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Lairage periods on temperament score and meat quality of beef cattle

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Abstract – The objective of this work was to evaluate the influence of lairage periods on the temperament, physiological indicators of stress, and meat quality of beef cattle. Thirty-two castrated Aberdeen Angus x Nellore crossbred steers were distributed into four lairage periods: 12, 18, 24, and 48 hours. The following variables were evaluated: serum levels of the physiological indicators of stress glucose and cortisol (upon arrival at the slaughterhouse, after the lairage period, and at bleeding), animal temperament (movement, resistance to approach, and aggressiveness), and meat quality (final pH, water-holding capacity, color parameters, and tenderness). With a longer lairage period, the temperament score was higher, indicating that the animals were more agitated and resistant to human approach. Serum cortisol and glucose levels correlate positively with animal temperament and negatively with meat quality. At bleeding, an increase was observed in glucose and cortisol levels, respectively, for lairage periods longer than 24 hours and of 12 hours. The lairage of 48 hours reduces meat tenderness and water-holding capacity.

Index terms: animal welfare, cortisol, meat tenderness, stress.

Tempos de descanso pré-abate no escore de temperamento e na qualidade da carne de bovinos de corte

Resumo – O objetivo deste trabalho foi avaliar a influência de diferentes tempos de descanso pré-abate no temperamento, nos indicadores fisiológicos de estresse e na qualidade da carne de bovinos de corte. Trinta e dois novilhos mestiços Aberdeen Angus x Nelore castrados foram distribuídos em quatro tempos de descanso pré-abate: 12, 18, 24 e 48 horas. Foram avaliados as seguintes variáveis: níveis séricos dos indicadores fisiológicos de estresse glicose e cortisol (na chegada dos animais ao matadouro, após o período de descanso e no sangramento), temperamento animal (movimentação, resistência à aproximação e agressividade) e qualidade da carne (valor final para pH, capacidade de retenção de água, parâmetros de coloração e maciez). Com um maior período de descanso pré-abate, a pontuação de temperamento foi mais alta, o que indica que os animais estavam mais agitados e resistentes à aproximação de humanos. Os níveis séricos de cortisol e glicose correlacionam-se positivamente com o temperamento animal e negativamente com a qualidade da carne. No sangramento, observou-se aumento nos níveis de glicose e de cortisol, respectivamente, no tempo de descanso de mais de 24 horas e no de 12 horas. O tempo de descanso de 48 horas reduz a maciez da carne e sua capacidade de retenção de água.

Termos para indexação: bem-estar animal, cortisol, maciez da carne, estresse.

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Introduction

The adaptability of an animal to a given environment can be defined as ability of competences (Prayaga & Henshall, 2005). Knowledge of this varying behaviour in different environments is important to maximize the sustainability of a production system, as well as to achieve the quality standards required in the international market (Sejian et al., 2018). In cattle production, for example, it is possible to examine the negative impact caused by stressful events by evaluating behavioral characteristics, coupled with physiological variables (Chulayo & Muchenje, 2015).

Pre-slaughter handling is the most stressful procedure inflicted on domestic animals. In cattle, there are reports of an increase in escape attempts, vocalization, and defecation and urination during confinement in the corridor to the stunning area (Hultgren et al., 2014). Another factor resulting in pre-slaughter stress is the lairage period, which directly affects animal welfare by altering animal temperament (Chulayo et al., 2016) and indirectly affects meat quality by increasing ultimate pH and shear force (del Campo et al., 2010; Coutinho et al., 2017). Animals exposed to stress in lairages usually produce dark, firm, and dry (DFD) meat that is visually characterized by a dark color of the cut muscle surface (Ponnampalam et al., 2017).

The adverse situations to which animals are subjected can be identified by clinical changes or physiological stress indicators, such as levels of cortisol; these levels are an important biomarker for a more accurate assessment of the reactions expressed by the organism when maintaining homeostasis (Sejian et al., 2018). According to Chulayo & Muchenje (2015), increases in the levels of free cortisol, glucose, and free fatty acids in the blood plasma cause changes in the physiological or behavioral state of the animal, indicating that its welfare has been compromised and its meat quality reduced.

The objective of this work was to evaluate the influence of lairage periods on the temperament, physiological indicators of stress, and meat quality of beef cattle.

Materials and Methods

The research was conducted after approval by the ethics committee on animal use of Universidade Federal de Pelotas (Pelotas, Rio Grande do Sul, Brazil), case number 8794.

