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Lairage time effect on meat quality in Hereford steers in rangeland conditions

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ABSTRACT - This study evaluated lairage time effects on carcass and meat quality traits of Hereford steers. Thirty Hereford steers fed on pasture were assigned to two treatments according to lairage time: 3 h (n = 15) and 12 h (n = 15). Individual temperament was assessed using crush score and flight speed. pH decline, glycogen content, meat color, and shear force were measured. pH was not different between treatments at any time point, showing a normal decline rate. Meat color and shear force did not differ between treatments, but muscle glycogen was lower in treatment with 3 h, not enough to affect quality but suggesting a higher level of stress in 3 h lairage time. Temperament did not have any impact on carcass and meat quality traits. No differences were found in pH, color, and tenderness between treatments, but the lower muscle glycogen concentrations for the shorter lairage suggest a higher risk regarding meat quality.

Keywords: crush score, flight speed, glycogen, pH, shear force

Introduction

Pre-slaughter handling procedures are critical for beef cattle, because the animals may be exposed to stressful situations in which fear, dehydration, hunger, increased physical activity, fatigue, and physical injury may occur and may negatively affect meat quality (for a review see Ferguson and Warner, 2008). Expenditure of glycogen reserve could increase due to these stressful situations, leading to the reduction of glycogen concentration in the muscle. In turn, this condition could impair proper meat acidification, sometimes negatively affecting meat quality (reviewed in Muchenje et al., 2009).

Lairage time is part of the pre-slaughter procedures and is related to a variety of stress factors challenging farm animals (Velarde and Dalmau, 2012). Some studies have shown that long lairage leads to a negative impact on meat quality (Gallo et al., 2003; Amtmann et al., 2006). Thus, some international bodies recommend that all livestock animals should be slaughtered immediately after their arrival at the slaughterhouse (European Union Council - Council Regulation (EC) Nº 1099/2009 of 24 September 2009; OIE, 2009). On the other hand, other authors have reported that longer lairage time allows cattle

to recover from the stress of transport with a positive effect on the meat acidification process (Mounier et al., 2006; del Campo et al., 2010).

The impact of lairage time on meat quality is mediated by stress physiology, and there is clear evidence that cattle with more excitable temperaments are more susceptible to stressful situations (Curley et al., 2008; Cafe et al., 2011a). Some studies show that more excitable cattle have higher ultimate pH and shear force values and higher incidence of dark cuts (Voisinet et al., 1997; del Campo et al., 2010; Hall et al., 2011; Coutinho et al., 2017). However, the relationship between temperament and meat quality seems to be more complex, since some authors have failed to find significant effects (Turner et al., 2011) or do not agree about which meat quality indicators are affected by which temperament traits (King et al., 2006; Behrends et al., 2009; Cafe et al., 2011b; Hall et al., 2011).

Most of the cited studies have explored the isolated effect of either lairage time or temperament on meat quality. Thus, the objective of this study was to evaluate the effects of lairage time on carcass and meat quality traits of Hereford steers in extensive conditions in Uruguay. The relationship between temperament and meat quality traits was also assessed in this experiment.

Material and Methods

The research protocol of the current study was approved by the Committee for the Ethical Use of Animals (Protocol number 2011/1).

Thirty castrated Hereford steers were kept on grazing system with continuous stocking in a single group at an experimental farm in Paysandú, Uruguay (32°00'24.21" S, 57°08'05.84" W, 110 m). The animals were weighed every 15 days during the finishing period to assess weight gain and set the time for slaughter. Slaughter was performed when the animals reached an average of 500 kg of live weight, being three years old at that time.

Temperament assessment was carried out simultaneously with weighing, evaluated by a trained observer, one week before the steers went to the slaughterhouse. Two temperament measurements were used: crush score (CS; adapted by Grandin, 1993) – assessed just after the entry of the animal into the squeeze chute (without using physical restraint in the head bail) by applying a visual score from 1 (calm) to 4 (excitable) considering movements of limbs, head, and tail, with audible breathing, vocalization, visible white of eye, as well as behavioral signs of tension and stress; CS records were taken 4 s after closing the squeeze chute gates; and flight speed (FS), defined by the speed (m/s) taken by each animal to cover a known distance (in this case, 2 m) just after being released from the squeeze chute, as per Burrow et al. (1988). Faster animals were considered to have a more excitable temperament (Burrow, 1997).

