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Article

Evaluation of animal welfare indicators of cattle in a Federal Inspection Type slaughterhouse

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Abstract:

The slaughter process is the last stage of management and represents a point at which the welfare of the cattle is compromised. The objective of this study was to evaluate the animal welfare indicators of cattle in a Federal Inspection Type slaughterhouse. The slaughter process of 1,167 animals [740 males (63.8 %) and 420 females (36.2 %)] was observed in a Federal Inspection Type slaughter plant in northwestern Mexico. Management and behavioral variables, as well as indicators of return to sensitivity were recorded, and the

bruises observed on the carcasses were characterized. 10 % of the cattle received electric shocks with prods, while 24.2 % were beaten by operators. Arching of the spine was observed (44.5 %), 62.2 % of the animals exhibited sensitivity during exsanguination. The prevalence of bruising was 88.8 %. Females had 1.62 % higher risk of bruising than males and the presence of large horns increased the risk of bruising by 1.46 %. The characteristics of the bruises observed were bright red (93.8 %), mottled (71.5 %), small (82.9 %), and grade 1 (95.7 %). The area most affected by bruises was the dorsal-lumbar area, with 58.3 %. It was concluded that the animals included in this study were exposed to conditions conducive to stress, including the use of the electric prod by the operators, undesirable behaviors of cattle during herding, ineffective stunning, and the presence of bruises on the carcasses.

Keywords: Animal welfare, Bruising, Slaughter, Stunning.

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Introduction

Pre-slaughter handling of cattle at slaughter plants can negatively compromise animal welfare[1] and stress affects the quality of the final product, causing undesirable changes in the meat[2]. The slaughter process is the last stage which, in terms of animal welfare, is important because it can cause fear, stress, and pain to the animals if it is not carried out correctly[3]. Therefore, and due to the growing interest of consumers in animal welfare, particularly for food animals, slaughter plants have implemented measures to reduce the risk of stress in animals[4]. Scientific evaluations of the performance of these processing plants have gained relevance, and more and more studies are focused on analyzing animal welfare indicators during the slaughter process.

In Mexico, the stunning of cattle before exsanguination is mandatory in Federal Inspection Type slaughterhouses and is performed with a penetrating bolt gun[5]; in industrialized countries, this is the most widely used method of humane desensitization, which aims to achieve a profound loss of consciousness through direct damage to brain tissue[6]; this should facilitate the handling of the cattle prior to hoisting them onto the transfer rail in the slaughter hall, as movements have been observed in stunned cattle, which impede safe handling[7]. From an animal welfare approach, this procedure is of utmost importance, since initiating a bleeding process without the animal having been properly desensitized beforehand entails

unnecessary suffering, as stunning causes a shock wave that brings about a failure in the brain due to neuronal damage and thus prevents pain[8].

On the other hand, the presence of bruises in the carcasses indicates a lack of animal welfare and can be detected at the post-mortem stage by visual inspection of the carcasses; therefore, a number of systems have been proposed for their evaluation[9]. Records and characterization of bruises provide information on pre-slaughter handling, where the handling on the farm, during transfer, or during the stay and the handling at the slaughter plant may be risk factors for their appearance[10]. For this reason, it is important to conduct research to determine the risks to which cattle are subjected and propose strategies to reduce bruising; in this sense, it has been reported that the use of instruments for herding is related to the occurrence of bruises and that a change in the use of such instruments as prods reduces the prevalence of bruises[11].

Given the importance of information on the evaluation of *ante mortem* handling in slaughter plants, the effectiveness of the stunning, and the presence of bruises on carcasses to ensure the welfare of cattle, the objective of this study was to evaluate the animal welfare indicators of cattle in a Federal Inspection Type slaughterhouse.

Material and methods

Study design and location

The study was observational and was conducted in the state of Sinaloa, Mexico, during the period from December 2017 to March 2018 in a Federal Inspection Type (FIT) slaughterhouse; these establishments meet the requirements of the Mexican Official Standards[5,12,13]. During the study, ten visits were made to the production line where variables were recorded, and 1,167 animals [740 males (63.8 %) and 420 females (36.2 %)] from 27 lots were evaluated.

