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Hematological, biochemical and histopathological changes in cattle submitted to emergency slaughter

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Abstract

This study aimed to evaluate the effects of stress in bovines submitted to emergency slaughter caused by lengthy transportation to slaughterhouses in the state of Tocantins, Brazil. Blood collections were performed individually during exsanguinations. Tissue samples were obtained after evisceration on the slaughter line. The animals were divided into the Experimental Group (EG), with 19 bovines destined for immediate emergency slaughter, and the Control Group (CG), with 24 bovines slaughtered normally at the abattoir flow. The EG showed lower values of erythrocytes, hemoglobin, hematocrit, platelets, neutrophilia with band neutrophils, and lymphopenia. Hyperalbuminemia, high levels of total proteins, glucose, and creatinine, and lower urea levels were observed in the EG. The globulins were equal between the groups. Histopathology revealed hepatocytes with vacuolated cytoplasm (87.5% of the cases) and liver congestion (83.33%). Interstitial emphysema (95.65%), alveolar emphysema (65.21%), and congestion (52.17%) were observed in the lungs. The kidneys showed congestion (80.95%) and hyaline casts (100%). The spleens showed rarefaction in the white (70%) and red pulp (65%), in addition to hemosiderosis (10%). This suggests that the animals transported over long distances are submitted to severe stress, with water and food deprivation, resulting in hematological and biochemical changes, with histological lesions and serious bruises affecting the musculoskeletal system. There is loss of homeostasis, severe debilitation, with destination for emergency slaughter with the conditional use of meat for consumption.

Key words: blood parameters; bovine; histopathology; injuries; stress; transport.

Introduction

The commonest way to conduct cattle to abattoirs is through highway transportation (39). Therefore, transport is considered the most important cause of stress, harming animal health and welfare (5). The transported herd is submitted to a series of changes physical and climatic stimuli, loading and unloading, vibrations, noises, unknown environment, water and food deprivation, temperature and humidity changes, and social resettlement (10, 24). These changes determine homeostasis alterations and, consequently, lead to an attempt by the body to adapt in search of physiological balance (12). Other factors such as vehicle design, load capacity, highway quality, prolonged transportation, and long distances traveled may influence cattle welfare during transportation (6).

Inside moving livestock trucks, cattle confinement generates high stress levels during displacement (24), leading to economic loss because of reductions in carcass weight, the presence of hematomas, and increases in pH, producing dark beef (13). Upon their arrival at abattoirs, some animals may be diseased, agonizing, on forced decubitus, have fractures or generalized bruises, and be hypo or hyperthermic. In such cases, the Brazilian Federal Inspection System determines that these animals be submitted to emergency slaughter, i.e., the immediate slaughter performed by Federal Inspection workers (4). The reasons for this kind of slaughter may be related to stressful situations, with higher occurrence among cows (42) and the leading cause being lesions to the locomotor system (32, 42).

To evaluate the effects of stress on the loss of homeostasis, several variables may be analyzed, including blood parameters, used as indicators of animal welfare (2) and the dynamics of which are influenced by transportation (7). During physical, the hypothalamus-pituitary-adrenal (HPA) axis is activated, with an increase in the plasma concentrations of cortisol and, consequently, in the immune system response to stress (9, 37). Stress and, thus, hypercortisolemia stimulate hepatic glycogenolysis, raising plasma glucose (21). The dehydration that also occurs during transport is made evident by alterations to the total protein and albumin concentrations as well as some haematometric indices, characterizing hemoconcentration (21).

Given this information, this study aimed to evaluate the effect of transportation time, distance traveled, and high load density on different hematological (erythrogram, leukogram, and platelet count) and biochemical (total protein, serum albumin, urea, creatinine, and plasma glucose) parameters, correlating them with stress, as well as to perform histopathological evaluations on cattle destined for emergency slaughter, thus generating important data from productive, technological, and scientific perspectives, considering the lack of scientific production on stress evaluation in this category. It is important to emphasize that any alterations observed in the welfare of the animals had no human interference.

Material and methods

The experiment was carried out in slaughterhouses inspected by the Brazilian federal government in Araguaína, located in the north of the state of Tocantins, Brazil (7° 11' 28" S, 48° 12' 26" W), from January to July 2013. The research was approved by the CEUA-UFT Ethics Committee (Process No.: 23101.003943/2012-01). All bovines used in this research were transported to the slaughterhouses in trucks (twenty cows) or wagons (thirty cows), obeying the maximum load capacity. Then, the animals were divided into two groups, with the Experimental Group (EG) being composed of a total of 24 animals destined for immediate emergency slaughter that had traveled approximately 695 km in 12 h of transportation and were slaughtered soon after they arrived at the abattoir, without being submitted to rest, food deprivation, or a water diet.