Animals from both lairage times (treatments) remained grazing until loading (without fasting on farm). The distance between the farm and the slaughterhouse (located in Salto, Uruguay, 31°24'14.63" S, 57°59'28.09" W, 29 m) was 178 km, and the length of the journey was 3.5 h on average, with three short stops for monitoring the animals. The transport was done in one non-articulated truck, with one deck and two load compartments, ensuring 1.2 m^2/animal, according to Uruguayan cattle transport requirements. The same driver did both trips, using the same truck, and no problems were recorded during the journeys.

The steers were randomly assigned to two treatments, according to lairage time in the slaughter plant: 3 h (from 7:00 to 10:00 h, n = 15) and 12 h (from 18:00 to 6:00 h of the next day, n = 15). Steers from each treatment remained in covered pens with concrete floors, divided in two groups (eight and seven animals) following the international requirements for space allowance (420 kg/2.5 m^2). The groups were not considered as replicates. Animals were fastened during the transport and lairage period and had *ad libitum* access to water in the lairage pen. Animals from both treatments were slaughtered during the morning of the same day in a slaughterhouse enabled for export, following animal welfare standards.

Carcasses were graded using the Uruguayan Grading System (INAC, 1997) based on the conformation and fatness scores. Carcass conformation was based on a visual assessment of muscle mass development, with lower numbers indicating better conformation (1 = good muscle development and 6 = poor muscle development), being classified as I, N, A, C, U, and R according to the conformation score system from Uruguay. Fat finishing was based on the amount and distribution of subcutaneous fat, using a five-grade scale, in which lower numbers indicate lack of fat cover and higher numbers, excessive covering, grading 0, 1, 2, 3, or 4 according to the methodology used in Uruguay.

Samples of approximately 20 g of the *Longissimus thoracis et lumborum* muscles (LTL; between the 11th and the 13th ribs) were extracted from 14 carcasses (seven from each treatment) at 45 min *post mortem* to determine glycogen content. Samples were wrapped in aluminum foil and frozen at −80 °C in nitrogen immediately after extraction. Glycogen content was determined in 2 g of muscle, heated to 100 °C in a test tube with 8 mL of 2 M HCl for 2 h, then filtered and neutralized with 2 N NaOH (del Puerto et al., 2011). Measurements were taken using the glucose oxidase procedure, in which the residues were measured (Passonneau and Lowry, 1993). Results were expressed as milligrams of glucose residue per gram of muscle (mg/g).

Carcass pH was measured on the left side of all carcasses (n = 15 for each treatment), in the LTL muscle (between the 11th and the 13th rib) at 1, 3, 6, 24, and 48 h *post mortem*, using a pH meter (Orion 210A) with gel device; assuming the 24 h *post mortem* measurement as the ultimate pH (pHu).

One steak per animal (n = 15 for each treatment) from the LTL muscle (between the 11th and the 13th ribs) was vacuum-packaged individually and transported to the laboratory. The steak (2.54 cm thickness) was aged for two days at 2-4 °C, and after that, meat color and shear force (WBSF) were measured. Meat color was measured on the LTL for L* (lightness), a* (redness), and b* (yellowness) color spaces (CIE, 1986), using a Minolta® C 400 colorimeter, after 1 h of blooming. Values were registered from three different locations on the upper side of the steak to obtain a representative average value.

The same sample was put into a polyethylene bag and cooked in a water bath for 1.5 h until an internal temperature of 70 °C was achieved (checked by a Barnant 115 thermometer with type E thermocouple). Six cores, 1.27 cm diameter, were removed from each steak parallel to the muscle fiber orientation. Shear force measurement was obtained for each core using a texturometer (Warner Bratzler – Model D 2000), and an average value was calculated for each steak.

To evaluate the effect of lairage time and temperament on carcass and meat quality traits, analysis of variance was used fitting a general linear model. The interactions between treatments and CS or FS were firstly tested using GLM procedure of SAS (Statistical Analysis System, version 9.4.). Because no significant interactions were found, the final models included only the main effects (lairage times, CS, and FS). Before the inclusion of both CS and FS together as fixed effects in the statistical models, Pearson's coefficient of correlation was first calculated between them, showing that both temperament indicators were not associated (r = −0.08; P = 0.698) and, therefore, express different facets of cattle temperament.