Chronological description of the slaughter process

The facility usually slaughters around 40 cattle per hour during a 10-h period, which results in 350 to 400 cattle per workday. The slaughter process began when the cattle entered the stunning ramp through a guillotine-type door, where they remained in groups of 4 to 5 animals lined up one behind the other. The cattle then passed through a second guillotine-

type door to access the stunning box, which does not have a head immobilizer and has a horizontal revolving door for the exit of the desensitized cattle. This stage is operated by two people, one who directed the cattle into the stun box, ensuring continuous advancement, and a second worker who desensitized the cattle using a pneumatic penetrating bolt gun (USSS-1 JARVIS® Jarvis Products Corporation; Middletown, CT, EE.UU). After stunning and once the horizontal door was opened, the desensitized animals were slid to the floor and then hoisted by the left hind limb. Consequently, they were made to go through bleeding, electrical stimulation, head washing, skinning, evisceration, and hot carcass washing workstations. At the end of the process in the production line, the carcasses were taken to a refrigerated room, where they remained until the temperature and time conditions established by the regulations were met.

Evaluation of management practices and animal welfare indicators in the ramp and stunning box

In the stunning ramp, the sex of the cattle was recorded and in order not to interfere with the slaughter process, the size of the horns was determined visually by personnel trained for this task (the facilities in the slaughter plant make instrumental measurement impossible due to the risk to the safety of the evaluators), defining the categories of small (≤10 cm) and medium to long (>10 cm)**.** The approximate age of the cattle was also considered and evaluated according to dentition, which was established according to two categories: ≤30 or >30 mo of age.

For herding the cattle, the operator had a plastic tube and an electric prod. During pre-slaughter operations at the stun ramp, the frequency of the use of the electric prod, the frequency of blows inflicted by workers on the animals with the plastic tube, and the frequency of tail twisting to move the cattle were evaluated. For the animal welfare indicators observed in the ramp, the frequency of vocalizations and slips presented by the cattle was recorded.

In the tunning box, the shocks caused to the animal when lowering the guillotine-type door, the release of compressed air from the gun to attract the attention of the animals, and the number of animals stuck in the hinged door when exiting the stunning box were recorded. As part of the evaluation of animal welfare indicators, the number of animals that bowed their heads and attempted to retreat and escape was recorded.

Evaluation of stunning effectiveness indicators

The effectiveness of stunning was assessed immediately after the cattle exited the stunning box through the flap door by recording when a bovine showed signs of returning sensitivity, such as eye movement, tonic and clonic phases, rhythmic breathing, arching of the spine, and sensitivity to bleeding. The stun-to-bleed interval was established according to the following categories: 0-30, 31-60, and >60 sec. Additionally, in the head inspection area, the number of holes caused by the penetrating bolt in the head of each animal was recorded. In addition, a transparent template placed in the frontal region of the skull was used to evaluate the precision of the shot (0-2, 2.1-5 and 5.1-8 cm), which corresponded to the distance from the center defined by the template to the location of the hole found; the template had a reference line that was made to coincide with the base of the eyeballs of the skull. The trajectory (perpendicular or diagonal with respect to the frontal bone of the skull) and the depth of the shot were evaluated by introducing a silicone tube graduated in centimeters through the hole produced by the bolt, with measurement categories of 0-3, 3.1-6, 6.1-9 and 9.1-12 cm. For shot orientation, the same transparent template was used to record the shot position (center, northeast, southeast, southwest, and northwest)[14]. In order to provide a metric of stun effectiveness (effective or ineffective stun), three criteria were considered including 1) The time interval from stun to bleed ≤60 sec, 2) A shot accuracy ≤2 cm, and 3) A perpendicular trajectory of the shot.

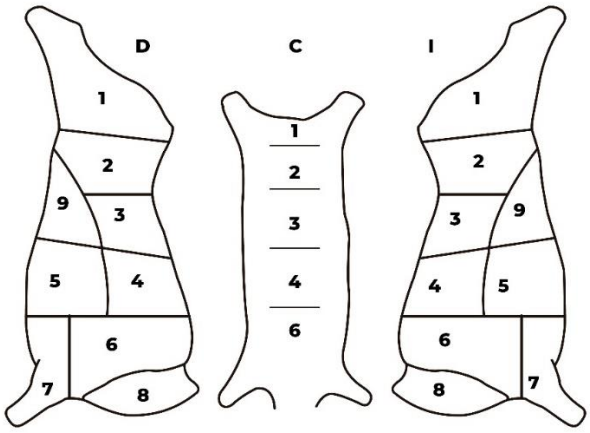
Prevalence and characteristics of bruises

The prevalence and characteristics of bruises on the carcasses were determined through visual and systematic inspection, after which the bruises were recorded and classified in a format designed for this purpose (Figure 1)[15]. Visual inspection was carried out systematically on the production line, starting on the right plane, followed by the dorsal-lumbar region (center), and ending on the left plane of the carcass and following a path from anterior to posterior.

