Conventional versus modern abattoirs in Colombia: Impacts on welfare indicators and risk factors for high muscle pH in commercial Zebu young bulls

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[1] *In memoriam*

Abstract

The aim of the study was to determine the effects of abattoir type (conventional abattoir -CA- versus modern abattoir –MA-) on stress parameters and risk factors for high muscle pH in Colombia. A total of 522 Zebu young bulls were studied in two groups: 285 at CA, and 237 at MA. Blood samples were taken to measure cortisol, glucose, lactate, creatine kinase, β-hydroxybutyrate, total protein, albumin, creatinine, urea, haematocrit, leukocytes and N/L ratio. Cattle were monitored during the unloading, lairage, handling and stunning. The logistic regression model showed that stocking density, transport time, abattoir type, and inefficient stunning were variables associated with the prevalence of dark cutting carcasses. This study demonstrated that modern improvements at abattoir level, proper infrastructure, and stunning equipment, do not always guarantee quality in terms of animal welfare. As a first attempt in the Colombian beef industry, this research suggested how handling practices could affect cattle welfare and the prevalence of high muscle pH even at MA.

Key Words: Abattoir assessment; Zebu welfare; DFD meat; pre-slaughter stress; Colombia

1. Introduction

During pre-slaughter operations, even under favourable conditions, livestock is exposed to a range of potential stressors which may compromise their welfare, health and performance including increased handling and human contact, transportation, loading and lairage, different or unfamiliar environments, food and water deprivation, alterations in weather conditions and also changes in social structure through separation, mixing and crowding, noise and environmental pollutants (Miranda-de la Lama, Villarroel, & María, 2014). These stressors will initiate a cascade of reactions in the organism, with activation of the nervous sympathetic-adrenomedullary system and the hypothalamic–pituitary–adrenocortical axis, causing an increase in levels of catecholamines and glucocorticoids, respectively (Eriksen et al., 2013). The increased physiological stress and physical activity of the animals during pre-slaughter operations can cause depletion of muscle glycogen, leading to high ultimate pH and therefore result in dark, firm and dry (DFD) meat also known as a dark cutting (Van De Water, Verjans, & Geers, 2003). This type of meat has poor processing characteristics, darker colour, great variations in tenderness and high water-holding capacity and high potential of microbial growth at an early age compared with normal meat (Franco et al., 2015).

Stress in the pre-slaughter logistic chain needs further consideration for ethical reasons

(Miranda-de la Lama, 2013). Several technical, legislative and political initiatives have been developed in Latin America as an attempt to promote cattle welfare, with an emphasis on training programs, the production of best management practices guidelines (Paranhos da Costa, Huertas, Gallo, & Dalla-Costa, 2012), and abattoir modernization (Romero, Uribe-Velásquez, Sánchez, & Miranda-de la Lama, 2013). The design of an abattoir can also have an indirect effect on animal welfare by encouraging or discouraging the stockpersons from optimal actions towards the animals (Hultgren et al., 2014). The establishment of modern abattoirs is a central aspect in developing countries as a strategy for exporting and building a modern meat industry. Global value chains are potential links between smallholder farmers in developing countries and lucrative markets in industrialised nations (Guarín, 2013).

Cattle production is one of the most important sectors of Colombian agribusiness and Colombia is the fourth largest Latin American beef producer with a commercial herd of 27.8 million heads (FAOSTAT, 2015). It is estimated that 90% of the national herd consists of Zebu (*Bos indicus*) breeds. Colombian abattoirs are in the process of modernization (or replacement) of their infrastructure and implementing Good Manufacturing Practices (GMP) and systems of Hazard Analysis and Critical Control Points (HACCP) within the gradual plan of compliance, having as deadline 2016 (MPS, 2007). Colombian commercial systems that include indirect sales via cattle markets or auctions, prolonged transportation, long and poor lairage conditions may result in even greater prevalence of DFD meats (Romero et al., 2013). Studies are generally conducted under experimental or commercial conditions in order to understand the causes and consequences of stress at slaughter. Both approaches are necessary to understand how animals perceive different aspects of the slaughter process and how they react to these in a field situation (Bourguet, Deiss, Tannugi, & Terlouw, 2011). The current study examined animal welfare indicators obtained under commercial conditions of Zebu young bulls from arrival to the slaughter period without any kind of interference or assistance during abattoirs' operations. The study is based on the hypothesis that a modern abattoir with improved pre-slaughter operations may reduce the stress responses of the cattle, which may optimise animal welfare and meat quality. Therefore, the aim of this study was to assess the effects of the abattoir procedures on certain stress parameters, in a conventional abattoir (CA) and a modern abattoir (MA) in Colombian beef cattle, and to determine which factors may increase risk for high ultimate muscle pH.

2. Material and methods

The study was carried out in two commercial abattoirs in Colombian Central Andes (distance of 300 km between facilities) from June to July 2013. Animals came from the same agro-ecologic region and the genetic background was Colombian commercial Zebu. Both abattoirs complied with the announcement 1500 that created the Official System of Meat Inspection, Surveillance and Control for all meat and meat products and established the sanitary and safety requirements for primary production, slaughtering, processing, storage, transport, sales, import and export of all meat and meat products. The animals were transported and slaughtered in compliance with national regulations applied in research and commercial slaughtering. The permission to conduct the study was approved by the Ethics Committee for Animal Experimentation of the University of Caldas (Act 1 13/02/2012, -*Activities with minimal risk*-).