The cows were from farms located in the states of Mato Grosso (MT), Pará (PA), and Tocantins (TO). Tissue samples were collected from all EG animals, yet blood samples were only collected from 19 animals (18 cows and 1 ox). The Control Group (CG) was constituted of 24 animals, with 19 cows and 5 oxen from farms located in TO that traveled approximately 100 km in 3 h of transportation and were slaughtered according to the ordinary slaughter flow, under 12 h of fasting, resting, and water diet. Only blood samples were collected for this group since it was expected only to contain healthy animals with no tissue lesions. Additionally, the collection would bring an economic loss to the slaughterhouses due to damage to tissues destined for consumption.

The desensitization of both groups was carried out with a pneumatic pistol or dart gun. Blood and tissue samples were collected for the EG in different slaughterhouses, according to the occurrence of emergency slaughter cases. In turn, the CG was submitted to random collections on scheduled days.

A 4 mL blood sample was obtained for each animal during the exsanguination using tubes containing EDTA K2 (BD Vacutainer, São Paulo, SP, Brazil) for the hemogram and tubes containing sodium fluoride added to EDTA (BD Vacutainer, São Paulo, SP, Brazil) for obtaining the plasma following glucose determination. An additional volume of 10 mL was collected in tubes free of anticoagulant, with clot activator, to obtain the serum destined for the biochemical analysis. Next, the tubes were identified, blood smears were made, and both the tubes and the blood smears were sent to the EMVZ-UFT Veterinary Clinical Pathology Laboratory in Araguaína for fast sample processing and analysis.

In order to perform the hemograms, the automatized veterinary hematologic analyzer (PocH-100iV Diff, Sysmex Co, Lincolnshire, IL, USA) was used, while Diff-Quick staining followed by optical microscopy (Nikon E200, Nikon Instruments Inc, Melville, NY, USA) at a 400X magnification was performed for the leucometric evaluation of the smears.

Plasma and serum were obtained from sample centrifugation (Centrifuge Centribio 80-2B, São Paulo, SP, Brazil) for 6 min at 3.605 g and used for the serum determination of urea, albumin, and total proteins and the plasma determination of glucose using a spectrophotometer (Biochrom Asys UVM340 spectrophotometer, Holliston, MA, USA), while the serum determination of creatinine was performed in a semi-automatic spectrophotometer (Bioplus S semi-automatic spectrophotometer, São Paulo, SP, Brazil) using commercial kits (Labtest commercial kits, Lagoa Santa, MG, Brazil). The globulin values resulted from subtracting the measured total protein from the measured albumin.

Heart, lung, liver, spleen, kidney, and small intestine samples were collected from bovines destined for emergency slaughter. The collection was performed after evisceration in the slaughter line. Then, the samples were sent to the EMVZ-UFT Veterinary Pathology Laboratory placed in flasks containing 10% formol, correctly identified, fixed for 72 h, and processed routinely, followed by the preparation of slides and staining with hematoxylin-eosin for optical microscope observation (Optical microscope Leica DM500 observation, Leica Microsystems, Heerbrugg, Switzerland). Liver samples were also subjected to periodic acid-Schiff (PAS) to assess glycogen stores in hepatocytes.

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Table 1. Effect of transport over long distances on red blood cell (RBC) counts, hemoglobin concentrations, packed cell volumes (PCV), mean corpuscular volumes (MCV), mean hemoglobin concentration volumes (MHCV), and platelets in animals destined for immediate emergency slaughter (EG) and animals slaughtered according to the ordinary slaughter flow (CG). The results are expressed in means.

Variables	CG	EG	SEM*	P†
RBC (x10^6/mL)	9.618 a	8.555 b	0.2491	0.0043
Hemoglobin (g/dL)	14.336 a	13.41 b	0.2864	0.0271
PCV (%)	41.991 a	38.605 b	10.195	0.0235
MCV (fL)	43.877 a	45.42 a	0.9279	0.2462
MHCV (g/dL)	34.209 a	34.86 a	0.2695	0.0951
Platelets (x10^3/mL)	257.54 a	177.9 b	239.936	0.0236

Means followed by lower-case letters on different lines differ 5% probability according to Student's t-test.
* Standard error of the mean.
† Probability type 1 error.

