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INDICADORES DE ESTRESSE E QUALIDADE DE CARNE DE BOVINOS TRANSPORTADOS EM DIFERENTES TIPOS DE CAMINHÕES (TRADICIONAL, CARRETA DE UM PISO E CARRETA DE DOIS PISOS) E DIFERENTES DISTÂNCIAS NA REGIÃO DE CUIABÁ/ MT/ BRASIL

(Stress indicators and meat quality of cattle transported over different distances and in differently designed trucks (truck, trailer and double deck) in the region of Cuiabá/MT/Brazil)

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RESUMO: O transporte de bovinos para o abatedouro constitui importante etapa do abate humanitário, fatores como: tipo de carroceria, distância e tempo de transporte e condições climáticas possuem elevada correlação com a qualidade da carne e carcaça e devem ser ajustadas para cada região produtora. Avaliou-se o efeito da distância de transporte (D1 = 75 a 130 km e D2 = 180 a 250 km) e tipo de caminhão (C1= Caminhão Boiadeiro; C2= Carreta de 1 piso; C3= Carreta de dois pisos) no bem-estar, qualidade de carne e carcaça de 120 nelores em um estudo aleatorizado. Observou-se diferenças significativas nos valores de pH 1h *post-mortem* ($p \leq 0.05$) entre os tratamentos, entretanto, não observou-se o mesmo resultado nos valores de pH 24 *post-mortem* no mesmo músculo avaliado (L. dorsi). Elevada frequência (1,09 e 1,02) e intensidade (1,81 e 1,65) de hematomas foram obtidas nas carcaças de animais transportados pelo caminhão (C3) comparativamente aos demais tratamentos ($p \leq 0.05$). Elevadas concentrações de cortisol sanguíneo foram obtidas nos animais transportados no caminhão (C3) e distância (D2), seguidos pelos animais transportados em (C1) na distância (D1). Concluiu-se que o caminhão (C3) e a distância (D2) quando associados proporcionaram baixo nível de bem-estar comparativamente aos outros tratamentos estudados.
Palavras-chave: estresse; índice de hematomas; pH; transporte

ABSTRACT: The transport of animals for slaughter is an important step in humane slaughter and factors such as truck design, transport distance and time and weather conditions have a high correlation with meat and carcass quality and must be adjusted for each producing region. The effect of transport distance (D1 = 75 to 130 km and D2 = 180 to 250 km) and truck design (C1= regular truck, C2= trailer and C3= double deck) were evaluated on welfare, meat and carcass quality of 120 Nellore cattle using a completely randomized design. Differences were observed in the pH values 1h *post-mortem* ($p \leq 0.05$) between treatments, but this was not true for the values of pH 24 *post-mortem* in the same muscle (L. dorsi). Higher bruising frequency (1.09 and 1.02) and intensity (1.81 and 1.65) were found in the carcasses of animals transported by C3 in relation to other treatments ($p \leq 0.05$). High blood cortisol levels were obtained in animals transported by C3 over distance D2, followed by C1 over distance D1. It was concluded that C3 over distance D2 showed poor welfare and carcass quality compared to the other treatments.
Key Words: bruise index; pH; stress; transport

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INTRODUCTION

Cattle transportation is an important stage in production and it is vital that this process is carried out properly, using methods and techniques that ensure animal welfare, meat quality and carcass and economic viability (Paranhos, 2000; Fike *et al.*, 2006, Fisher *et al.*, 2009; De Witte e Hons, 2009).

Road transport of cattle, under unfavorable conditions that include density, fasting, dehydration, fatigue, temperature, relative humidity and distance traveled may lead to losses related to stress such as bruises, fractures, weight loss and death (Ishiwata *et al.* , 2008; Gupta *et al.*, 2007).

The physiological responses to transport stress are usually observed through high plasma levels of cortisol, creatine phosphokinase (CPK), lactate dehydrogenase (LDH) body temperature and cardiac frequency (Ferguson e Warner, 2008; De Witte e Hons, 2009; Fike *et al.*, 2006).

The welfare of animals transported by truck depends on several factors, such as: vehicle maintenance, driver training, weather, distance, road quality, animal temperament, loading and unloading platform, sex, density, fasting and truck design (Luchiari Filho, 2000).

