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Stress indicators in cattle submitted to different pre-slaughter rest times

Indicadores de estresse em bovinos submetidos a diferentes tempos de descanso no pré-abate

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Highlights

Animal welfare measures were compared with different pre-slaughter times.
The physiological values used as welfare measures were different.
Animal welfare techniques influence product quality.

Abstract

The objective of this study was to determine the effect of pre-slaughter time about some blood constituents used as stress indicators in the evaluation of animal welfare in cattle. In the practice of ethology, animal welfare is assessed through physiological and behavioral indicators. For the determination of serum biomarkers of animal stress, a total of 180 animals was used, divided into two major groups according to the time of pre-slaughter. In the acceptable group, the animals had a pre-slaughter time up to 24 hours and in the not acceptable group the pre-slaughter time was greater than 24 h. These two groups were split up into three animal categories (males, females and castrated males). In this study, 30 samples were collected from each animal category of the two groups, acceptable and not acceptable, totaling 180 samples. Samples were separated for analysis of muscle enzymes creatine kinase and lactate dehydrogenase (CK and LDH), glucose and cortisol. Physiological values, regardless of the animal category and pre-slaughter time, were higher than the normal values of reference, expressing the prior management effect on animal welfare. The data clearly show a need to update and adapt the entire production chain to animal welfare practices, with the objective of producing competitive quality meat in the world market.

Key words: Cortisol. Creatina kinase. Lactate dehydrogenase.

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Resumo

O objetivo do estudo foi avaliar o efeito do tempo de pré-abate sobre alguns constituintes sanguíneos utilizados como indicadores de estresse na avaliação do bem-estar animal em bovinos. Na prática da etologia, o bem-estar animal é avaliado por meio de indicadores fisiológicos e comportamentais. Para a determinação sérica de biomarcadores de estresse animal utilizaram-se 180 animais, divididos em dois grupos de acordo com o tempo de pré-abate: até 24 horas ou acima de 24 horas. Esses dois grupos subdividiram-se em três categorias animais (machos não-castrados, machos castrados ou fêmeas). Foram coletadas 30 amostras de cada categoria animal nos dois grupos, totalizando 180 amostras. Foram separadas amostras para análise de enzimas musculares creatina quinase e lactato desidrogenase (CK e LDH), glicose e cortisol. Os valores fisiológicos, independentemente da categoria animal e do tempo de pré-abate, foram superiores aos valores de referência, expressando o efeito do manejo prévio sobre o bem-estar dos animais. Os dados evidenciam claramente a necessidade de atualização e adequação de toda a cadeia produtiva às práticas de bem-estar animal, com o objetivo de produzir carne de qualidade competitiva no mercado mundial.

Palavras-chave: Cortisol. Creatina quinase. Lactato desidrogenase.

Introduction

The concept of animal welfare and the fulfillment of its principles have generated generating increasing concern in most of the countries, especially when associated to the development of consumer awareness relative to agricattle exploitation. In this concept, production with the desirable quality attributes is a complex task that involves all the participants in the beef cattle chain. In a competitive and demanding market, which requires the necessary from each of the involved parts, the aspects related to animal welfare (AWF) appear as a demonstration of special attention objects. The human actions over animal production should, by themselves, be a sufficient reason for consideration, not only for ethic and moral concerns, but also for the clear impact over the productive economy (Ohl & Van Der Staay, 2012).

In the pratic of etology, animal welfare is evaluated by means of physiological and behavioral indicators. Physiological measures associated to stress are based in the lowering of animal welfare when stress increases. Behavioral indicators are especially based on the demonstration of abnormal behaviors and behaviors that go far from that ones that are own from natural environment (Marchant-Forde, 2015).

Glicocorticoids levels in the plasma catecholamines, endorphins and prolactin, as well as heart frequency, have been used as physiological parameters for the study of animal stress before slaughter (Broom, 2018). Cortisol is the main and most powerful glicocorticoid secreted by the córtex, characterizing an adrenal response to the adrenocorticotrofic hormonal liberation (ACTH) by the pituitary gland (Svete et al., 2012), turning it into a good indicator of acute stress (Souza et al., 2016) and psychological stress (Jones et al., 2010).

Different authors have used blood constituents as cortisol, glicosis and creatine phosphokinase (CK) concentration and lactate dehydrogenase (LDH) as stress indicators associated with the management, transportation and sacrifice to which

animals are submitted in the pre-slaughter management (Cafazzo et al., 2012; Earley et al., 2012; Silva, 2016; Ferlazzo et al., 2018).

