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RESEARCH ARTICLE

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The effect of transport density and gender on stress indicators and carcass and meat quality in pigs

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Abstract

A total of 168 finishing pigs were used to investigate the effects of gender (barrows and gilts) and transport densities for slaughter (236, 251, and 275 kg/m²) on stress indicators and carcass and pork quality. The animals transported at 251 kg/m² (T251) presented cortisol values below those at 236 kg/m² (T236), but no different from those at 275 kg/m² (T275). The lactate dehydrogenase (LDH) values in pigs transported at T236 were the lowest. The blood components did not differ between T236 and T275. The pH values at 45 min (pH_{45}) and at 24 h (pH_{24}) *postmortem* were higher for pigs subjected to T236. However, the pH_{45} was higher at T251 than at T275, but pH_{24} was lower at T251 than at T275. The lightness values in the muscles of the pigs transported at T236 and T251 were higher than those at T275. Lower drip loss values were observed in the muscle of animals at T251. Carcasses of pigs at T236 contained more 1–5 cm lesions while those at T275 contained more 5–10 cmlesions in sections of loin. No significant effects of gender were found on the stress indicators, blood components, pH_{45}, pH_{24}, color, drip loss or carcass lesions in general. These results indicate that the pre-slaughter transport of pigs at densities of 251 kg/m² generates less physiological damage and smaller losses on carcass and pork quality irrespective of gender.

Additional keywords: slaughter; color; cortisol; pH; skin lesions.

Abbreviations used: a* (red/green); b* (yellow/blue); CK (creatine kinase); EOS (eosinophils); HCT (hematocrit); HGB (hemoglobin); L* (lightness); LDH (lactate dehydrogenase); LT (*longissimus thoracis*); LYM (lymphocytes); MON (monocytes); NEUT (segmented neutrophils); pH_{24} (pH at 24 h *postmortem*); pH_{45} (pH at 45 min *postmortem*); PLT (platelet); PSE (pale, soft and exudative); RBC (red blood cells); WBC (white blood cells).

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Introduction

Aspects of animal welfare have directly influenced the practices adopted in pig farming, especially those related to pre-slaughter handling procedures. The animal transport, an important part of the process, is a complex stressing factor that involves fluctuations of temperature and density, withdrawal of food and water, mixing with strangers, movement, noises, unknown smells, and interaction with humans. Such factors lead to behavioral and physiological responses that can contribute to reducing animal welfare and the meat quality (Araújo, 2009; Ochove *et al*., 2010; Bryer *et al*., 2011; Ludtke *et al*., 2012).

Research has demonstrated that the animal density during transport is a key factor that can lead to negative effects on mortality rate, welfare, blood components (Sutherland *et al*., 2009; 2012) and meat quality (Chai *et al*., 2010; Ludtke*et al*., 2010). Transport density has also shown influence on physiological parameters during transport such as elevated levels of LDH and creatine kinase (CK), as observed by Chai *et al*. (2010).

The genders have shown different responses to stress during transport, as gilts display lower loss by dehydration, fatigue and fewer skin lesions (Mota-Rojas *et al*., 2006; 2012) and greater lactate values and a lower percentage of hematocrit (HCT) compared with barrows (Herrera *et al*., 2010).

Pereira & Corassa (2014) observed that the transport of pigs for slaughter in Mato Grosso State (Brazil), is on average distance of 205 km and 26.53% of the animals are transported between 200 and 2,000 km. However, given that production of pork in Brazil reached 3.43 million tons in 2013 (ABPA, 2014), the pre-slaughter handling procedures are estimated to have a great impact on the transport losses in the country, but these losses are not yet well known.

No consensus has been reached for recommendations of transport density of pigs for slaughter. In the United States, the recommended area for the transport of pigs is ~ 0.33 m^2/animal (National Pork Board, 2008). The European Union, however, regulates that pigs near 100 kg should be transported at no more than 235 kg/m^2 or 0.42 m^2 per pig (EC, 2005), but allows the area to be adjusted according to the meteorological conditions, travel time, or genetics, size, and physical condition of the animals. The Brazilian legislation does not specify the density for the transport of pigs.

Thus, the objective of the present study was to evaluate the effect of gender and transport density of pigs during their transport to the abattoir on indicators of stress, blood components and carcass and meat quality.

Material and methods

Animal husbandry and diets

The experiment was conducted from July to September 2013, in the north of Mato Grosso State, Brazil (coordinates 11°52'23" S, 55°29'54" W and 380 m altitude). The climate in the region is characterized as Aw and B2wA'a' according to the Köppen & Thornthwaite classifications, having an average temperature of 24.70ºC (Souza *et al*., 2013).