Appropriate truck design is essential to ensure good practice in the transport of cattle. Tips of wood, exposed nails or screws, ceiling and walls of inadequate dimensions, improperly inclined ramp, irregular ventilation conditions and dirty and slippery floors are usually the main factors related to the problems of welfare and meat quality and carcass resulting from transportation by cattle truck (Strappinia *et al.*, 2010).

Cuiabá city receives most of the cattle production in Mato Grosso state, which requires that animals are transported for various distances and face scarcity, high temperatures and extremely low or high air humidity conditions on the roads, which usually vary depending on the season of the year.

The distances and the number of animals influence the design of truck used for cattle transport in the region studied. Trucks with one or two floors are usually used for long distances while and the double deck truck is used for shorter distances. Slaughterhouses generally choose to use the double deck truck for freight economy, but it should be noted that many farms have no access conditions or cattle docking facilities suitable for this type of transport.

The objective of the present research was to study the influence of distance and truck design on the welfare, meat quality and carcass of cattle transported in the region of Cuiabá-MT.

MATERIAL AND METHODS

One hundred and twenty Nelore crossbred cattle were assessed, average age 24 months, tracked, from different production systems (extensive, confined or semi-confined), transported by three types of trucks: regular truck, trailer and double deck over two distances: distance 1 (75 to 130 km) and distance 2 (180 to 250 km) to the slaughterhouse located in the city of

Cuiabá/MT with slaughter capacity of 600 to 800 head / day and under the Federal Inspection Service (SIF) (Table 1).

Table 1 - Identification scheme truck design and distances.

Truck design	Distance 1 / Location	Distance 2 / Location
Regular truck	75 Km (Nossa Senhora do Livramento)	250 km (Cáceres)
Trailer	130 km (Rosário Oeste)	180 km (Nobres)
Double Deck	82 Km (Cuiabá)	230 Km (Cáceres)

The regular truck (10.60 x 2.40 m) had three compartments: back (2.65 x 2.40 m), mid (5.30 x 2.40 m) and front (2.65 x 2 40 m) with an average capacity of 20 animals. The trailers (14.8 x 2.60 m) also had three compartments, back (4.16 x 2.60 m) mid (7.10 x 2.60 m) and front (3, 43 x 2.60 m) with an average capacity of 27 animals while the double deck truck had two floors (14.80 x 2.60 m) and six compartments: front (3.95 x 2.45 m), lower front, lower back, all measuring 3.55 x 2.45 meters, and back (3.60 x 2.45 meters) with capacity for 42 animals.

In regular trucks were transported 18 cattle, already in the trailers were transported average of 28 animals and double deck trailers 40 heads.

After transportation, the cattle were driven to a rest area (2.5 m^2/450kg), separated by sex, with free access to water for 24 hours and were then slaughtered, following the welfare slaughter protocol defined by Instruction n^0 3/2000 of the Ministry Agriculture, Livestock and Supply (Brazil, 2000).

After stunning by non-penetrating captive bolt (concussion) the animals were submitted to bleeding, at this stage blood samples were collected to measure stress blood indicators (lactate dehydrogenase - LDH, creatinine phosphokinase - CPK and cortisol). The samples were collected in plastic cups and then stored in Falcon 15 mL tubes with anticoagulant (EDTA) and without anticoagulant. After collection, the tubes were homogenized, stored on ice and then centrifuged (3,500 rpm for 10 minutes) to obtain the serum and plasma, which were stored at -20 ° C for subsequent analysis of LDH (serum) and CPK (plasma) using the commercial kit) and cortisol (serum) by the immunofluorescence technique (optimized UV method (LABTEST, **Coat**-A-**Count® kit**, **Siemens Medical** Solutions **Diagnostics**, **Los Angeles**, **CA).**

The methodology proposed by AUS-MEAT (2001) was used to evaluate skin damage and bruises on the carcass surface, measuring the intensity as: I (only subcutaneous tissue), II (subcutaneous tissue and muscle) and III (subcutaneous, muscle and bone) and the extent of affected tissue (level I: 1-10 cm; level II: 11-20 cm and level III: more than 20 cm). The evaluations were carried out visually on the slaughter line, immediately after removing the leather, using a report form and standard photos.

