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Article

Evaluation of the components of management before, during and after slaughter and their association with the presence of DFD beef in cattle from northeastern Mexico

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Abstract:

A total of 27 management variables (before, during and after slaughter) in 394 bovines were analyzed and used to determine their association and explanatory value with the presence of DFD (Dark, Firm, Dry) beef, using probability ratios in a multiple logistic regression model. The study was conducted from November 2016 to August 2017 on a Federal Inspection Type slaughterhouse located in northeastern Mexico. The presence of DFD beef was 13.45%. A contrast was made between classes for the factors evaluated by means of Student's t and Chi-square according to the nature of the variable as a criterion for inclusion in logistic modeling. Ten of the variables showed statistical significance ($P<0.05$) in these tests, but only four of them presented explanatory value in the final multiple logistic model ($P<0.01$), which were: the waiting time prior to death, poor desensitization, the thickness of the subcutaneous fat and pH differential of the carcass established with 24 h of difference. The first two increased the possibility in the presence of DFD beef, on the contrary, the fat thickness and pH differential were inversely proportional. The four variables included in the final model were present at different stages and are of a different nature. For this reason, to effectively prevent this problem, a multicausal evaluation is needed throughout the slaughter process.

Key words: Meat, Dark cutting, DFD, Bovine.

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Introduction

DFD (Dark, Firm, Dry) beef is a problem that affects the sanitary, physicochemical and sensory quality of the product[1,2], due to a high final pH (>5.8) that favors the growth of bacterial flora and decreases shelf life[3,4]. In addition, DFD beef exhibits a dark red color and sticky texture, which gives the appearance of being meat from old animals or that has been stored for a long time[5,6]. This leads to low consumer acceptance and causes economic losses to producers[7,8]. In Mexico, it is estimated that the loss for each carcass with DFD characteristics is 88.58 US dollars[9], higher than the 5.43 dollars of loss per carcass found in the USA[10]. DFD beef has the following characteristics: increase in water retention capacity, poor palatability, less tenderness and greater light absorption, which affects its technological aptitude for the production of various meat products[11,12,13].

The speed in the decrease of the *post-mortem* pH is directly related to the level of stress suffered by the animal before slaughter[14,15]. Chronic stress and long-term exposure to acute stress, just before being slaughtered, cause muscle glycogen stores to be consumed quickly[2], reducing the amount of lactic acid that is formed by anaerobic glycolysis in the muscle after the death of the animal, which causes the presence of DFD beef, also called Dark Cutting[16,17]. There are different intrinsic factors that increase the risk of a greater presence of DFD beef such as: breed origin (more frequent in *Bos indicus*)[18,19], sex (greater in entire males)[7,20], weight (less common in cattle of greater live weight)[21,22], amount of fat (it occurs more frequently with low values in the thickness of subcutaneous fat)[4,23] and age (it is more frequent in old animals)[24,25]. Environmental conditions also influence the presence of DFD beef, extreme temperatures cause heat or cold stress[26,27].

Improper handling in *ante-mortem* processing is one of the main triggers of dark cutting, long distances in transport and high animal density in small spaces influence their presence, as well as long times in waiting pens, sleeves and in the slaughter drawer[15,20], the use of stressful instruments (electric prods, lariats, sticks, etc.) in the herding towards the desensitization drawer and poor effectiveness of the latter has also been reported as risk factors[28,29]. The post-slaughter process also affects the presence of DFD beef, the rate of decrease in pH and muscle temperature interact continuously during *rigor mortis* and are probably two of the most important *post-mortem* factors that affect the properties of the meat, such as: color, final pH, water retention capacity and tenderness[30,31,32], some characteristics of the carcass such as its weight and the thickness of subcutaneous fat influence this interaction[33,34].

Studies such as the above have been conducted to establish the association between the presence of DFD beef and the factors evaluated; however, most of them have established this association by analyzing these factors individually and in isolation, which does not allow analyzing the effect of the variables together or their interactions. Therefore, the objective of this work was to evaluate the association of factors with explanatory value, as well as their interactions, related to the management before, during and after slaughter, on the presence of DFD beef.

