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Bleeding Time and False Aneurysm Incidence on Cattle Slaughtering Using Non-Penetrative Pre-Slaughter Stunning in Indonesia

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ABSTRACT

The use of non-penetrating pre-slaughter stunning (NPPSS) in ruminants remains a contentious issue in Indonesia since it is thought to interfere with the bleeding. This paper evaluated the bleeding time (BT), incidence of the false aneurysm (FA), and factors influencing these parameters in 460 NPPSS and 50 non-NPPSS slaughters in Indonesia. In this research, bodyweight, neck skin fold, sex, incisors condition, stunner strength, door closed-final stunning interval, number of shots, stunning success rate (SSR), stunning final-slaughter interval, broken skull, shooting placement, stunner-men and slaughter-men competencies, incision direction, incision location, BT, and FA were assessed for NPPSS slaughter. The same parameters were also assessed for non-NPPSS except the stunning parameter. The results showed that the BT of the non-NPPSS slaughters (187±47.37 seconds) was faster than that of the NPPSS slaughters, with the BT for the NPPSS dependent upon the level of unconsciousness. The longest BT was found in fully unconscious cattle shot once (304.32±69.76 seconds). The risk of FA incidence in non-NPPSS cattle was higher than in the NPPSS cattle. The FA incidence was 2.64 times higher in non-NPPSS cattle than in unconscious NPPSS cattle. The BT of non-NPPSS cattle was affected by FA, whereas the NPPSS cattle were affected by SSR, number of shots, broken skull, and FA incidence. The primary influence factor of FA in non-NPPSS cattle was incision location, whereas the NPPSS cattle were DCFS, incision location, and incision direction. Based on the results of this study, it can be concluded that the use of NPPSS prolongs BT and reduces the risk of FA in the slaughter of Australian Brahman cross cattle.

Keywords: bleeding time; cattle; false aneurysm; slaughter; stunning

INTRODUCTION

Stunning is a technical process performed on cattle to induce unconsciousness and loss of sensitivity so that they can be slaughtered without fear, anxiety, pain, or excessive stress (Fuseini *et al.*, 2017; Limon *et al.*, 2010; Nakyinsige *et al.*, 2013). As a Muslim-majority country, Indonesia only allows electrical stunning and non-penetrating pre-slaughter stunning (NPPSS) based on the Halal Assurance System 23103 (Indonesian Ulema Council (MUI), 2012) and Indonesian National Standard (SNI) 99003:2018 (Indonesian National Standardization Board (BSN), 2018). According to MUI, the requirements of NPPSS are only to render the animal unconscious, not dead before slaughter, and not cause pain and permanent physical damage, especially to the central nervous system.

Even though NPPSS is legally allowed in Indonesia, it remains controversial in the community, especially regarding the effect of NPPSS on death status, bleeding rate, and total blood loss (Abdullah *et al.*, 2019; Fuseini *et al.*, 2016). In contrast, the non-NPPSS slaughter controversy related to animal welfare due to prolonged consciousness after severed blood vessels (Bozzo *et al.*, 2017), high level of stress-related hormone concentration during slaughter (Bozzo *et al.*, 2018; Barrasso *et al.*, 2020), and animal cruelty occur during handling and slaughtering (Farouk *et al.*, 2016; Rahman, 2017; Alam *et al.*, 2019). Opponents of the stunning process believe that stunning causes death in the animals before slaughtering and decreases the bleeding rate and total blood loss. Blood that remains in the meat is a crucial factor affecting meat quality and shelf life (Nakyinsige *et al.*, 2014). Residual blood in the meat has an adverse effect since blood is a natural medium for bacterial growth, and hemoglobin is a potent initiator of lipid oxidation (Farouk *et al.*, 2014; Khan *et al.*, 2018). Some studies have shown that stunning and level of consciousness do not affect bleeding efficiency (Sabow *et al.*, 2015; Terlouw *et al.*, 2015) or the total volume of blood loss (Anil *et al.*, 2006; Agbeniga & Webb, 2012; Khalid *et al.*, 2015). However, application of head-heart electric stunning following NPPSS decrease blood yield in calf slaughtered (Bartz *et al.*, 2015).

