BJVP
Brazilian Journal of Veterinary Pathology
ISSN 1983-0246

Original Full Paper

Deaths of Cows During Long Distance Transportation to the Slaughterhouse. Changes on Histopathological Examination, Muscle and Liver Glycogen Storage and Muscle pH

Leonardo Vaz Burns[1*], Sandro Estevan Moron[1], Fabiano Mendes de Córdova[1], Luciano Fernandes de Sousa[1], Angela Patricia Medeiros Veiga[2], Sílvia Minharro Barbosa[1], Fredson Ronei Cândido[3], Orliomar Martins da Cruz[3], Dagoberto Machado Prata[3], Adriana Carla Floresta Feitosa[3], Nádia Regina Stefanine[1], Katharine Brandão Monteiro[1], Andressa Karollini e Silva[1], Francielli Cordeiro Zimermann[2], Adriano Tony Ramos[2]

[1]Universidade Federal do Tocantins (UFT) - Escola de Medicina Veterinária e Zootecnia, Araguaína – TO
[2]Universidade Federal de Santa Catarina (UFSC) - Campus Curitibanos, Curitibanos – SC
[3]Serviço de Inspeção Federal (SIF), Ministério da Agricultura, Pecuária e Abastecimento, Palmas - TO.
*** Corresponding Author:** BR. 153, km 112, 77.000-00, Araguaína, TO, Brazil. E-mail: leovazburns@hotmail.com

Submitted February 20th 2014, Accepted March 10th 2014

Abstract

The present experiment aimed to perform histopathological, muscle and liver glycogen and muscle pH analyses of cows dead during transportation, and submitted to necropsy in slaughterhouses inspected by Federal Agencies, in Araguaína – TO, Brazil, from January to July 2013. Six affected animals dead during transportation and submitted to necropsy constituted the Experimental Group (EG); a Control Group (CG), was composed by 6 cows slaughtered ordinarily following the slaughter flow. Gross and histopathological evaluations were accomplished only on EG. The main gross lesions observed at necropsy were extensive subcutaneous and muscle hemorrhage and hematomas, interstitial (83.3% of cases) and alveolar (66.6% of cases) lung emphysema, lung congestion (66.6% of cases) and edema (16.6% of cases), kidney (83.3% of cases) and liver congestion (16.6% of cases). On histopathological evaluation, the lungs showed interstitial (100% of cases) and alveolar emphysema (66.6% of cases), congestion (66.6% of cases), edema (16.6% of cases) and blood aspiration (16.6% of cases). Renal congestion (83.3% of cases), hyaline casts (50% of cases) and tubular degeneration were the kidneys changes (16.6% of cases). Rarefaction of the white pulp (66.6% of cases), red pulp (66.6% of cases) and hemosiderin (16.6% of cases) were detected on spleen. The hepatocytes showed turve and finely vacuolyzed cytoplasm (lace-like aspect) possibly caused by a reduction on glycogen (50% of cases) and congestion (16.6% of cases). Autholysis was observed on 50% of the livers and 16.6% of the spleens and kidneys. The animals from the EG presented higher mean of degenerate muscle fibers. The intercostal muscle was more affected when compared with other muscles on the same group. Muscle pH values were higher on EG than on CG. Differently, glycogen average values were similar between groups (EG 58.97 µmol/g and CG 57.05 µmol/g). The results obtained in the present study allow to confirm that the cows, transported through long distances, and exposed to intense stress and water and food deprivation, show an homeostasis fall. Thus, stress and time of transportation affect glycogen concentrations and muscle pH. Bruises caused miofibrillar degeneration and hemorrhages that, when associated with loss of fluids through dehydration, lead to a clinical picture of hypovolemic shock evidenced by irreversible gross and microscopic changes causing death.
Key Words: hypovolemic shock, glycogen and pH changes, stress, transportation, cattle.

Introduction

Meat cattle are commonly conducted to slaughterhouses through highway transportation (35) although this is the most important cause of stress among animals, causing somatic and welfare injuries (26).

Transport stress is a very complex situation in which a number of factors, such as pretransportation problems, noises, vibrations, agglomeration, social reorganization and climate factors (temperature, humidity and vapors), restraint, load and unload, duration of the travel, food and water deprivation are involved (34). These factors alter homeostasis as an attempt to a body adaptation, aiming to restore the physiologic balance (11).

Cattle transportation to the abattoir may induce subclinical and clinical manifestations, even death (22), since stress has a direct impact on animal health (39). Thus, the number of dead animals in association with transportation to slaughterhouses can be an indication of their welfare (39). Transport deaths are more common among chickens and pigs than among ruminants and the affected animals have emaciation, salmonellosis, trauma (14), asphyxiation, and heart failure (7) as main causes. Some researchers report mortality rates during transport in different places and periods: South Africa in 1980: 0.01%; in 1990: 0% (16); USA: 1% (17); Australia [Victoria] from 1998 to 2000: 0.64% (6); Hungary: 0.01%; Australia [Queensland]: 0.25% (7).