In a study to determine the effect of 36 hours of land transportation with or without rest in blood variables indicators of bovine stress, a significant increase in plasmatic concentration of cortisol was observed, except in those animals that had access to a period of rest (Tadich et al., 2000; Navarro et al., 2019).

In general, a longer waiting period negatively affects the temperament of beef cattle, which become more agitated and resistant to human approach, where the serum levels of cortisol and glucose are positively correlated with the animal's temperament and negatively with the quality of the meat.

Pre-slaughter time is a fundamental factor, during which many stressing situations are generated for the animals. Fasting before slaughter has some advantages, besides facilitating evisceration, reducing the possibility of carcass contamination, as well as facilitating skin remotion for rehydratation and making bloodening more abundant. Notwithstanding, it is important to consider that a very long fasting period can cause very negative effects over welfare because of hunger sensation, it can also increase the incidence of low quality meat and decrease carcass weight (Santos, 2018).

Some authors consider that pre-slaughter time permits the rehydratation of the animals, as well as the recovering of fatigue probably caused by the journey, allowing the recovering of the levels of muscle glycogen (Rault, 2012; Ferlazzo et al., 2018). On the other side, many authors stand that waiting time is a negative factor, not allowing the animals to recover from privation of food and water (Mendonça et al., 2019). For longer waits, it is associated to the depression of carcass and meat quality. Those conflicting views could be explained by many factors among which: duration, type of transportation and waiting time evaluated, the history of the animals (genotype, temper, feeding), and the infrastructure conditions of the abbatoir, among others. Other authors have stated that times without resting resulted in deterioration of the quality in bovines for slaughter (Losada-Espinosa et al., 2018).

The objective of this study was to determine the effect of pre-slaughter time about some blood constituents used as indicators of animal welfare in cattle.

Materials and Methods

The realization of this work was made possible by the acquiescence of a slaughterhouse-fridge exporter located in Northern region of the brazilian state of Mato Grosso, which is enabled to export *"in natura"* meat for several countries, registered in the Federal inspection service.

In this first phase of the experiment, with the aid of a semi-structured questionnaire and through visits, a characterization survey of the eight breeding properties and a finishing confinement was carried out, all of which were them usual suppliers of animals for slaughter at the slaughterhouse in question. The facilities, production system, animal category and phases of exploitation were evaluated, as well as a subjective evaluation of the management practiced. Those information that could not be found at the time of the visit were filled in according to the responses of those responsible for the establishment that carried out the follow-up.

For the determination of serum biomarkers of animal stress, a total of 180 animals was used, divided into two major groups according to the time of pre-slaughter, being: up to 24 hours; or more than 24 hours. These two groups were split up into three animal categories (males, females and castrated males). 30 samples were collected from each animal category of up to 24 hours; or more than 24 hours, totaling 180 samples. The pre-slaughter time (24h or more than 24h of pre-slaughter) was calculated from loading on the farm to arrival at the refrigerator.

Data were collected during the rainy season between November and March. The climate of the region is characterized as having average temperatures above 27 °C in the hottest months (November to February), average temperatures above 18 °C in the coldest months (June to August) and average annual rainfall between 1000 and 1500 mm distributed in two well-defined periods in terms of precipitation: period of intense rains between the months of October to March and clear period of drought between the months of April to September (Marchi et al., 2019).

The data on the kilometers traveled were estimated from their origins on the farms that were included in the mandatory documentation required (Animal Transit Guide - GTA), while the data on the duration of transport in hours and the occurrence of eventual problems were carried out by applying a checklist to the drivers responsible for transporting the animals at the time of disembarkation.

After unloading the animals were taken to the arrival and selection pens, separated by slaughter batch according to breed, sex and category. The arrival and selection pens had an area of 2.5 m^2/animal constructed of reinforced concrete, with non-slip flooring, the sanitary cord had a height of 0.30 m (thirty centimeters), with rounded corners and edges, the constant level drinkers , type reinforced concrete trough.

Stunning was performed using a captive dart pistol fired by an explosion cartridge. The dart passed through the skull at high speed (100 to 300 m/s) and force (50 kg/mm2) producing a temporary cavity in the brain.

Animal blood samples analyzed were collected in the mornings on channel of sangria from fridge/slaughterhouse. Blood was collected at the time of bleeding after opening the dewlap, through the linea Alba and by sectioning the jugular veins of the carotid arteries, using a previously sterilized knife. After collecting, they were placed in vials of 10 ml, without anticoagulant. The samples were properly identified and stored in isothermal boxes with dry ice getting to stand for 60 minutes to clot retraction. Then the samples were centrifuged for 10 minutes at 5,000 rpm, the serum was then withdrawn and transferred to plastic tubes of 1.5 ml eppendorf kept under refrigeration and then analyzed in a laboratory.