Efficient and rapid bleeding is essential in the slaughter process to reduce suffering and increase animal welfare in cattle. According to Mdletshe *et al*. (2020), bleeding time (BT) significantly correlates with the time to loss of sensibility in goats slaughtered without stunning. In addition, Gregory *et al*. (2006) found that enlargement at the end of a severed artery was associated with the occurrence of a false aneurysm (FA). Histologically, at the end of the severed artery, there is blood coagulation between the outer wall of the artery and the inside of the connective tissue surrounding it (Bozzo *et al.*, 2020). These blood clots cause blockages in arterial blood vessels so that the blood flow is blocked or even stopped. In non-NPPSS cattle, FA is associated with time to loss of consciousness (Bozzo *et al.*, 2020; Gibson *et al.*, 2015).

The location of the incision site affects the risk of FA formation and final collapse in cattle slaughtered without stunning. Incisions at the first cervical bone level can reduce the risk of FA incidence, then the incidence increases caudally (Gregory *et al.*, 2012). Gibson *et al*. (2015) stated that an incision in the tracheal ring position (T) ≤ 2 presents a shorter final collapse time than an incision in the T ≥ 2.5 positions. In halal slaughter, the incision must sever the trachea, esophagus, and common carotid arteries, with the incision made at the caudal end of the larynx.

BT and FA are both critical and affect one another. In the context of animal welfare, rapid and profuse bleeding and the absence of FA hastens death. An invasive dressing procedure should be performed after the animal has lost consciousness or died completely. In regards to meat quality, rapid and profuse bleeding causes maximum blood loss and hastens the loss of consciousness and death, which allows the cattle to struggle less and minimize bruising to the meat. In Indonesia, there are several different slaughter methods and styles of slaughtering and dressing procedures related to non-NPPSS and NPPSS; however, there are limited data on BT and FA incidence in NPPSS and non-NPPSS slaughters in Indonesia. The purpose of this study is to determine BT and FA prevalence in non-NPPSS, and NPPSS slaughters in Indonesia and the factors that influence them. The results of this study also provide a reference for dressing procedure times after complete death based on the final BT.

MATERIALS AND METHODS

Materials

This research is a field study of 24 slaughterhouses in Java, Sumatra, and Kalimantan, Indonesia. For ethical and confidentiality reasons, the names of the slaughterhouses have been withheld. Observations were made on 460 Australian Brahman cross cattle (45 bulls, 12 cows, 279 steers, and 124 heifers) with a bodyweight range of 350–700 kg using the Magnum Cash Knocker Concussion Stunner (0.25-caliber; Accles and Shelvoke, Sutton Coldfield, UK), and 50 non-NPPSS slaughters. The stunning process was performed using simple restraining boxes (MLA-Livecorp Mark I Australia), while the non-NPPSS slaughter process was performed using Mark IV (MLA-Livecorp Mark IV, Australia).

Methods

The study was conducted on the regular activity of the slaughterhouses without any intervention from the researchers. Two researchers observed and recorded the processes performed by the animal handlers, stunner-men, and slaughter-men before, during, and after slaughtering, until death was confirmed. In addition, morphometric measurements of the head and incision location (IL) were performed after the head was separated from the body. For the NPPSS slaughters, bodyweight, neck skin fold, sex, condition of incisors, stunner-men competencies, stunner strength (SS), door closed-final stunning interval (DCFS), number of shots (NS), stunning success rate (SSR), stunning final-slaughter interval (SFS), slaughter-men competencies, incision direction (ID), IL, BT, and FA were recorded. After the head was separated from the body, the researchers also examined the broken skull (BS) and shooting placement (SP). The same observations were also conducted for the non-NPPSS cattle slaughters, except for the stunning parameter.