Thirty-two castrated Aberdeen Angus x Nellore crossbred steers, with 24 months of age and an average weight of 450 kg, were used. The animals were born in the spring of 2016, being raised and managed, during their lives, as a single lot on the same property in the municipality of Pinheiro Machado, in the state of Rio Grande do Sul (53°43"92.86'W, 31°53"25.38'S, at 117 m altitude), in an exclusive grazing system. The animals were slaughtered in late spring in November 2018, in a commercial slaughterhouse in the municipality of Pelotas, in the same state (52°28"80.69'W, 31°69"46.20'S, at 12 m altitude). The climate of the region is Cfa, humid subtropical, with a mean annual temperature of 18°C according to Köppen-Geiger's classification. The steers were transported for slaughter in the same articulated double-axle vehicle, at a loading density of 415 kg live weight per square meter (1.20 m^2 per animal), on a 3-hour journey over a distance of 127 km from the farm to the slaughterhouse. Upon arrival at the slaughterhouse, the animals were randomly distributed into four lairage periods (12, 18, 24, and 48 hours), with eight animals each, and housed in uncovered pens with concrete floors, observing the international requirements of 420 kg per 2.5 m^2 of space allowance. The steers were fastened during the lairage period and had ad libitum access to water in the lairage pen.

Upon unloading, the steers were removed from the lorry in stages, walking down the unloading ramp and entering a curved chute with a capacity for eight steers. At the opposite end of the chute, before the access corridor to the pens, the animals were restrained with a headgate; at their rear, the handler collected blood (pre-rest) from the caudal vein region. During collection management, neither the animal whose blood was being collected nor the others were able to view the handlers or the other animals, and the location of the restraining chute cancelled out other potential stressors such as noise from machinery and equipment, vehicle traffic, and company employees. Every two new animals whose blood was collected were identified with numbers painted on their loin area and taken to their respective lairage pens, each being destined to a different treatment. After the lairage period established for each treatment, all animals in the lot were returned to the restraining

chute and a new blood collection was performed (post-rest), following the same procedure described previously.

The blood samples were taken to evaluate physiological stress indicators, being collected in test tubes, containing: sodium fluoride, for glucose analysis; or no anticoagulants for serum extraction, for cortisol analysis by electrochemiluminescence using the cobas and 411 Elecsys Roche analyzers (Roche Diagnostics GmBH, Mannheim, Germany).

The tubes containing the blood and added sodium fluoride were centrifuged to obtain plasma. Glucose levels were later determined using commercial Glucose PAP Liquiform kits (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil), through the glucose oxidase method. For the analysis, the test tubes were properly packed, transported, and stored in specific laboratories.

Each animal was considered an experimental unit. The parameters measured as variables were: animal temperament; pH value at 24 hours; water-holding capacity (WHC); the instrumental color parameters lightness (L*), redness (a*), and yellowness (b*); meat tenderness; and glucose and cortisol levels, which were evaluated pre-lairage, post-lairage, and at the time of bleeding.

In the pens, after the lairage period, a behavioral assessment was carried out based on the temperament shown by the animals, by testing their reaction when approached. For this, a scale from zero to five, adapted from Hearnshaw & Morris (1984), was used, where: 0, animal static, quiet, with no resistance to approach; 1, some resistance and constant movement; 2, light movements, withdrawal attempt; 3, excited, escape attempts; 4, very agitated, frightened movements, constant jumping; and 5, fully approach resistant, dangerous. For this evaluation, the animals remained in groups to avoid behavioral changes caused by isolation. Temperament assessments were also carried out in the same pens. For this, the evaluator would approach, observe the shown behaviour, and assign a score to each animal under the identification they received during blood collection.

Carcass pH was measured on the left side of all carcasses 24 hours after slaughter, using the PM 602 portable pH meter (Analion Aparelhos e Sensores, Ribeirão Preto, SP, Brazil). Measurements were taken along the *Longissimus thoracis* muscle between the twelfth and thirteenth ribs at the time the carcass was removed from the cold room.

One steak of the *Longissimus thoracis* muscle (between the eleventh and the thirteenth ribs) was collected per animal, vacuum-packaged, and transported to the laboratory for subsequent preparation of the samples and later evaluation of color, tenderness, and WHC.