One hundred and ninety two pigs of the Agroceres PIC® strain were fed a diet containing 5 mg/kg ractopamine during the finishing phase until they had an average body weight of 115.54 ± 6.03 kg and they were then feed-deprived for 12h. All animals originated from one farm located 290 km from the experimental area, with a travel time of 5.21 ± 0.11 h to the meat packing plant, totaling 17 h without feed intake.

The treatments comprised two genders (barrows and gilts) and three densities of pre-slaughter transport (0.518, 0.453 or 0.403 m^2/animal which corresponded to 236, 251, or 275 kg/m^2, respectively). The densities were adjusted in each shipment without changing the structure of the truck, only altering the number of animals transported in the compartments, which were seven, eight, and nine animals per compartment, respectively. Only one density was used per shipment, with pigs distributed in the four compartments of the front part of the truck body, barrows being in the upper right and lower left compartments, and gilts in the upper left and lower right compartments.

Upon arrival at the abattoir, all pigs were weighed and housed in holding pens at the stocking density of up to 0.77 m^2/animal, according to the current law. The number of animals in the holding pens subsequently slaughtered was standardized based on the lowest density, which corresponded to seven pigs per compartment. Upon unloading, one animal was randomly selected from each compartment of T251 and two animals from T275 and divided into other holding pens; their data were not recorded. All activities before slaughter were performed in the same manner, according to the routine procedures established by the abattoir. The distribution of animals is shown in Table 1.

Table 1. Distribution of pigs during transport, in holding pens and at slaughter according to the treatments

Transport density (kg/m^2)	Gender	During transport			Holding pens and slaughter	
		Compartments	Trips	Total	Pens	Total
236	Barrows	7	14	28	7	28
	Gilts	7	14	28	7	28
251	Barrows	8	16	32	7	28
	Gilts	8	16	32	7	28
275	Barrows	9	18	36	7	28
	Gilts	9	18	36	7	28
	Total			192		168

All transport events were performed by the same driver in only one vehicle, a Scania P310 truck –2.57 × 11.86 m, 4-axle, 30,000 kg capacity, aluminum body, two floors, and 12 2.97 × 1.22 m compartments (six on each floor).

The animal procedures adopted were reviewed by the Animal Experimentation Ethics Committee of Universidade Federal do Mato Grosso, protocol no. 23108.700436/14-7.

Slaughtering and sampling

The animals boarded the truck through a wooden chute with closed sides, with a ramp angle less than 20°, grooveless floor, and in a good state of preservation. The animals were led to the vehicle in groups of up to five using a handling board and non-aversive behaviors practiced by the boarding team, which consisted of six people. The shipments and unloading events occurred at similar times of the day, during the morning.

Unloading in the abattoirwas carried out through a masonry chute with closed sides, ramp with an angle less than 20°, grooved floor, without radiation- or rain-shielding structure, 4.07 m long and 0.90 m wide without sharp curves and in a good state of preservation.

After the application of water spray for 30 min and a rest period of 3 h, the pigs were handled into a 35 m long corridor to the stunning pen. Subsequently, electrodes were placed in the region of the temples and heart, and the animals were stunned for 3 sec with an electric discharge of 340 V, a frequency of 50 Hz, and an electric current of 1.0 A.

Blood collection and analysis of stress indicators

Blood measurements were carried out in order to assess pre-slaughter stress. Blood samples were collected at exsanguination and quickly separated into two tubes that were kept refrigerated until processed immediately on arrival at the laboratory. One tube (8 mL) containing serum clot activator with micronized silica particles was placed on ice immediately and subsequently centrifuged at 1300×g at 4°C for 15 min to obtain plasma. Plasma samples were stored at -20°C for subsequent analysis of enzymatic activity of CK, LDH and concentration of cortisol. The CK and LDH activity was determined by commercial kits (Labtest®) in a biochemical analyzing machine (Celm, model E225-D®), while plasma cortisol was analyzed by chemiluminescence (Ochove*et al*., 2010).

The other tube (4 mL) containing ethylenediaminetetraacetic acid (EDTA) was used to measure hematological parameters including white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), HCT and platelets (PLT); it was analyzed immediately with an automatic counter (BC 2800 vet®), according to the methodology described by Chai *et al*. (2010). For each sample a blood smear was prepared on a pre-cleaned glass slide for differential WBC counting. Blood smears were air-dried and stained using the panoptic staining method, and 100 cells (segmented neutrophils (NEUT), lymphocytes (LYM), eosinophils (EOS) and monocytes (MON)) were counted under microscope magnification.

Carcass and meat parameters

A total of 28 carcasses per treatment chosen randomly were eviscerated and sawn; they were transferred to a cold room at 4ºC for the physicochemical and visual assessments. The other procedures of the slaughter line were performed according to Normative Instruction 711 from 1 November, 1995 (BRASIL, 1995).