Cattle necropsy in abattoirs is performed at the “Necropsy Department”, being recommended always when animals are found dead in trucks, wagons, corrals or within factories dependencies (5).

The present experiment aimed to perform histopathological evaluation of a number of tissues, muscle and liver glycogen analysis and muscle pH measurement in cows that died during transportation, submitted to necropsy in slaughterhouses inspected by Federal Agencies in Araguaína-TO, Brazil, from January to June 2013. Since data on evaluation of changes that cause death on such a category are scarce, our data constitute important informations, from a productive, technologic, and scientific point of view. The research was approved by the Ethics Committee -CEUA-UFT (Process number: 23101.003943/2012-01).

Materials and methods

Animals and Treatment

The present experiment was developed on slaughterhouses under Federal Inspection, in Araguaína, North of Tocantins State (coordinates 7º11’28” latitude, 48º12’26” longitude), Brazil, from January to July 2013. The autopsies were routinely proceeded according to rules of inspection services including examinations for surveillance of bovine spongiform encephalopathy (BSE). The collection of data for this work did not affect the inspection service. The experiment consisted on necropsies of six cows that died during transportation-constituting the Experimental Group (EG)- proceeding from farms located in Mato Grosso-MT and Pará-PA States, submitted to necropsy immediately after their entrance in the slaughterhouse. The Control Group (CG) consisted of six cows arising from Tocantins (TO) farms, slaughtered according to the normal flow of slaughter, being randomly selected. All cows used in this research were transported to the slaughterhouse in trucks or wagons, obeying the load capacity; therefore EG animals were transported through a distance of approximately 800 km with a 12-hour duration. The animals from CG went through about 150 km for a 2-hour period.

Histopatologycal Analysis

Samples of heart, lung, liver, spleen, kidney, small intestine, and intercostal, psoas major, abdominal internal oblique, and semitendinosus muscles were collected during necropsy. The procedure took place in the necropsy room, in different days, considering the specific cases mentioned in the text above. Healthy cows did not show damage of tissues proper for consumption, thus CG animals were submitted only to muscle fragment collections, in order to avoid any economic loss to the slaughterhouses.

Soon after collection, the samples were sent to the Veterinary Pathology Laboratory of the School of Veterinary Medicine and Animal Sciences of the Universidade Federal do Tocantins (UFT), Araguaína Campus, placed in flasks containing 10% neutral formalin, properly identified, fixed for 72 hours, routinely processed, prepared on glass slides, and stained with the hematoxylin/eosin (HE) method for light microscopy observation.

Morphometric evaluation of muscles was performed on transversal cuts through a 300-fibers count per muscle, following a classification on normal or degenerate. The images were directly captured for a monitor through a computer program and an optical microscope with a 40X objective. The quantification of fibers was accomplished on different fields of the muscle section, initiating on the left lower field, following diagonally in a ladder shape, until the count of 300 fibers was reached.

Muscle and Liver Glycogen Analysis

Twelve liver samples (CG= 6; EG= 6) measuring 4 x 4 cm and 48 muscle samples (psoas major, semi-tendinosus, abdominal internal oblique, and intercostal; CG= 24; EG= 24) measuring 4 x 4 cm were collected immediately after necropsy of EG animals, wrapped in plastic bags, properly identified and refrigerated at collection, followed by freezing at -20C, and sent to the Biochemistry and Morphology Laboratory for glycogen measurement. Fragments of 0.1g of liver and 0.2g from each muscle were removed from the frozen samples, placed in individual centrifuge tubes containing 2 mL 30% KOH in order to analyze glycogen concentrations (9).

pH Measurements

The freezed muscle samples (CG= 24; EG= 24) were placed in the refrigerator during 36 hours in order to promote a slow and complete thawing, following by the pH determination of each muscle, using a meat pHmeter

Statistical analysis

Data on glycogen concentration, pH, and percentage of muscle fibers degeneration were arranged and analyzed through an entirely randomized design, arranged as subdivided parcels, the treatments being considered as parcels (CG and EG) while the different muscles collected (intercostal, psoas major, abdominal internal oblique, and semitendinosus) were considered as subparcels. The means of the referred factors were compared through Student's t test with a 5% error probability. Histological data were analyzed considering percentage rates of abnormality changes only on EG animals.