2.1. Study Description

Data were recorded from 522 Colombian comercial Zebu young bulls (Brahman, Guzerat, and Nellore crossbreds), that ranged from 18 to 24 months of age, average live weight of 429±29.03 kg and non-castrated. These comercial phenotypes are very commun in extensive grazing systems; approximately 70% of slaughtered cattle come from this genetic background in Colombia. Animals were divided into 285 animals slaughtered at the conventional abattoir -CA- (20 journeys), and 237 young bulls at the modern abattoir -MA- (17 journeys). The study was performed in two stages at two abattoirs: (1) effects of abattoir type on physiological welfare indicators, stunning, and meat quality, and (2) pre-slaughter risk factors as possible causes for high muscle pH. Four recording visits at each abattoir were carried out during June and July for CA and MA, respectively. Animals were fattened under similar conditions, mainly on native pastures with mineral supplements offered. The livestock trucks monitored during this study complied with Colombian standards for cattle transport and these were the most commonly used in Colombia (14-15 animals capacity), 10-tonne capacity, two-axle vehicles with a rigid chassis (combined wood and steel), passive ventilation and a canvas roof. The young bulls were individually observed during the unloading process, in the slaughter corridors, in the stunning boxes and during bleeding (Fig.1).

2.1.1. Conventional abattoir (CA)

The CA is located in the Caldas Department (5°06′N; 75°33′O, central Colombia), which is characterized by a tropical rain forest climate, a mean annual rainfall of 1878 mm, a mean annual temperature of 15.9°C and an altitude of 2038 m.a.s.l. The abattoir operated from Monday to Friday (0900–1800 hours) with a slaughter capacity of 300 heads/day at a rate of 30 heads/hour. The concrete unloading ramps (20°) had nonslip floors that were as wide as the livestock trailers. They were connected by a series of corridors to a lairage area that consisted of 18 pens (3.65 m wide × 20 m long; 39.9 m^2), without roof and nonslip concrete floors. At the lairage, the animals had access to water *ad-libitum* while resting. A concrete straight passageway guided animals from the lairage area to a stunning box without a head fixation system. Access to the stunning box was through a guillotine door and ejected from the side of the box. After being stunned by a non-penetrating captive bolt, the cattle were slaughtered, suspended by a hind leg, bled and transferred to the production line to begin the process of removing the head, feet, skin, viscera, and quartering of the carcass.

2.1.2. Modern abattoir (MA)

The MA is situated in the Department of Antioquia (6°13′00″N 75°34′00″O; central north western of Colombia), which is characterized by a tropical rain forest climate, a mean annual rainfall of 2060 mm, a mean annual temperature of 16.6°C and an altitude of 2550 m.a.s.l. The abattoir operated from Monday to Friday (0600–1600 hours) with a slaughter capacity of 300 heads/day at a rate of 30 heads/hour. The concrete unloading ramps (45°) had nonslip floors that were as wide as the livestock trailers. They were connected by a series of corridors to a lairage area that consisted of 22 pens (5.9 m wide × 9.8 m long; 57.8 m^2), with roof and nonslip concrete floors. Animals from different livestock trucks were not mixed at the plant and each group was housed in separate pens. Water was freely available, but there was not access to feedstuffs. A concrete curved passageway guided animals from the lairage area to a stunning box with a head fixation system. Access to the box was through a guillotine door and a rotating iron exit door. After being stunned by a non-penetrating captive bolt, the bovines were slaughtered, suspended from a hind leg, and after bleeding were transferred to the production line to begin with the process of removing the head, feet, skin, viscera, and quartering of the carcasses.

2.2. Handling and stunning assessment

The behavioural events recorded were falls (when an animal dropped down from a higher level to a lower level), aggression/fight (antagonistic behaviour observed among animals), slips (when an young bull lost the balance temporarily), jumps (when an animal passed over something by jumping), baulks (when an animal stopped suddenly and refused to walk for more than 10 seconds), reversing (when an animal moved backwards), mounting (when an animal mounted another animal), and vocalization (bellow, moo). The unloading time was considered as the time in which the door of the truck was opened to unload animals until the last animal entered into the lairage at the abattoirs. The same behavioural events were recorded in the slaughter corridors and in the stunning boxes. The frequencies of human-animal interactions were recorded as tactile, auditory and visual. Tactile interactions of humans included pushing, hitting and electric shocks. Additionally, tail twisting and prods to sensitive parts of the animal such as the eyes, ears, nose, anus or testicles were recorded as negative human interactions. Auditory interactions included talking, shouting, whistling and the use of artificial noises, such as banging of pen fittings. Waving was the only visual interaction recorded.

Animals after stunning were considered unconscious if signs of unconsciousness or death were detected (absence of corneal reflex, absence of blinking reflex and absence of rhythmic breathing). These clinical signs are indirectly associated with brain functions involved in consciousness, particularly the reticular formation (Terlouw, Bourguet, & Deiss, 2016) and if consciousness signs were absent (standing posture, head-up reflex, voluntary vocalisations, spontaneous blinking, and eye movements). Other indicators were assessed: delay until stunning (s) (interval between the arrival to the stunning box and the last shot), interval of stunning to bleeding (s) (time interval since the last stunning shot occurred and the beginning of the bleeding), percentage of cattle stunned effectively in the first attempt, shot in the right location of the head and number of shots.