The pH was measured at 45 min (pH_{45}) and at 24 h (pH_{24}) *postmortem* using a portable pH meter AK86 (CE RS232, Akso®) with digital identification system, temperature compensation sensor and a proper glass electrode (sc18). The tissue used to measure pH was taken from the LT muscle, located between the 13th and 14th intercostal spaces, perpendicular to the midline of the left half-carcass, with an average depth of 3.5 cm.

In addition, at 24 h *postmortem*, a sample of 100 g of the LT muscle was taken from each selected carcass, from between the 6th and 7th thoracic vertebrae, weighed on a semi-analytical scale, and used to analyze color and drip loss. Meat color parameters were analyzed according to the CIELAB system (colorimeter Minolta DL65, CR400) by reading the light reflectance in three dimensions as lightness (L*), red/green (a*) and yellow/blue (b*) at the Food Technology Laboratory of the Federal University of Mato Grosso, Sinop Campus, after a standard period of exposure to atmospheric air for 30 min. Drip losses were evaluated by suspension of the samples in inflated plastic bags under the action of gravity for 48 h at 4ºC, according to the method described by Honikel (1998).

The incidence of lesions on the skin was determined in the shoulder, loin, and ham on the left half-carcass of the pigs 24 h *postmortem* in the cold chamber at 4 ºC, by visual assessment, counting the number of lesions of 1–5, 5–10, and 10–15 cm and the presence of non-linear abrasions. Only recent lesions were considered in this evaluation (Araújo, 2012).

Statistical analysis

The experiment was conducted in a completely randomized design, in a 3 × 2 factorial arrangement, with 28 replicates per treatment, considering one animal as the experimental unit. The variables related to stress indicators, blood components, and meat quality traits were subjected to variance analysis (SAS, 2003) and treatment means were compared by Tukey's test, considering 5% probability for type I error (α=0.05). The LDH and CK variables were subjected to logarithmic transformation. To evaluate the lesions, a non-parametric analysis was performed with Kruskal-Wallis (α=0.05).

Results

Stress indicators

No interactions between gender and transport density were observed for stress indicators. The pigs subjected to different pre-slaughter transport densities did not show differences for the enzymatic activity of CK (Table 2). The LDH concentration in the pigs transported at T236 was higher than at the other densities ($p<0.01$), while at T251 and T275 this did not differ. The pigs transported at T251 presented cortisol values below that shown by those at T236 ($p=0.03$), but no different from T275. None of the physiological indicators of stress in the present study differed between the two highest densities. No significant differences were found in these variables between genders.

Blood components

No interactions between gender and transport density were observed for any of the blood components studied. The parameters WBC, RBC, HGB, HCT and NEUT (Table 3) differed ($p\leq0.05$) with transport density. In general, the blood components did not differ between T236 and T275 at pre-slaughter. The mean values of WBC and NEUT in animal transports at T251 were higher than those at T236. The RBC, HGB and HCT values in pigs at T236 were higher than those at T251. Different transport density of pre-slaughter pigs did not influence the PLT, MON, LYM and EOS values. The blood components were not influenced by gender, except the NEUT which were higher in barrows than in gilts.

Table 2. Mean values (± standard deviation) of stress indicators in pigs according to gender and transport density

	Transport density (kg/m²)			Gender		Pd	Pg	Pd×g
	236	251	275	Barrows	Gilts			
Creatine kinase (IU/L)	7.9±0.76	8.0±0.62	8.0±0.47	7.9±0.16	8.0±0.03	0.36	0.46	0.43
Lactate dehydrogenase (IU/L)	7.4±0.21[a]	7.2±0.19[b]	7.2±0.28[b]	7.3±0.16	7.3±0.11	<0.01	0.92	0.46
Cortisol (µg/mL)	6.3±1.65[a]	5.6±1.38[b]	6.2±1.64[ab]	6.1±0.63	6.0±0.29	0.03	0.70	0.11

[1] Pd: significance of density; Pg: significance of gender; Pd×g: significance of density × gender. Different superscripts within the same row indicate significant differences by Tukey's test ($p<0.05$).