Bruises were visually classified according to color (age), shape, size in cm and degree of severity. Bruise color was categorized as bright red (0 to 10 h), dark red (24 h) and yellowish (3 days)[16]; the shape of the bruise was categorized as mottled (bruise where the affected area appears covered with spots in the form of defined dots), irregular (bruise without clear dimensions and with irregular margins), linear (bruise in the form of a line), circular (bruise in the shape of a circle or almost a circle), and train tracks (two parallel linear bruises separated by a paler undamaged area); size was categorized as small (≥2 cm to ≤8 cm),

medium (>8 cm to ≤16 cm), and large (>16 cm). The severity of the bruise was classified as grade 1 (affects subcutaneous tissue and shows slight hemorrhage), grade 2 (affects subcutaneous and muscular tissue and shows significant hemorrhage), grade 3 (affects bone tissue and looks like a fracture)[17], and generalized bruise, assigned to carcasses with multiple, diffuse, and large hematomas that could not be classified individually. Furthermore, in order to anatomically locate each of the bruises, the carcass was divided into the following regions: 1) leg, 2) sacrum, 3) dorso-lumbar, 4) dorso-costal, 5) rib, 6) back, 7) arm, 8) lateral neck, 9) lateral abdominal, and 10) caudal.

Figure 1: Scheme adapted and used to anatomically locate and characterize the bruises in the carcasses[15]



Statistical analysis

The data obtained from each observation were coded in an Excel spreadsheet and filtered in order to detect recording or data entry errors. Minitab 16 software was used to prepare frequency tables, expressed as percentages and confidence intervals at 95%. The association of the risk factors with the return to sensitivity indicators was determined using a multivariate logistic regression analysis LOGISTIC, of SAS. The risk factors associated with the presence of bruises in the carcass were determined by multivariate logistic regression analysis using the LOGISTIC procedure of SAS[18]. A factor was considered to be included in the regression model when it had an alpha value of 0.20 for both groups of variables.

The multivariate logistic regression model was:

$$\pi(y) = \frac{exp\ (\alpha + \Sigma \beta i \chi i)}{1 + exp\ (\alpha + \Sigma \beta i \chi i)}$$

Where: $\pi(y)$ is the probability of a positive outcome, ßi are the regression values, and χi represents the vector of the independent variable.

Results

Of the 1,167 animals slaughtered during the study, sex and presence of horns were recorded in 1,160, of which 63.8 % were males (740) and 36.2 % were females (420). Regarding the presence of horns, cattle with small horns (<10 cm) were the most frequent (50.6 %, 587), while 45.0 % had large horns (>15 cm) (522). On the other hand, hornless cattle were less frequent, amounting to 4.4 % (51). As for the age of the cattle, out of 1,090 cattle evaluated, 85.2 % were less than 30 mo old (929), and 14.8 % were over 30 mo old (161).

Evaluation of management practices and animal welfare indicators in the ramp and stunning box

According to Table 1, of the 371 cattle evaluated in the stun chute, 10 % received an electric shock with a cattle prod, 24.3 % received at least one blow to the animal's body with the plastic tube by the operator, and tail twisting was recorded in 0.5 % of the cattle. Regarding the welfare indicators, it was observed that out of 371 animals evaluated, only 1.3 % slipped, and 3.0 % vocalized inside the stun chute.

In the 1,160 animals evaluated in the stunning box 10.9 % were hit with the guillotine door during their passage from the stun chute to the stunning box, while the practice of releasing compressed air from the stunning gun to get the animal's attention was very frequent (41.2 %) and 1.4 % of the cattle were caught in the flap door after stunning; As for the animal welfare indicators recorded in the stunning box, recoil was the most observed behavior (30.9 %), followed by tilting the head to avoid the bolt of the gun (15.5 %), and the attempted escape of animals (20.0 %).