2.3. Physiological assessment

Blood samples were collected during bleeding for lab analysis (two 10-ml tubes per animal - BD Vacutainer, Franklin Lakes, N.J.-, with and without anticoagulant, EDTA). Samples were kept on ice during sampling (up to 2 h) and then taken to the laboratory for routine haematological measurements. For the analysis of blood cellular components (haematocrit value, haemoglobin content, erythrocyte and leukocyte counts), EDTA was placed within the blood collection tubes as anticoagulant, the proportion was 2 mg/ml of blood; on the other hand, the tubes without EDTA were used for blood biochemical components analysis (glucose, lactate and cortisol), these samples were centrifuged at 2500 rpm for 10 min (room temperature) and the serum was separated into 5 ml sterile vials and stored at 0°C for later analyses. The samples used for blood cellular components analysis were stored at 4°C.

Packed cell volume (PCV) values were obtained using the micro haematocrit technique. Leukocyte profile (WBC) and neutrophil: lymphocytes ratio (N/L) were performed during the microscopic observation of blood sample slides stained with Wright's stain. Serum cortisol concentrations (μg/dl) were measured in duplicate using a radioimmunoassay -RIA- (Clinical Assays GammaCoat Cortisol 125I RIA Kit, DiaSorin, Minnesota, USA), the coefficient of variation inter-assay was 9.31%. The concentrations of glucose (mmol/L), urea (mmol/L), total protein (g/L), creatine kinase (CK, U/L), creatinine (mmol/L) were determined using a Biosystem kit (Biosystems®, Barcelona, Spain), and spectrophotometer BTS-330 (Biosystems®, Barcelona, Spain). The evaluation of the β-hydroxybutyrate (ßHB, mmol/L) levels and lactate (mmol/L) concentration were performed with Randox kits (Randox Laboratories Limited®, Crumlin, United Kingdom), and spectrophotometer BTS-330 (Biosystems®, Barcelona, Spain).

2.4. pH measurements and pre-slaughter risk factors for DFD meat

A portable pH meter was used (fitted with a penetrable electrode pH/mV/temperature meter Model IQ150, I. Q. Scientific Instruments, Loveland, CO) to determine carcass pH 24 h *post-mortem* (pH24). The electrode was inserted into a small incision made in the *M. longissimus* in the left side of the carcass ($14^{th}/15^{th}$ rib interface). After every five samples, the pH meter was re-calibrated using two standard buffer solutions at pH 7.0 and 4.0 at room temperature (5 °C). Carcasses that showed pH24>5.8 were classified as dark meat and pH24<5.8 were considered as normal quality. An initial list of pre-slaughter operations-level risk factors for bruising and high muscle pH was created based on a scientific review (Miranda-de la Lama et al., 2014) and discussed by a group of experts from two countries (Colombia and México) in terms of both animal welfare and meat science. Pre-slaughter operations-level risk factors included (1) abattoir type (conventional versus modern abbatoir), (2) journey time (three times: 7-10 h; 10.1-12 h; 12.1-15 h), (3) stock density during lairage (<300 kg/m^2; 300-350 kg/m^2; 351-400 kg/m^2), and (4) effective stunning (yes or not).

2.5. Statistical Analysis

The software Stata Version 12.0 (College Station, Texas, EU) was used for all the statistical analyses. Firstly, a normality test of the evaluated variables was carried out and the variables with non-normal distribution were transformed by means of the natural logarithm and these values were used for later statistical analysis. The replicate effects according to day of transport were not significant and therefore were omitted from the final statistical model.

Multiple linear regressions were used in order to test the relationship between haematological stress indicators and independent variables (abattoir type, transport time, stocking density, lairage time, flooring space at lairage, meat pH, and effective stunning) and models' fit was tested by means of the adjusted R^2. A *t-test* was used in order to test the mean difference between abattoir types (CA vs MA) of the behavioural and animal-human interactions recorded during unloading and handling in the corridor. Pearson correlation (r) was used to measure the degree of relationship of the variables whistles, artificial noise and hits; and with behavioural variables such as slips and falls.

The pH24 values were analysed as a binomial response variable with values of pH24>5.8 and pH<5.8. Logistic regression analyses were performed on higher ultimate pH carcasses. The general model was:

$$Y = \frac{e^{(\beta o + \sum \beta i X i)}}{1 + e^{\beta o + \sum \beta i X i}}$$

Where *Y* is the probability of the presence of higher ultimate pH, *ßo* is the intercept, *ßi* is the regression coefficient, *Xi* is the explanatory variable included in the analysis. Each analysis began with a univariate analysis of each predictor variable in order to explore the data. A full model containing all predictor variables was then used to estimate their effects and significances. Non-significant ($P \geq 0.05$) variables were removed from the model starting with the variable that showed the highest overall *P*-value. The final statistical model included abattoir type (CA vs MA), transport time (h), stocking density (kg/m^2), and effective stunning (immediate collapse). Variables were considered significant when $P \leq 0.05$. The model was run several times in order to assess confounding factors. This procedure consisted in comparing the estimates of the new model with those of the previous model. Confounding was deemed present when estimates changed at least 25%; confounders were forced in the model irrespective of their significance in order to obtain less biased estimates. The goodness-of-fit of the models was checked by the Hosmer–Lemeshow statistic test. The logistic regression model was used to estimate the odds ratio (OR), which is a measure that quantifies the risk in terms of occurrence of a binary variable or dependent variable based on independent variables (abattoir type, transport time, stocking density, lairage time, flooring space at lairage, and effective stunning) on the occurrence of higher ultimate pH. An OR equal to 1 indicated that higher ultimate pH was not affected by an independent variable, an

OR greater or smaller than 1 indicated that higher ultimate pH variable had higher or lesser probability of occurrence in a specific category of the independent variable.