Material and methods

Twenty-seven (27) intrinsic and extrinsic variables were evaluated before, during and after slaughter, in the four seasons of the year. The work was carried out between November 2016 and August 2017 in a Federal Inspection Type (TIF) slaughterhouse located in Cd. Victoria, Tamaulipas. Records of the variables were taken in 16 periods of 3 d each: on arrival, during slaughter and processing of the carcass. The genotype of the animals corresponded, mainly, to commercial crosses of *Bos taurus* x *Bos indicus*. The bovines arrived from different parts of the region and in different types of vehicles.

Sample size

The number of animals was determined for simple random sampling by attributes, considering a finite population(35). The components of the formula were a confidence value of 95% (Z = 1.96), accuracy of 5%, an estimator of variance equal to 0.25 [$\sigma^2 = \pi(1-\pi)$] and an N value generated from the slaughterhouse of the last three years (n= 38,950 animal/year). The sample size obtained (n= 394) was distributed proportionally by the number of slaughters in the seasons of the year: spring= 95, summer= 110, autumn= 78 and winter= 110. Data collection was carried out in November 2016, February, May and August 2017.

Information gathering

Ante-mortem variables

Upon arrival at the slaughterhouse, intrinsic and extrinsic variables such as transport practices, form of acquisition of the animal, season of the year, temperature and relative humidity were recorded (thermohygrometer with a probe, Hanna instruments, model HI9565). In the rest pens, the presence of visible lesions and variables concerning the space and time of permanence was recorded. The separation of an animal in individual pens for behavioral or health reasons (surly, mounts or lesions) was also recorded. Before the slaughter, the day of the week and the individual data referring to the time spent in the sleeve that leads the animals to the desensitization drawer and the conditions of the herding of the cattle were recorded. Temperature and relative humidity were measured before entering the slaughter drawer. The temperature-relative humidity index (ITHR) was obtained by the following formula: $ITH = [0.81*T] + HR/100*(T–14.4) + 46.4$, where T= Ambient temperature (°C) and HR = Relative humidity (%)[36].

Variables during slaughter

The stunning was carried out by means of a captive bolt gun. During the hanging of the animal's body, the effectiveness of desensitization was assessed by recording the following behavioral indicators: spontaneous blinking, total rotation of the eyeball, rhythmic breathing, attempt to get up, straightening and vocalizations. It was considered an incorrect desensitization of the animal when it presented any of the previous signs. The stunning-bleeding interval from the time the animal collapsed to the slaughter was also determined[37,38].

Variables in the hot carcass

The weight of the hot carcass was recorded; in addition, 45 min after slaughter, the pH value (pH_{45min}) was recorded (in triplicate) in order to establish a differential (ΔpH) between the pH_{45min} and the last pH (pHu) evaluated 24 h later, the pH was measured with a potentiometer that had a puncture device for meat (Hanna instruments, model HI99163). The temperature of the carcass was also recorded (in triplicate) at 45 min, in the *Longissimus dorsi* muscle at 5 cm penetration (thermometer with penetration probe, Hanna instruments, model HI935007N).

Variables in the cold carcass

In the cold room (2 °C) 24 h after slaughter, the values of the final pH, the thickness of the subcutaneous fat and the colorimetric parameters were recorded: L* = Luminosity (0 to 100), a* = red index (-60 to 60) and b* = yellow index (-60 to 60). All these records were made in triplicate in the area of the *Longissimus dorsi* muscle between the 10th and 12th rib of the left half carcass, 30 min after having made the cut. The thickness of the subcutaneous fat was determined with a stainless-steel Vernier calibrator and the color values with a Minolta spectrophotometer with 5 cm aperture, illuminant C and 2° observer (Model CR-410, Minolta Co., Ltd., Osaka, Japan,). The Chroma (0 to 200) was calculated using the following equation: $C^* = (a^*2 + b^*2)^{1/2}$[39]. Finally, the density in the cold chamber (number of carcasses/m^2) was recorded.