Results and Discussion

Gross and Histopathological Evaluations

During necropsy the main alterations observed were extensive subcutaneous and muscular hemorrhages and hematomas (Figure 1), mainly on the lateral areas of all animals, interstitial lung emphysema in 83.3% (5/6 cows) and alveolar emphysema in 66.6% (4/6 cows), lung congestion in 66.6% (4/6 cows) and edema in 16.6% (1/6 cows; Figure 2), renal congestion in 83.3% (5/6 cows) and liver congestion in 16.6% (1/6 cows), all confirmed histologically (Figures 3 and 4). The most important histopathological changes were found in lungs, kidneys, spleen, and liver. Autolysis was observed on 50% of livers and 16.6% of spleens and kidneys collected during necropsy, being one of the major problems for diagnosis determination (21).

Figure 1. Bovine, subcutaneous tissue, extensive hemorrhages on lateral thoracic region.

Interstitial emphysema is a very common lesion in cattle (20). These animals could have been submitted to very agonic situations with increase in cardiac frequency moments before death, leading to interstitial emphysema. Rupture and distension of alveoli with presence of air bubbles in lung parenchyma are seen in alveolar emphysema and may be caused by agonic situations as well (20).

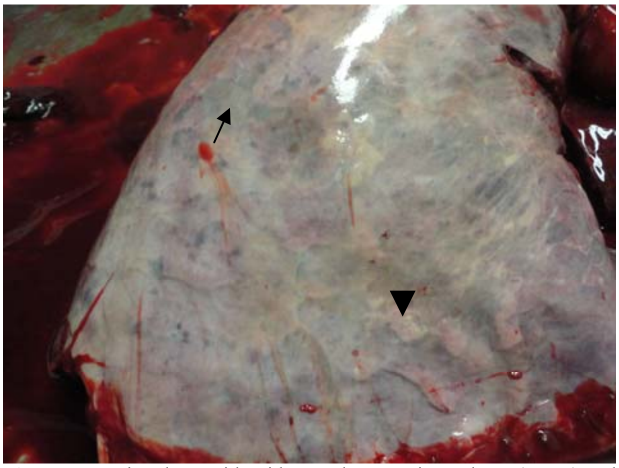

Figure 2. Bovine, lung with widespread congestion, edema (arrow) and emphysema (arrow head).

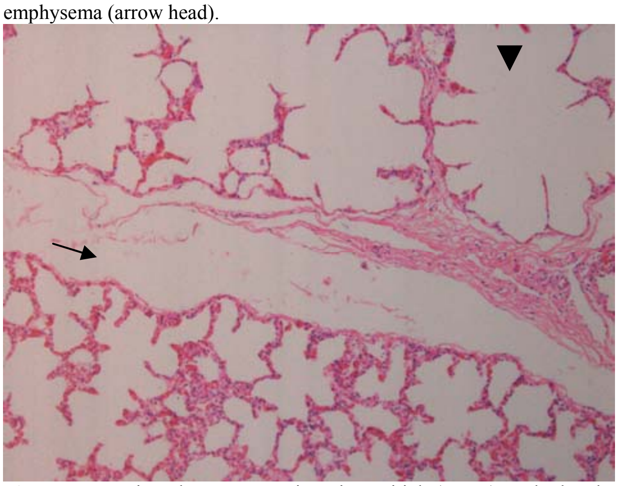

Figure 3. Bovine, lung, congestion, interstitial (arrow) and alveolar emphysema (arrow head). HE, 10X.

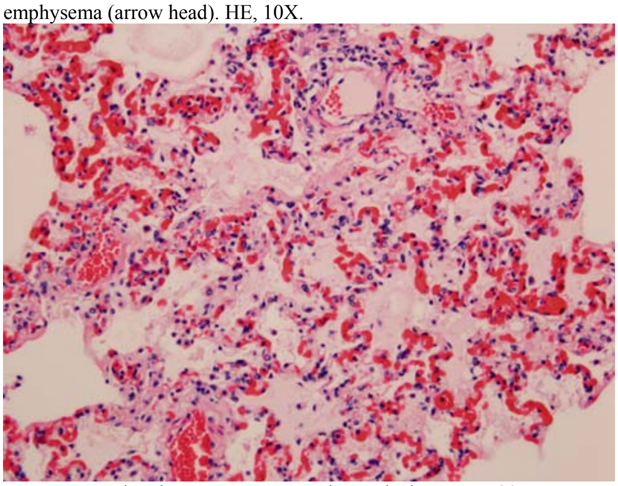

Figure 4. Bovine, lung, severe congestion and edema. HE, 20X.

Lung congestion may occur by gravity action and circulatory deficit, causing hypostatic congestion (20). Dead cows were on lateral decubitus at arrival in the abattoir, which facilitated the development of hypostatic congestion. During transportation, the animals were kept under water deprivation; the dehydration verified in animals submitted to long periods of transportation (10), leads to hypovolemia and hemoconcentration, with inadequate tissue perfusion (13) that cause pulmonary congestion and edema (20).