3. Results

The means of pre-slaughter management practices such as transport time, stocking density, lairage time, flooring space at lairage, and weight were not significantly (P≥0.05) different and suggested homogeneity between conventional abattoir (CA) and modern abattoir (MA) (Table 1).

3.1. Handling and stunning assessment

Table 2 shows the averages of behavioural events observed during unloading and handling in the slaughter corridors. The average unloading time at both abattoir types was 3.8±0.4 min per lot that grouped 14 to 15 steers (CA: 3.1±0.4 min, and MA: 4.7±0.7 min; P≤0.01). Slips variable was significantly (P≤0.05) different between abattoirs; the young bulls at MA presented higher proportion of slips than the group in CA during unloading process. Average handling time in the slaughter corridors of both abattoirs was significantly (P≤0.05) different (CA: 4.7±0.9 versus MA: 2.8±0.3 min), and it showed a positive and significant (P≤0.01) relationship with the handling variables such as whistles (r= 0.52), artificial noise (r =0.63) and hits (r = 0.71); and with the behavioural variables such as slips (r= 0.57) and falls (r=0.68).

The slips, falls and vocalizations were significantly (P≤0.05) different during handling in the slaughter corridor, the Zebu young bulls showed more slips, falls and vocalizations at MA than young bulls at CA. The human–animal interactions evaluated at unloading operations showed significant (P≤0.05) differences, the young bulls at MA had more prevalence of hits than animals at CA. Artificial noises were used in grater proportion during loading in conventional abattoir (P≤0.05). The young bulls during handling in the slaughter corridors at MA showed higher stocking density, hits, electric shocks, puncture wounds in sensitive parts, whistling/shouting, artificial noises and waving of hands (P≤0.05) than young bulls at CA throughout the observed period.

Table 3 shows the variables evaluated during stunning and bleeding stages. A low percentage of young bulls were stunned effectively in the two abattoirs (CA: 38.6% and MA: 23.6%) and 11.4% of young bulls experimented more than one shot (n=37). Although a high proportion of animals collapsed with the first shot (CA: 89.5% and MA: 100%), 26% of the shots were located in the right side of young bulls' head at MA and 34% to the left side at CA. A high proportion of young bulls had long time intervals from stunning to bleeding. No significant ($P \geq 0.05$) differences were found in vocalizations, falls and jumps within the stunning box in the two abattoir types.

3.2. Physiological assessment

The means of the physiological welfare indicators of Zebu young bulls at two-abattoir types are presented in Table 4. The values of cortisol and creatinine kinase, creatinine and urea were higher in young bulls at CA than in MA ($P \leq 0.05$). Abattoir type had no significant effect on glucose, lactate, N/L ratio, βHB, total protein, albumin, haematocrit and leucocytes. Results from the multiple linear regressions for transformed blood values of cortisol, hematocrit (PCV), β-hydroxibutyrate (ß-HB), creatinine and leucocytes showed significant ($P \leq 0.05$) relationships among abattoir, lairage time, and stunning (R^2 ranged from 0.11 to 0.21).

3.3. Pre-slaughter risk factors for DFD meat

The overall rate of dark cutting carcasses in the study was 69%, and the prevalence between abattoirs was significantly ($P \leq 0.01$) different (CA: 56.9% versus MA: 83.1%). The results of the regression model (Table 6) indicated that ultimate pH was affected significantly ($P \leq 0.01$) by abattoir type, transport time, stocking density and effective stunning. The Zebu young bulls slaughtered at MA had greater risk of dark cutting carcasses (OR=4.5; $P \leq 0.01$). A longer transport time (12-15 h), increased prevalence of DFD meat (OR=7.0; $P \leq 0.01$) compared with journey time between 7 and 10 h. Stocking density during lairage between 300 and 400 kg/m^2 presented low risk factor for DFD meat (OR=0.14 and 0.12 respectively; $P \leq 0.01$), nonetheless ineffective stunning showed high risk factor (OR=1.6; $P \leq 0.05$).

4. Discussion

From the perspective of abattoir type, this study is the first to address animal welfare and meat quality in Colombian abattoirs. The abattoirs' modernization in countries like Colombia has been cataloged as a progress mechanism within meat industry and as an access opportunity to international markets with high commercial values. This modernization is promoted by public and private entities with the argument that would ensure an improvement in terms of safety, product quality and recently, animal welfare. Our results indicated that the latter is not necessarily true in terms of animal welfare. Both abattoirs had a deficient management according to international guidelines (i.e. Grandin, 2013). However, each abattoir had its own critical and differentiated points; the critical points for MA were related to a deficient management during animal unloading, lairage and driving to slaughtering process whereas the critical points for CA were stunning and bleeding processes.

4.1. Handling and stunning assessment

Often, unloading demands more physical effort than the journey itself (Maria, Villarroel, Chacon, & Gebresenbet, 2004). Our study indicated that the unloading times were short during arrival at both CA and MA that could be related to the Zebu young bulls escape behaviour when are confronted with different environments and acoustic human interactions, which increased the number of jumps and slips during unloading (Hemsworth et al., 2011). However, our results showed that young bulls at MA had higher proportion of slips than young bulls at CA at unloading operations. The possible reasons could be related to the violent handling for unqualified personnel and the inadequate design of the ramp. At MA, the ramp had 45º angle, urine and faeces that increased the possibility of slips whereas at CA, ramps' design had 20º angle that allowed an unloading with lower slips (Grandin, 2008).