The data related to the hematological and biochemical variables were submitted to a normality test (Kolmogorov-Smirnov) and variance homogeneity (erythrogram, leukogram, thrombogram, TP, albumin, urea, creatinine, and glucose). The normal variables were submitted to an analysis of variance in an entirely casualized design with two treatments (CG and EG), with 24 and 19 repetitions, respectively. The means of the treatments were compared by Student's t-test with a 5% error probability. The variables that did not present normality or homogeneity were subjected to a logarithmic transformation.

Finally, the samples that did not normalize even after the transformation were subjected to the non-parametrical Mann-Whitney test with a 5% error probability. Spearman's correlation was calculated between spleen changes, which are subjective and categorical, and hematological variables (RBC count, hemoglobin, and PCV). Regarding the histopathological analysis, percentages of abnormality occurrences were calculated for the EG samples.

Results

There was a significant difference in the red blood cell (RBC) counts, hemoglobin concentrations, and packed cell volume (PCV) between groups. The RBC, hemoglobin, and PCV values were lower for the EG than the CG (Table 1). The mean corpuscular volume (MCV) and mean hemoglobin concentration volume (MHCV) did not differ between the groups. There was a significant difference in the platelet counts between the studied groups (Table 1). The EG animals showed lower platelet values than CG animals. The correlation between categorical variables (white and red pulp rarefaction) and hematological variables (RBC count, hemoglobin, and PCV) was not significant.

The white blood cell counts (WBC) were within the reference range for both groups. No significant differences in WBC were detected between the studied groups; however, the neutrophil count differed, with results above the reference interval for the EG. There was a difference in the lymphocyte counts, with an increasing

Table 2. Effect of long transport distances on white blood cell (WBC), neutrophil, band neutrophil, and lymphocyte counts and the neutrophils-to-lymphocytes ratio in animals destined for immediate emergency slaughter (EG) and animals slaughtered according to the ordinary slaughter flow (CG). The results are expressed in means.

Variables	CG	EG	SEM*	P†	CG	EG
WBC (x10^3/mL)	8.87 a	9.40 a	0.5468	0.5039	-	-
	Relative values (%)				**Absolute values (x103/mL)**	
Neutrophils	43.44 a	55.50 b	27.815	0.0037	3.965	5.427
Band neutrophils	0.05 a	7.9 b	0.5811	0.0001	0.0035	0.7226
Lymphocytes	49.14 a	34.1 b	28.336	0.0005	4.258	3.043
N:L ratio‡	0.88	1.62	-	-	-	-

Means followed by lower-case letters on different lines differ 5% probability according to Student's t-test.
* Standard error of the mean.
† Probability type 1 error.
‡ Neutrophils-to-lymphocytes ratio.

Table 3. Effect of long transport distances on total protein, albumin, globulin, urea, and creatinine serum levels and glucose plasmatic levels in animals destined for immediate emergency slaughter (EG) and animals slaughtered according to the ordinary slaughter flow (CG). The results are expressed in means.

Variables	CG	EG	SEM*	P†
Total protein (g/dL)	6.703 a	7.138 a	0.2000	0.1672
Albumin (g/dL)	3.859 a	3.867 a	0.0242	0.7452
Globulins (g/dL)	2.844 a	3.271 a	0.1943	0.3898
Urea (mg/dL)	68.119 a	62.394 b	13.707	0.0036
Creatinine (mg/dL)	2.34 a	2.65 b	0.0945	0.0253
Glucose (mg/dL)	123.516 a	185.139 b	92.927	0.0000

Means followed by lower-case letters on different lines differ 5% probability according to Student's t-test.
* Standard error of the mean.
† Probability type 1 error.

Table 4. Quantification of the degrees of histopathological injuries related to stress changes during long transport distances observed in the livers, lungs, kidneys, and spleensof bovines destined for immediate emergency slaughter (EG).