The kidneys showed congestion in 83.3% of cases (5/6 cows), with findings of an irregular eosinophilic substance inside tubular lumens (hyaline casts) in 50% (3/6 cows), as well as tubular degeneration in 16.6% (1/6 cows) (Figure 5). Renal congestion could have emerged as a consequence of the hypovolemia caused by dehydration, as discussed previously for pulmonary congestion. Hyaline casts are being caused by an increase in concentrations of albumin or other proteins (19). Animals submitted to long term transportation show high serum levels of albumin and total proteins, indicating dehydration (10) what may explain the presence of hyaline casts in the animals of the present study.

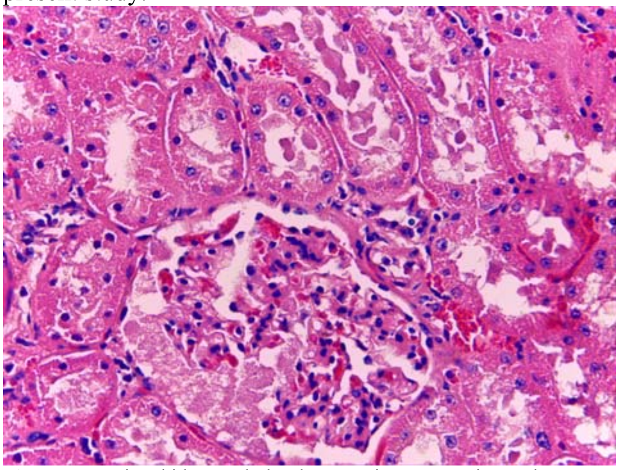

Figure 5. Bovine, kidney, tubular degeneration, congestion and presence of hyaline casts inside tubular lumens. HE, 40X.

Tubular hydropic degeneration, a reversible change, occurs due to alterations on mitochondrial membranes, with vacuoles formation (23), having hypovolemia as a possible cause, with implications on renal perfusion and acute kidney failure (28).

Four out of six cows (66.6%) showed splenic white (lymphoid follicles) as well as red pulp rarefaction, while hemosiderin was detected in 16.6% (1/6 cows) of the tissues evaluated. Animals transported through long distances are submitted to high levels of severe stress that can cause degenerative spleen lesions, such as lympholysis and decrease of cells in germinal centers (38), as the observed in our investigation.

Hepatocytes with cloudy cytoplasm and fine vacuolization (lace-like aspect) were observed on liver sections, possibly due to a reduction on glycogen in 50% (3/6 cows) and congestion in 16.6% (1/6 cows) of the investigated animals (Figure 6). The cows used in this study went through food deprivation during the way to the slaughterhouse, approximately 12 hours, which is possibly associated with decreased levels of liver glycogen (24, 33), these animals submitted to transport stress show high levels of plasma glucose due to liver glycogen depletion (1) (Figure 7).

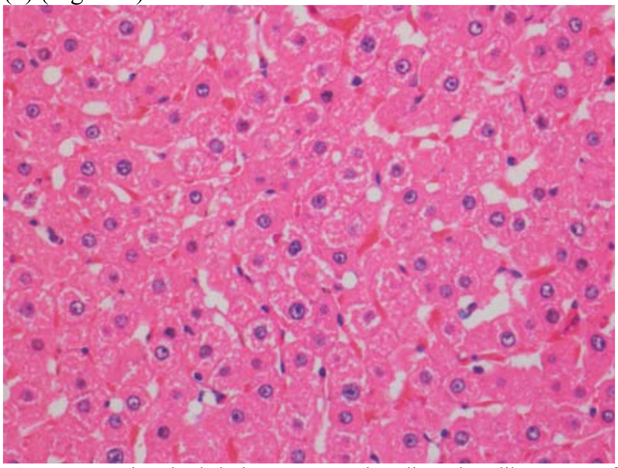

Figure 6. Bovine dead during transportation, liver, lace-like aspect of hepatocytes cytoplasm due to glycogen depletion, and congestion. HE, 40X.

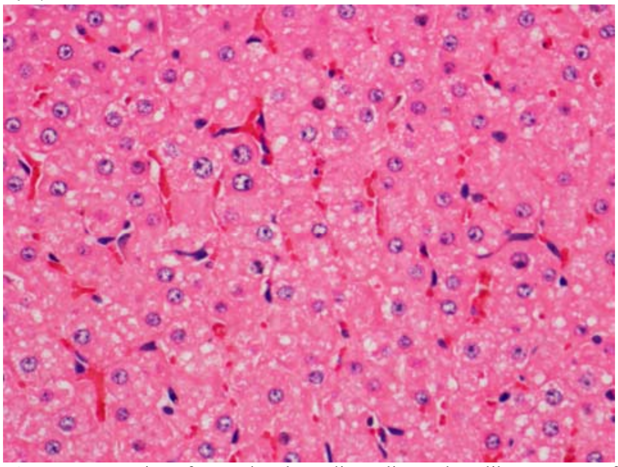

Figure 7. Bovine from slaughter line, liver, lace-like aspect of hepatocytes cytoplasm due to glycogen depletion, and congestion. HE, 40X.