Table 1: Handling practices and animal welfare indicators in the stun chute and the stunning box

Place	Action	Frequency (%)
Stun chute (n=371):		
Management practices	Use of electric prod	37 (10.0)
	Blow by operator	90 (24.3)
	Tail twisting	2 (0.5)
Animal welfare indicators	Slip	5 (1.3)
	Vocalization	11 (2.9)
Stunning box (n=1,160):		
Management practices	Guillotine-type door blow	127 (10.9)
	Compressed air release	478 (41.2)
	Bolted on hinged door	16 (1.4)
Animal welfare indicators	Recoiling	358 (30.9)
	Bowed head	180 (15.5)
	Escape attempt	23 (1.9)

A bovine could presented one or more actions in the same place.

Evaluation of stunning effectiveness indicators

Table 2 summarizes the results of the evaluation of the cattle immediately after they left the stunning box. The results showed that 0.4 % of the cattle exhibited eye movements, 96.7 % showed tonic phase, 28.2 % exhibited clonic phase, 0.9 % showed rhythmic breathing, while 4.5 % exhibited arching of the spine, and 62.2 % showed sensitivity to bleeding. On the other hand, the evaluation showed that the stunning-to-bleeding interval was in the range of 31-60 sec in 54.6 % of the cattle, while in the remaining 45.4 % the interval exceeded 60 sec. In addition, when evaluating cattle skulls, it was observed that 96.5 % of cattle were shot, 82.3 % of the shots deviated 0-2 cm from the center, and 64.4 % of the shots followed a perpendicular trajectory. In addition, it was observed that impacts with a depth between 6.1 and 9.0 cm were present in 79.7 % of the skulls evaluated, with shots oriented to the southeast and southwest being the most frequent (31.9 and 24.6 %, respectively), followed by 18.7 % of the heads that exhibited an impact in the center of the skull.

Table 2: Results of the evaluation of post-stunning indicators

Indicator	Category	Frequency (%)
Eye movements, n=1,160	-	5 (0.4)
Tonic phase, n=1,160	-	1 122 (96.7)
Clonic phase, n=1,160	-	327 (28.2)
Rhythmic breathing, n=1,160	-	10 (0.9)
Spine arching, n=1,160	-	516 (44.5)
Sensitivity to bleeding, n=1,160	-	722 (62.2)
Stunning-to-bleeding interval (n=1,142)	0-30 sec	0 (0)
	31-60 sec	624 (54.6)
	>60 ssec	518 (45.4)
Number of shots (n=1,158)	1	1,118 (96.5)
	2-3	40 (3.5)
Shot accuracy (n=1,139)	0-2 cm	937 (82.3)
	2.1-5 cm	196 (17.2)
	5.1-8 cm	6 (0.5)
Shot trajectory (n=1,147)	Perpendicular	739 (64.4)
	Diagonal	408 (35.6)
Shot depth (n=1,139)	0-3 cm	0 (0)
	3.1-6 cm	132 (11.6)
	6.1-9 cm	908 (79.7)
	9.1-12 cm	99 (8.7)
Shot orientation (n=1,156)	Center	216 (18.7)
	Back of neck	24 (2.1)
	Northeast	124 (10.7)
	Northwest	139 (12.0)
	Southeast	369 (31.9)
	Southwest	284 (24.6)

Indicators of return to sensitivity of animals by sex and age were also evaluated according to the effectiveness of stunning (Table 3). Males had higher odds of exhibiting spinal arching

(OR= 1.46, 47.8 %) and sensitivity to bleeding (OR= 1.31, 64.6 %) compared to females (38.6 and 58.1 %, respectively).

Younger cattle (<30 mo of age) showed a higher incidence of spinal arching and had 1.44 (1.03 to 2.05) higher odds of having this indicator compared to older cattle (45.5 and 36.6 %, respectively). While the incidence of bleeding sensitivity was similar (OR= 1.05, 0.74 to 1.48) for both age categories (60.9 and 62.1 % for >30 and <30 mo, respectively). In 1,108 cattle, 29.5 % were observed to have effective stunning, indicating that ineffective stunning was more frequent in 70.5 %. Finally, cattle that were ineffectively stunned had a 1.47 higher probability (1.13 to 1.92) of having spinal arching compared to cattle that were effectively stunned (47.4 and 37.9 %, respectively). In contrast, there was no association (OR= 1.04, 0.79 to 1.36) between the effectiveness of stunning and the incidence of sensitivity to bleeding.