The Observation of Pre-stunning (NPPSS)

Cattle weight was grouped into weights under 450, 450–550, and weights over 550 kg based on purchase order data. Standard cartridges were classified based on body weight, with orange cartridges used for cattle weighing less than 450 kg, black cartridges for cattle weighing between 450 and 550 kg, and red cartridges for cattle weighing more than 550 kg. The SS were divided into three groups based on body weight and cartridge classification: cattle stunned with low, standard, and high strength. Cattle neck skin folds were grouped into four categories (1–4): no neck skin fold; narrow and thin; wide but thin or thick but narrow; wide and thick. The sexes were grouped into four categories (1–4): bull; cow; steer; and heifer. Animal age was grouped into five categories according to teeth examination: those who had no permanent incisors; and those who had changed one, two, three, and four incisors, respectively. The stunner-man competencies were grouped into two categories (1–2): untrained/uncertified and certified.

The Observation of Stunning Procedure (NPPSS)

In NPPSS, the DCFS interval observations were grouped into five categories (1–5): ≥121, 91–120, 61–90, 31–60, and ≤30 seconds. The NS was grouped into two categories (1–2): one and >1 shot. We used posture, righting reflexes, vocalization, eye reflexes, and rhythmic breathing to indicate the level of unconsciousness.

The SSR was based on Atkinson *et al*. (2013), which used the indicator of unconsciousness reported by Terlouw *et al*. (2015), Verhoeven *et al*. (2015), and Verhoeven *et al*. (2016). The SSR was grouped into four groups (1–4): >1 shot, not fully unconscious; >1 shot, unconscious; 1 shot, not fully unconscious; and 1 shot, unconscious. The SFS intervals were grouped into five categories (1–5): ≥41, 31–40, 21–30, 11–20, and ≤10 seconds.

The Observation of Slaughtering Process (Non-NPPSS and NPPSS)

The slaughtering process was carried out with a slaughter knife that met the requirements of the Indonesian National Standard (SNI) 99003 (BSN, 2018) in a lying position on the floor or on a retaining panel. The ID of each slaughter was grouped into three categories (1–3): pushing from top to bottom with the position of the slaughter-man behind the cattle; from the front of the cattle neck with the position of the slaughter-man in front of the cattle; and pulling from the bottom to the top with the position of the slaughter-man behind the cattle. The NI was calculated based on the change in the ID and was grouped into four categories (1–4): >3, 3, 2, and 1 incision. The BT was calculated starting when the knife cut through the carotid artery until the blood stopped pumping due to cardiac arrest, following exsanguination after stunning (Aghwan *et al.*, 2016).

The Observation after Slaughtering Process

After death was confirmed, the head was separated from the body. The IL was determined by calculating the trachea ring (T) attached to the larynx and was grouped into five categories (1–5): >T6, T0 (just right/in front of the larynx); T5-6, T3-4, and T1-2. Observations of the BS were based on HAS 23103 (MUI, 2012), and observations of SP were conducted based on Gilliam *et al*. (2016).

Data Analysis

The data obtained were written on observation cards and scored according to their categories. The data were then scored and statistically analyzed using IBM SPSS software (Statistical Package for the Social Science, NY, USA) version 21, with p-values <0.05 considered statistically significant. Bleeding time was analyzed using One Way ANOVA and Duncan's tests to determine the BT differences between non-NPPSS and NPPSS. The factors affecting BT were analyzed using linear regression tests. FA risk factors were analyzed using the Chi-Square test and continued with ordinal regression for the parameter with p<0.2.

RESULTS

Bleeding Time

The average BT in non-NPPSS cattle was faster than in NPPSS cattle, which was 187±47.37 seconds. The BT in the non-NPPSS group was significantly different (p<0.05) from the NPPSS groups, both in not fully unconscious and unconscious cattle (Table 1). In the NPPSS group, the cattle that were not fully unconscious had a faster BT than the unconscious cattle, which was 258.39±63.98 seconds in 1 shot cattle and 258.94±75.22 seconds in the cattle that got more than 1 shot. The unconscious cattle showed the longest BT of 304.32±69.76 seconds (1 shot) and 300.67±59.49 seconds (>1 shot) (Table 1).