	Degree of injury*					
Tissue/Injury	**G −**	**G +**	**G ++**	**G +++**	**G ++++**	**G +++++**
Liver (24 samples)						
Vacuolar degeneration	3	4	3	9	4	1
Congestion	4	4	10	6	0	0
Lung (23 samples)						
Interstitial emphysema	1	4	5	7	5	1
Alveolar emphysema	8	5	7	2	0	1
Congestion	11	1	5	4	2	0
Kidney (21 samples)						
Congestion	4	4	6	7	0	0
Hyaline casts	0	7	8	6	0	0
Tubular degeneration	19	0	2	0	0	0
Spleen (20 samples)						
White pulp rarefaction	6	4	5	3	2	0
Red pulp rarefaction	7	2	7	4	0	0
Hemosiderin	19	2	0	0	0	0

* G− = absence; G+ = mild; G++ = mild to moderate; G+++ = moderate; G++++ = moderate to severe; G+++++ = severe.

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trend observed for the EG, although the results were within the reference interval in both groups. There was an increase in the N/L ratio in both groups, with an emphasis on the EG, for which an inversion of the mentioned ratio was observed along with a significant appearance of band neutrophils (Table 2).

No significant differences were found between the mean TP values in the studied groups; nevertheless, the EG showed an increasing trend compared to the CG (Table 3). In this study, the mean values for albumin and globulins did not differ between the groups. A significant difference in glucose levels between groups was noticed, with an increase in glucose for the EG compared to the CG (Table 3). Considering the urea concentrations, there was a significant difference between groups, with higher values observed in the CG. The creatinine levels showed a statistically significant difference, with results for the EG being higher than for the CG (Table 3).

The most prominent histopathological changes were detected in the livers, lungs, kidneys, and spleens (Table 4). Among all 24 cows submitted to immediate emergency slaughter, 87.5% (21/24 cows) showed hepatocytes with a turbid cytoplasm with fine vacuolization (lace-like aspect) and also PAS-positive hepatocytes significantly reduced due to a glycogen decrease (Fig. 2A and B), and 83.33% (20/24 cows) had hepatic congestion (Fig. 1A). The lungs showed interstitial emphysema in 95.65% (22/23 cows), alveolar emphysema in 65.21% (15/23 cows), and congestion in 52.17% (12/23 cows) (Fig. 1B). The kidneys were congested in 80.95% of the cases (17/21 cows), with an irregular eosinophilic substance inside the tubular lumen (hyaline casts) in 100% (21/21 cows) (Fig. 1C). The alterations observed in the spleens were white pulp rarefaction (lymphoid tissue) in 70% of the animals (14/20 cows), red pulp rarefaction in 65% (13/20 cows), and the presence of hemosiderin in only 10% of the cases (2/20 cows) (Fig. 1D).