Table 3: Indicators of sensitivity recovery by sex, age and effectiveness of stunning

Variable	Category (n)	Spine arching		Sensitivity to bleeding	
		Frequency (%)	OR (CI)	Frequency (%)	OR (CI)
Sex (n=1,160)	Female (420)	162 (38.6)	Reference	244 (58.1)	Reference
	Male (740)	354 (47.8)	1.46 (1.14-1.86)	478 (64.6)	1.31 (1.03-1.68)
Age (n=1,090)	>30 m (161)	59 (36.6)	Reference	98 (60.9)	Reference
	≤30 m (929)	423 (45.5)	1.44 (1.03-2.05)	577 (62.1)	1.05 (0.74-1.48)
Stunning* (n=1,108)	Effective (327)	124 (37.9)	Reference	201 (61.5)	Reference
	Ineffective (781)	370 (47.4)	1.47 (1.13-1.92)	488 (62.5)	1.04 (0.79-1.36)

* An effective stunning was considered when the following three criteria were met: 1) stunning-to-bleeding interval ≤60 sec, 2) shot accuracy ≤2 cm, and 3) perpendicular trajectory of the shot. OR= Odd ratio. CI = 95% confidence interval.

Prevalence and characteristics of the bruises

In the evaluation of bruises, a prevalence of 88.8 % (95% CI, 86.8 to 90.5) was observed. Female carcasses showed a higher incidence of bruising than male carcasses (P<0.05). In addition, animals with medium to long horns (>10 cm), had more bruising compared to those

with small horns (≤10 cm; *P*<0.05). Sex was found to be a risk factor for the presence of bruises; female carcasses were 1.6 times more likely to exhibit bruises than male ones (*P*<0.05). A second risk factor for bruising was horn size, with animals with large horns having a 1.5 times greater risk of bruising than animals with small horns (*P*<0.05) (Table 4).

Table 4: Prevalence of bruises observed in carcasses and their risk factors

Risk factor	Category (n)	Frequency of bruising (%)	OR (CI)
	(n=1,167)	1,036 [(88.8), CI: 86.8–90.5]	
Age (n=1,090)	≤30 mo (929)	820 (88.3)	Reference
	>30 mo (161)	150 (93.2)	1.0
Sex (n=1,160)	Male (740)	645 (87.2)	Reference
	Female (420)	385 (91.7)	1.62 (1.061-2.433)
Horn size, cm (n=1,160)	Small ≤10 (638)	556 (87.1)	Reference
	Medium to long >10 (522)	474 (90.8)	1.46 (1.003-2.13)

OR = Odd ratio. CI= 95 % confidence interval.

In relation to the characteristics of the bruises on the cattle carcasses (Table 5), in terms of color it was observed that bright red was found in 93.8 % (960 carcasses) and dark red in 29.9 % (306 carcasses), while 5.2 % (54 carcasses) exhibited yellowish bruises. In terms of shape, carcasses with mottled bruises (71.5 %) were predominant, followed by circular (56.5 %), irregular (29.9 %), linear bruises (4.0 %), and train track-like bruises (1.8 %).

As for the size, small bruises were observed in 82.9 % of the carcasses, medium ones in 51.6 %, and large ones in 26.5 %. In terms of severity, grade 1 occurred in 980 carcasses (95.7 %), grade 2 occurred in 233 carcasses (22.7 %), and only one carcass with grade 3, and 12 carcasses exhibited generalized bruises. Finally, regarding the distribution of bruises by anatomical planes, it was observed that the carcasses exhibited more bruises on the right side (73.1 %), followed by the center (62.1 %), while the left side of the carcasses was affected with 58.6 %.

Table 5: Characterization of bruises found in carcasses

Type and location of the bruises		No bruises	Frequency (%)
Color	Bright red	64	960 (93.8)
	Dark red	718	306 (29.9)
	Yellowish	970	54 (5.2)
	Mottled	291	733 (71.5)
Shape	Irregular	717	307 (29.9)
	Linear	983	41 (4.0)
	Circular	445	579 (56.5)
	Train track-like	1,005	19 (1.8)
Size (cm)	Small (>2 a ≤8)	175	849 (82.9)
	Medium (>8 a ≤16)	495	529 (51.6)
	Large (>16)	752	272 (26.5)
Degree of severity	1	44	980 (95.7)
	2	791	233 (22.7)
	3	1,023	1 (0.1)
Carcass map	Right	275	747 (73.1)
	Center	387	635 (62.1)
	Left	423	599 (58.6)

Table 6 shows the results of the anatomical area affected by the bruises, as well as the degree and number of bruises per carcass. A total of 58.3 % of the carcasses exhibited bruises in the dorsal-lumbar area. Regarding the distribution by degree of severity, 93.2 % of the carcasses exhibited grade 1 bruises, followed by grade 2 (6.8 %); the number of bruises registered in this area was 1,106, which corresponds to 26.4 %. It is also worth mentioning that 95.5 % of the bruises were classified as grade 1, and the rest (4.4%), as grade 2. The sacral area was the next most important area with 52.8 % of the carcasses with bruises; the third and last was the dorsal-costal area, with 45.1 %.