Based on a separate linear regression analysis, four variables significantly influenced the BT in the NPPSS group (p<0.05): SSR, NS, BS, and FA. The non-NPPSS cattle group showed that only the FA significantly influenced BT. Based on the linear regression analysis results for the BT, the linear equation formula for BT in NPPSS slaughter is 323.758 + (39.50 × SSR) − (60.30 × NS) − (7.38 × BS) + (19.92 × FA), while the non-NPPSS follows the linear equation 154.62 + 17.81FA.

Incidence of False Aneurysm

Observations of FA incidence showed that the non-NPPSS cattle had a 2.64 times higher risk than unconscious NPPSS cattle with 1 shot (p<0.05) (Table 2). In the NPPSS cattle group, the risk of FA incidence was higher in the not fully unconscious groups with 1 shot and the cattle that got more than 1 shots, but this difference was not significant (p>0.05). Based on the Chi-Square test results, two factors affected the FA incidence in non-NPPSS cattle: the NI and IL; however, the risk factors having significant associations with IL were only incisions at the T0 and T > 6 positions (Table 3). In non-NPPSS cattle, the risks of FA at T0 and T > 6 were

Table 1. Bleeding time in non- and NPPSS cattle and various stunning success rates

Variables	Bleeding time (second)			
	N	Average ± Sd	Minimum	Maximum
Non-NPPSS	50	187.04 ± 47.37[a]	116	293
NPPSS				
>1 shot not fully unconscious	18	258.94 ± 75.22[b]	140	416
> 1 shot unconscious	33	300.67 ± 59.49[c]	195	414
1 shot not fully unconscious	67	258.39 ± 63.98[b]	175	531
1 shot unconscious	342	304.32 ± 69.76[c]	104	545
Total	510	284.95 ± 75.79	104	545

Note: Means in the same column with different superscripts differ significantly (p<0.05). NPPSS= non-penetrating pre-slaughter stunning

Table 2. False aneurysm incidence in non-NPPSS cattle and various stunning success rates

Variables	Incidence of false aneurysm (N (%))			Total	Odds ratio	p
	No FA	1 Artery	2 Arteries			
Non-NPPSS	20 (40.0)	19 (38.0)	11 (22.0)	50 (9.8)	2.64 (1.50–4.63)*	0.00
>1 shot not fully unconscious	12 (66.7)	0 (0.0)	6 (33.3)	18 (3.5)	1.45 (0.57–3.69)	0.43
>1 shot unconscious	20 (60.6)	7 (21.2)	6 (18.2)	33 (6.5)	1.38 (0.68–2.80)	0.37
1 shot not fully unconscious	40 (59.7)	18 (26.9)	9 (13.4)	67 (13.1)	1.32 (0.78–2.23)	0.30
1 shot unconscious	229 (67.0)	72 (21.1)	41 (12.0)	342 (67.1)	Reference	
Total	321 (62.9)	116 (22.7)	73 (14.3)	510 (100)		

Note: * There is a significant association at p<0.05. NPPSS= non-penetrating pre-slaughter stunning

Table 3. Analysis of false aneurysm incidence of 50 Australian Brahman cross cattle slaughter using non-NPPSS

Variables	Incidence of false aneurysm (N (%))			Total	Odds ratio	P
	No FA	1 Artery	2 Arteries			
Number of incision(s)						
>3 times	6 (37.5)	4 (25.0)	6 (37.5)	16 (32.0)	1.99 (0.30–13.35)	0.48
3 times	9 (42.9)	10 (47.6)	2 (9.5)	21 (42.0)	1.08 (0.18–6.55)	0.94
2 times	0 (0.0)	2 (40.0)	3 (60.0)	5 (10)	9.20 (0.65–130.55)	0.10
1 time	5 (62.5)	3 (37.5)	0 (0.0)	8 (16)	Reference	
Incision location (tracheal ring)						
> T 6	4 (22.2)	6 (33.3)	8 (44.4)	18 (36.0)	11.20 (2.16–58.09)*	0.00
Larynx (T0)	0 (0.0)	1 (25.0)	3 (75.0)	4 (8.0)	72.41 (4.23–1240.68)*	0.00
T 5-6	3 (60.0)	2 (40.0)	0 (0.0)	5 (10)	1.60 (0.19–13.52)	0.67
T 3-4	3 (37.5)	5 (62.5)	0 (0.0)	8 (16)	3.30 (0.55–19.81)	0.19
T 1-2	10 (66.7)	5 (33.3)	0 (0.0)	15 (30.0)	Reference	
Total	20 (40.0)	19 (38.0)	11 (22.0)	50 (100)		

Note: * There is a significant association at p<0.05. NPPSS= non-penetrating pre-slaughter stunning

72.41(4.23–1240.68) and 11.20(2.16–58.09) times higher than the FA incidence at T1-2.