Classification variables

According to the established criteria, the carcass was classified into dark, firm and non-exudative (DFD) based on the following: pHu ≥ 5.8, $L^* < 40$ and $C^* < 30$[40]. Carcasses that presented different criteria were classified as normal.

Analysis of variables

The contrast between the DFD and normal classes for the studied variables was made according to the nature of the variable: Student's t was used for the continuous quantitative variables, while Chi-square and Fisher's exact test (for frequency <5 in one box) were used for the categorical variables. Significance was established when $P<0.05$.

Association study

The association of the study factors with the classification of meat (dependent variable) of binomial nature (1 = DFD, 0 = normal) was carried out by applying a logistic model with multiple independent variables, as well as their interactions. As a first step in the use of the logistic model, the variables with statistical significance ($P<0.05$) in the comparison between DFD and normal classes were included. Factors that were not significant ($P\geq0.05$) according to Wald's test were excluded from the complete model. This allowed obtaining the final model with its probability ratios (OR), standard errors (EE) and confidence intervals (95% IC). The final model underwent the Hosmer-Lemeshow goodness of fit test[41,42]. The contrasts between DFD and normal classes for the studied variables, as well as the analysis of the logistic model with multiple independent variables were performed when applying the TTEST, FREQ and LOGISTIC procedures of the SAS 9.4 statistical package[43].

Results and discussion

The percentage of DFD beef found in this study was 13.45 %, lower than the 38.99 % observed in the last study conducted in another region of Mexico[41]. Regional differences between the presence of DFD beef suggest that the factors influencing this condition are multiple and varied[44]. In addition, an increase in the frequency of this problem has been observed in other North American countries: in the US, it went from 1.9 % in 2005[45] to 3.2 % in 2012[46] and in Canada from 1.0 % to 1.3 % in a span of just over a decade[47,48].

Of the variables included in this study, only 10 showed significance in DFD *vs* normal contrast (Tables 1 and 2). However, when performing the analysis of the multiple logistic regression model, only four of them showed explanatory value ($P<0.05$), which were: time in the waiting pen, stunning efficiency, subcutaneous fat thickness (EGS) and the pH differential (ΔpH) (Table 3). None of the interactions between these variables or with the remaining ones showed significant value within the model (*P*>0.05). In the Hosmer–Lemeshow goodness of fit test, the null hypothesis was not rejected (*P*=0.963). Table 4 presents the OR values, along with their 95% confidence interval.

Table 1: Effect of quantitative, intrinsic and extrinsic variables on the type of beef

Variable	DFD		Normal		
	Mean	**SE**	**Mean**	**SE**	***P*-value**
Unloading					
Temperature, °C	30.4	1.00	32.5	0.28	0.010
HR–T Index	77.1	10.5	79.7	0.28	0.934
Transport					
Animal density, m^2/ head	3.1	0.29	2.6	0.09	0.061
Rest pen					
Animal density, m^2/ head	9.6	1.17	11.5	0.55	0.214
Time, h	15.4	0.23	14.8	0.10	0.021
Conductive sleeve to the slaughter drawer					
N° of people in herding	1.5	0.13	1.7	0.07	0.449
Temperature, °C	23.6	0.80	25.4	0.24	0.009
HR–T Index	71.2	1.12	74.1	0.33	0.002
Time, min	68.2	6.47	54.4	2.18	0.023
Slaughter					
Stunning-bleeding interval, sec	179.8	10.04	147.7	4.49	0.008
Hot carcass					
Weight, kg	284.7	10.32	295.8	3.78	0.289
pH_{45min}	7.0	0.03	6.9	0.02	0.131
Temperature, $°C_{45min}$	33.2	0.31	33.5	0.11	0.351
Cold carcass					
ΔpH	0.95	0.04	1.45	0.02	< 0.001
Fat thickness, cm	0.40	0.04	0.55	0.02	0.007
Density in the cold room, m^2/ carcass	2.3	0.06	2.2	0.03	0.667

P-value of Student’s t test.