Table 6: Distribution of bruises found in carcasses by anatomic region

Anatomical area	Bruised carcasses (%)	1st Degree (%)	2nd Degree (%)	Number of bruises (%)
Lumbar dorsum	604 (58.3)	563 (93.2)	41 (6.8)	1,106 (26.4)
Sacrum	547 (52.8)	500 (91.4)	47 (8.6)	1,053 (25.1)
Rib dorsum	467 (45.1)	432 (92.5)	35 (7.5)	736 (17.6)
Legs	331 (31.9)	259 (78.3)	72 (21.7)	450 (10.7)
Back	285 (27.5)	245 (85.9)	40 (14.0)	367 (8.8)
Caudal	133 (12.8)	120 (90.2)	13 (9.8)	215 (5.1)
Rib	102 (9.9)	84 (82.4)	18 (17.7)	126 (3.0)
Front leg	82 (7.9)	61 (74.4)	21 (25.6)	90 (2.1)
Lateral abdominal	38 (3.7)	24 (63.2)	14 (36.8)	42 (1.0)
Side of neck	3 (0.3)	3 (100)	0	3 (0.1)

Discussion

Evaluation of management practices and animal welfare indicators in the stun chute and stunning box

In the present study, when evaluating the handling practices in the stunning chute, it was observed that 10.0 % of the cattle used the electric prod (37/371), and 24.3 % received at least one blow by the operator (90/371). On the other hand, tail twisting to force cattle forward was a rarely observed activity (0.5 %). Thus, the use of an instrument such as the electric prod and pain-inducing activities such as tail twisting cause stress in animals; therefore, reducing the use of electric prods could improve livestock management[19]. In the stunning chute, there was a low proportion of cattle that slipped (1.3 %) or vocalized (3.0 %). Slipping and vocalizations occurred with a relatively low frequency; however, they are related to the moments prior to slaughter, as, although good management calmed the cattle, they were still exposed to fear-causing situations [20]. In addition, slips can be related to poorly designed facilities; for example, in a study conducted in two slaughter plants, it was observed that, in the first one, 17.6 % of the animals slipped inside the stunning box, while in the second one slips reached up to 31.7 %. These studies also reported that 11.2 % of the animals

vocalized in the first plant, and 9.7 % in the second slaughterhouse[21]. In bovines, vocalizations prior to slaughter are related to severe stress[22].

Once inside the stun box and because this did not have an immobilizer, the animals were able to move, 10% of them were hit with the guillotine door while trying to recoil towards the stun chute. Also, because the cattle were restless inside the stunning box, the sound of the compressed air released by the gun was used to attract their attention and thus the shot was fired with the penetrating bolt gun. On the other hand, after being shot, 1.4 % of the cattle got stuck in the flap door when released from the stunning box. This could be improved with the proper training and education of personnel responsible for handling the animals[23], as well as by implementing a full body restraint system and head immobilizer for the animals and improving animal welfare indicators[24]. In the present work, a considerably high proportion of cattle were observed to recoil in the stun box (30.9 %, 358/1,160), and 15 % bowed their heads inside the box, making it difficult to position the penetrating bolt gun for firing; in addition, a small number of animals tried to escape from this. Setbacks and attempts to escape from the stun box are related to states of reactivity and fear which, in addition to stress, can delay activities and slow down the flow in production processes[25]. In this sense, in a study conducted with 40 steers, 50 % moved their heads up and down inside the stun box; in addition, at the time of aiming, all steers showed behaviors in which they avoided the stun gun, none was calm inside the stunning box, and 5 % vocalized[26].