Table 3. Mean values (± standard deviation) of blood components in pigs according to gender and transport density

Blood components	Transport density (kg/m²)			Gender		Pd	Pg	Pd×g
	236	251	275	Barrows	Gilts			
WBC (10⁹/L)	20.0±3.24[b]	22.0±4.16[a]	20.6±3.76[ab]	20.8±3.26	20.7±3.93	0.03	0.70	0.87
RBC (10¹²/L)	8.8±0.86[a]	8.3±0.78[b]	8.6±0.58[ab]	8.7±0.82	8.5±0.70	0.02	0.11	0.12
HGB (g/dL)	14.3±1.48[a]	13.5±1.12[b]	14.0±1.20[ab]	14.0±1.37	14.0±1.27	0.02	0.97	0.62
HCT (%)	51.8±5.19[a]	48.3±3.85[b]	51.5±3.78[a]	50.9±4.78	50.4±4.38	<0.01	0.76	0.61
PLT (10⁹/L)	6.1±0.24	6.1±0.21	6.0±0.34	6.1±0.28	6.1±0.27	0.37	0.97	0.33
MON (10⁹/L)	7.1±0.86	6.6±1.37	6.9±0.87	6.8±0.99	7.0±1.07	0.09	0.07	0.77
LYM (10⁹/L)	9.4±0.23	9.5±0.28	9.3±0.86	9.4±0.24	9.4±0.79	0.38	0.60	0.62
EOS (10⁹/L)	5.8±1.38	6.0±1.40	6.0±1.10	5.8±1.32	6.0±1.26	0.54	0.27	0.22
NEUT (10⁹/L)	8.2±1.08[b]	8.6±0.55[a]	8.4±0.62[ab]	8.6±0.42[a]	8.2±1.01[b]	0.02	<0.01	0.36

WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; PLT: platelets; MON: monocytes; LYM: lymphocytes; EOS: eosinophils; NEUT: segmented neutrophils. Pd: significance of density; Pg: significance of gender; Pd×g: significance of density × gender.

Meat quality parameters

No interactions between gender and transport density were observed for pork quality parameters. The mean values of pH_{45} and pH_{24}, L*, a*, b*, and drip loss of the LT muscle were affected by density, but there were no differences between barrows and gilts (Table 4).

The pH_{45} and pH_{24} values of the LT muscle were higher ($p \leq 0.01$) for pigs subjected to T236 than for pigs subjected to other densities. However, pH_{45} in the LT muscle was higher at T251 than T275, but pH_{24} was lower atT251.

In general, the color parameters showed lower values in the LT muscle of pre-slaughter pigs transported at T275 ($p>0.01$). The L* values in the LT muscle of the pigs transported at T236 and T251 were higher ($p \leq 0.05$) than those at T275. For the a* parameter the higher values were observed at T251. However, b* values decreased as the density increased. The drip loss of the LT muscle showed no difference ($p>0.05$) between T236 and T275 but both were higher than the intermediate density ($p<0.01$).

Carcass quality parameters

No interactions between gender and transport density were observed for any of the carcass quality parameters studied. A large number of lesions of 1–5 cm and 5–10 cm extent were counted on the shoulder, loin and ham regions in the left half-carcass 24 h *postmortem*, observed independently of transport density or gender (Table 5).

In general, carcass quality parameters were not affected by gender, although the number of 1–5 cm lesions in shoulders of the T275 and non-linear abrasions in loins of 251 kg/m² were higher ($p<0.01$) for barrows than for gilts. Moreover, lesions of 5–10 cm in shoulders of pigs transported at 251 kg/m² were higher ($p<0.05$) for gilts than for barrows.

More lesions of small extent (1–5 cm) were observed in the loins of pigs of the 236 kg/m² treatment independent of gender. Barrows transported at 275 kg/m² showed a lower number of 1–5 cm lesions in ham than pigs of other treatments. However, carcasses of pigs of the 275 kg/m² treatment contained more 5–10 cm lesions on the loins of barrows and gilts and on the ham of barrows. Similarly, the loins of T275 gilts contained more 10–15 cm lesions in comparison to other densities.

The number of non-linear abrasions was higher in carcasses of pigs submitted to the intermediate density of pre-slaughter transport regardless of the location.

Discussion

Stress indicators

The CK enzyme is involved in the metabolic process of obtaining energy (Warriss *et al.*, 1998) and is commonly used to assess muscle damage (bruising) caused by vigorous physical exercise (Correa *et al.*, 2010). In general, the CK values were high compared to those of Araújo (2012) but within the normal levels (Kaneko *et al.*, 1997). The lack of effect on serum CK concentrations could indicate that the treatments did not produce muscle damage in pigs, but were a source of some distress with effects until before slaughter. Correa *et al*. (2010) also obtained higher values (11.80, 11.89, 11.90 IU/L, respectively) but without significant effect in the evaluation of compressed air, paddles and electric prods in loading.