Eight factors were suspected to influence the risk of FA incidence in NPPSS-slaughtered cattle based on the Chi-Square test: sex, SS, SSR, DCFS, BS, ID, NI, and IL, but only three factors having significant associations with the occurrence of FA ($p<0.05$): DCFS, ID, and IL (Table 4). For DCFS, the risk of FA occurrence in NPPSS increased by 2.48 (0.98–6.29) times at intervals ≥121 seconds compared to ≤30 seconds. The second factor that had a significant association with FA incidence was ID. When the incisions were performed with the slaughterman positioned in front of the cattle neck, the risk of FA occurrence was 0.43 (0.20–0.93) times lower than when the ID was from the bottom to the top. While not significantly different, the ID from the top to the bottom did indicate a lower FA risk. The third factor associated with FA is the location of the incision (IL). More caudal incisions increased the FA incidence risk to 3.52 (1.94–6.38); 12.84 (6.47–25.49); and 15.43 (6.91–34.43) times higher at T3-4, T4-5, T6-7, respectively, than an IL at position T1-2 ($p<0.05$). An IL at T0 also had a high FA risk of 13.97 (6.17–31.61) times higher than an IL at the T1-2 position ($p<0.05$).

DISCUSSION

In this study, the BT for non-NPPSS cattle was longer than that reported by Pisestyani *et al*. (2015) at 127.8 seconds in the non-NPPSS group and 181.2 seconds in the NPPSS group. The BT found in the present study also differed from those reported by Anil *et al*. (2006). The total blood loss between the stunned and not-stunned cattle only differed at the beginning of the slaughter, when estimated blood loss was as much as 25%; thereafter, it did not significantly vary at blood losses of 50%, 75%, or 90% (Anil *et al*., 2006). In the non-NPPSS group, the cattle were slaughtered in a conscious state so that coordination between the brainstem, the heart, and lungs was still functioning. When the common carotid artery is severed, there is a drastic decrease in blood flow to the brain (Johnson *et al*., 2015); however, the brainstem, the center of breathing and heart rate regulation, receives its blood supply from the vertebral artery (Baldwin & Bell, 1963; Johnson *et al*., 2015). The decrease in blood flow to the brain due to the severed common carotid artery induces the heart and lungs to increase the blood and oxygen supply, causing blood flow to accelerate through the severed cardiac end of the carotid artery in the non-NPPSS cattle.

In addition, we found a unique bleeding pattern during two bleeding phases. At the beginning of bleeding, blood spurts per the heart rate pattern, then decreases even it stops flowing. Shortly afterward, the blood flow increases again, then decreases until the blood stops gushing. This second bleeding phase may have been missed during previous studies. The heart and lungs are assumed to re-establish function once

Table 4. Analysis of false aneurysm incidence of 460 Australian Brahman cross cattle slaughter using NPPSS