For these analyses, the flight speed variable was categorized into three classes based on terciles, as follows: low FS (calmer animals; 0.04 to 0.41 m/s), medium FS (medium temperament; 0.42 to 0.46 m/s), and high FS (more excitable animals; 0.50 to 0.90 m/s). Due to the low occurrence of animals classified with extreme scores for CS (N = 2 for CS 1 and CS 4), this indicator was categorized into two classes: low CS (calmer animals; scores 1 + 2) and high CS (more excitable animals; scores 3 + 4).

The following model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk},$$

in which Y_{ijk} = the observation; μ = overall mean; α_i = the fixed effect of lairage time (3 and 12 h); β_j = the fixed effect of CS (low and high); γ_k = the fixed effect of FS in classes (low, medium, and high);

and e_{ijk} = the error. Adjusted means were compared by the Tukey test and the results were considered statistically significant when P<0.05.

Results

Based on the Uruguayan classification system of carcass conformation, there were no differences between treatments, as it was expected (P>0.05), with 90% of the animals graded as A and 10% graded as C. For fatness cover, 86.67% of the carcasses was scored as grade 2 (fat moderately abundant and with uniform distribution) and 13.33% as grade 1 (scarce cover with large areas without fat). No differences were found in hot carcass weight between treatments (mean±SD = 268.79±2.97 kg for 3 h and 266.59±2.97 kg for 12 h lairage; P>0.05).

The muscle glycogen content differed between treatments. Animals kept in lairage for 3 h showed lower muscle glycogen content than those kept for 12 h (Table 1).

pH values did not differ between treatments at any of the evaluated times, with an adequate rate of pH decline and with pHu (24h) values below 5.8 (means and SE are shown in Table 1). In addition, there were no significant effects of lairage time on either meat color or WBSF (Table 2).

In the present study, the FS±SD was 0.44±0.19 m/s (n = 29; min = 0.04 and max = 0.90 m/s). Considering the terciles, 34.48% of the animals had low FS (calmer animals; 0.04 to 0.41 m/s), 34.48% had medium FS (medium temperament; 0.42 to 0.46 m/s), and 31.04% showed a high FS (more excitable animals; 0.50 to 0.90 m/s). Regarding CS, 66.67% of the animals showed a low CS (calmer animals; scores 1 + 2) and 33.33% had a high CS (more excitable animals; 3 + 4).

Crush score and FS did not affect muscle glycogen concentration, meat color, and WBSF (Table 3). Regarding pH, there was a significant effect of CS on pH at 6 and 24 h *post mortem* and of FS on pH at 24 h *post mortem* (Table 4). In general, more excitable animals (high CS and FS) produced meat with lower pH when compared with the calmer ones (low CS and FS). However, all pHu values were below 5.8 and, therefore, had no potential to compromise meat quality.

Table 1 - Effect of lairage time on muscle glycogen concentration and pH values of Hereford steers collected at different times *post mortem*

Lairage	Glycogen (mg/g)	pH 1 h	pH 3 h	pH 6 h	pH 24 h	pH 48 h
3 h	3.30b±0.85	6.47±0.05	6.12±0.05	6.03±0.05	5.57±0.01	5.52±0.01
12 h	10.36a±1.11	6.57±0.07	6.25±0.10	6.10±0.08	5.57±0.03	5.54±0.01
N	14	29	29	29	29	29
F-value	16.33	0.92	1.17	0.65	0.03	1.44
P-value	0.003	0.346	0.291	0.427	0.865	0.242

Data show average ± standard error.
Means in the same column followed by different letters are different by the Tukey test (P<0.05).

Table 2 - Effect of lairage time on meat color and Warner Bratzler Shear Force values of Hereford steers

Lairage	L*	a*	b*	WBSF (N)
3 h	29.38±0.30	13.11±0.30	4.94±0.15	39.11±3.00
12 h	29.54±0.30	13.14±0.24	5.36±0.16	36.86±2.65
Number	29	29	29	29
F-value	0.11	0.01	2.63	0.31
P-value	0.748	0.942	0.118	0.586

L* - lightness of meat; a* - redness of meat; b* - yellowness of meat; WBSF - Warner Bratzler Shear Force (in Newton).
Data show average ± standard error.
Means in the same column followed by different letters are different by the Tukey test (P<0.05).