Evaluation of stunning effectiveness indicators

In the evaluation of the effectiveness of stunning, a relatively low percentage (0.4 %) of cattle exhibited eye movements, while 96.7 % of the animals experienced tonic phase, 28.2 % exhibited clonic phase, and less than 1 % of the animals showed rhythmic breathing. On the other hand, the most obvious signs of ineffective stunning were arching of the spine and sensitivity to bleeding. The fact that the stunning box lacks a system to properly immobilize the animal implies that the operator in charge of stunning the animal cannot aim the gun in an accurate way, due to excessive movement of the animal. In this regard, in a study conducted on cattle stunned with a penetrating captive bolt gun, using two different air pressures, they found that less than 2 % of the animals exhibited some type of ocular reflex. In addition, 58 to 62 % of the cattle exhibited tonic phase, and 20 %, clonic phase; breathing was rhythmic in 27 % of the animals with high pressure of captive penetrating bolt gun, and 8 %, with low barrel pressure[27]. Effective stunning involves immediate collapse, no attempt to stand up, with the body and muscles rigid (tonic phase), no normal rhythmic breathing, eyelids remain open with the eyes facing forward, and no ocular rotation[28].

In this study, a relatively long time interval, of over 60 sec from stunning to bleeding, was also observed in at least 45.4 % of the animals. The operator must be skilled in order to correctly perform the cut that causes the cattle to bleed, especially not to exceed 60 sec between stunning and bleeding, since this period is crucial to avoid a possible return to sensitivity. In the same sense, a study reported a similar percentage, with 41.6 % of the animals having bled in less than 60 sec after stunning in one of three slaughter plants that were evaluated[29]. In the present work, a relatively high percentage (96.5 %) of the animals received a single shot with the captive penetrating bolt gun, while for shot accuracy the distance to the ideal target (this was considered as a hole at a distance ≤2 cm from the center) was observed at a high frequency (937/1,139, 82.3 %); on the other hand, the trajectory followed by the bolt and the depth of the impacts were considered to be relatively acceptable, as the frequency of skulls with a perpendicular trajectory was 739/1,147 (64.4 %). Most of the holes measured in the skulls (79.7 %) were 6 to 9 cm deep and were mainly oriented to the southeast of the ideal point. 18.7 % of the heads evaluated had the hole in the center of the skull (without deviation). Although the tunning box did not have a cattle immobilizer, the operator in charge of firing the captive penetrating bolt gun was apparently skilled in doing so, as the number of shots per cattle, the accuracy of the shot and the depth of the shot were relatively acceptable. In this regard, the use of the captive penetrating bolt stunning method has been shown to be effective in desensitizing cattle in comparison with the non-penetrating bolt stunning method because the former causes more damage to specific brain structures[30]. In a study conducted in a slaughter plant without a head and neck restraint in the stun box, 87.5 % of the cattle were reported to have received a single shot, and 92 % of the holes were in the ideal spot. The authors of the study also mention that when the captive penetrating bolt gun is not placed completely perpendicular to the head, the force of the impact is reduced, affecting the depth of the hole, which contributes to decrease the stun effect[31].

The sex of the animals was related to the presence of certain signs of return of sensitivity, with males showing greater arching of the spine and sensitivity to bleeding than females. Older male cattle have thicker skulls at the forehead, resulting in greater resistance to the kinetic force released by the bolt, which reduces the effectiveness of stunning[31]. However, in the results of the present study, cattle <30 mo were more likely to exhibit arching of the spine; this factor could be related to the return to sensitivity due to a longer time interval between stunning and bleeding. Ineffective stunning increased the likelihood of a bovine exhibiting arching of the spine (OR= 1.47). In this sense, it has been documented that the damage to the brain structures involved in the desensitization of the cattle must be done effectively. Thus, a poorly performed stun would cause the cattle to experience involuntary reflexes unrelated to sensitivity for some time after stunning due to partial unconsciousness, and therefore a second shot must be fired immediately[32]. This could explain the fact that no association was observed between ineffective stunning and sensitivity to bleeding. In addition to the measurements described above, three criteria were considered to evaluate the effectiveness of stunning, which included the time interval between the stunning and the

bleeding, the accuracy of the shot, and the trajectory followed by the shot. This resulted in a relatively high percentage (70.7 %) of animals with ineffective stunning. Effective stunning of animals is essential to avoid unnecessary suffering because, when performed correctly, the animal will become unconscious and will no longer feel pain[33]. There is now constant pressure on food companies to ensure animal welfare, especially in slaughterhouses, because improper handling during slaughter is known to result in poor meat quality[34].