Each steak, 2.5 cm thick and weighing approximately 350 g, was aged for two days at 2–3ºC. Afterwards, meat color and shear force were measured. Instrumental color was evaluated after 1 hour of exposure, using the CR-300 Chroma Meter (Minolta Corp., Ramsey, NJ, USA), by estimating the values of L*, a*, and b* of the CIELab system (International Commission on Illumination) (Ramos & Gomide, 2017). In the system, L* is the chroma associated with brightness (L* = 0 black, 100 white), a* is the chroma ranging from green (-) to red (+), and b* is the chroma ranging from blue (-) to yellow (+) (Houben et al., 2000). Values from three different locations on the upper side of the steak were recorded.

Shear force was used to evaluate tenderness, following the methodology described by American Meat Science Association (AMSA, 2015). Samples of the *Longissimus thoracis* muscle were roasted until reaching an internal temperature of 71°C, then cooled until the interior of the muscle reached 20°C, at which time the measurement was taken using a Warner-Bratzler machine with an analogue meter (G-R Manufacturing, Manhattan, KS, USA). For this assessment, four cores, 1.27 cm in diameter, were removed from each steak parallel to the orientation of the muscle fiber.

WHC was evaluated by the pressure method (Warner, 2017) and expressed as percentage of retained water. Five grams of the *Longissimus thoracis* muscle were compressed for 5 min, between two filter papers with 5 cm in diameter. The weight difference was used to determine the amount of water not retained by the meat.

The data were subjected to the analysis of variance using the PROC GLM procedure of the SAS, version 9.4, statistical software package (SAS Institute Inc., Cary, NC, USA), using the following model: $Y_{ij} = \mu + P_i + e_{ij}$, where Y_{ij} is the response variable, μ is the overall mean, P_i is the effect of the lairage period ($i = 1, ... 4$ classes), and e_{ij} is the residual term. When

means differed significantly, they were compared by Tukey's test ($p<0.05$). The variables were also subjected to Pearson's correlation analysis, to check their combined behaviour.

Results and Discussion

The mean values of serum glucose (93.45 mg dL^{-1}) and cortisol (8.7 mcg dL^{-1}) upon the arrival of the steers at the slaughterhouse were higher than those reported for animals with no acute or chronic stress response (Table 1). Under stress-free conditions, serum glucose in healthy Pantaneiro (*Bos taurus*) cattle fluctuated around 69 mg dL^{-1} (Pogliani & Birgel Junior, 2007), whereas cortisol levels in Holstein-Gir crossbred animals were 3.01 mcg dL^{-1} during winter and 4.77 mcg dL^{-1} at higher temperatures (Ferreira et al., 2009).

The higher glucose and cortisol levels found in the present study are probably due to the fact that the steer lots were evaluated after being handled at the farm and then transported and unloaded at the slaughterhouse, representing environmental changes and, consequently, stress conditions (Dawkins, 2017). For most animals, pre-slaughter transport inside a lorry is the first experience of this sort in their lives, often causing acute stress due to the generated anxiety, which can be a determining factor for carcass losses (Mendonça et al., 2018, 2019; Bethancourt-Garcia et al., 2019).

In the pre- and post-rest assessments, the serum glucose and cortisol levels did not differ ($p>0.05$) between the different lairage periods (Table 1). However, higher glucose values were observed at bleeding for the animals subjected to the longest lairage periods of 48 and 24 hours, differing from those obtained at 18 hours, but not at 12 hours. Moreover, the animals subjected to a lairage period of 12 hours showed a higher concentration of cortisol at bleeding.

The higher values of the physiological indicators glucose and cortisol at bleeding for the animals that rested for 12 hours can be explained by the stress caused during transport. Another contributing factor is that the animals had not yet adapted to the environments, since the blood levels of these indicators tend to drop after 18 hours of rest if the lairage process is properly carried out by the meat industry.

The higher serum glucose levels with an increased lairage period of 24 and 48 hours was expected, since the minimum period of 12 hours and water fasting (ad libitum supply) are aimed at allowing the animals to recover from the strain from handling during loading on the farm, as well as from transportation, reception, and handling at the slaughterhouse (Chulayo et al., 2016). In addition, muscle glycogen can be recovered by the transformation of lactic acid into glycogen, through the process of gluconeogenesis in the liver (Costa et al., 2019). That management, using physiological indicators, is adequate for the meat industry; however, as the lairage period increases to 24 and 48 hours, the blood glucose levels of the animals increase as they wait for slaughter. This is not only due to the stress prior to their arrival at the slaughterhouse, which may decrease or normalize after 12 hours of rest, but also to the hunger process associated with an increase in metabolism to maintain activity as the lairage period advances, which can cause chronic stress to the animals under unfamiliar handling and facilities (Grandin, 2013).