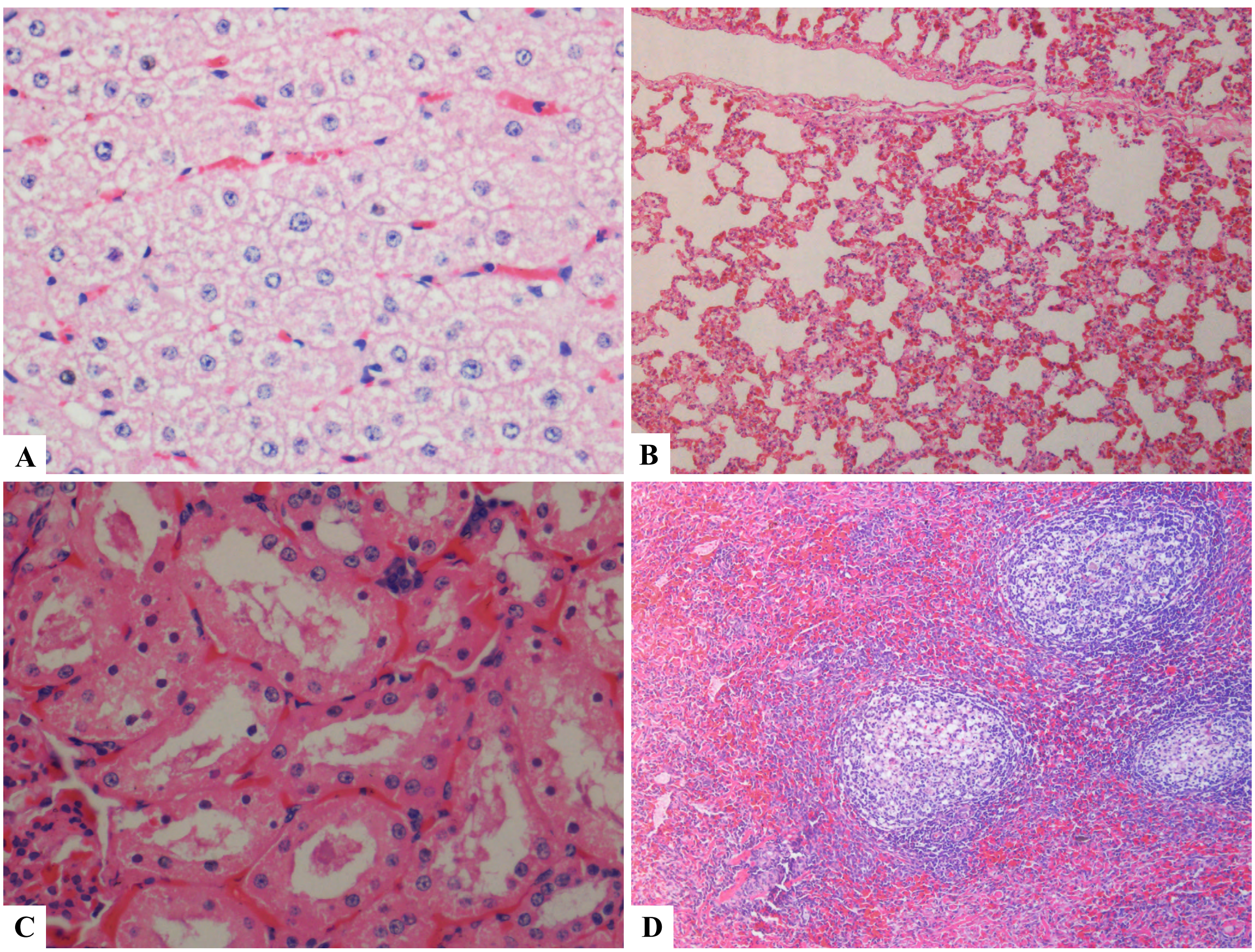

Figure 1. Histopathological evaluation of different tissues in bovines submitted to immediate emergency slaughter. A: liver, lace-like aspect of hepatocytes cytoplasm due to glycogen depletion and congestion, HE, 40X. B: lung, congestion, interstitial, and alveolar emphysema, HE, 10X. C: kidney, congestion and presence of hyaline casts inside tubular lumens, HE, 40X. D: red and white pulp (lymphoid tissue) rarefaction, HE, 10X.

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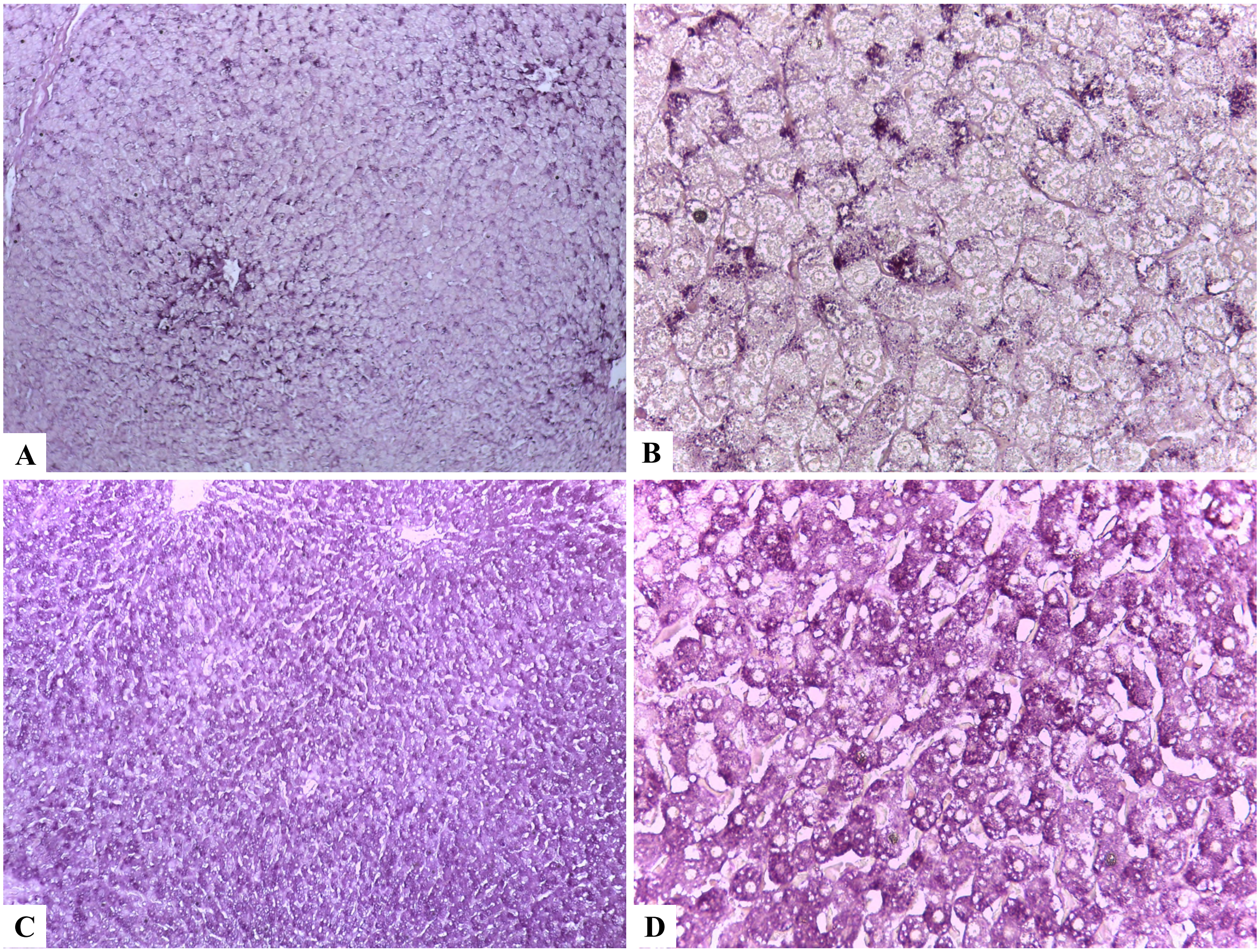