Table 2: Effect of categorical, intrinsic and extrinsic variables on the type of beef

Variable	**DFD**		**Normal**		
	Frequency	**Percentage**	**Frequency**	**Percentage**	***P*-value**
Animal					
Sex					0.186(1)
Male	11	20.00	44	80.00	
Female	42	12.39	297	87.61	
Origin					
Form of acquisition					0.895(1)
Auction	18	14.17	109	88.89	
Farm	35	13.11	232	86.49	
Unloading					
Season					0.097(1)
Spring	6	6.32	89	93.68	
Summer	15	13.51	96	86.49	
Autumn	14	17.95	64	82.05	
Winter	18	16.36	92	83.64	
Transport					
Distance					0.048(1)
>60 min	9	25.71	26	74.29	
30-60 min	6	8.45	65	91.55	
<30 min	38	13.19	250	86.81	
Type of transport					0.191(1)
<2m long	24	13.87	149	86.13	
2-4 m long	6	25.00	18	75.00	
>4m long	23	11.68	174	88.32	
Rest pens					
Separation					0.741(2)
Yes	3	15.00	17	85.00	
No	50	13.37	324	86.63	
Visible lesions					0.293(2)
Yes	2	25.00	6	75.00	
No	51	13.21	335	86.79	
Conductive sleeve to the slaughter drawer					
Herding instrument					0.070(1)
Prod	25	15.92	132	84.08	
Other	6	6.38	88	93.62	
None	22	15.38	121	84.62	
Falls					0.089(2)

Yes	2	50.00	2	50.00	
No	51	13.08	339	86.92	
Slaughter					
Day of the week					0.771(1)
Monday	30	14.15	182	85.85	
Thursday	23	12.64	159	87.36	
Efficacy in stunning					< 0.001(1)
Correct	25	9.29	244	90.71	
Wrong	28	22.40	97	77.60	

P-value of χ^2 test (1) and Fisher's exact test (2).

Table 3: Coefficient, standard error and *P*-value of the variables included in the multiple logistic model

Variable	**Coefficient**	**Standard error**	***P*-value**
Time in corral, h	0.522	0.126	<0.0001
Stunning efficacy	1.251	0.375	0.0009
Subcutaneous fat thickness, cm	-1.883	0.636	0.0031
pH differential [ΔpH]	-4.554	0.695	<0.0001
Constant	-5.308		

Table 4: Probability ratio (OR) and confidence interval (CI) of the variables included in the logistic model

Variable	**OR**	**95% CI**
Time in pen, h	1.686	1.317 a 2.159
Incorrect stunning	3.492	1.674 a 7.287
Subcutaneous fat thickness, cm	0.152	0.044 a 0.529
pH differential [ΔpH]	0.011	0.003 a 0.041

The time prior to slaughter that cattle spend in the waiting pens is associated with the presence of DFD beef, the OR value indicates that the possibility of this defect occurring in the carcasses is 1.69 times greater for each hour that elapses. Some authors recommend a rest time of 3 hours as sufficient for the animal to recover from the negative effects derived from transport(15,49), however, the regulations of Mexico and other countries indicate that the rest time of the animals in the slaughterhouse should be from 12 to 24 h(50,51), considering the OR value obtained in this study, the application of the maximum time of these standards implies a significant increase in the risk of presence of DFD beef. Waiting times higher than 15.8 h and 12.0 h in retention pens, evaluated in two different studies, have resulted in OR values of 2.20 and 2.03 respectively, estimated by applying logistic regression models for carcasses with final pH $\geq$5.8[7,52]. The results of this work, as well as those referred to above, show that the longer the animal spends in the rest pen, the more stressful elements may occur, increasing the possibility of greater frequency of DFD beef.