Table 1. Physiological indicators of cattle (Aberdeen Angus x Nellore) subjected to different lairage periods[(1)].

Physiological indicator	Lairage period (hours)			
	48	24	18	12
Pre-lairage glucose (mg dL^{-1})	95.3±9.6a	88.3±6.8a	97.1±7.6a	93.1±6.8a
Post-lairage glucose (mg dL^{-1})	107.6±11.8a	97.2±9.6a	83.7±9.1a	91.8±9.0a
Bleeding glucose (mg dL^{-1})	125.3±15.3a	131.6±12.4a	89.5±11.7b	115.6±11.7ab
Pre-lairage cortisol (mcg dL^{-1})	8.8±1.32a	9.7±0.93a	8.2±1.04a	8.1±0.93a
Post-lairage cortisol (mcg dL^{-1})	10.6±1.5a	12.4±1.2a	10.6±1.1a	12.5±1.1a
Bleeding cortisol (mcg dL^{-1})	9.8±1.5b	8.0±1.2b	8.5±1.2b	14.2±1.2a

[(1)]Means ± standard deviation followed by equal letters, in the rows, do not differ by Tukey's test, at 5% probability.

During lairage, variations and fluctuations in serum hormone levels may occur so that the animal can adapt to stressors (Galán et al., 2018). The few available studies in the literature show contradictory results of fasting on blood glucose concentration. Chulayo et al. (2016), evaluating lairage periods of 18, 20, and 24 hours in a slaughterhouse after travel periods shorter than 10 hours, reported an effect on blood glucose concentration, with the highest levels at the lairage period of 24 hours and the lowest ones at 20 hours. Tadich et al. (2005) observed that glucose levels increased up to 6 hours of lairage in steers transported over a period of 16 hours, but only after 24 hours in steers transported for 3 hours. However, it should be considered that the lairage period generally has a weaker effect on the metabolism of ruminants than in that of other species, as the rumen acts as a reservoir of nutrients and volatile fatty acids (Tadich et al., 2005).

Animal temperament increased significantly with the lairage period, except from 18 to 24 hours (Table 2). The animals that rested for only 12 hours were classified as not agitated, exhibiting constant movement and some resistance. However, when the animals were subjected to a lairage period of 48 hours, the mean classification for the lot was very agitated, frightened, with wild movements, always attempting to escape. It is important to note that this group included animals that were totally resistant to approach, intractable, and dangerous at handling. Temperament was positively correlated with the physiological indicators of stress: glucose at pre-rest (r=0.40237) and bleeding (r=0.33567); and cortisol at pre- (r=0.39872) and post-rest (r=0.62444). The greater temperament observed in animals after a long lairage period is probably caused by the higher stress to which they were subjected (Grandin, 2016; Braga et al., 2018). This physiological response was likely due to the change in animal environment, routine, and confinement facilities (Dawkins, 2017). Excitable animals show a greater susceptibility to stress (Grandin, 2013), as well as higher plasma cortisol concentrations (Chulayo et al., 2016), which was not verified in the present study, although the correlations between cortisol and temperament were significant in the pre- ($p<0.0227$) and post-rest periods ($p<0.0001$). Therefore, the methods to evaluate stress based on behaviour scales and physiological stress indicators can be considered quite accurate, and the possibility of incorporating them into the routine of a slaughterhouse should be considered (Stockman et al., 2012).

Despite the variations in the studied physiological indicators and animal temperament, the lairage period did not influence meat pH values (Table 2). In the experiment led by Costa et al. (2019), temperament also had no impact on meat quality, although meat shelf life could have been affected by a lower concentration of muscle glycogen in the animals that were subjected to shorter lairage periods. It should be noted that pH values greater than 5.8, as observed for the lairage periods of 24 and 48 hours, are a limiting parameter in the routine of meat-exporting slaughterhouses. Additionally, pH levels greater than 5.8 tend to produce darker, firmer, and drier meat, drastically reducing the shelf life of the product.

During the lairage periods of 12 and 18 hours, the animals had the opportunity to ruminate the feed

Table 2. Temperament and meat quality variables of cattle (Aberdeen Angus x Nellore) subjected to different lairage periods[1].