The elevation of LDH in the pigs transported at T236 in comparison to other densities is related to physical stress or fatigue, with greater release of this enzyme into the bloodstream, characterizing muscle exhaustion,

Table 4. Mean values (± standard deviation) of pH at 45 min and at 24 h *postmortem*, color parameters, and drip loss in the *longissimus thoracis* muscle in pigs according to gender and transport density

	Transport density (kg/m²)			Gender		Pd	Pg	Pd×g
	236	251	275	Barrows	Gilts			
pH_{45}	6.6±0.18^{a}	6.5±0.20^{b}	6.4±0.21^{c}	6.5±0.20	6.5±0.24	<0.01	0.87	0.21
pH_{24}	5.5±0.21^{a}	5.1±0.51^{c}	5.3±0.33^{b}	5.3±0.43	5.4±0.38	<0.01	0.44	0.28
Color L*	50.1±2.75^{a}	51.8±2.34^{a}	48.7±2.11^{b}	49.9±2.78	50.5±2.61	<0.01	0.09	0.79
Color a*	7.0±1.22^{b}	8.0±1.33^{a}	7.1±1.44^{b}	7.5±1.47	7.3±1.33	<0.01	0.34	0.46
Color b*	2.6±0.84^{a}	1.5±0.69^{b}	1.0±0.69^{c}	1.6±1.01	1.8±1.01	<0.01	0.21	0.58
Drip loss (%)	6.5±1.52^{a}	5.1±1.81^{b}	6.2±1.75^{a}	6.2±1.74	5.7±1.82	<0.01	0.06	0.09

Pd: significance of density; Pg: significance of gender; Pd×g: significance of density × gender. Different superscripts within the same row indicate significant differences by Tukey's test ($p<0.05$).

Table 5. Mean number of lesions of different extent counted on the shoulder, loin and ham regions in the left half-carcass 24 h *postmortem* in pigs according to gender and transport density

Extent of lesions	Location	Gender	Transport density (kg/m^2)			Pd	Pg (T236)	Pg (T251)	Pg (T275)
			236	251	275				
1–5 cm	Shoulder	Barrows	2.4	2.7	3.3^{A}	0.56	0.95	0.98	0.03
		Gilts	2.8	2.9	1.6^{B}	0.20			
	Loin	Barrows	3.2^{a}	0.9^{b}	1.0^{b}	<0.01	0.92	0.53	0.41
		Gilts	3.2^{a}	0.7^{b}	0.8^{b}	<0.01			
	Ham	Barrows	2.6^{a}	1.6^{a}	0.4^{b}	<0.01	0.44	0.49	0.20
		Gilts	1.6	1.2	1.0	0.41			
5–10 cm	Shoulder	Barrows	1.7	0.8 B	0.4	0.06	0.33	0.04	0.30
		Gilts	0.9	2.0 A	0.8	0.16			
	Loin	Barrows	3.9^{ab}	1.4^{b}	4.5^{a}	0.02	0.11	0.28	0.42
		Gilts	2.6^{b}	1.5^{c}	5.3^{a}	<0.01			
	Ham	Barrows	0.6^{c}	0.8^{b}	2.2^{aA}	<0.01	0.23	0.22	<0.01
		Gilts	1.1	0.9	0.5^{B}	0.21			
10–15 cm	Shoulder	Barrows	0.2	0.0	0.7	0.22	0.98	0.17	0.90
		Gilts	0.2	0.1	0.3	0.84			
	Loin	Barrows	0.8	1.2	0.7	0.63	0.15	0.09	0.10
		Gilts	0.3^{b}	0.1^{b}	0.9^{a}	<0.01			
	Ham	Barrows	0.5^{ab}	0.8^{a}	0.3^{b}	0.04	0.07	0.95	0.19
		Gilts	0.5	0.3	0.8	0.17			
Non-linear abrasions	Shoulder	Barrows	0.1	0.8	0.0	0.05	0.09	0.83	0.33
		Gilts	0.0^{b}	0.6^{a}	0.0^{b}	<0.01			
	Loin	Barrows	0.0^{b}	3.4^{Aa}	0.2^{b}	<0.01	0.34	<0.01	0.17
		Gilts	0.0^{b}	0.6^{Ba}	0.0^{b}	<0.01			
	Ham	Barrows	0.0^{b}	1.2^{a}	0.5^{b}	<0.01	1.00	0.10	0.54
		Gilts	0.0^{b}	0.9^{a}	0.0^{b}	<0.01			

Pd: significance of density effect; Pg: significance of gender effect in 236, 251 or 275 kg/m² densities. Treatments followed by uppercase letters in the rows (gender) and lowercase letters in the columns (density) do not differ statistically by the Kruskal-Wallis test at 5% probability.

which causes the formation of large amounts of lactic acid, a result of intense degradation of the muscle glycogen. The data from the present study differ from those reported by Pérez *et al*. (2002) and Chai *et al*. (2010), who observed an increase in the lactate levels as the transport density was elevated.The elevation in the blood lactate levels at the lowest density may be related to impacts against the truck body, frequent changes of position, and greater occurrence of tired animals (Pilcher*et al*., 2011), due to the larger free space in the compartment.

