between PCB placements for a sex. (x:y, *P* = 0.0478; z:z, *P* = 1.000).

boars; however, the exact PCB placement site is not clearly described and the specific regions of the brain where damage occurred were not reported.

Bolt Penetration Force and Energy

Bolt penetration force and energy values can be observed in Table 5. There was no evidence to support significant differences ($P > 0.05$) in either the force used to break through the skull or energy required to break through the skull between PC MARKET BARROWS and IC BOARS. There was no evidence to support a significant difference ($P = 0.7699$) in maximum force between PC MARKET BARROWS (7130.32 ± 483.23 N) and IC BOARS (6974.60 ± 463.70 N). Additionally, there was no evidence to support a difference ($P = 0.6153$) in penetration energy,

Table 5. Maximum force and penetration energy required to penetrate the cranial cavity of tissue samples of cadaver heads from physically castrated market barrows (PC MARKET BARROWS) and immunocastrated boars (IC BOARS)

Dependent variable	Treatment						
	PC MARKET BARROWS			IC BOARS			
	LS means	*n*	SE	LS means	*n*	SE	*P*-value
Maximum force, N	7,130.32	18	483.23	6,974.60	18	463.32	0.7699
Penetration energy, J	33.37	21	2.77	32.04	17	2.50	0.6153

between PC MARKET BARROWS (33.37 ± 2.77 J) and IC BOARS (32.04 ± 2.50 J). These results indicate that the force and energy required to pass through the skull and reach the brain is similar for IC BOARS and market hogs. It should be noted that the velocity of the test machine used in the present study was 7.62 cm/min, much slower than the bolt velocity of PCB devices. For example, the PCB device used by Anderson et al. (2021b) had an estimated bolt velocity of 310,920 ± 18,300 cm/min (M. Abdul, Jarvis Corp., personal communication). PCB devices recommended to be effective for the euthanasia of market hogs are likely to be effective for IC BOARS, based on the force and energy required for the bolt to penetrate the skull and reach the brain.

Significant linear relationships were not identified between penetration energy and maximum deflection distance for PC MARKET BARROWS ($R^2 = 0.0396$, $P = 0.3870$) or IC BOARS ($R^2 = 0.0305$, $P = 0.5023$). Significant linear relationships were not identified between penetration energy and HCW for PC MARKET BARROWS ($R^2 = 0.0938$, $P = 0.1769$) or IC BOARS ($R^2 = 0.0834$, $P = 0.3168$). Significant linear relationships between penetration energy and head weight for PC MARKET BARROWS ($R^2 = 0.0590$, $P = 0.2886$) and IC BOARS ($R^2 = 0.0798$, $P = 0.2719$) were not identified. There were no significant ($P > 0.05$) linear relationships identified between penetration energy and tissue thicknesses for either PC MARKET BARROWS or IC BOARS. Specifically, for PC MARKET BARROWS, there were not significant linear relationships between penetration energy and cranial thickness ($R^2 = 0.0637$, $P = 0.2598$) or total tissue thickness ($R^2 = 0.0663$, $P = 0.2695$); for IC BOARS, there were not significant linear relationships between penetration energy and cranial thickness ($R^2 = 0.0104$, $P = 0.6964$) or total tissue thickness ($R^2 = 0.0798$, $P = 0.3939$). These findings indicate that physical characteristics, such as maximum deflection distance, HCW, head weight, as well as tissue thicknesses are not predictors of the penetration energy required for a bolt to penetrate the skull and reach the brain for either PC MARKET BARROWS or IC BOARS.

Significant linear relationships were not identified between maximum force and penetration energy for PC MARKET BARROWS ($R^2 = 0.0086$, $P = 0.6819$) or IC BOARS ($R^2 = 0.0181$, $P = 0.5944$). In addition, there were not significant linear relationships identified between maximum force and HCW for PC MARKET BARROWS ($R^2 = 0.1465$, $P = 0.0787$) or IC BOARS ($R^2 = 0.1287$, $P = 0.1891$). Significant linear relationships were not identified between maximum force and head weight for either PC MARKET BARROWS ($R^2 = 0.0011$, $P = 0.8857$) or IC BOARS ($R^2 = 0.0202$, $P = 0.5733$). There were no significant ($P > 0.05$) linear relationships identified between maximum force and tissue thicknesses for either PC MARKET BARROWS or IC BOARS. Specifically, for PC MARKET BARROWS, there were not significant linear relationships between penetration energy and cranial thickness ($R^2 = 0.0026$, $P = 0.8203$) or total tissue thickness ($R^2 = 0.0129$, $P = 0.6150$); for IC BOARS, there were not significant linear relationships between penetration energy and cranial thickness ($R^2 = 0.0223$, $P = 0.5543$) or total tissue thickness ($R^2 = 0.0003$, $P = 0.9415$). These findings indicate that physical characteristics, such as maximum deflection distance, HCW, head weight, as well as tissue thicknesses are not predictors of the maximum force required for a bolt to penetrate the skull and reach the brain for either PC MARKET BARROWS or IC BOARS.

The use of cadaver heads allowed this research to be done without testing on live animals; however, it is not known whether the responses of IC BOARS are similar to that of PC Market Barrows once the PCB has been applied. Additionally, physiological responses such as hind limb kicking, and involuntary muscle movements have not been explored in IC BOARS.

Implications

Determining the differences between PC MARKET BARROWS and IC BOARS helps to ensure that the PCB tools that are currently being used to render PC MARKET BARROWS insensible, should also work in ensuring a humane death for IC BOARS, which safeguards the welfare of the animal. PCB use has been evaluated in market hogs and mature sows and boars, but no research exists regarding the head morphology of IC BOARS. In this study, the tissue thicknesses, head morphology, and force and penetration energy requirements for both PC MARKET BARROWS and IC BOARS were explored. Within all of these areas of research, there were no differences discovered between PC MARKET BARROWS and IC BOARS. Future studies evaluating the insensibility and death outcomes, as well as exploring the physiological reactions following PCB application for IC BOARS are warranted. However, refinement of PCB placement is necessary to ensure consistent bolt-thalamus contact before testing on live animals. Since there are no differences between the tissue thicknesses and force and penetration energy requirements between PC MARKET BARROWS and IC BOARS, it can be concluded that the devices that are effective in rendering PC MARKET BARROWS insensible should also be effective for IC BOARS.

Acknowledgements

The authors thank the commercial slaughter establishment that provided the cadaver heads for this research. The authors greatly appreciated the data collection assistance of B. Dittrich,

R. Heap, A. Matzek, A. Schwark, S. Sigl, A. Tomandl, and R. Walske (all affiliated with the University of Wisconsin—River Falls at the time of this study). The provision of a specially machined captive bolt by the Jarvis Corporation for force testing was greatly appreciated. The authors would also like to thank the commercial meat processing company that provided the bandsaw used for this research. The comments and suggestions of the manuscript reviewer were also appreciated. Funding was provided by the University of Wisconsin – River Falls Falcon Scholars Program.

Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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