Temperament and meat quality variable	Lairage period (hours)			
	48	24	18	12
Temperament (score)[2]	3.80±0.44a	2.80±0.31b	2.88±0.35b	1.60±0.31c
Meat pH at 24 hours	5.91±0.16a	5.94±0.13a	5.81±0.12a	5.80±0.12a
Water-holding capacity (%)	0.47±0.11c	0.88±0.09a	0.70±0.08b	0.65±0.08b
Lightness (L)	33.80±1.70a	36.70±1.4a	35.30±1.30a	35.30±1.30a
Redness (a*)	18.60±1.10a	19.70±0.9a	19.10±0.9a	18.40±0.90a
Yellowness (b*)	6.89±0.79a	4.17±0.64b	3.66±0.60b	2.32±0.60c
Meat tenderness (kgf)	12.19±0.64a	9.02±0.68b	9.24±0.64b	8.59±0.83b

[1]Means ± standard deviation followed by equal letters, in the rows, do not differ by Tukey's test, at 5% probability. [2]Adapted from Hearnshaw & Morris (1984).

ingested before loading, increasing the production of muscle glycogen. In this way, they were able to restore their muscle reserves from mobilized liver glucose (Costa et al., 2019). Despite the possibility of a greater acclimatization to the environment in longer lairage periods, such as those of 24 and 48 hours, without feed supply, the effect of hunger can be very harmful to the animal (Costa et al., 2019), whose meat loses commercial value due its undesirable characteristics and susceptibility to microbial deterioration (Strappini et al., 2013). Although there is evidence that the main factor inducing the appearance of DFD meat is inappropriate pre-slaughter handling (Grandin, 2013), the difference imposed on the animals in the present study was lairage period, whose purpose is to restore the energy reserves levels in the animal, normalizing the blood metabolites that indicate temperament.

The WHC of the meat (percentage of retained water) differed ($p<0.05$) between lairage periods. The highest value was found in the meat from the animals that rested for 24 hours (Table 2), followed by those that rested for 18 and 12 hours ($p>0.05$), which were superior ($p<0.05$) to those that rested for 48 hours before slaughter. The increasing WHC in the meat of the animals subjected to a lairage period of 24 hours is attributed to a greater recovery of muscle glycogen levels due to the rumination of the feed consumed at the farm. Decreases in muscle glycogen reserves lead to muscle fiber shortening and protein denaturalization, reducing WHC (Ponnampalam et al., 2017).

The instrumental color tests showed no difference between lairage periods for L* or a* (Table 2). The b* parameter, however, increased gradually, differing ($p<0.05$) between lairage periods, with superior results at 48 hours. For this variable, the 24 and 18 hour lairages did not differ from each other ($p>0.05$), but showed results higher than that of 12 hours. Despite the similar L* and a* values ($p>0.05$) between lairages, L* was correlated negatively with animal temperament and cortisol levels at bleeding, whereas a* was correlated negatively only with cortisol levels at bleeding, with respective values of -0.4062, -0.4853, and -0.4569.

Although linked to consumer preference and possibly varying over time and space, the muscle L* and a* color variables are determinant of meat quality, with lower values translating into meat that is darker and has an inferior appearance (Hughes et al., 2019; Ijaz et al., 2020). The absence of significant differences in final pH values may also have been responsible for the lack of significant differences in L* and a* in the muscle (Table 2), since the redness of meat is also influenced by its final pH value (Hughes et al., 2017).

There was an effect ($p<0.05$) of lairage periods on meat tenderness (Table 2). When the animals were exposed to a lairage period of 48 hours, the kilogram of force necessary to shear the meat was greater. However, Costa et al. (2019) found no effect of lairage periods of 3 and 12 hours on meat tenderness. In contrast, del Campo et al. (2010), comparing lairage periods of 3 and 15 hours, reported that shorter lairages resulted in higher meat pH and tenderness, suggesting that differences in pH decline are influenced by animal temperament and breed, ultimately determining differences in meat tenderness (Coutinho et al., 2017).

Conclusions

1. Longer lairage periods have a negative effect on the temperament of beef cattle (Aberdeen Angus x Nellore), which become more agitated and resistant to human approach.

2. The lairage period does not influence the levels of the physiological indicators of stress glucose and cortisol in beef cattle.

3. Serum cortisol and glucose levels correlate positively with animal temperament and negatively with meat quality.

4. At bleeding, there is an increase in animal glucose and cortisol levels at a lairage period longer than 24 hours and of 12 hours, respectively.

5. The lairage of 48 hours reduces meat tenderness and water-holding capacity.

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