Poor desensitization of the animal on this slaughterhouse showed a 3.49 times greater chance of resulting in dark-cutting type meat; therefore, in the slaughter of cattle, it is important to determine if the animal is insensitive after the shot, since the bleeding and processing of the carcass cannot begin without having carried out this stage correctly[16,53]. For the efficacy of desensitization in hoisting to be recognized as ‘’acceptable’’, a percentage of no more than 0.2 % of animals with signs of sensitivity must be present[29]. In this study, the percentage of animals with signs of sensitivity in hoisting was 31.7 %, which indicates that, in addition to negatively affecting the quality of meat, there is a serious animal welfare problem; this problem is not exclusive to the slaughterhouse evaluated, since the percentage found was less than the 49.0 % reported in another TIF slaughterhouse in northwestern Mexico[54] and 66.9 % in another slaughterhouse in Chile[15]. In relation to the number of shots, the following percentages were observed: 1 (88.1 %), 2 (9.6 %) and 3 or more (2.3 %). It is considered as ‘’acceptable’’ when the percentage of animals instantly stunned with a single shot is 95 % or more, and as “serious problem’’ when it does not reach 90 %[29]; in this slaughterhouse this last figure was not reached, evidencing the problem of animal welfare at this stage. The most frequent causes of the low efficacy in desensitization by firing with retractable bolt are improper maintenance of the gun or fatigue that the operator experiences due to a high speed of the flow of animals in the stunning drawer[55]. Although there are studies that have examined the impact of a poor desensitization on the presence of DFD beef[28], most research on desensitization in cattle has paid greater attention to behavioral and physiological reactions related to animal welfare[29,56]. However, the efficiency of stunning in the quality of the carcass should be assessed more thoroughly[57], as desensitization is a very important part of the slaughter process and therefore can affect the quality of the final product[58].

The EGS showed an inversely proportional relationship on the presence of DFD beef. The value of OR of 0.15 indicates that it is a protective factor. Its inverse indicates that for each cm of increase in EGS, there is a 6.67 times greater chance of resulting in meat normal. This result was similar to that obtained in another research that applied the SEUROP carcass fatness grade classification system, where an OR of 0.18 was observed for carcasses with a good fatness grade[7]. It is estimated that carcasses with an EGS of less than 0.76 cm have a higher probability of presenting DFD beef[4]. Carcasses with greater fatness maintain a temperature similar to the live animal for longer when they are introduced to the cold room[23], accelerating muscle metabolism and presenting a greater decrease in pH in the process of establishing *rigor mortis*[30].

The rate of pH decrease in the muscle *post-rigor* has a direct influence on the pHu and the color of the carcasses. The relationship observed between ΔpH and the presence of DFD beef was inversely proportional, with a value of 0.011 for OR. Its inverse indicates that, for each increment by a unit, the chance of normal meat being presented will be 90.9 times greater. There is a direct relationship between the rate of pH decline and the temperature of the carcass[32,59]. Carcasses with higher temperatures in the *pre-rigor* period generate higher ΔpH values, therefore, with less possibility of resulting in dark cutting[30].

Conclusions and implications

The percentage in the presence of DFD beef obtained in this study was 13.45 %. Of the 27 variables evaluated, 10 of them, intrinsic and extrinsic, revealed statistical association with the presence of DFD beef, however, only four of these ten showed explanatory value to quantify the risk of dark cutting within the mathematical model used; these were: time in the waiting pen, efficacy of the desensitization (where animal welfare problems were observed), ΔpH and EGS. The first three are present throughout the slaughter process; from the handling that is given to animals before and during death, as well as in *post-mortem* metabolism, the latter is typical of the animal. Therefore, a multicausal evaluation is necessary throughout the slaughter process to adequately prevent this problem. Overall, this study presents concrete data on what factors actually favor the presence of DFD beef, with a direct interest for the slaughterhouse itself and for those working under similar conditions (TIF), but also for scientific purposes.