For determination of glucose enzyme, an automated methodology was used, Wiener Lab® Appliance Kit Cobas Mira n. For the determination of lactate, dehydrogenase (LDH) Optimised Uv Methodology was used (Sfbc), Wiener Lab Kit®. For the determination of creatine kinase (CK) Uv method was optimized (Ifcc), Wiener Lab® Kit, both in Appliance Cobas Mira s. For determination of glucose enzyme automated methodology was used, Wiener Lab® Appliance Kit Cobas Mira S.

For analysis of cortisol, the methodology of chemiluminescence was followed, in automated IMMULITE 2000 device with the use of commercial Kit. The data obtained for glucose, CK, LDH and cortisol were submitted to variance analysis by F-test and the comparison of the averages held by t test the 5% probability, using the statistical program 5.3 Sisvar (Build 75).

Results and Discussion

The average values of glucose (Table 1) regardless of category animal and pre-slaughter time were higher than the normal values found in the literature reference. This coincides with the described by other authors who found a significantly high concentration of glucose as 36-hour effect of land transport, with or without the rest period (Tadich et al., 2000). A significant increase of this variable during and after the transport of cattle due to muscular contraction produced by Catecholamines and cortisol released in response to stress (Ferlazzo et al., 2018).

There was a difference in the concentration of glucose between the animal categories according to the pre-slaughter waiting time, in which it is verified that for times less than 24 hours non-castrated males showed lower glucose concentrations in relation to females and males neutered. For pre-slaughter waiting time greater than 24 hours, females had a lower glucose concentration than non-castrated males, with an intermediate behavior for castrated males.

In relation to the pre-slaughter times, up to 24 h and more than 24 h, in general no large differences were observed, except in the category of males that presented slightly less value than others in acceptable weather condition and which difference was significant ($p< 0.05$).

Em relação ao cortisol plasmático foram observados valores sempre superiores aos normais citados na literatura, mesmo para condições excepcionais de estresse agudo. Mais uma vez, houve agrupamento entre fêmeas e machos castrados diferindo de machos inteiros no tempo pré-abate até 24 h, e completa distinção entre todas as categorias para tempo de pré-abate acima de 24 h, com diferenças significativas ($p<0,05$).

Table 1
Medium values of glucose and blood cortisol of refrigerated carcasses determined for the diferente animal categories, due to pre-slaughter time

	Glucose enzyme ($mg.dL^{-1}$)		Cortisol ($ng.mL^{-1}$)	
	Time		Time	
Animal Category	up to 24 h	More than 24 h	up to 24 h	More than 24 h
Female	156.16 aB	128.17 aA	73.0 aB	75.8 aC
Castrated males	171.72 aB	154.63 aAB	68.3 aB	61.9 aB
Males	124.12 bA	158.37 aB	40.1 aA	46.2 aA
C.V. (%)	37.11		35.81	

Medium followed by the same small letter in the line or capital in the column do not statistically differ between themselves by the t test t the 5% probability level.

During transportation, there is an increase in heart rate and plasma levels of cortisol, creatine kinase, free fatty acids and lactate dehydrogenase (Cafazzo et al., 2012; Mendonça et al., 2019). Cortisol levels increase during the first 30-60 minutes and then remained stable (Sartorelli et al., 1992). Those physiological changes are most probably related to the stress due to the unfamiliar situation and physical effort related to the load to maintain balance in the truck in relation to movement and vibration (Bourguet et al., 2010).

Tadich et al. (2000) noted that the plasma concentrations of cortisol in the time of bleeding were higher for animals transported without rest, indicating the need for a period of rest when the transport is extended. According to Hagenmaier et al. (2017), high blood concentrations of cortisol are more a reflection of the effect of the previous management in which animals are subjected. Earley et al. (2012) assessed the physiological effects indicative of stress in cattle transport trucks of the trick type, obtained mean values of creatine kinase and cortisol close to those found in this study, approximately 3 hours after landing.

To CK and LDH enzymes, indicative of tissue damage, the data are presented in table 2 below. The observed values were systematically far above those of reference to the normality of the species. As verified by other authors (Tadich et al., 2000; Ferlazzo et al., 2018). there was an increase in plasma CK activity in function of transport, indicating that it is a physically stressful event, probably due to the stress that animals perform to keep their composure in the moving vehicle, causing great muscular fatigue and in some cases bruising, which also justifies the high number of injuries found in this study.