Figure 2. The analysis of liver glycogen by periodic acid-Schiff (PAS) stainedin purple-magenta color. Liver glycogen in the experimental group (EG) with PAS-positive hepatocytes weresignificantly reduced, A (PAS, 10X) and B (PAS, 40X),when compared to the control group (CG) that showed higher number of PAS-positive hepatocytes,C (PAS, 10X) and D (PAS, 40X).

Discussion

Unlike the animals in the CG, those in the EG had been through situations that could have caused changes to the RBC and platelet counts, hemoglobin concentrations, and PCV values. Those changes to the erythrocyte dynamics are in line with the spleen red pulp rarefaction observed in the EG. The animals presented for emergency slaughter had extensive bruises and subcutaneous as well as muscular hematomas caused mainly by trampling (trauma) during transportation (data not shown); such lesions were not observed on the carcasses of the CG.

Unfavorable transportation conditions (1), high-density loads (24), and long transportation periods (6) are situations related to an increase in bruises (24), given that debilitated cows remain decumbent longer, enabling the appearance of muscular traumas due to the increased risk of muscle hypoxia and contusions (17). Hematomas are formed by hemorrhages confined to a determined space (subcutaneous space and muscles) because of physical trauma to blood vessels (26) due to contusions, which are a possible cause of the lower RBC counts, hemoglobin levels, and PCV for the EG. This information is relevant since the EG animals were transported for approximately 12 h at the maximum load for trucks (20 bovines) or wagons (30 bovines).

Although cases of relative erythrocytosis caused both by dehydration or excitation and fatiguing activity are common (adrenergic discharge) (35), even with both groups undergoing these events, there is no corroboration with such hypothesis in any of the groups.

Regarding the MCV and MCHC, the absence of anemia in both groups agrees with other studies that evaluated the effect of different transportation times on bovine hematological parameters (10) and showed no differences in these indices. Features of thrombocytosis resulting from spleen contraction due to adrenergic stimuli and in the presence of inflammatory cytokines, especially IL-6, are common (35). Although the animals in both groups were submitted to stress, traumas, or bruises during transportation, the mean platelet numbers were within the reference range for the species. The decrease in the platelet counts verified for the EG compared to the CG was possibly caused by their consumption due to severe contusions resulting in hemorrhages (data not shown). The results of this analysis agree with the literature that describes that blood loss does not result in significant thrombocytopenia since platelet concentrations secondary to hemorrhages are rarely lower than 100,000/µL (3).