Literature cited:

1. Alende M, Volpi-Lagreca G, Pordomingo AJ, Pighín D, Grigioni G, Carduza F, *et al*. Efectos del tiempo de transporte, espera pre-faena y maduración en novillos sobre indicadores de estrés, calidad instrumental y sensorial de la carne. Arch Med Vet 2014;(46):217-227.

2. Adzitey F, Nurul H. Pale soft exudative (PSE) and dark firm dry (DFD) meats: causes and measures to reduce these incidences - a mini review. Int Food Res J 2011;(18):11-20.

3. Lawrie RA, Ledward DA. Lawrie's meat science. 7th ed. Abington Hall, England. Published by Woodhead Publishing Limited; 2006.

4. Page JK, Wulf DM, Schwotzer TR. A survey of beef muscle color and pH. J Anim Sci 2001;79(3):678-687.

5. Hughes J, Clarke F, Purslow P, Warnerd R. High pH in beef *Longissimus thoracis* reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. Food Res Int 2017;(101):228-238.

6. Sawyer J, Apple J, Johnson Z, Baublits R, Yancey J. Fresh and cooked color of dark cutting beef can be altered by post-rigor enhancement with lactic acid. Meat Sci 2009;83(2):263-270.

7. Mach N, Bach A, Velarde A, Devant M. Association between animal, transportation, slaughterhouse practices, and meat pH in beef. Meat Sci 2008;(78):232–238.

8. Viljoen HF, de Kock HL, Webb EC. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. Meat Sci 2001;(61):181–185.

9. Leyva-García IA, Figueroa-Saavedra F, Sánchez-López E, Pérez-Linares C, Barreras-Serrano A. Economic impact of DFD beef in a Federal Inspection Type (TIF) slaughterhouse. Arch Med Vet 2012;(44):39-42.

10. Miller M. Dark firm and dry beef. Beef facts product enhancement. Texas Tech University. 2007 https://fyi.extension.wisc.edu/wbic/files/2011/04/Dark-Firm-and-Dry-Beef.pdf. Accessed Aug 20, 2019.

11. Apple JK, Kegley EB, Galloway DL, Wistuba TJ, Rakes LK. Duration of restraint and isolation stress as a model to study the dark-cutting condition in cattle. J Anim Sci 2005;(83):1202-1214.

12. Zhang SX, Farouk MM, Young OA, Wieliezko KJ, Podmore C. Functional stability of frozen normal and high pH beef. Meat Sci 2005;(69):765–772.

13. Huff-Lonergan E, Lonergan SM. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci 2005;(71):194–204,

14. Ferguson DM, Warner RD. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? Meat Sci 2008;(80):12–19.

15. Gallo C, Teuber M, Cartes M, Uribe H, Grandin T. Improvements in stunning of cattle with a pneumatic stunner after changes in equipment and employee training. Arch Med Vet 2003;(35):159-170.

16. Chambers PG, Grandin T. Guidelines for humane handling, transport and slaughter of livestock. FAO-HIS. 2001.

17. Mota D, Huertas SM, Alarcón AD, Pérez C, Guerrero I, Carrasco A, *et al*. Músculo oscuro, firme y seco: mecanismos involucrados. En: Mota D, *et al*, editores. Bienestar animal. Una visión global en Iberoamérica. 3a ed. Barcelona, España: Elsevier; 2016: 447-493.

18. Curley KO, Paschal JC, Welsh TH, Randel RD. Technical note: Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J Anim Sci 2006;(84):3100-3103.

19. King DA, Schuehle CE, Randel RD, Welsh TH, Oliphint RA, Baird BE, *et al*. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci 2006;74(3):546-556.

20. Panea B, Ripoll G, Olleta JL, Sañudo C. Efecto del sexo y del cruzamiento sobre la calidad instrumental y sensorial y sobre la aceptación de la carne de añojos de la raza Avileña-negra ibérica. ITEA. 2011:107(3):239-250

21. Mahmood S, Basarab JA, Dixon WT, Bruce HL. Can potential for dark cutting be predicted by phenotype? Relationship between sex, carcass characteristics and the incidence of dark cutting beef. Can J Anim Sci 2016;(96):19-31.