As for the animal category, for the enzyme CK there was no significant difference for up to 24 h time of pre-slaughter, observing differences only to unacceptable time, when the group of males differed from females. Analyzing the data for weather condition of pre-slaughter, up to 24 h and more than 24 h, it turns out that there was no significant change of the parameter for the category of females, while for others, the values were not only significant ($p<0.05$), but much larger than those observed in the pre-slaughter up to 24 h time. Similar behavior was observed for the enzyme LDH, with a single exception that there was shared response behavior of both females and males integers to the castrated male category in more than 24 h pre-slaughter weather condition.

Table 2
Medium values of creatinina quinase (CK) and lactato desidrogenase (LDH) determined for the different animal categories, due to pre-slaughter time

	CK (UI.L^{-1}) Time		LDH (UI.L^{-1}) Time	
Animal Category	up to 24 h	More than 24 h	up to 24 h	More than 24 h
Female	842.83 aA	646.92 aA	2750.97 aA	2809.96 aA
Castrated males	572.50 aA	1143.35 bB	2752.29 aA	3239.76 bAB
Males	734.66 aA	1046.04 bB	2833.78 aA	3454.93 bB
C.V. (%)	65.34		28.89	

Medium followed by the same small letter in the line or capital in the column do not statistically differ between themselves by the *t* test t the 5% probability level.

Other authors have not found significant differences in the activity of CK before and after slaughter. As creatine kinase has a short half-life, it is immediately related to the pre-slaughter period. Pre-slaughter operations should not be reflected in the muscle of cattle, and consequently changes in the values of CK. High values mean the persistence of factors that are capable of inducing tissue damage. Among them, inadequate management, poor condition of transportation, long journeys and/or behavioral reasons of certain groups or individuals (Tadich et al., 2000).

Schwartzkopf-Genswein et al. (2012) observed that stress blood indicators varied significantly during transport as compared to control samples taken a week before shipping. The values of cortisol were higher after 1-2 hours of travel compared with shorter and longer days. The glucose values did not vary significantly, however the CK values were significantly lower on shorter trips, as well as noted on the values of LDH. No significant changes were observed in pH or staining of the flesh in relation to transport. The authors concluded that if the transport conditions are appropriate, there is little effect on animal welfare and meat quality.

Bourguet et al. (2010) found elevated cortisol values of serum creatine kinase and lactate dehydrogenase after transportation, as observed in the present study. The increase of these variables makes it clear that the transport, involving loading, landing, handling, travel time and strange environments is stressful for the animals and can affect the quality of the meat.

Conclusion

Regardless of the pre-slaughter waiting time and animal category, stress indicators in cattle were above baseline values.

References

Bourguet, C., Deiss, V., Gobert, M., Durand, D., Boissy, A., & Terlouw, E. M. C. (2010). Characterising the emotional reactivity of cows to understand and predict their stress reactions to the slaughter procedure. *Applied Animal Behaviour Science, 125*(1-2), 9-21. doi: 10.1016/j.applanim.2010.03.008

Broom, D. M. (2018). The scientific basis for action on animal welfare and other aspects of sustainability. In J. D.'Silva & M. Carol (Eds.), *Farming, food and nature* (pp. 93-100). Routledge. https://www.taylorfrancis.com/chapters/edit/10.4324/9781351011013-13/scientific-basis-action-animal-welfare-aspects-sustainability-donald-broom

Cafazzo, S., Magnani, D., Calà, P., Razzuoli, E., Gerardi, G., Bernardini, D., Amadori, M., & Costa, L. N. (2012). Effect of short road journeys on behaviour and some blood variables related to welfare in young bulls. *Applied Animal Behaviour Science, 139*(1-2), 26-34. doi: 10.1016/j.applanim.2012.03.009

Earley, B., Murray, M., Prendiville, D. J., Pintado, B., Borque, C., & Canali, E. (2012). The effect of transport by road and sea on physiology, immunity and behaviour of beef cattle. *Research in Veterinary Science, 92*(3), 531-541. doi: 10.1016/j.rvsc.2011.04.002

Ferlazzo, A., Cravana, C., Fazio, E., & Medica, P. (2018). The contribution of total and free iodothyronines to welfare maintenance and management stress coping in ruminants and equines: physiological ranges and reference values. *Research in Veterinary Science, 118*, 134-143. doi: 10.1016/j.rvsc.2018.01.025

Hagenmaier, J. A., Reinhardt, C. D., Bartle, S. J., Henningson, J. N., Ritter, M. J., Calvo-Lorenzo, M. S., Vogel, G. J., Guthrie, C. A., Siemens, M. G., & Thomson, D. U. (2017). Effect of handling intensity at the time of transport for slaughter on physiological response and carcass characteristics in beef cattle fed ractopamine hydrochloride. *Journal of Animal Science, 95*(5), 1963-1976. doi: 10.2527/jas2016.0821