Cortisol value differences can be explained by the fact that a lower density (236 kg/m²) creates a space within the compartment in which the animals would attempt to balance, and at a greater density (275 kg/m²) the movements would tend to be more restricted; both situations can bring about greater secretion of the cortisol hormone in response to the release of the adrenocorticotropic hormone by the hypophysis in situations of stress. In a similar study, Chai *et al*. (2010) showed no difference in cortisol concentrations of pigs transported at 230 and 275 kg/m²; however, animals transported at 325 kg/m² presented higher values of cortisol than at the intermediate density. Sutherland *et al*. (2009) and Bryer *et al*. (2011) found no difference in cortisol concentrations as an effect of density in pig transport, although as the latter recorded higher concentrations after the trip, it suggests that these pigs experienced stress during transport. The inconsistency of the data can be related to the influence of other factors such as transport time (Chai *et al*., 2010) and distance (Ochove *et al*., 2010) on cortisol production.

In this study the greater lactate and cortisol concentrations of pigs at different pre-slaughter transport densities seem to be indicators of fatigue resulting from the long-term effect of poor transport on the physiological state of pigs at slaughter, as observed at T236 and T275.

The stress indicators were not influenced by the gender; nevertheless, Averós *et al*. (2007) reported higher basal cortisol levels in barrows compared with gilts (3.68 ± 0.22 and 3.00 ± 0.21 µg/mL, respectively). In this same study, they observed that there was no difference in the CK and LDH levels. The absence of effect of gender on the studied parameters also contrasted with the studies of Pérez *et al*. (2002) and Piñeiro *et al*. (2007). These contradictions can be related to the specific conditions of this study, supposedly with stress level that can cover up the effect related to the gender, which can be seen from the high levels of stress indicators in comparison to those in the literature.

Blood components

The results of the analysis of blood components showed a polycythemia in animals at T236 and T275; in other words, an increase of the number of circulating RBC. This could occur due to the splenic contraction, as an alert response that is activated in situations of fear or excitement, which may have been promoted by elevated levels of cortisol in these animals (Table 2); releasing catecholamines, which can cause rapid glycogenolysis; breakdown of muscle glycogen; and increase in the concentration of lactate (Ludtke *et al*., 2010). The changes of those blood parameters were clearest at the intermediate transport density in comparison to lower transport density; therefore, T251 might have been more favorable than T236. Lowest values of RBC, HGB, and HCT were found in pigs transported at T251 when compared to those of T236, and the intermediate values of those at T275 leads us to speculate that pigs need plenty of oxygen-carrying capacity in the blood during periods of high energy demand in the high stress condition, since the HCT, the proportion of RBC in the blood, is altered when animals are transported (Chai *et al.,* 2010).

The elevated HCT values in the blood are linked to relative polycythemia, a result of reduction in the plasma level and hemoconcentration, caused by dehydration during transport as reviewed by Mota-Rojas *et al*. (2012). The presented values may imply that the loss of body water at T251 was reduced in comparison to the other treatments. Alterations in HCT, HGB and RBC count indicate a situation of adaptation or resistance, in which pigs, after being exposed to the stressful factors for some time, tend to release more hormones. This occurs by contraction of the spleen and release of a greater number of oxygenated RBC, which allowed the animals to increase their physical activity, as observed in the levels of LDH and cortisol at T236 and T275.

The occurrence of a high level of cortisol, reduction in the number of LYM, and increase in the number of NEUT suggests that the pigs were under stress, which was not the case in the present study, because the levels of LYM at the proposed densities remained within the normal range (Sutherland *et al*., 2009). Similarly, changes in the populations of WBC types in response to stressors, particularly the relative decrease in LYM compared with NEUT numbers, have been measured in studies relevant to animal welfare. Chai *et al*. (2010) reported changes in WBC trafficking and release from the bone marrow by elevated concentrations of glucocorticoids from the adrenal medulla during stress; however we did not find differences in this parameter in different transport times, but there was a tendency toward increasing the number of WBC with higher transport density. In opposition, no differences were found in WBC and LYM counts of gilts transported at equivalent 220 and 270 kg/m², but both values were lower compared with non-transported gilts by reporter of Bryer *et al*. (2011).

Thus, the elevation in the number of NEUT is explained by endocrine factors that are released in stress situations, in which there is modulation of the immune system, described by the occurrence of neutrophilia, lymphopenia and monocytosis, and then a redistribution of leukocytes between the blood and other compartments of the immune system (Bryer *et al*., 2011).