22. McGilchrist P, Alston CL, Gardner GE, Thomson KL, Pethick DW. Beef carcasses with larger eye muscle areas, lower ossification scores and improved nutrition have a lower incidence of dark cutting. Meat Sci 2012;(92):474–480.

23. Sañudo C, Monsón F, Campo MM, Beltrán JA, Bello JM. Variación del pH en canales comerciales de cordero. En: Casasús-Pueyo I editor. XXXVII Jornadas de estudio y XI Jornadas sobre producción animal. Zaragoza, España 2005:703-705.

24. Hopkins DL, Stanley DF, Martin LC, Toohey ES, Gilmour AR. Genotype and age effects on sheep meat production. 3. Meat quality. Aus J Exp Agric 2007;47(10):1155-1164.

25. Vestergaard M, Oksbjerg N, Henckel P. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, *Longissimus dorsi* and supraspinatus muscles of young bulls. Meat Sci 2000;54(2):177-185.

26. Kadim IT, Mahgoub O, Al-Ajmi DS, Al-Maqbaly, RS, Al-Mugheiry SM, Bartolome DY. The influence of season on quality characteristics of hot-boned beef *Longissimus thoracis*. Meat Sci 2004;66(4):831-836.

27. Gregory NG. How climatic changes could affect meat quality. Food Research Int 2010;43(7):1866-1873.

28. Chulayo AY, Bradley G, Muchenje V. Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, HSPA1A and how they relate with meat quality in cattle. Meat Sci 2016;(117):89-96.

29. Grandin T. Return sensibility problems after penetrating captive bolt stunning of cattle in commercial beef slaughter plants. J Am Vet Med Assoc 2002;(221):1258-1261.

30. Cadavez VAP, Xavier C, Gonzales-Barrona U. Classification of beef carcasses from Portugal using animal characteristics and pH/temperature decline descriptors. Meat Sci 2019;(153):94-102.

31. Jacob RH, Surridge VSM, Beatty DT, Gardner GE, Warner RD. Grain feeding increases core body temperature of beef cattle. Animal Prod Sci 2014;(54):444–449.

32. Warner RD, Thompson JM, Polkinghorne R, Gutzke D, Kearney GA. A consumer sensory study of the influence of rigor temperature on eating quality and ageing potential of beef striploin and rump. Animal Prod Sci 2014;(54):396–406.

33. Hargreaves A, Barrales L, Peña I, Larraín R, Zamorano L. Factores que influyen en el pH último e incidencia de corte oscuro en canales de bovinos. Cien Inv Agr 2004;31(3):155-166.

34. Lonergan HE, Zhang W, Lonergan SM. Biochemistry of postmortem muscle - lessons on mechanisms of meat tenderization. Meat Sci 2010;86(1):184-195.

35. Daniel WW, Cross CL. Biostatistics: A foundation for analysis in the health sciences. Tenth edition. New York, NY, USA; 2013.

36. Hahn GL. Dynamic responses of cattle to thermal heat loads. J Anim Sci 1999;77(2):10–20.

37. FAO. Manual de buenas prácticas para la industria de la carne. Organización de las Naciones Unidas para la Agricultura y la Alimentación. Roma, Italia; 2007.

38. HSA. Aturdimiento de animales por perno cautivo. Humane Slaughter Association. Wheat hampstead, Herts. AL4 8AN, UK; 2016.

39. Konica-Minolta. Precise color communication. Konica Minolta Sensing Inc. Japan. 2007.https://www.konicaminolta.com/instruments/knowledge/color/pdf/color_communication.pdf. Accessed 21 Aug, 2019.

40. Pérez-Linares C, Barreras A, Sánchez E, Herrera B, Figueroa-Saavedra F. The effect of changing the pre-slaughter handling on bovine cattle DFD meat. Rev MVZ Córdoba. 2015;20(3):4688-4697.