The pH1 (1^0h *post-mortem)* was measured in a cold chamber (5^0C), in the *longissimus dorsi* muscle between the 11^{th} and 12^{th} vertebra on the right side of the carcass, using a portable Hanna pH meter with automatic calibration, electrode FC 232D and temperature sensor. Carcasses remained in the cooling chamber for 24 hours and then the pH24 (24^0 h post-mortem) was measured at the same location.

After measuring the pH24, the *Longissimus dorsi* muscle samples were

collected and placed in plastic bags and taken to the UFMT / FAMEV Food Technology Laboratory for color measurements using a Minolta CR 400 colorimeter, in the CIE, parameters L * (lightness), a * (red), b * (yellow) by performing three replicates per point at three different points on the muscle surface (Honikel, 1998).

A completely randomized design was used and the data were analyzed by analysis of variance (ANOVA) and the SNK test at 5% significance.

The values of creatinine phosphokinase and skin damage (bruises) were analyzed by nonparametric statistics using the Kruskal-Wallis test at 5% probability.

RESULTS AND DISCUSSION

Evaluating the influence of the distance which the animals travelled (D1 ≤ 130 km) and (D2 ≥ 180 km) for the quality parameters shown in Table 2, is displayed larger than the distance afforded a pH 1h pm significantly higher for all types of trucks evaluated which showed higher energy expenditure of the cattle subjected to this treatment, but between 24h *pm* no significant differences were observed.

It was found that the carcasses of cattle transported on regular truck and trailer low, initial pH exhibited differences between the two distances , and in both cases the minimum distance (D1) held 1 hour post- a pH significantly lower mortality . As for the carcasses of animals transported for double deck, also statistical differences were observed between the distance, but the greater distance (D2) showed higher pH drop.

Differences were found for the pH of the carcasses of cattle transported by all kinds of bodies, from the distance 1, and animal meat transported in double deck had the highest initial pH value, followed later by the lower cart and presenting the lowest pH , animal meat transported in regular truck. The opposite occurred in the distance 2, in which the higher initial pH values were observed for the animals of meat transported by regular truck and trailer low, differing from the animals transported by double deck.

Based on the initial pH of the carcasses of animals found in this study, it is suggested as the best, the value of the regular truck animals in the distance 1.

Joaquim (2002) points out that the unsatisfactory decline after slaughter the pH is indicative of the influence of the physiological conditions of the animal at the time of sacrifice. This is due to depletion of muscle glycogen by intense physical activity or physical stress.

The initial decrease in pH was due primarily to the release of H + ions, which occurs before the reduction of pyruvate to lactate. The rate of decrease in pH and the final pH of the meat after 24-48 hours is highly variable (Lawrie, 2004). For cattle, glycolysis typically develops slowly, the initial pH (0 hours) around 7.0 drops to 6.4 to 6.8 after 5 hours after slaughter and from 5.5 to 5.9 after 24 hours (Forrest *et al.*, 1979).

In the study it was observed that the mean pH 1 hour post-slaughter were above 6.4 (mean 6.57) for all castings examined in such treatments. It should be noted that the meat of bovine carcasses transported by regular truck in D1 had a mean pH one hour after

slaughtering of 6.42, the largest drop in pH, which is correlated with the highest concentration of muscle glycogen, which in turn is related to lower stress condition in the pre slaughter.

The unsatisfactory drop post-mortem pH, due to muscle glycogen depletion by intense physical activity or psychological stress, indicated the influence of physiological conditions of the animal at slaughter, and the conditions of well-being in the pre-slaughter (De Witte *et al.*, 2009; Ferguson e Warner, 2008).

Significant variations between the (a*) values between D2 and D1 were observed for the trailer truck treatment, which did not occur in the other treatments. This variation in the red pigment concentration is generally related to 24pm pH and/ or red pigment concentrations that can vary depending on genetics and animal nutrition (Felício, 1997) (Table 2). In the study, although there was a standardization of the cattle evaluated, there was a certain genetic variability because the animals were Nelore crossbred.

Table 2 - Evaluation of meat quality parameters and blood stress indicators of cattle transported by trucks of different designs and different distances.