Dehydrated animals may manifest circulatory failure (36) and a decreased blood flow through hepatic veins may cause congestion (18, 33). Some cases may have implications on liver venous circulation because of lung emphysema, resulting in congestion (18). The causes of liver congestion on EG animals may be related to dehydration, what is confirmed by elevations in total protein, hyperalbuminemia and high levels of creatinine on these animals, as well as by the lung emphysema that was verified in all animals with congested liver, what may have favored such tissue alteration.

According with the results obtained from gross and histological evaluations, one can infer that the most significant changes (lung, liver, and kidney congestion; and lung edema and emphysema) underlie hypovolemic shock. The loss of circulatory homeostasis that can emerge with a reduction of circulating blood volume caused by hemorrhage or loss of fluids from dehydration, characterizes hypovolemic shock (27). The extended subcutaneous and muscle hemorrhages associated with a probable state of dehydration could have acted as factors on the establishment of hypovolemic shock and tissue low perfusion. A series of chained events resulted in EG cows death. Among them, we can point out initially the stress due to a long time of transportation (overloaded trucks, social reorganization, high temperatures, noises, curves, and water and food deprivation), falls and extended bruises, and dehydration, crucial factors on the establishment of irreversible hypovolemic shock and death (Figure 8).

Figure 8. Bovine dead inside the truck during transportation showing dark marks of treading by other animals.

When the percentage of fibers degeneration between CG and EG were compared, a significant difference was found (P=0.0001), the higher mean of degenerated fibers being observed on EG animals (Figures 9 and 10). The individual comparison of muscles between groups (EG intercostal x CG intercostal; EG psoas major x CG psoas major; EG abdominal internal oblique x CG abdominal internal oblique; EG semitendinosus x CG semitendinosus) revealed a significant difference and the higher means of fiber degeneration were identified on EG animals. On the comparison between means of percentage of degenerate fibers of EG muscles, semitendinosus muscle differed from all others, with lower values. No difference was detected on CG (Table 1). Although intercostal muscle did not differ significantly from psoas major and abdominal internal oblique, it was the one that presented higher amounts of degenerate muscle fibers. The most frequently reported lesions during transport are bruises and lacerations, mainly on thoracic and abdominal regions (25), coinciding with the places of bruises observed in this research.

During the necropsy, extensive subcutaneous and muscle hemorrhages were found, mainly on the lateral areas of the body, due to bruises caused by treading during transportation. As a consequence, hemorrhages and vasculitis may lead to muscle ischemia and anoxia, and the fall on blood supply causes myofibrillar alterations, since muscle fibers are extremely sensitive to lack of oxygen. Ischemia may occur due to prolonged decubitus as well, causing blood vessels to compress and thus decrease blood flow, leading to myofibrillar degeneration (37), as the observed in the present study.

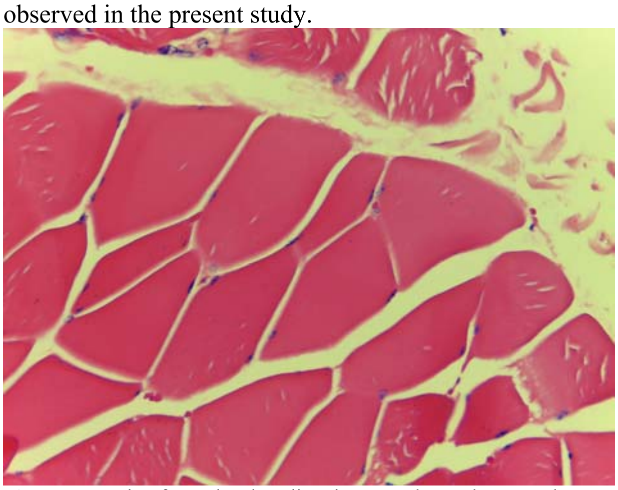

Figure 9. Bovine from slaughter line, intercostal muscle, normal aspect of fibers. HE, 40X.

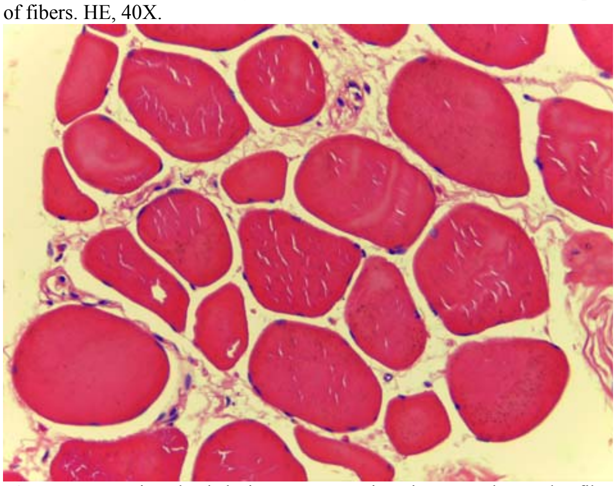

Figure 10. Bovine dead during transportation, intercostal muscle, fiber degeneration showing discoloration and round profiles. HE, 40X.