Both management and flock transportation are considered stressful conditions, leading to hematological changes (33). Studies on the effects of 12 h transportation through highways on bovine leukocyte parameters have detected neutrophilia and lymphopenia persisting up to 24 h after transportation (16). Leukocytosis with marked neutrophilia and reduced concentrations of other cells, e.g., eosinophils and lymphocytes, was also observed when the consequence of a 24 h transportation through highways on the defense cells of bovines was studied (10). Such changes are associated with high levels of glucocorticoids acting directly on bone marrow, causing alterations to different leukocyte populations (8).

In this study, the animals from the EG, submitted to approximately 12 h of transportation, also showed neutrophilia. Still, despite the reduced lymphocytes levels, this group did not present lymphopenia. The bovine neutrophil-to-lymphocyte ratio (N:L) is approximately 0.5 and raises under excitatory circumstances because of epinephrine, inflammatory, and stressful (cortisol) stimuli. Nevertheless, the stress-induced neutrophilia (hypercortisolemia) observed in other species is uncommon in bovines (44).

One may attribute a higher N:L ratio in the EG than the CG to traumas and lesions caused by transportation, leading to pain, higher excitation, and inflammatory stimulus, also associated with the release of band neutrophils from the marrow storage pool. The animals from the EG showed more effects of stress due to the longer distance traveled and travel time, in addition to the fact that they had suffered more severe bruises during transportation, leading to a feature of acute inflammation that may explain the left shift for this group.

Both groups had serum TP results within the reference range (19). The increase in TP, albumin, and PCV are indicative of dehydration during transportation (11). The TP values were similar to those found by other researchers, who reported progressive dehydration of the animals according to the travel time (43). Still, the TP proved to be a stress-sensitive variable in animals transported for 6 h to 24 h (10). The serum albumin concentration in both groups showed a slight increase compared to the reference range (19).

The animal transportation is determinant of the relative increase of albumin in blood since the animals are submitted to water restriction during the whole period (11), which also occurred with both studied groups, characterizing some degree of dehydration. Also, the increase in albumin after transport may be a form of compensation by the organism of the metabolic acidosis caused by fluid loss and food deprivation (9). The albumin and globulin results differ from those in other studies, in which physiologically reduced albumin levels and increased globulin levels in bovines were found to be associated with different transportation times (10).

The release of glucocorticoids and catecholamines induced by transportation stress is responsible for stimulating liver glycogenolysis, increasing blood concentrations of glucose (21). However, some studies did not find changes to plasma glucose as a response to transportation (29). The significant response of cortisol on glycemia is expected from 3 h to 16 hours of transportation (38) and is related to the duration of the displacement and fasting period in this study.

Nevertheless, blood metabolites such as glucose increase after food and water deprivation during highway transportation. Such an increase in glycemia during long periods of starvation is caused by insulin inhibition and glucagon activation since the latter is responsible for the increment of liver gluconeogenesis and glycolysis and for reducing glycogen synthesis and glucose degradation in the liver (28). Thus, one may infer that short or long-term hyperglycemia due to transport stress indicates a metabolic depletion associated with the mobilization of energy reserves (23). Given the exposed, one may conclude that, although both groups underwent stressful situations during transportation, the animals submitted to immediate emergency slaughter were submitted to rougher conditions of stress, water, and food restriction than the conventionally slaughtered animals. Thus, the feasible origin of the glycemia amplitude of the EG compared to the CG was caused by the increase in cortisolemia and adrenergic stimulus.

Azotemia, i.e., increased serum levels of nitrogenous compounds, especially urea and creatinine, may be prerenal, renal, or postrenal in origin (36). In this study, events such as dehydration, leading to a decrease in renal perfusion, a serum increase of cortisol that influences protein catabolism, and physical stress, would justify the high levels of urea surpassing the reference range (19) for the CG, characterizing prerenal azotemia, and being near the maximum limit for the species in the EG. Psychological stress may induce diuresis (30) due to the effect of hyperglycemia on the increase of the glomerular filtration rate (25), consequently leading to an increase in tubular flow, which decreases urea reabsorption, which in turn would justify the lower levels found in the EG compared to the CG.

Creatinine is a non-protein nitrogenous product that originates from the muscle metabolism of creatine and phosphocreatine (15). A blood creatinine increase in stressed animals may be caused by the decrease of renal perfusion resulting from the reduction of extracellular fluid, decreasing its depuration by the kidneys (34), or by the increased non-enzymatic breakdown of muscle creatine due to physical stress (31). Thus, one may deduce that the EG animals showed a higher degree of dehydration since they were not submitted to fasting and a water diet as the CG animals were. Besides, another dehydration index such as albumin was also higher for the EG than the CG, and the EG animals presented a higher musculoskeletal impairment resulting from bruises during transportation.