Variable	N	Regular truck		Trailer		Double deck		Sig
		D1	D2	D1	D2	D1	D2	
pH 1 h *PM*	120	6.42 cB	6.71 aA	6.53 bB	6.64 aA	6.63 aA	6.50 bB	*
pH 24 h *PM*	120	5.55	5.69	5.57	5.61	5.60	5.60	NS
L*	120	38.64ab	37.10b	39.62a	38.50ab	38.86ab	37.86ab	*
a*	120	24.51 a	25.25 a	25.59 aA	23.10 bB	25.51 a	24.63 a	*
b*	120	10.77	10.59	10.86	10.47	11.50	10.03	NS
Cortisol (µg/dL)	60	7.51 ab	5.61 bc	5.08 bc	3.99 c	5.88 abcA	8.63 aB	**
LDH (U/L)	120	537.45 ab	593.38 a	605.41 aA	432.23 bB	494.01 b	471.42 b	*

Lowercase letters on the same line represent the differences between the truck designs; bold capital letters on the same line represent differences between the distances in the same truck; * (p = 0.005), ** (p = 0.001), NS (not significant) by the SNK and Tukey tests; LDH (lactate dehydrogenase), D1 = short distance; D2 = long distance.

Olivo (2006) reported an inverse correlation between pH and surface lightness (L*), in the other words, the lower the pH the greater the (L*) value but no significant variation among the trucks types or transport distances evaluated were observed, demonstrating that stress from the truck design or distance was not enough to change the (L*) values as shown (Table 2).

According to Ramos and Gomide (2007), the quality of meat can be promoted with as indicating the combination of pH, light, and water holding capacity (WHC). So the brightness values (L *), or brightness measurement is an objective analysis that provides information about the paleness of flesh, predisposing its quality.

In this study (in accordance with the cortisol, values), the D2 distance also resulted in greater psychological stress in cattle transported in double deck trucks, in the other treatments the transport distance did not influence the cortisol concentrations, although the regular truck treatment presented a similar performance (Table 2).

The concentration of cortisol have been a measure of stress response in humans and animals. Negative or undesirable temperaments have been associated with increased serum concentrations of cortisol in cattle (Stahringer; Randel; Neuendorff, 1990; Curley *et al.*, 2006). The release of cortisol by the adrenal cortex is associated with stress stimuli and has significant negative impact on animal performance, immunity, meat quality and tenderness of the meat (King *et al.*, 2006).

It can be said that the animals of that experiment showed cortisol levels within the normal range (average of all the analyzes of 6.11 µg/dL). This is confirmed when we compare the values with those of the literature, where normal

cortisol average values in cattle of zebu breeds are up to 3.68 mg/dL, and animals that have high concentration of this hormone in the bloodstream are considered stressed (Reis *et al.*, 2006) .

With respect to blood tests, it was found that both blood samples of cattle transported by regular truck, as for the high wagon were not found among the distances statistical differences with respect to LDH activity in the blood serum of animals. Since the serum samples of animals transported by the trailer at low greatest distance (D2) had significantly lower enzyme activity compared to the concentration of blood serum samples of animals which traveled distance 1.

Saad *et al.* (2006) pointed out that the LDH enzyme catalyzes the conversion of lactate and pyruvate with concomitant conversion of NADH and NAD + whereas an increased LDH activity indicates a higher conversion of pyruvate to lactate.

Variations in the lactate dehydrogenase enzyme values (LDH) between the animals transported over the distances (D1) and (D2) were observed in the treatment using the trailer where the animals subjected to distance (D2) presented lower values than the animals subjected to distance (D1), an expected contradiction, whereas, at a greater distance, the energetic expenditure by glycolysis is higher, but muscles composed of a large amount of red fiber most frequently obtain energy by oxidation (Table 2).

When the stress indicators and meat quality (Table 2) of cattle subjected to different truck designs for the same transport distance were observed, the pH1*pm* values showed significant difference but the same data behavior was not observed during 24h*pm*, demonstrating that the truck design was not enough to change the pH 24*pm*, so with what happened with the treatments with different distance.

During the conversion of muscle meat (onset of rigor mortis) due to anaerobic glycolysis occurs pH lowering, alteration can occur in the meat water retention capacity, depending on the severity speed of installation and the final pH (Shimokomaki *et al.*, 2006).

Considering the influence of the truck designs for the same distance, the cortisol values of the animals transported by double deck truck were significantly higher compared to those transported by regular truck and trailer, respectively, for the distance (D2), which demonstrated probably this type of truck can offer more stressful conditions for the animals over long distances. But it must be stressed the need for observation at all stages of the pre- slaughter.