Variables	Incidence of false aneurysm (N (%))			Total	Odds ratio	p
	No FA	1 Artery	2 Arteries			
Sex						
Bull	26 (57.8)	15 (33.3)	4 (8.9)	45 (9.8)	0.91 (0.42–1.96)	0.82
Cow	6 (50.0)	4 (33.3)	2 (16.7)	12 (2.6)	3.32 (0.86–12.79)	0.08
Steer	192 (68.8)	53 (19.0)	34 (12.2)	279 (60.6)	0.87 (0.52–1.46)	0.60
Heifer	77 (62.1)	25 (20.2)	22 (17.7)	124 (27.0)	Reference	
Stunning strength						
Low	93 (70.5)	28 (21.2)	11 (8.3)	132 (28.7)	0.95 (0.48–1.88)	0.87
Recommendation	166 (67.5)	43 (17.5)	37 (15.0)	246 (53.5)	0.79 (0.44–1.40)	0.41
High	42 (51.2)	26 (31.7)	14 (17.1)	82 (17.8)	Reference	
Stunning success rate						
>1 shot not fully unconscious	12 (66.7)	0 (0.0)	6 (33.3)	18 (3.9)	1.01 (0.33–3.06)	0.99
>1 shot unconscious	20 (60.6)	7 (21.2)	6 (18.2)	33 (7.2)	1.66 (0.72–3.82)	0.23
1 shot not fully unconscious	40 (59.7)	18 (26.9)	9913.4)	67 (14.6)	1.10 (0.59–2.03)	0.77
1 shot unconscious	229 (67.0)	72 (21.1)	41 (12.0)	342 (74.3)	Reference	
Door closed-final stunning interval						
≥121 seconds	13 (52.0)	4 (16.0)	8 (32.0)	25 (5.4)	2.48 (0.98–6.29)*	0.05
91–120 seconds	10 (47.6)	4 (19.0)	7 (33.3)	21 (4.6)	1.39 (0.52–3.68)	0.51
61–90 seconds	25 (58.1)	12 (27.9)	6 (14.0)	43 (9.3)	1.04 (0.49–2.20)	0.91
31–60 seconds	58 (66.7)	18 (20.7)	11 (12.6)	87 (18.9)	0.92 (0.52–1.63)	0.77
≤30 seconds	195 (68.7)	59 (20.8)	30 (10.6)	284 (61.7)	Reference	
Broken skull criteria						
No damage	65 (59.6)	29 (26.6)	15 (13.8)	109 (23.7)	0.87 (0.17–4.42)	0.87
Bruise skull	79 (63.2)	32 (25.6)	14 (11.2)	125 (27.2)	1.20 (0.59–2.45)	0.62
Crack of bone skull	112 (75.2)	19 (12.8)	18 (12.1)	149 (32.4)	0.80 (0.43–1.47)	0.47
Crack of bone skull and shifted	41 (58.6)	16 (22.9)	13 (18.6)	70 (15.2)	1.02 (0.55–1.89)	0.95
A hole in the skull	4 (57.1)	1 (14.3)	2 (28.6)	7 (1.5)	Reference	
Incision direction						
Top to bottom	64 (66.7)	13 (13.5)	19 (19.8)	96 (20.9)	0.66 (0.32–1.38)	0.27
In front of neck	79 (79.8)	14 (14.1)	6 (6.1)	99 (21.5)	0.43 (0.20–0.93)*	0.03
Bottom to top	158 (59.6)	70 (26.4)	37 (14.0)	265 (57.6)	Reference	
Number of incisions						
>3 times	22 (50.0)	10 (22.7)	12 (27.3)	44 (9.6)	1.66 (0.70–3.91)	0.25
3 times	78 (69.6)	18 (16.1)	16 (14.3)	112 (24.3)	1.20 (0.58–2.49)	0.62
2 times	59 (70.2)	14 (16.7)	11 (13.1)	84 (18.3)	1.60 (0.78–3.28)	0.20
1 time	142 (64.5)	55 (25.0)	23 (10.5)	220 (47.8)	Reference	
Incision location (tracheal ring)						
> T 6	8 (22.2)	15 (41.7)	13 (36.1)	36 (7.8)	15.43 (6.91–34.43)*	0.00
Larynx	8 (25.8)	13 (41.9)	10 (32.2)	31 (6.7)	13.97 (6.17–31.61)*	0.00
T 5-6	23 (39.0)	17 (28.8)	19 (32.2)	59 (12.8)	12.84 (6.47–25.49)*	0.00
T 3-4	78 (63.9)	28 (23.0)	16 (13.1)	122 (26.5)	3.52 (1.94–6.38)*	0.00
T 1-2	184 (86.8)	24 (11.3)	4 (1.9)	212 (46.1)	Reference	
Total	301 (65.4)	97 (21.1)	62 (13.5)	460 (100)		