Prevalence and characteristics of bruises

Several indicators have been used to estimate animal welfare, one of which is the assessment of carcass bruising in cattle[35]. A bruise is an accumulation of blood caused by a focal hemorrhage that is caused by the impact of a blunt instrument on the body of the animal; this constitutes a *post mortem* finding, and therefore it is observed on the surface of the carcass[36]. Bruises in cattle carcasses concern to meat industry because of their negative impact on animal welfare and because they reduce the quality of meat products[37]. The prevalence of 88.8 % of bruises in this study was lower than that reported in a study conducted in Mexico (92 %)[23], but higher than that found in another study which reported a prevalence of 75.8 %[38]. On the other hand, in the United States, a percentage of 42.6 % was observed, which is lower than that of the present study[39].

In the present study, a relationship was observed between the prevalence of bruising in the carcasses and the sex of the animals; females presented more bruises than males. Also, there was an influence of horn size, as cattle with medium to long horns exhibited more bruises; these were significant risk factors. Different studies have reported the sex of the cattle as a risk factor for the occurrence and severity of bruises, these studies agree that females present a higher risk than males[15,40,41]. On the other hand, the presence of horned animals in transport trailers has shown a direct correlation with the prevalence of carcass bruising[42]. Females and the presence of cattle with horns longer than 10 cm as factors related to the presence of bruises is partly due to the animals' reactivity as a reflex in response to the management practices performed in the pre-slaughter. Physical and structural differences between the sexes such as skin thickness and fat coverage may influence the perception of bruising in the carcasses. Cattle with horns tend to be dominant, increasing the likelihood of bruised carcasses[43]. Since bruises can originate in the slaughter plant due to deficient installations (overhangs, hinged doors, etc.) and improper handling such as blows by operators, blows with guillotine-type doors and the use of electric prods, bruises can exhibit a variety of characteristics such as differences in color, shape, size and degree of severity, as well as variation in the anatomical site where they are observed[44]. The variation in color is used as a reference to the time elapsed since the bruise occurred; bright red indicates a recent

bruise (0 to 10 h); dark red, approximately 24 h, and a yellowish bruise is older (3 d)[16]. In this study, bright red (93.8 %), mottled (71.5 %), small (82.9 %) and grade 1 bruises prevailed, suggesting that some bruises with these characteristics may have been caused prior to slaughter, in the herding chutes when the cattle were going to the stunning box, due to the use of such devices as plastic tubes and electric prods. The shape and size of the bruises at the time of carcass inspection indicate the type of object that inflicted the bruise; in addition, the degree of severity is related to the intensity with which the bruise occurred[45]. In this regard, a study found 61.3 % of the bruises were circular in shape, 73.7% were small in size, and 54.3 % of the hematomas were grade 2[46].

As for the location of the bruises by anatomical planes of the carcass, the results obtained in the present study showed that the right plane was the most affected. The fact that a greater number of bruises are present in the right plane of the carcass could be due to the positions of the operators in the cattle race and in the stun chute, given the observed frequent use of droving instruments. In this regard, a study in Uruguay showed that 58 % of the carcasses were bruised on only one side, while 42 % of the carcasses were bruised on both sides[47]. Another study points out that the bruises observed in the back area (90.5 %) were inflicted inside the stunning box[48]. In another study, they observed that the loin (29.7 %), rib (14.4 %), flank, and shoulder were the anatomical areas of the carcasses with the highest frequency of bruises[49]. The anatomical regions that were most frequently affected by bruises are those that are most exposed to handling in the slaughterhouse due to the use of devices and to being hit by the slaughterhouse infrastructure, such as guillotine-type doors. Some of the bruises found on the carcasses are related to handling in the slaughter plant and infrastructure, especially if it is take into account that bruises caused by low-intensity blows (mottled, small, and grade 1) and in well-defined anatomical areas prevailed.

Conclusions and implications

At the slaughterhouse, there were events related to poor management practices that impact the welfare of slaughtered cattle. In addition, infrastructure failures led to inadequate animal behavior on the stunning chute and in the stunning box, which prevented effective stunning, reflected in the fact that the cattle showed signs of returning to sensitivity. On the other hand, the findings of bruises on the carcasses suggest that some were caused during pre-slaughter handling at the plant. The implications of the results of this research indicate that constant training of operators is necessary and investments in infrastructure improvements are required to improve the process of stunning cattle and reduce economic losses caused by the presence of bruises, thus ensuring animal welfare and better quality and safety of the meat from slaughtered cattle.

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