In the present study, the histopathological changes observed in the livers of the EG animals were vacuolar degeneration, congestion and PAS-positive hepatocytes significantly reduced. The amount of hepatocyte glycogen of animals with food restriction is reduced (27), and the transportation stress leads to liver glycogen exhaustion, increasing plasma glucose concentrations (21). Given these data, one may infer that hepatocyte vacuolar degeneration and higher plasma glucose levels in the EG indicate that these animals were exposed to intense stress associated with a longer period of food deprivation due to the more extended transportation time.

Dehydrated animals may show circulatory insufficiency (40), and the decreased blood flow in hepatic veins may lead to congestion (18). In some cases, the venous circulation of the liver may be compromised by lung emphysema, resulting in congestion (18). The causes of liver congestion in the EG may be related to dehydration, confirmed by the trend of a TP increase, hyperalbuminemia, and high levels of creatinine in this group, as well as to the lung emphysema verified in all animals with congested livers, which may have favored such tissue alterations.

Studies evaluating the causes of emergency slaughter in different categories obtained 1.05% of respiratory causes in cows, 16.33% in heifers, 16.71% in bulls, and 47.43% in calves (42). The changes observed in the lungs during histopathological examinations in this study were interstitial and alveolar emphysema and congestion. Emphysema is secondary to lung alterations such as death-struggle, appearing in all species. The distention and rupture of the alveoli are evident in alveolar emphysema, with the development of air bubbles inside the pulmonary parenchyma (22). Interstitial emphysema, on the other hand, is more common in bovines and may be caused both by agony or violent breathing movements, resulting in the accumulation of air in alveoli and bronchiole walls, forcing its passage to the interlobular conjunctive tissue, leading to a significant increase in the interlobular septal thickness (22).

During the observation of the EG animals in moments preceding the emergency slaughter, it was stated that almost all animals manifested intense agony and forced breathing movements, which were determinant factors in the establishment of emphysema. Pulmonary congestion may be caused by the action of gravity and circulatory deficit, with the deposit of blood in lower regions of the body in animals that remain laying down during long periods, characterizing hypostatic congestion, which is common in bovine species (22). Besides, dehydration causes hypovolemia and hemoconcentration with inadequate tissue perfusion (14), and the decreased pulmonary blood perfusion leads to lung congestion with tissue blood stasis (22).

Because the EG animals were in lateral or sternal decumbency upon their arrival at the abattoir, they would not go down from the trucks to the waiting corral along with the rest of the bovines, favoring hypostatic congestion. Nevertheless, the increase in albumin and creatinine levels in the EG indicates dehydration, which could also have favored pulmonary congestion.

Renal congestion may appear secondarily to hypovolemia, as discussed earlier. On some occasions, proteinaceous substances (hyaline casts) may be microscopically visualized as dense formations that stain intensely inside renal tubules due to the high contents of albumin and other proteins (20). The EG animals showed higher levels of TP and albumin than the CG animals, which could be the feasible cause of the presence of hyaline casts inside renal tubules. This information is relevant because, although urinalysis was not performed, the presence of hyaline casts indicates possible proteinuria, and this urinary loss of protein prevented an increase in their serum values caused by the dehydration observed in the EG bovines.

Severely stressed animals may have spleen degeneration such as lympholysis and changes to hypocellular and epithelioid germinative centers (41). This fact justifies the alterations observed in the white pulp and agrees with the hematological findings, in which the EG showed lower lymphocyte counts than the CG since the animals were submitted to high stress levels during transportation.

Conclusion

From the results, it is concluded that animals referred to immediate emergency slaughter suffer severe stress due to transportation during long periods and distances and to undergoing water and food deprivation, causing hematological, biochemical, and histopathological changes, breaking homeostasis, and leading them to suffer from severe bruises that affect the musculoskeletal system, rendering them extremely debilitated. The importance and relevance of experimental findings in Tocantins, Brazil, show that improvements are needed in the inspection of the cattle road transport system and reduction of the distance and time of transport to slaughterhouse to mitigate the effects of transport on cattle welfare.

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Declaration of conflicting interests

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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