NEUT values observed in gilts can be related to the hormonal factor, since estrogen reduces the production of immunoregulatory factors, limiting the production of macrophages, granulocytes, fibroblasts and endothelial cells. Values lower than those obtained in this study were found by Sutherland *et al*. (2012) in the transport of gilts: $5.7 \cdot 10^9$/L in comparison to non-transported gilts, which showed $4.7 \cdot 10^9$/L. Opposite results to ours were described by Herrera *et al*. (2010) and Mota-Rojas *et al*. (2012), who observed that gilts were more susceptible to transport stress compared with barrows.

These blood components are sensitive indicators of physiological or physiopathological responses of animals to stress (Chai *et al*., 2010). Hence, associating the elevation in the concentrations of HGB, HCT and the number of RBC in the present study at T236 and T275 with the cortisol analysis, we can infer that there were stress factors in these treatments above the density of 251 kg/m². Therefore, the stress factors affected neither the normality range of the total leukocyte parameters nor the counts of LYM and NEUT, as they could be responses to transport as observed by Sutherland *et al*. (2012).

In terms of gender, the data obtained for RBC, HGB and HCT showed no significant differences (p>0.05). Pérez *et al*. (2002), however, observed mean values of 14.97 and 14.28 g/dL HGB for gilts and barrows, respectively, which were significantly different (p<0.05). Nevertheless, Herrera *et al*. (2010) observed no differences in the HCT levels between gilts and barrows, obtaining 29.51 ± 0.45 and 30.95 ± 0.44%, respectively. The mean values of total leukocytes varied from (20.01 ± 3.24)·10^9/L to (22.00 ± 4.16)·10^9/L, which is within the normal values for pigs, from 11·10^9/L to 22·10^9/L according to Pérez *et al.* (2002).

Meat quality parameters

Significant differences of pH_{45} and pH_{24} can be noted between the animals at different transport density, but they presented different trends. The present results produced a lower pH in pork as transport density increased. In general, early *postmortem* pH is mostly related to the level of muscle activity before slaughter. The muscle metabolic state pre-slaughter depends directly on the hormonal state of the organism (Ludtke *et al*., 2012), *i.e.* at the highest density (275 kg/m²) the animals showed a pH_{45} and cortisol level which may characterize a situation of discomfort for the animals in the truck, which stimulated the activity of stressor agents, thus having an effect on the meat quality. Greater pH_{45} values (6.22 ± 0.02) were recorded at the densities equivalent at 256 and 222 kg/m² in comparison to lower or higher densities, whose mean pH was 6.18 ± 0.02 (Perre *et al*., 2010).

The pH_{24} of the intermediate density showed the lowest value followed by values of T275 which in turn were lower than at T236. In this way the results seemed to indicate a low chance of production of pale, soft and exudative (PSE) pork when the density decreases from 275 to 236 kg/m². Previous studies of Warriss *et al*. (1998) and Chai *et al*. (2010) showed no effect of density on pH_{24}. A large drop in pH between initial and final measurements was observed in the animals at T251. A possible explanation for understanding the results of this study is that densities of 251 and 275 kg/m² characterized more stressful situations to animals causing depletion of the muscle glycogen, which interfered with the muscle metabolism rate *postmortem*, due to the requirement of ATP and production of lactic acid in the muscle, causing a drop in pH. This might be related to the lack of space for the pigs to rest, resulting in behavioral alterations during the transport. The pH_{24} values appear slightly lower than the normal range (5.6–6) in both groups.

These findings may be related to the transport time of this study, as reported by Pérez *et al*. (2002) and Chai *et al*. (2010), who concluded that pH_{24} was strongly increased by long transport time. The genetic background of the pigs under study also may be the source for these results. Indeed, Dalla Costa *et al*. (2007) showed that genotypes characterized by high muscle glycolytic potential (*i.e.* RN gene carriers) produce low pH pork regardless of the stress applied.

The L* values in the LT muscles of the pigs transported at T236 and T251 can be explained by the influence of the reduction in muscle pH promoting a correlation with the meat-color aspect, because drops in pH_{24} are commonly linked to increased L* and are indicative of PSE pork (Gajana *et al*., 2013). In our study we obtained results close to those found by Correa *et al*. (2010), who reported L* values varying from 49.83 to 50.32 with a transport density equivalent at 293 kg/m².

The L* value observed at T275 can be considered normal, as it is within the 42≤ L* ≤50 range, according to Ludtke *et al*. (2010). However, associating the decrease in the pH value with elevated levels of cortisol and LDH, it is understood that there were several stress factors during transport that did not allow the animals to lie down and rest for a short time. At the same proposed density, Gajana *et al*. (2013) observed a negative effect, with the occurrence of PSE pork.