Muscle pH and Glycogen Analysis

The results regarding muscle pH and glycogen are described on Table 1. There was significant difference on pH values between EG and CG (P=0.0014), the highest mean being observed on EG. When the muscles were individually compared between groups, only psoas major was significantly different (P=0.0370) with the highest mean on EG compared with CG, while muscle glycogen measurement was similar between groups (P=0.4908).

Muscular glycogen concentration varies according to species, nutritional status, type of muscle fiber, stress previously to slaughter (15), distance crossed and time of transportation, time of remaining on waiting corrals (12) and gender (32). Muscle pH is associated with lactic acid synthesis from glycolysis (30) and deposition of lactic acid leads to a reduction on pH after death, which is not uniform among animals from the same species (29). Transport stress may cause postmortem biochemical changes on muscles from glycogen depletion and consequently decrease of muscle pH (3). Nevertheless, such change was not observed in the present study, once EG had a glycogen general mean of 19.54 µmol/g and pH of 6.16. The present study found muscle glycogen concentrations lower than the values found in resting cattle, which is 45 µmol/g (4), possibly by the action of transport stress and food restriction, promoting the consumption and thus reduction on the stored glycogen, although not enough to cause a reduction on EG pH. At early postmortem, ATP used on muscle metabolism originates from phosphocreatine breakdown and only after its depletion, glycogen reserves are degraded, that could be one of the causes of non-acidifying muscle pH observed on EG (2). Besides, the glycolysis occurs slowly in cattle and pH, which soon after death is 7.0, will suffer an initial decrease only after 5 hours (31).

Table1. Effect of long distances transportation of cows on muscle degeneration, pH, and glycogen values between animals dead during transport (EG) and animals from the slaughter line (CG).

	Groups	Intercostal	Psoas Major	Abdominal Internal Oblique	Semitendinosus	Means
Degenerate fibers (%)	EG	35.26 Aa	31.67 Aa	31.26 Aa	16.33 Ab	28.21A
	CG	7.40 Ba	2.72 Ba	3.20 Ba	2.72 Ba	3.89B
	MPM*	21.33 a	17.20 ab	17.23 ab	9.52 b	16.05
pH	EG	6.17 Aa	6.16 Aa	6.17 Aa	6.08 Aa	6.14 A
	CG	5.80 Aa	5.54 Ba	5.70 Aa	5.53 Aa	5.65 B
	MPM*	5.99 a	5.86 a	5.92 a	5.81 a	5.89
Glycogen (µmol/g)	EG	20.21 Aa	18.69 Aa	19.44 Aa	19.63 Aa	19.53 A
	CG	20.22 Aa	20.31 Aa	16.92 Aa	17.05 Aa	18.62 A
	MPM*	20.21 a	19.58 a	17.92 a	18.23 a	19.04

Means followed by minuscule letters on different lines and capital letters on different columns differ 5% probability of type 1 error by Student's t-test; *Mean per muscle

Another relevant fact is that rigor mortis, a muscle alteration characterized by ATP and glycogen depletion that begins after 1 to 6 hours after death (24), was not observed during necropsy. Its absence may explain those increased values of pH on EG. It is important to emphasize that, due to early death of EG animals, muscle pH measurements were performed before conversion of muscle into meat, also contributing for pH raise. Lower values of pH were observed on CG animals, possibly because of their maintenance in waiting corrals under hydric diet for 12 hours and, during this period, there was a slow consumption of glycogen, with pH reduction. Since muscle collections on these animals were performed immediately after slaughter, there was not enough time to convert muscle into meat. Both EG and CG did not have significant depletion of muscle glycogen storages with reduction of pH, expected to be caused by stress, distance, and transport time**.**

Liver Glycogen Analysis

Liver glycogen was similar between groups (P=0.8618) (Table 2). Aspects related with liver carbohydrates and nutritional factors influence liver glycogen concentrations (31). Both EG, with values of 58.98 µmol/g, and CG (57.06 µmol/g) showed lower levels of glycogen than the results found on studies performed previously, on the effects of supplementation in dairy cows, which varied from 86.8 to 168.4 µmol/g (8). Such fact emphasizes the effect of food restriction and transport stress on lower concentrations of liver glycogen. An early death, still during the transport, occurred on EG animals, while CG animals traveled for a short period, in addition of being maintained under resting conditions before the slaughter. This proves that glycogen consumption was accelerated on EG if compared to CG, although no significant difference was found between groups.