Note: * There is a significant association at p<0.05. NPPSS= non-penetrating pre-slaughter stunning

the NPPSS effect has decreased due to the returning consciousness during bleeding, resulting in the second bleeding phase. These two stages of bleeding are critical because some of the World Halal Certification Bodies use heart rate to determine whether or not the animal is dead (Grandin, 2015). The difference in BT between the non-NPPSS and NPPSS groups has implications for determining the death status following bleeding before invasive dressing procedures, such as separating heads, legs, evisceration, and skinning, should begin. Based on these findings, we recommend that invasive dressing techniques not be performed for at least three minutes for non-NPPSS cattle and six minutes for NPPSS animals following slaughter.

The parameters used to determine SSR are posture, vocalization, rhythmic breathing, and eye reflexes based on failure stunning and consciousness (Atkinson *et al.*, 2013; Terlouw *et al.*, 2015; Verhoeven *et al.*, 2015; Verhoeven *et al.*, 2016). Although cattle that show indicators of a stunning failure should be subjected to additional stunning based on the stun quality rate criteria (Atkinson *et al.*, 2013), the limitation of cartridges in Indonesian slaughterhouses results in the second stunning only being performed on cattle that have not collapsed. Thus, there were 67 cattle slaughtered in a not fully unconscious state after receiving one shot and 18 cattle slaughtered in a state that was not fully unconscious even though they had received more than one shot. This is a very critical finding that has implications for animal welfare. The not fully unconscious NPPSS cattle experienced twice the pain due to the use of NPPSS and the slaughtering itself.

The not fully unconscious NPPSS cattle (≥ 1 shot) had BTs that were significantly shorter than unconscious cattle. The faster BT indicates that the cardiovascular system of the not fully unconscious cattle was better than in the unconscious group. The SSR score showed that unconscious cattle with less NS had increased BTs. These animals displayed corneal and palpebral reflexes that indicate the coordination between the afferent nerve fibers of the trigeminal nerve and the efferent nerve fibers of the facial nerve. This phenomenon stipulates coordination between the cerebral cortex, thalamus, and brainstem, which includes the respiratory and circulatory systems (Adams & Sheridan, 2008). Increased FA incidence also prolonged BT due to the presence of FA-inhibiting blood flow due to a blood clot at the end of the incision (Bozzo *et al.,* 2020; Gibson *et al.,* 2015). This condition contrasts with the effect of NS and BS, which accelerates bleeding.

The stunning effectiveness of NPPSS on cattle is lower than that of penetrative stunning, while in horned goats there is no significant difference between the effectiveness of NPPSS and non NPPSS (Collins *et al.,* 2017; Gibson *et al.,* 2019; Neves *et al.,* 2016; Oliveira *et al.,* 2018). The SSR of the unconscious cattle with one shot was 83.8%, while the SSR of the unconscious cattle >1 NS was 64.7%. This lower SSR in NPPSS cattle with >1 NS shortened the BT. The increase in the BS score also shortened the BT, which is related to the low SSR in cattle with >1 shot. If the second stunning was performed at the exact location of the first stunning, the BS score increased, but did not increase SSR. We suggest that this level of consciousness affects the FA incidence. Thus, FA incidence affects BT both in non-NPPSS and NPPSS cattle. The highest FA risk occurs in non-NPPSS cattle, followed by not fully unconscious and >1 shot NPPSS cattle. FA relates to animal welfare, since FA incidence is related to a longer collapse time in cattle experiencing FA (Bozzo *et al.,* 2020; Gibson *et al.,* 2015).

The level of consciousness of the cattle at the time of slaughtering affected the FA incidence. The conscious, non-NPPSS cattle group had a significantly higher FA incidence risk ($p<0.05$). Although not significant ($p>0.05$), the not fully unconscious NPPSS cattle group also showed a higher FA incidence risk than the unconscious cattle. In the non-NPPSS cattle group, the FA incidence relates to aspects of animal welfare since it correlates with longer collapse times in cattle experiencing FA (Bozzo *et al.,* 2020; Gibson *et al.,* 2015). FA increases suffering for cattle that are not fully unconscious due to the stunning failures, slaughter processes, and prolonged conscious state. To reduce the effect of FA on the length of suffering due to a prolonged conscious state and BT, a slaughter-man should take corrective action by cutting the ends of blocked arteries so that profuse bleeding will occur.