Although average handling time in the slaughter corridors was faster at MA than CA, due to the use of whistles, artificial noise and hits, the handling in the slaughter corridors at MA was a critical point due to the high frequency of vocalizations, slips, falls, tail twisting, and wounds on the skin. Acoustic signals like whistles and other noises affected the whole group (Waynert, Stookey, Schwartzkopf-Genswein, Watts, & Waltz, 1999). Another possible explanation for the frequent acoustic interventions in the CA may be the presence of driveways that caused steers to be unwilling to enter the stunning box and therefore resulted in aggressive handling. Similar results were obtained by Hultgren, Wiberg, Berg, Cvek, & Kolstrup (2014). In contrast to the CA, the MA had a curved corridor for ease handling of cattle (Grandin 2005), although this abattoir showed many negative interactions. Although the handlers at each abattoir were consistent throughout the study, the skill, training level and temperament of individual handlers could have influenced handling, perhaps contributing to differences between the abattoirs (Jarvis, Selkirk, & Cockram, 1995). These results indicated that a proper training of the handlers in both abattoirs is urgently required. Human presence and negative interventions may cause fear reactions, increase the risk of bruised meat (Romero et al., 2013), and occupational hazards (Drudi, 2000). Additionally, the results showed a very frequent use of electric prods in the slaughter corridors, with even higher use in the MA compared to the CA. In both abattoirs, frequency of use was much higher than the 5% allowedd in audits of slaughter plants in the USA (Grandin, 2010). Vocalizations and falls were also more frequent in the MA than in the CA, possibly as a result of the frequent use of electrical prod (Miranda-de la Lama et al., 2012).

The frequent occurrence of the guillotine door hitting the back of the animals, fear behaviour in the stunning box in both abattoirs and the frequent use of the electrical prod in the CA illustrated that improvements are urgently needed. For example, indicators such as slips, reversing, falls and jumps could be considered for the design of the stunning boxes (Gallo, Teuber, Cartes, Uribe, & Grandin, 2003). An earlier work showed that negative interventions before or during entry in the stunning box increases the risk of stunning failures (Probst et al., 2014). In addition, in the present study these practices were associated with an increase of ultimate pH, leading to economic losses. Similarly poor handling practices have been described in abattoirs in Chile (Muñoz, Strappini, & Gallo, 2012) and Sweden (Hultgren et al., 2014). Vocalizations during stunning exceeded acceptable levels (3-5%) proposed by Grandin (2013). The increase in vocalization could have been related to inefficient gun calibration (do not calibrated according to the size of cattle), lack of maintenance, deficient personnel training, the presence of very excited cattle and the excessive pressure of the head brace (Grandin, 2010). There was a significant correlation between the delay between entering the stunning box, stunning, and occurrences of vocalization. Vocalizations in the stunning box are indicative of stress (Grandin, 1998), illustrating that animals should be stunned without delay (Muñoz et al., 2012).

Animal restraint systems are required by the authorities in some countries to restrict animals' movements in all directions, with the aim of improving precision of the stunning shot (Gallo et al., 2003). In this vein, 100% of animals in MA showed immediate collapse with the first shot which showed that animal restraint systems have positive benefits compared to 89.5% obtained at CA. These results were consistent with other studies in Latin America, for example in Chile, a program for the evaluation of modernization of stunning box with animal restraint system of cattle based and trained abattoir staff, revealed a significant improvement in management conditions, with better first-shot stunning efficiency (from 72.8 to 97.8%) and an increase percentage of animals correctly stunned from 0.0 to 99.8% (Gallo et al., 2003). In Brazil, a comparative study was carried out between two types of stunning boxes (Bertoloni

& Andreola, 2010), the authors recorded a higher immediate collapse in the stunning box with restraint system (94%) vs. conventional system (84%) when the same pneumatic gun was used. However, not only the use of such systems is part of the improvement of an abattoir, but also a better design of stunning boxes is needed in order to facilitate animals' access and staff training.

At both abattoirs the occurrence of rhythmic breathing after the stunning was higher than considered acceptable in certain abattoirs audits (Grandin, 2013), the highest levels were present at MA. Such levels may be explained by insufficient power of the non-penetrating captive bolt, incorrect shot with respect to the recommended site, or both. The brain controlling breathing is located in the medulla of the brain stem, i.e. at a greater distance from the place of impact of the bolt. Although breathing may occur in correctly stunned steers, it is considered unacceptable because it is an indicator of an increased risk of a poor stunning and the risk is even greater if the occurrence of breathing after stunning is relatively high (Terlouw et al., 2016) as it occurred in the present study. Additionally, the stunning-bleeding interval was often longer than 60 seconds in both abattoirs, increasing even more the risk of a return of consciousness or prolonged suffering (Gregory, Fielding, Von Wenzlawowicz, & Von Holleben, 2010).