Table 3 - Effect of temperament traits of Hereford steers on muscle glycogen concentration, meat color, and Warner Bratzler Shear Force values

Trait	No.	Glycogen (mg/g)	L*	a*	b*	WBSF (N)
CS						
Low	19	8.42±1.34 (N = 8)	29.42±0.26	12.94±0.26	5.14±0.15	34.06±2.09
High	10	5.25±1.78 (N = 6)	29.50±0.37	13.31±0.21	5.16±0.17	41.91±4.15
F-value		2.80	0.02	0.69	0.01	3.49
P-value		0.129	0.881	0.415	0.922	0.074
FS						
Low	10	6.81±2.27 (N = 5)	29.33±0.19	13.08±0.30	5.01±0.18	37.38±2.30
Medium	10	7.90±1.50 (N = 5)	29.52±0.49	13.06±0.29	5.13±0.21	38.85±3.91
High	9	5.79±1.66 (N = 4)	29.53±0.43	13.24±0.42	5.31±0.24	37.73±3.66
F-value		0.47	0.08	0.08	0.52	0.05
P-value		0.641	0.927	0.926	0.602	0.951

CS - crush score; FS - flight speed; L* - lightness of meat; a* - redness of meat; b* - yellowness of meat; WBSF - Warner Bratzler Shear Force (in Newton).
Data show average ± standard error.
Means in the same column followed by different letters are different by the Tukey test (P<0.05).

Table 4 - Effect of temperament traits of Hereford steers on pH at different times *post mortem*

Trait	No.	pH 1 h	pH 3 h	pH 6 h	pH 24 h	pH 48 h
CS						
Low	19	6.57±0.06	6.28±0.05	6.17a±0.05	5.61a±0.02	5.53±0.01
High	10	6.47±0.07	6.09±0.13	5.96b±0.09	5.53b±0.02	5.53±0.01
F-value		0.98	2.50	4.39	8.43	0.01
P-value		0.333	0.127	0.047	0.008	0.905
FS						
Low	10	6.53±0.05	6.27±0.09	6.12±0.08	5.61a±0.02	5.53±0.01
Medium	10	6.58±0.06	6.29±0.06	6.12±0.06	5.59ab±0.02	5.54±0.01
High	9	6.45±0.12	6.00±0.13	5.95±0.10	5.52b±0.03	5.52±0.01
F-value		0.59	2.70	1.62	4.67	0.30
P-value		0.565	0.087	0.219	0.019	0.743

CS - crush score; FS - flight speed.
Data show average ± standard error.
Means in the same column followed by different letters are different by the Tukey test (P<0.05).

Discussion

According to Warriss (2003), values lower than 10 mg/g of muscle glycogen are critical for suitable muscle acidification. Brown et al. (1990) reported that muscle glycogen concentration of 8 to 9 mg/g can cause elevation of pH. In the present study, both treatments showed glycogen concentration values even under those proposed thresholds. Nevertheless, pH was not adversely affected.

Muir et al. (1998) considered that pasture-raised animals are more susceptible to pre-slaughter stress, because they may have less muscle glycogen stores and, therefore, higher risk of increased meat pH when compared with grain-fed steers. The animals evaluated in the present experiment were raised on pasture, which may have contributed to the low muscle glycogen content registered for both treatments and especially for treatment of 3 h of lairage time.

Even considering that both treatments had a proper acidification process, the low muscle glycogen concentration registered with 3 h lairage may be associated with stress during the pre-slaughter handling (Ferguson and Warner, 2008). Brown et al. (1990) also showed that some pre-slaughter stress factors reduced glycogen concentrations in 30% of the evaluated animals, but these were not enough to affect meat pHu values. In this case, the authors speculated that if these individuals had been subjected to a little extra effort, there could have been detrimental consequences to meat quality, with reduced

glycogen stores and higher pHu. It is worth noting that the exact glycogen level threshold needed for proper acidification may depend on factors related to the muscle characteristics and factors related to molecular processes involving apoptosis (Laville et al., 2009; Ouali et al., 2013). Thus, regardless of whether muscle acidification remains appropriate, low glycogen concentration should be considered as a warning flag regarding animal welfare.

Is important to mention that the digestive process has a longer lag phase when animals are pasture-fed. In the present experiment, animals from treatment with 12 h lairage ruminated during the night (Costa, 2013); thus, glycogen levels after 12 h could probably have been an important component of glucose availability. They probably had the opportunity to rest overnight when the environment of the slaughterhouse was quieter and could also have achieved some control over possible stress-induced energy intake caused by the new environment. In addition, these animals could have restored their muscle reserves from mobilized liver glucose and would have had greater time to restore muscle glycogen from gluconeogenesis during the resting period.