Jones, T. A., Waitt, C., & Dawkins, M. S. (2010). Sheep lose balance, slip and fall less when loosely packed in transit where they stand close to but not touching their neighbours. *Applied Animal Behaviour Science, 123*(1-2), 16-23. doi: 10.1016/j.applanim.2009.12.006

Losada-Espinosa, N., Villarroel, M., Maria, G. A., & Lama, G. C. M. (2018). Pre-slaughter cattle welfare indicators for use in commercial abattoirs with voluntary monitoring systems: a systematic review. *Meat Science, 138*, 34-48. doi: 10.1016/j.meatsci.2017.12.004

Marchant-Forde, J. N. (2015) The science of animal behavior and welfare: challenges, opportunities, and global perspective. *Frontiers in Veterinary Science, 2*(1), 16. doi: 10.3389/fvets.2015.00016

Marchi, S. R., Silva, H. M., Ferreira, C. F., Marques, R. F., & Moraes, J. B (2019). Interference of noxious shrubs on grazing behavior by bovines. *Planta Daninha, 37*(1), 1-10. doi: 10.1590/S0100-83582019370100009

Mendonça, F. S., Vaz, R. Z., Vaz, F. N., Leal, W. S., Silveira, I. D. B., Restle, J., Boligon, A. A., & Cardoso, F. F. (2019). Causes of bruising in carcasses of beef cattle during farm, transport, and slaughterhouse handling in Brazil. *Animal Science Journal, 90*(2), 288-296. doi: 10.1111/asj.13151

Navarro, G., Bravo, V., Gallo, C., & Phillips, C. J. C. (2019). Physiological and behavioural responses of cattle to high and low space, feed and water allowances during long distance transport in the south of Chile. *Animals, 9*(5), 229. doi: 10.3390/ani9050229

Ohl, F., & Van Der Staay, F. J. (2012). Animal welfare: at the interface between science and society. *The Veterinary Journal, 192*(1), 13-19. doi: 10.1016/j.tvjl.2011.05.019

Rault, J. L. (2012) Friends with benefits: social support and its relevance for farm animal welfare. *Applied Animal Behaviour Science, 136*(1), 1-14. doi: 10.1016/j.applanim.2011.10.002

Santos, C. T. (2018). *Jejum pré-abate de bovinos confinados: indicadores de estresse e qualidade da carne.* Tese de doutorado, Universidade Estadual Paulista, Botucatu, SP, Brasil. http://hdl.handle.net/11449/152857

Sartorelli, P., Dominoni, S., & Agnes, F. (1992). Influence of duration of simulated transport on plasma stress markers in the calf. *Journal of Veterinary Medicine Series A, 39*(1-10), 401-403. doi: 10.1111/j.1439-04 42.1992.tb00198.x

Schwartzkopf-Genswein, K. S., Faucitano, L., Dadgar, S., Shand, P., González, L. A., & Crowe, T. G. (2012). Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: a review. *Meat Science, 92*(3), 227-243. doi: 10.1016/j.meatsci.2012.04.010

Silva, M. B. (2016). *Influência do período de transporte pré-abate de bovinos nos níveis séricos de creatinoquinase, hemograma e pH final da carcaça.* Tese de doutorado, Universidade Federal de Mato Grosso, Cuiabá, MT, Brasil. https://cms.ufmt.br/files/galleries/64/DISSERTA%C3%87%C3%95ES/2016/MAIANNE%20BORGES%20DA%20SILVA.pdf

Souza, M., Oliveira, F., Ferreira, C. R., Sangoi, C., Gasperin, B. G., & Lucia, T., Jr. (2016). Avaliação do cortisol agudo e crônico em bovinos submetidos a diferentes técnicas de esterilização. *Anais do Congresso de Iniciação Científica,* Cuiabá, MT, Brasil, 25. https://cti.ufpel.edu.br/siepe/arquivos/2016/CA04254.pdf

Svete, A. N., Cebulj-Kadunc, N., Frangez, R., & Kruljc, P. (2012). Serum cortisol and haematological, biochemical and antioxidant enzyme variables in horse blood sampled in a slaughterhouse lairage, immediately before stunning and during exsanguination. *Animal, 6*(8), 1300-1306. doi: 10.1017/S1751731112000079

Tadich, N., Gallo, C., & Alvarado, M. (2000) Efectos de 36 horas de transporte terrestre con y sin descanso sobre algunas variables sanguíneas indicadoras de estrés en bovinos. *Archivos de Medicina Veterinaria, 32*(2), 171-183. doi: 10.4067/S0301-732X2000000200004