4.2. Physiological welfare indicators

The high values of cortisol in blood observed during bleeding showed the effects of management at abattoir level on stress rather than the transport itself. The young bulls at CA had greater levels of cortisol and creatine kinase, suggesting that they experienced more stress than animals at MA. Rough handling during pre-slaughter has been related to increased plasma cortisol levels in cattle particularly in poorly designed abattoirs (Schwartzkopf-Genswein, Faucitano, Dadgar, Shand, González, & Crowe, 2012). In this study, the plasma cortisol levels were 58 and 47 ng/ml when young bulls were slaughtered at CA and MA, respectively. These results were above basal values generally measured in similar commercial categories of *Bos Indicus* (14 ng/ml in Brahman -Agado, 2011) and *Bos Taurus* (i.e. 22.3 ng/ml in Gascon -Miranda-de la Lama et al., 2013; 11 ng/ml in Blond d'Aquitaine, 6.8 ng/ml in Limousin and 6.8 ng/ml Angus -Bourguet, Deiss, Boissy, & Terlouw, 2015), showing higher cortisol values under Colombian slaughter conditions.

The multiple linear regression analysis showed that plasma cortisol levels depended on the abattoir type and were higher after a long waiting at lairage area and due to an efficient rather than an inefficient stunning. An increase in cortisol levels with increasing lairage time have been reported by Bourguet et al. (2011). In the lairages, steers are expected to recover the energy lost during transportation. However, the rate which energy is gained depends upon the amount of stress from transportation and the conditions of the lairages at the abattoir (Chulayo, Bradley, & Muchenje, 2016). These conditions are achieved by controlling the microclimate, do not mixing animals from different origins, gentle handling quality, acoustic isolation, clean pens, and water *ad libitum* (Miranda-de la Lama, 2013). The authors believe that the increase in cortisol concentrations found in the present study was mainly a response to poor lairage conditions in both evaluated abattoirs. Although a significant relationship was observed, the R^2 value was low indicating that the effects were small. One explanation for the latter may be that cortisol sampling occurred relatively quickly after the stunning while cortisol secretion is often a slow process and may take up to 20 min (Apple, Kegley, Galloway, Wistuba, & Rakes, 2005), as a result the measured effects were relatively small.

In the current study, young bulls at CA had greater CK, creatinine and urea levels compared to MA. These levels may express higher levels of physical activity and/or stress (Mpakama, Chulayo, & Muchenje, 2014). The CK leaves the sarcoplasm of muscle cells, especially when they are active, due to a high permeability of the sarcolemma muscle cell membrane (Earley, & Murray, 2010). Creatinine and urea is used to assess the effect of physical stress on the functioning of kidneys in ruminants (Das, Mani, Kaur, Kewalramani, & Agarwal, 2012). The higher levels of stress and physical effort may be related to aspects of the equipment of CA, such as the use of a metal fence bordered straight passageway leading to the stunning box, therefore, struggling with the driving of cattle (Grandin, 2013).

4.3. Pre-slaughter risk factors for DFD meat

The ultimate pH is used to measure meat quality at the commercial level (Villarroel, Maria, Sierra, Sanudo, Garcia-Belenguer, & Gebresenbet, 2001). The ultimate pH cut-off for classifying meat as DFD has been traditionally thought to be above pH 6.0, yet some argue as low as 5.8 (England et al., 2016).). Overall, 69% of the meat was DFD type. Our results were similar to those reported in Mexico (Leyva-García, Figueroa-Saavedra, Sánchez-López, Pérez-Linares, & Barreras-Serrano, 2012), but higher than those reported in Chile (Amtmann, Gallo, Van Schaik, & Tadich, 2006). Furthermore the results showed that MA had higher prevalence of DFD meat than CA. The reason for this might be that steers in MA were under stressful handling during unloading and lairage area, therefore the animals could have been consumed partially their glycogen reserves which suggested an increase in the risks of DFD meat, even if the stunning process was efficient. High ultimate pH (low amplitude of pH decline) is explained by reduced glycogen reserves leading to reduced production of protons and lactate. Reduced glycogen reserves may occur due to an increase in activity and possibly psychological stress during the hours preceding slaughter (Terlouw et al., 2008). Specifically, stress increases adrenaline secretion from the adrenal and causes physical reactions. Adrenaline is known to increase glycogen breakdown in the exercising muscle (Foury et al., 2011). In both abattoirs the prevalence was high, which indicated that the slaughter process, including transport, lairage time and handling in the passageways to the stunning box were demanding for the steers (Warner, Ferguson, Cottrell, & Knee, 2007). In accordance with the latter, the results indicated that a higher prevalence of DFD meat was associated with longer transport times and higher stocking densities during lairage. A higher prevalence was further observed when the stunning was not effective. This was unexpected because stress at the moment of slaughter accelerated early post-mortem pH decline (Bourguet et al., 2011; 2015) and we are unaware of reports about an effect on ultimate pH. Although there are no earlier reports about a relationship between stunning efficiency and prevalence of DFD meat, it is possible that inefficient stunning triggered stress and physical reactions resulting in further glycogen deficiency, thus increasing the risk of DFD meat. These results showed the need for national policies for actions helping to increase animal welfare while simultaneously reducing the incidence of DFD meat.

Lairage is a common commercial practice that allows animal resting after transport. Animals must have access to water and in the EU animals spending more than 12 h in lairage must be fed (Liste et al., 2011). If conditions are good, lairage may allow the replenishment of muscle glycogen concentrations, mitigates dehydration and carcass weight loss (Teke, Akdag, Ekiz, & Ugurlu, 2014). The present study suggests that stocking density was an important aspect of lairage conditions because higher stocking densities were associated with an increase in the risk of DFD meat and possibly the animal welfare was compromised due to restrictions of self-grooming, substrates, water access, shaded places, and dry-comfortable resting places (Miranda-de la Lama et al., 2013). Overall, the results indicated that efforts are required in order to improve animal handling and slaughter. Adequate training programs need to be implemented for slaughterhouse personnel, both in the lairage and stunning areas. Specific protocols for stunning should be designed in order to standardise assessments that allow benchmarking of stunning quality (Atkinson, Velarde, & Algers, 2013) and therefore, contributing to set up standards as a safeguard for cattle welfare during stunning at Colombian abattoirs.