The factors that affected FA incidence in the NPPSS group were DCFS interval, IL, and ID. The first factor influencing the incidence of FA was the DCFS interval. In Indonesia, almost all slaughterhouses still use simple restraining boxes without neck braces and head restraints. The lack of a head restraint results in a low SP accuracy, thus increasing the risk of stunning failure because the cattle head can still move freely to avoid stunning (Von Wenzlawowicz *et al.,* 2012), causing the stunner-man to take longer time to perform the stunning. Poor handling when animals enter the restraining box and the time frame of the stunning action can have different effects on the psychological status of the animal in relation to stress-related hormone assessments (Khan *et al.,* 2018. Maghfiroh *et al.,* 2014; Zulkifli *et al.,* 2014). As social animals, the isolation of cattle alone in a restraining box and the stunner-man activity at shooting are potent stressors. A DCFS interval ≥121 seconds causes excessive stress that activates the sympathetic-adrenal medullary axis system, causing arterial constriction and increasing FA risk.

The results of this study agreed with those of Bozzo *et al*. (2020), Gibson *et al*. (2015), and Gregory *et al*. (2012), which stated that the more caudal the IL, the greater the risk of FA incidence. In this research, incisions that severe the larynx/cranial to the larynx also increased the risk of FA incidence. High FA incidence of IL in the larynx/cranial of larynx proves the truth of halal and Kosher slaughter methods, which sever the trachea, esophagus, and common carotid artery (incision should be done behind the larynx). Compared to NPPSS slaughter, the risk factors for FA incidence in the non-NPPSS group only had a significant effect on IL T > 6 and T0 (larynx/cranial of the larynx), whereas NPPSS cattle had a considerable impact on T0 incisions (larynx/cranial of the larynx) and above T3. This indicates that an IL on NPPSS cattle at T1-2 is very important to reduce the FA incidence. In the caudal IL position, the position of the carotid artery is in the profundal of the brachiocephalic muscle, with the cardiac end of the severed carotid artery closer to the heart. A combination of muscle pressure and the heart suction pump increases the risk of FA incidence.

The ID also influenced the FA incidence in the NPPSS group. The higher FA incidence in incisions cut from the bottom to the top may have been due to the more substantial pressure compressing the blood vessels, which increased the risk of FA formation. Incisions made from the bottom to the top produced more significant pressure on the neck. In this position, the shoulder angle is flexed to 180°, and the elbow joint angle is fully extended to create the most unyielding grip strength (Parvatikar & Mukkannavar, 2009). The combination of the gripping force and the force of the concentric contraction of the bicep brachial muscle produces intense pressure on the neck. This pressure is thought to cause the artery to compress and collapse, increasing the risk of FA incidence.

To reduce FA incidence, DCFS should be done as quickly as possible, and the slaughter incision should be just behind the larynx at T1-2, with the slaughter-man positioned in front of the cattle. When the FA occurs, corrective action should be done as soon as possible by cutting the blocked cardiac end of the carotid artery. Delayed corrective action implies prolonged consciousness and increases residual blood in the meat.

CONCLUSION

Based on our results, we concluded that NPPSS influences BT, with non-NPPSS cattle having the shortest BT. The BT in the non-NPPSS groups was affected by FA, whereas in the NPPSS groups were affected by SSR, NS, BS, and FA incidence. Thus, non-NPPSS cattle have a higher risk of FA than NPPSS cattle. The IL influenced the FA incidence in non-NPPSS cattle, with the highest risk of incisions at the T0 and caudal to the sixth tracheal ring. Factors that influenced FA incidence in NPPSS cattle were DCFS, ID, and IL. A DCFS <30 seconds, an ID from the front of the cattle's neck, and an IL at T1-2 level had the lowest FA incidence risk.

CONFLICTS OF INTEREST

We certify there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript. This research has been approved by IPB University ethics commission No. 111a/SKE/KEH/X/2018.

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