A higher bruising score incidence (intensity and extension) was also obtained in the animals transported by this tuck (Figures 1 and 2).

The cortisol concentrations of animals transported over the distance (D1) were higher compared to those of animals transported by regular truck (Table 2) unlike what happened in the distance (D2), but the same behavior for the bruising incidence in the carcass (intensity and area) was not similar in the animals transported by double deck truck (Figures 1 and 2).

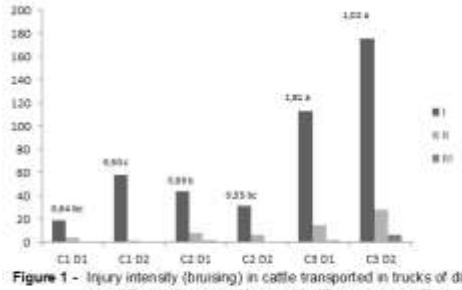

Figure 1 - Injury intensity (bruising) in cattle transported in trucks of different design and over different distances. Means with different letters differ by the Kruskal-Wallis test (P <0.05)

Where: C1 = Regular truck; C2= Trailer; C3 = Double deck; _D1= short distance; D2= long distance;A: Bruise (1 a 10 cm); B = Bruise(11 – 20 cm) e C= Bruise greater than 20 cm.

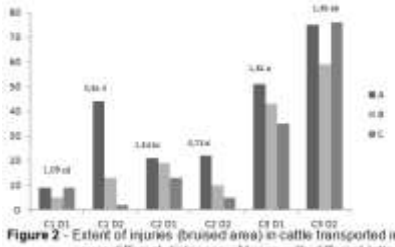

Figure 2 - Extent of injuries (bruised area) in cattle transported in trucks of differentdesign and over different distances. Means with different letters differ by the Kruskal-Wallis test (P < 05).

Where:
C1 = Regular truck; C2= Trailer; C3 = Double deck; D1= short distance; D2= long distance;A: Bruise (1 a 10 cm); B = Bruise(11 – 20 cm) e C= Bruise greater than 20 cm.

The creatine phosphokinase enzyme (CPK) catalyzes the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CK enzyme reaction is reversible, so that ATP can also be generated from PCr to ADP and (HANEY *et al.* 2004). Evaluation of the physical stress from the cattle truck designs and distances studied shows (Table 3) that the distance (D2), as expected, resulted in greater CPK concentration in all treatments but the double deck truck treatment was not statistically different.

Table 3 - Effect of the truck designs on the creatinine phosphokinase concentration in cattle transported over different distances (N = 120 animals).

Truck design	Ordering average	Creatinine phosphokinase (mg/dL)
Regular truck D1	51.38^{b}	1.68
Regular truck D2	90.75^{a}	2.63
Trailer D1	65.37^{ab}	1.85
Trailer D2	57.17^{b}	1.77
Double deck D1	34.00^{b}	1.46
Double deck D2	54.31^{b}	1.78

Means with different letters in the same column differ according to the Kruskal-Wallis test at 5% level of significance; D1 = short distance; D2 = long distance.

Comparison of the high cortisol levels and bruising indices of animals transported by double deck truck over distance (D2) (Table 2) with CPK levels (Table 3) showed that the CPK values performed differently, whereas higher concentrations of CPK were obtained in animals transported by regular truck over distance (D2).

The creatinine phosphokinase in muscle anaerobic conditions, catalyzes the phosphorylation of adenosine diphosphate (ADP) creatine phosphate, making the adenosine triphosphate (ATP) available to the muscle contraction (Chaney *et al.*, 2004).

CONCLUSIONS

Long distance (180-250 km) transportation caused greater muscle fatigue characterized by high CPK blood levels, but the higher energy expenditure was not high enough to cause changes in meat quality indicators such as pH and surface brightness (L *) 24h *pm* in the conditions of the study.

Severe bruising covering a larger area of muscle were observed in animals transported over long distances when two floors of the truck were, as well as higher levels of psychological stress characterized by high cortisol concentrations, demonstrating that this type of truck should be avoided for long distances under the conditions evaluated.

NOTAS INFORMATIVAS

Reservado ao parecer CEUA.

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