5. Conclusions and implications

This study reported novel information about animal handling, pre-slaughter logistic operations, meat quality and human-animal interactions in Colombian cattle abattoirs. Both abattoirs require major improvements in terms of animal management during unloading, lairage, handling and slaughtering. Specifically for MA, the animal management during unloading, lairage and conduction to the slaughter facility were identified as major critical points whereas stunning and bleeding processes were identified for CA. Both abattoirs showed a high prevalence of DFD meat that were explained to a larger extent by transport time, incorrect cattle handling and stunning. Although the results were specific for the abattoirs studied, they illustrated that steers handling, infrastructure facilities and equipment of an abattoir affected steers welfare and meat quality. In order to reduce the risk of steers' welfare to a minimum, not only the improvement of abattoirs' facilities should be taken into account but also the staff training for proper handling of steers, proper use of equipment and innovations in the design of abattoir facilities. Scientific support for monitoring programmes and risk assessment of animal welfare in Colombian abattoirs should be a prerequisite in modern infrastructure programs. Colombian abattoirs should adopt higher animal welfare standards in terms of handling, design and measurements *post-mortem* in order to increase the commercial benefits like higher revenues in domestic markets or meat trading in international markets.

Conflict of interest

All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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Figure

Fig. 1. Conventional versus modern abattoirs in Colombia: Lairage area (A), concrete passageway leads from the lairage area to a stunning box (B) and stunning box with a head fixation system (C).

Table

Table 1.
Least square means (±SE) of the pre-slaughter management practices at two abattoir types (Conventional Abattoir -CA- vs Modern Abattoir -MA-).

Practices	*Abattoir type*		*Both groups*	*P*
	CA (n=285)	*MA (n=237)*		
Transport time (h)	9.9±1.7	8.8± 1.8	9.4±1.9	NS
Lairage time (h)	15.3±1.3	16.6± 1.5	21.3±1.3	NS
Stocking density (kg/m^2)	330.7±20.1	343.3±20.5	336.4±20.3	NS
Flooring space (lairage; animal/m^2)	0.33±0.09	0.5±0.11	0.41±0.13	NS
Live weight at slaughter (kg)	439.7±20.5	418.2±22.3	429.3±21.3	NS

NS: not significant.

Table 2.

Least square means (±SE) of the behavioural welfare indicators and handling operations during pre-slaughter of Zebu young bulls at two abattoir types (Conventional Abattoir -CA- vs Modern Abattoir -MA-).

Variables	*Unloading*		*Handling in the corridor*	
	CA	*MA*	*CA*	*MA*
Slips	$3.0^{a} \pm 0.4$	$7.1^{b} \pm 0.6$	$4.33^{a} \pm 0.4$	$13.8^{b} \pm 2.3$
Falls	0.7 ± 0.2	0.7 ± 0.2	$2.40^{a} \pm 0.4$	$8.8^{b} \pm 1.6$
Reversing	1.1 ± 0.8	0.7 ± 0.3	26.3 ± 2.2	25.5 ± 4.0
Vocalization	0.5 ± 0.2	0.5 ± 0.1	$10.0^{a} \pm 1.08$	$19.7^{b} \pm 4.1$
Baulks	0.05 ± 0.04	0.06 ± 0.04	1.2 ± 0.4	1.0 ± 0.4
Jumps	0.84 ± 0.2	0.52 ± 0.2	5.3 ± 0.6	4.5 ± 1.1
Mounting	0.03 ± 0.03	0.07 ± 0.03	0.9 ± 0.2	0.7 ± 0.2
Aggression/fight	0.2 ± 0.1	0.1 ± 0.08	-	-
Human–animal interactions				
Compress	-	-	$4.4^{a} \pm 0.5$	$18.2^{b} \pm 3.5$
Tail twisting and wounds on the skin	5.8 ± 1.4	3.6 ± 1.5	-	-
Hits	$5.4^{a} \pm 2.1$	$10.3^{b} \pm 2.4$	$0.5^{a} \pm 0.3$	$27.1^{b} \pm 11.1$
Electric shocks	-	-	$66.9^{a} \pm 4.1$	$152.4^{b} \pm 25.1$
Prods to sensitive parts	3.6 ± 1.5	5.8 ± 1.1	$3.7^{a} \pm 0.7$	$9.7^{b} \pm 3.4$
Whistling/shouting	35.0 ± 5.2	35.3 ± 2.8	$57.4^{a} \pm 4.1$	$68.1^{b} \pm 5.3$
Artificial noises	$7.3^{b} \pm 1.3$	$0.7^{a} \pm 0.3$	$0.4^{a} \pm 0.1$	$20.5^{b} \pm 8.5$
Waving of hands	1.1 ± 0.5	0.6 ± 0.2	$2.6^{a} \pm 0.5$	$5.4^{b} \pm 1.3$

[a,b]: Different lower-case superscripts in the same row indicate differences between abattoir type ($P \leq 0.05$).