Table2. Effect of long distances transportation of cows on liver glycogen concentrations between animals dead during transport (EG) and animals from the slaughter line (CG).

Variable	Groups	
	EG	CG
Glycogen (µmol/g)	58.97	57.05

Probability of type 1 error by F-test.

Conclusion

The results suggest that some animals, when transported through long distances, are submitted to intense stress, as well as water and food deprivation, leading to homeostatic changes, thus affecting glycogen concentrations and muscle pH. Bruises cause myofibrillar degeneration and hemorrhages that, in association with fluid loss from dehydration, determine a picture of irreversible hypovolemic shock.

Acknowledgements

The authors are thankful to the Fundação de Amparo à Pesquisa do Tocantins (FAPT), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Universidade Federal do Tocnatins (UFT) for the fellowship conceded respectively to Burns LV, Silva AK, and Monteiro KB.

References

1. AVERÓS X., MARTIN S., RIU M., SERRATOSA J., GOSÁLVES LF. Stress response of extensively reared Young bulls being transported to growing-finishing farms under Spanish summer commercial conditions. **Liv. Sci.,** 2008, 119, 174-182.
2. BATE-SMITH EC., BENDALL JR. Factors determining the time course of rigor mortis. **J. Physiol.,** 1949, 110, 47-65.
3. BELTRÁN JA., JAIME I., SANTOLARIA P., SANUDO C., ALBERTI P., RONCALÉS P. Effect of stress-induced high post-mortem pH on protease activity and tenderness of beef. **Meat Sci.,** 1997, 45, 201-7.
4. BENDALL JR. Post-mortem changes in muscle, In: The structure and function of muscle, ed. BOURNE GH. New York: Academic Press. 3v, 227-274, 1965.
5. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **RIISPOA (Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal)**. Brasília: Ministério da Agricultura, Pecuária e Abastecimento, 1950. (Aprovado pelo decreto número 30691 de 29.03.52, alterado pelo decreto no 1.255, de 25.06.62).
6. CAVE JG., CALLINAN APL., WOONTON WK. Mortalities in bobby calves associated with long distance transport. **Aust. Vet. J.,** 2005, 83, 82-84.
7. CONNELL, J. International transport of farm animals intended for slaughter. **Commission of the European Communities,** 1984, Report EUR 9556, 67.
8. DAMGAARD BM., WEISBJERG MR., LARSEN T. Priming the cow for lactation by rapeseed supplementation in the dry period. **J. Dairy Sci.,** 2013, 96, 3652-61.
9. DUBOIS M., GILLES KA., HAMILTON JK., REBERS PA., SMITH F. Colorimetric method for determination of sugars and related substances. **Anal. Chem.,** 1956, 28, 350-6.
10. EARLEY B., MURRAY M., PRENDIVILLE., PINTADO B., BORQUE C., CANALI E. The effect of transport by road and sea on physiology, immunity and behaviour of beef cattle. **Res. Vet. Sci.,** 2012, 92, 531-41.
11. FERGUSON DM., WARNER RD. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? **Meat Sci.,** 2008, 80, 112-9.
12. GALLO C. Carnes de corte oscuro en bovinos. **Revista Vetermas.,** 2003, 2, 16-21.
13. GELBERG HB. Sistema digestório, In: Bases da Patologia em Veterinária, ed. McGAVIN MD., ZACHARY JF, 4th ed. Rio de Janeiro: Elsevier, 2009, 301-92.
14. GREGORY NG. Preslaughter handling, stunning and slaughter. **Meat Sci.,** 1994, 36, 45-56.
15. HARGREAVES AB., BARRALES LV., BARRALES DZ., RIVEROS JLF., PEÑA IR. Glycogen determination in bovine muscle: a proposal for rapid determination. **Chil. J. Agr. Res.,** 2009, 69, 366-72.
16. HENNING, P. A. Transportation of animals by road for slaughter in South Africa. In: **Livestock environment IV.** Coventry. Proceedings of 4th International Symposium on Livestock Environment. Coventry: American Society of Agricultural Engineers, 1993, 536-41.
17. IRWIN MR., GENTLEMAN WR. Transportation of cattle in a rail car containing feed and water. **South Western Veterinarian.,** 1978, 31, 205-8.