Greater water losses at T236 and T275 characterize a situation of intense stress, verified by the high levels of cortisol, with greater denaturation of the sarcoplasmic and myofibrillar proteins during the conversion of muscle to meat. The water drip loss in the *postmortem* period probably occurs due to alterations in the interaction between myosin and actin; the reduction of the myofibrillar volume within the cells, related to the effect of changes in their electrostatic charges originating from the elevated internal concentration of lactate, which directs the free water to the intercellular space; and the reduction of solubility of the sarcoplasmic and myofibrillar proteins, due to the rapid drop in pH (Araújo, 2009). Thus, denaturation occurred at the lowest and highest densities, causing the water to lose its ability to bind to proteins and allow protein solubility.

Analyzing the data, it was found that none of the proposed densities provided red/pink, firm, and non-exudative meat, which is considered ideal, with a pH_{24} of 5.6 to 5.8, drip loss of 2 to 5%, and L* of 42–50 (Ludtke *et al*., 2010).

We did not observe differences between barrows and gilts in pork quality traits, in agreement with Pérez *et al*. (2002) and Robina *et al*. (2013).

Carcass quality parameters

Skin lesions may necessitate trimming, which results in downgrading of the commercial value of the carcass and represents a problem for the industry. Dalla Costa *et al*. (2009), in a study conducted in Brazil, reported that 53.7% of pigs already presented skin lesions at the farm, before loading. This proportion further increased through the marketing process from 80.7% (after loading) to 95.8% (in lairage at the slaughter plant). Therefore, stocking density in the transport vehicle is an important factor contributing to variation in skin damage of carcasses (Faucitano, 2001).

The higher frequency of short lesion length (1–5 cm) in animals transported at T236 can be related to an attempt to stabilize the animals within the carrying compartment, causing the animals to rely on each other and generate injuries by contact of the foreclaws of one animal on the back of another. However, the increased supply of space caused the animal to remain on the other for a very brief period of time, subjected to the destabilizing effects of the vehicle movements, generating small extension injuries. Also, the restlessness at the lowest density can be a result of agitation, struggles or fights among pigs in the same compartment (Ludtke *et al*., 2012). Thus, this type of lesion may be related to the fact that when there is a large amount of space in the vehicle, animals tend to move more due to vibration, the speed of the vehicle and the total load, in addition to the greater impact in the compartments and lack of stability among pigs as well as impacts of the animals in the vehicle.

Moreover, animals transported at T275 had a higher frequency of medium (5–10 cm) and long (10–15 cm) lesions, suggesting that this type of lesion is caused by fore claws when pigs mount one another and scratch the back with the claws of the forelimbs, and usually occurs in overcrowded conditions in the pen, at loading, unloading or along the stunning chutes (Faucitano, 2001). Thus, there is reduced space for animals which remain supported for a longer period of time than those of subtly lower densities, producing lesions of longer length. Nevertheless, an inappropriate density results in low stability, as reported by Chai *et al*. (2010), who stated that the ideal density for animal welfare and meat-quality aspects should be lower than 275 kg/m². Therefore, at a high density (325 kg/m²) animals show difficulty in maintaining their balance in the transport vehicle.

The pigs in inadequate stocking densities displayed a higher number of lesions on the carcass due to the occurrence of antagonistic or mounting activities, suggesting they are related to elevated cortisol levels (Warriss *et al*., 1998) as observed at T236 and T275.

The lengths of injuries in barrows and gilts together were inconclusive for the proposed densities. Ludtke *et al*. (2012) reported that the occurrence of antagonistic interactions among animals allocated to the same compartment can increase the levels of aggression in barrows due to the establishment of a new social hierarchy. In contrast, Athayde *et al*. (2013) did not observe differences in carcass lesions between gilts and barrows, similar to this study.

The recommended densities to ensure welfare of pigs are still debatable, with the purpose of preventing the occurrence of skin lesions by handling, density or fights, and maintaining normal physiological levels and especially obtaining good meat quality. In fact, the space for the animals during the transport to the abattoir should be adjusted based on transport conditions such as time, loading chute, distance, and duration, among others, respecting the physiology of the pigs and improving welfare of the animals in the transport vehicle.

In conclusion, pigs transported at a stocking density of 251 kg/m² have less physiological damage at slaughter, with carcasses presenting a lower incidence of evident density-related injuries compared with those transported at the densities of 236 and 275 kg/m². Barrows and gilts did not differ in terms of stress indicators, total blood count, and meat quality when transported at the densities of 236, 251 and 275 kg/m² in the pre-slaughter handling.

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