Table 3.
Stunning procedures and behaviour of Zebu young bulls at two abattoir types (Conventional Abattoir -CA- vs Modern Abattoir -MA).

Variables	*Abattoir type* *CA* *(n = 285)*	*Abattoir type* *MA* *(n = 237)*
Behaviour and handling in the stunning box		
Slips %	17.6[a]	31.7[b]
Reversing %	17.5[a]	31.6[b]
Falls %	7.7	8.8
Vocalization %	11.2	9.7
Jumps %	9.5	9.3
Electric shocks %	41.8[b]	0[a]
Guillotine door to hit the young bulls %	68.7[b]	34.6[a]
Stunning		
Delay until stun (s)*	30.7[b] ± 1.0	18.3[a] ± 1.0
Shot in the right side %	58[b]	28[a]
Re-shot %	9.2[b]	0.1[a]
Immediate collapse %	89.5[a]	100[b]
Conscious signs		
Corneal reflex (yes)	2.5[b]	0[a]
Rhythmic breathing (yes)	33.0[a]	67.0[b]
Attempt to stand up (yes)	1.41[b]	0[a]
Vocalization (yes)	1.2	0
Interval between stunning (2nd stun if shot twice) and bleeding		
<60 sec %	4.8[b]	1.0[a]
60 – 120 sec %	74.4[b]	43.2[a]
>121 sec %	20.8[a]	55.7[b]

[a,b]: Different lower-case superscripts in the same row indicate differences between abattoir type ($P \leq 0.05$).

Table 4.
Least square means (±SE) of the physiological welfare indicators of Zebu young bulls at two abattoir types (Conventional Abattoir -CA- vs Modern Abattoir -MA).

Variables	*Abattoir type*		*P*
	CA (n=285)	*MA (n=237)*	
Cortisol (ng/ml)	58[b] ± 22	47 [a] ± 19	*
Glucose (g/L)	7.0 ±2.3	6.8 ± 1.8	NS
Lactate (mmol/L)	5.5 ±2.4	5.2 ± 1.8	NS
CK (U/L)	970.6[b] ± 841.5	815.3[a] ± 937.1	*
βHB (mmol/L)	0.36 ± 0.2	0.39 ± 0.15	NS
Total Protein (g/L)	78.2 ±14.5	83.5 ± 7.6	NS
Albumin (g/L)	36.5 ±4.1	33.7 ± 5.2	NS
Creatinine (mmol/L)	168.2[b] ± 24.4	142.6[a] ± 19.4	*
Urea (mmol/L)	8.4[b] ± 1.8	7.7[a] ± 3.6	*
Haematocrit (%)	46.2 ± 5.6	48.6 ± 4.7	NS
Leukocytes (miles/µl)	10.80 ± 4.5	11.65 ± 3.3	NS
N/L ratio	0.77 ± 1.4	0.7 ± 0.3	NS

NS: not significant. * $P \leq 0.05$. [a,b]: different lower-case superscripts in the same row indicate differences between abattoir type. CK: creatinine kinase, βHB: β-hydroxybutyrate, Ratio N/L: neutrophil: lymphocyte ratio.

Table 5.
Results of the multiple linear regressions for transformed blood values of Cortisol, hematocrit (PCV), β-hydroxibutyrate (ß-HB), creatinine and leucocytes.

Variables	*Cortisol* $R^2=0.14$			*PCV* $R^2=0.11$			*Creatinine* $R^2=0.21$			*ß-HB* $R^2=0.12$			*Leucocytes* $R^2=0.13$		
	ß	*Error*	*P*	*ß*	*Error*	*P*	*ß*	*Error*	*P*	*ß*	*Error*	*P*	*ß*	*Error*	*P*
Abattoir	-0.4	0.05	<0.01	-	-	-	-0.2	0.02	<0.01	0.44	0.05	<0.01	0	0	0
Transport time (h)	0	0	0	-0.01	0.002	<0.01	-0.01	0.004	<0.01	0.04	0.011	<0.01	-0.06	0.009	0.02
Stocking density (kg/m^2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lairage time (h)	0.009	0.003	<0.01	0.004	0.0006	<0.01	0.004	0.002	<0.01	0.03	0.004	<0.01	0	0	0
Flooring space (lairage; $animal/m^2$)	0	0	0	0	0	0	0.20	0.001	<0.01	0	0	0	0.52	0.13	<0.01
pH carcass	0.15	0.07	0.04	-0.03	0.017	0.04	0	0	0	0	0	0	-0.15	0.06	0.02
Effective stunning	0.003	0.0007	<0.01	-0.02	0.01	0.03	0	0	0	0	0	0	-0.26	0.1	0.02

Table 6.
Risk factors for DFD meat in Zebu young bulls (n= 522) assessed by multivariable logistic regression.

Variable	*Category*	*OR**	*SE*	*P value*
Abattoir	CA	1.0		Ref.
	MA	4.5	0.9	<0.01
Transport time (h)	7 - 10	1.0		Ref.
	10.1 - 12	6.6	2.8	<0.01
	12.1 - 15	7.0	3.7	<0.01
Stocking density at lairage (kg/m^2)	< 300	1.0		Ref.
	300 – 350	0.14	0.1	<0.01
	351 – 400	0.12	0.9	<0.01
Effective stunning	Yes	1.0		Ref.
	No	1.6	0.4	<0.01

*OR= odds ratios; Ref: category considered as reference. CA: Conventional Abattoir, MA: Modern Abattoir.