41. Lattin J, Green PE, Carroll D. Analyzing multivariate data. Pacific Grove, CA, USA. Brooks/Cole Editor; 2003.

42. Hosmer DW, Lemeshow S. Applied logistic regression. 2nd ed. New York, NY, USA. Wiley-Interscience publication; 2000.

43. SAS Inc. Base SAS® 9.4 Procedures Guide: Statistical procedures. 2nd ed. SAS Institute Inc. Cary, NC, USA: 2013.

44. Sánchez E, Navarro C, Sayas ME, Sendra E, Fernández J, Pérez JA. Análisis de diferentes factores que afectan la calidad de la carne: factores intrínsecos y *ante mortem*. En: Mota D, *et al,* editores. Bienestar animal. Una visión global en Iberoamérica. 3a ed. Barcelona, España: Elsevier; 2016: 495-510.

45. Garcia LG, Nicholson KL, Hoffman TW, Lawrence TE, Hale DS, Griffin DB, *et al*. National Beef Quality Audit-2005: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. J Anim Sci 2008;(86):3533- 3543.

46. Moore MC, Gray GD, Hale DS, Kerth CR, Griffin DB, Savell JW, *et al*. National beef quality audit–2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers. J Anim Sci 2012;90(13):5143-5151.

47. Donkersgoed JV, Jewison G, Bygrove S, Gillis K, Malchow D, McLeod G. Canadian beef quality audit 1998-99. Can Vet J 2001;(42):121-126.

48. Beef Cattle Research Council. National beef quality audit 2010/11 plant carcass audit. Canadian Cattlemen's Association; 2013.

49. Tadich N, Gallo C. Echeverría R, Van Schaik G. Efecto del ayuno durante dos tiempos de confinamiento y de transporte terrestre sobre algunas variables sanguíneas indicadoras de estrés en novillos. Arch Med Vet 2003;35(2):171-185.

50. NOM-033-SAG/ZOO-2014. Norma Oficial Mexicana NOM-033-SAG/ZOO-2014, Métodos para dar muerte a los animales domésticos y silvestres. Diario Oficial de la Federación; 2015.

51. MINAGRI, Reglamento sobre estructura y funcionamiento de mataderos, establecimientos frigoríficos, cámaras frigoríficas y plantas de desposte y fija equipamiento mínimo para tales establecimientos. Diario Oficial. Ministerio de Agricultura de Chile; 2009.

52. Amtmann VA, Gallo C, Van-Schaik G, Tadich N. Relaciones entre el manejo *ante-mortem*, variables sanguíneas indicadoras de estrés y pH de la canal en novillos. Arch Med Vet 2006;38(3):259-264.

53. Grandin T. Recommended animal handling guidelines & audit guide: a systematic approach to animal welfare. American Meat Institute Foundation; 2013.

54. Miranda-de-la-Lama GC, Leyva-García IG, Barreras-Serrano A, Pérez-Linares C, Sánchez-López E, Figueroa-Saavedra F, *et al*. Assessment of cattle welfare at a commercial slaughter plant in the northwest of Mexico. Trop Anim Health Prod 2012;(44):497–504.

55. Méndez-Medina RD, de Aluja AS, Rubio-Lozano MS, Braña-Varela D. Bienestar animal para operarios en rastros de bovinos. 1ª ed. DF, México. SAGARPA-CONACYT-COFUPRO; 2013.

56. Muñoz D, Strappini A, Gallo C. Animal welfare indicators to detect problems in the cattle stunning box. Arch Med Vet 2012;(44):297-302.

57. Velarde A, Gispert M, Diestre A, Manteca X. Effect of electrical stunning on meat and carcass quality in lambs. Meat Sci 2003;63(1):35-38.

58. Ríos-Rincón FG, Acosta-Sánchez DC. Sacrificio humanitario de ganado bovino e inocuidad de la carne. Nacameh 2008;2(2):106-123.

59. Warris PD. Ciencia de la carne. 1a ed. Zaragoza, España: Editorial Acribia; 2003.