As previously mentioned, there are different results on the effect of lairage time on pHu, with some authors showing that longer lairage times result in meat with higher pHu (Gallo et al., 2003; Amtmann et al., 2006) and others indicating that longer lairage times result in lower pHu meat (Brown et al., 1990; Mounier et al., 2006; del Campo et al., 2010). These varying results should be expected, given the multifactorial character of these traits, leading the different study designs (physiological status of the animals and lairage conditions) to produce different outcomes. Results from the present experiment suggest that local or regional conditions should be considered when lairage duration has to be defined.

In the present study, there were no significant effects of lairage time on either meat color or WBSF. These results may be directly influenced by the pH values for both treatments, since meat color and tenderness are directly affected by this variable (Abril et al., 2001). Similar results were reported by Ferguson et al. (2007), who did not find significant effect of lairage time (3 and 18 h) on meat color and WBSF. On the other hand, del Campo et al. (2010), comparing lairage times of 3 and 15 h, using conditions similar to those evaluated in the present experiment (productive systems, transportation duration, slaughter plant facilities, and handling procedures) but including Braford animals, reported that the shorter the lairage time, the higher the pH and WBSF values. Those authors suggested that early differences in pH rate decline, probably in that case more influenced by animal temperament and breed (Braford breed), could have determined differences in meat tenderness between treatments. Again, these differences may reflect the changes in pH values found in each of these studies. Therefore, in both experiments that were developed in the same context, the shorter lairage suggested a higher level of animal stress, which should be considered.

Individual temperament could directly affect meat quality. In excitable animals, the stress responses related to the activation of hypothalamic-pituitary-adrenal axis (HPA) and also the sympatho-adrenal-medullary responses can be more intense (Cafe et al., 2011a). Thus, in more "excitable/reactive" individuals, the depletion of muscular glycogen concentration would be more intense than in calmer animals, increasing the risk of meat quality defects (Grandin, 1997; Petherick et al., 2002; King et al., 2006). However, in the present experiment, cattle temperament traits (CS and FS) did not affect muscle glycogen concentration and meat color, consistent with other studies (McGilchrist, 2011; Coombes et al., 2014).

Several authors have reported that excitable temperaments are related to higher WBSF (King et al., 2006; Behrends et al., 2009; del Campo et al., 2010; Hall et al., 2011; Coutinho et al., 2017). One of the factors that may underlie the relationship between temperament and WBSF is the *post mortem* proteolysis during aging, which may vary depending on cattle temperament, although Magolski et al. (2013) suggested that other alternative mechanisms may also explain this relationship.

For *Bos taurus* (Aberdeen Angus and Limousin bulls) and frequently handled cattle, Turner et al. (2011) did not find significant effects of temperament on several meat quality traits, only some trends for the effect of FS and CS on instrumentally measured meat quality attributes (FS on L* and b* color parameters and CS on shear force). They proposed that the temperament variation of the evaluated

animals was not enough to cause negative effects on meat quality. The Hereford animals studied herein are considered even more docile than the other breeds of British origin (Tulloh, 1961; Stricklin et al., 1980). Moreover, they were handled using best management practices, contributing to their low level of reactivity (Waiblinger et al., 2006; Petherick et al., 2009). Thus, our results support previous findings reported by Turner et al. (2011), in which for calm cattle, the detrimental effects of temperament on meat quality are not pronounced. Conversely, for purebred and crossbred *Bos indicus* or for tropically adapted crossbred cattle with higher levels of reactivity to handling, the risks of meat quality defects in function of cattle temperament are considerable, as reported by several authors (Voisinet et al., 1997; Petherick et al., 2002; Kadel et al., 2006; Behrends et al., 2009; Coutinho et al., 2017).

Conclusions

The lower muscle glycogen storage in the short lairage is not enough to adversely affect pH, color, and tenderness. However, it suggests a higher risk regarding meat quality, implying that it could be better to maintain animals in pens for 12 h before slaughter.

Individual temperament differences in Hereford steers do not have any effect on meat quality.

Controversial experimental results have been reported regarding the effects of lairage time on animal welfare and meat quality, depending on the production systems and the general context of the meat production chain. Therefore, international entities such as the European Union and the World Organization for Animal Health (OIE) should consider those differences and therefore, contextualized scientific information, when writing worldwide regulations or recommendations. The findings presented herein may be relevant for such decisions and may help further research required to establish a proper lairage duration before slaughter, considering different situations and factors, like feeding systems, pre-slaughter handling procedures, distances, and transport duration, among others.

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