18. JONES TC., HUNT RD., KING NW. Sistema digestivo, In: Patologia veterinária, ed. JONES TC., HUNT RD., KING NW. 6th ed. Barueri: Manole, 2000, 1063-130.
19. KING NW., ALROY J. Deposições intracelulares e extracelulares; degenerações, In: Patologia veterinária, ed. JONES TC., HUNT RD., KING NW, 6th ed Barueri: Manole, 2000, 27-62.
20. LÓPEZ A. Sistema respiratório. In: Bases da Patologia em Veterinária, ed. McGAVIN MD., ZACHARY JF, 4th ed. . Rio de Janeiro: Elsevier, 2009, 463-558.
21. LUCENA RB., PIEREZAN F., KOMMERS GD., IRIGOYEN LF., FIGHERA RA., BARROS CSL. Doenças de bovinos no Sul do Brasil: 6.706 casos. **Pesq. Vet. Bras.,** 2010, 30, 428-34.
22. MALENA M., VOSLÁŘOVÁ E., KOZÁK A., BĚLOBRÁDEK P., BEDÁŇOVÁ I., STEINHAUSER L., VEČEREK V. Comparison of mortality rates in different categories of pigs and cattle during transport for slaughter. **Acta Vet. Brno.,** 2007, 72, 109-16.
23. MAXIE MG., NEWMAN SJ. Urinary system, In: Pathology of domestic animals, ed. JUBB KVE, PALMER NC, KENNEDY PC, 5th ed., Filadelfia: Saunders Elsevier, 2007, v 2, 426-521.
24. McGAVIN MD., ZACHARY JF. Respostas celulares e teciduais à lesão, In: Bases da Patologia em Veterinária, ed. McGAVIN MD., ZACHARY JF, 4th ed. Rio de Janeiro: Elsevier, 2009, 3-62.
25. MINKA NS., AYO JO. Physiological responses of food animals to road transportation stress. **Afr. J. Biotechnol.,** 2009, 8, 7415-27.
26. MORMEDE P., SOISSONS J., BLUTHE RM., RAOULT J., LEGARFF G., LEVIEUX D., DANTZER R. Effect of transportation on blood serum composition, disease incidence and production traits in young calves. Influence of the journey duration. **Ann. Rech. Vet.,** 1982, 13, 369-384.
27. MOSIER DA. Desordens vasculares e trombose, In: Bases da Patologia em Veterinária, ed. McGAVIN MD., ZACHARY JF, 4th ed. Rio de Janeiro: Elsevier, 2009, 63-100.
28. NEWMAN SJ., CONFER AW., PANCIERA RJ. Sistema urinário, In: Bases da Patologia em Veterinária, ed. McGAVIN MD., ZACHARY JF, 4th ed. Rio de Janeiro: Elsevier, 2009, 613-92.
29. PARDI MC., SANTOS IF., SOUZA ER., PARDI HS. Fundamentos da ciência da carne, In: Ciência, higiene e tecnologia da carne, ed. PARDI MC., SANTOS IF., SOUZA ER., PARDI HS, 2 ed. Goiânia: Editora da UFG, 2005, 39-134,
30. RAMOS EM., GOMIDE LAM. Análise de metabólitos, In: Avaliação da qualidade de carnes: fundamentos e metodologia, ed. RAMOS EM., GOMIDE LAM. Viçosa: Editora da UFV, 2007, 89-150.
31. ROÇA, R.O. Modificações pós-morte da carne. Acessado em <http://www.fca.unesp.br>, fevereiro de 2014.
32. SCANGA JA., BELK KE., TATUM JD., GRANDIN T., SMITH GC. Factors contributing to the incidence of dark cutting beef. **J. Anim. Sci.,** 1998, 76, 2040-7.
33. STALKER MJ., HAYES MA. Liver and biliary system. In: Pathology of domestic animals, ed. JUBB KVE, PALMER NC, KENNEDY PC, 5th ed., Filadelfia: Saunders Elsevier, 2007, v 2, 298-387.
34. SWANSON JCJ., MORROW-TESCH J. Cattle transport: Historical, research, and future perspectives. **J. Anim. Sci.,** 2001,79, 102-9.
35. TARRANT PV., KENNY FJ,. HARRINGTON D. The effect of stocking density during 4 hours transport to slaughter on behaviour, blood constituents and carcass bruising in Friesian steers. **Meat Sci.**, 1988, 24, 209-22.
36. THOMSON RG. Distúrbios circulatórios, In: Patologia geral veterinária, ed. THOMSON RG. Rio de Janeiro: Guanabara Koogan, 1983, 90-143.
37. VALENTINE BA., McGAVIN MD. Músculo esquelético, In: Bases da Patologia em Veterinária, ed. McGAVIN MD., ZACHARY JF, 4th ed. Rio de Janeiro: Elsevier, 2009, 973-1040.
38. VALLI VEO. Hematopoietic system, In: Pathology of domestic animals, ed. JUBB KVE, PALMER NC, KENNEDY PC, 5th ed. Filadelfia: Saunders Elsevier, 2007, v 2, 109-323.
39. VEČEREK V., MALENA M., MALENA M jr., VOSLÁROVÁ E., CHLOUPEK P. The impact of the transport distance and season on losses of fattened pigs during transport to the slaughterhouse in the Czech Republic in the period from 1997 to 2004. **Vet. Med-Czech.,** 2006, 51, 21-8.

