QUANTIFYING THE EFFECT OF LOADING DENSITY AND GENDER ON SELECTED BLOOD COMPONENTS AND CARCASS BRUISES OF SLAUGHTER HORSES

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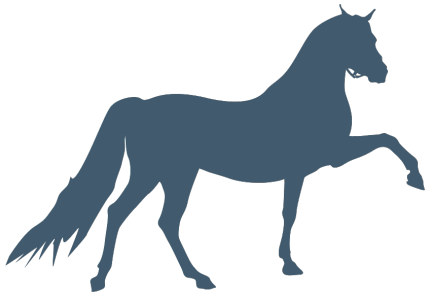
INTRODUCTION

- Too high and too low loading density negatively affects welfare of slaughter horses (*Collins et al., 2000; Iacono et al., 2007*)
- Consensus has not been reached for specific recommendations for space allowance in the lorry during transit of slaughter horses (*Driessen et al., 2022*)
  - In European Union - 1.75 m^2 per horse during a long journey (*European Regulation 1/2005*)
  - In Australia - only 1.20 m^2 per horse during a long journey (*Australian Animal Welfare Standards and Guidelines, 2021*)
- Horses of different genders (mares, geldings and stallions) may react differently to various stressful situations on the day of slaughter (*Auen et al., 2020; Razmaitė et al., 2021*)

THE AIM OF THE STUDY

- **The aim of this study was to determine the effects of loading density and gender on blood welfare indicators and carcass bruises of slaughter horses**

MATERIALS AND METHODS



× 89

Genders: 35 mares, 31 geldings and 23 stallions
Live weight: ~300 kg
Age: ~3 years old
Breed: Domestic mountain pony
Collecting point: Ruma, Srem district, Autonomous Province of Vojvodina, Serbia

11 shipments during spring
The same lorry and driver
Transportation time: about one hour (63.33 ± 5.41 minutes)
Loading density: from 198 to 236 kg/m^2 based on the live weight and number of horses

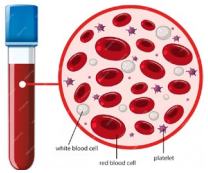
The same accredited slaughter plant and slaughterhouse personnel
Lairage time: ~3 hours
Stunning: penetrating captive bolt pistol
Exanguination: cutting the neck blood vessels (*a. carotis communis* and *v. jugularis*)
Legislation: Council Directive of the European Union 95/221EC

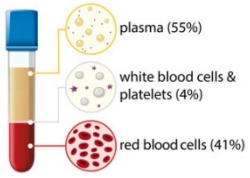
MATERIALS AND METHODS

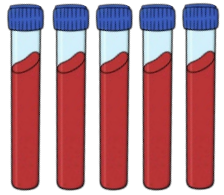
- **Blood analysis**

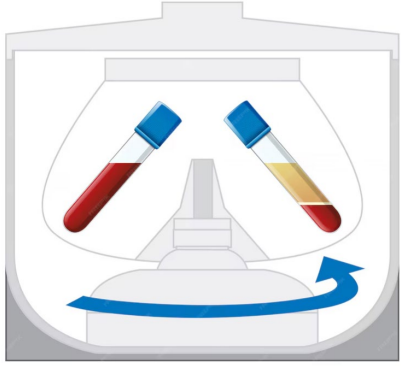
Blood glucose
(Accu-chek® Performa, Roche Diagnostics, Mannheim, Germany)

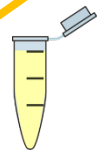
Blood lactate
(Accutrend Plus, Roche Diagnostics, Roche, Mannheim, Germany)











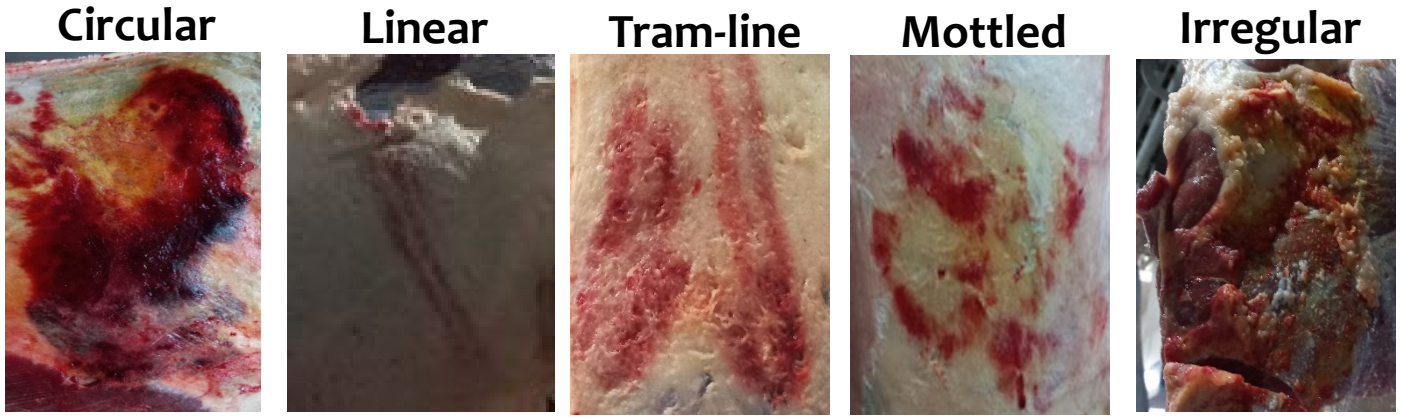
Plasma

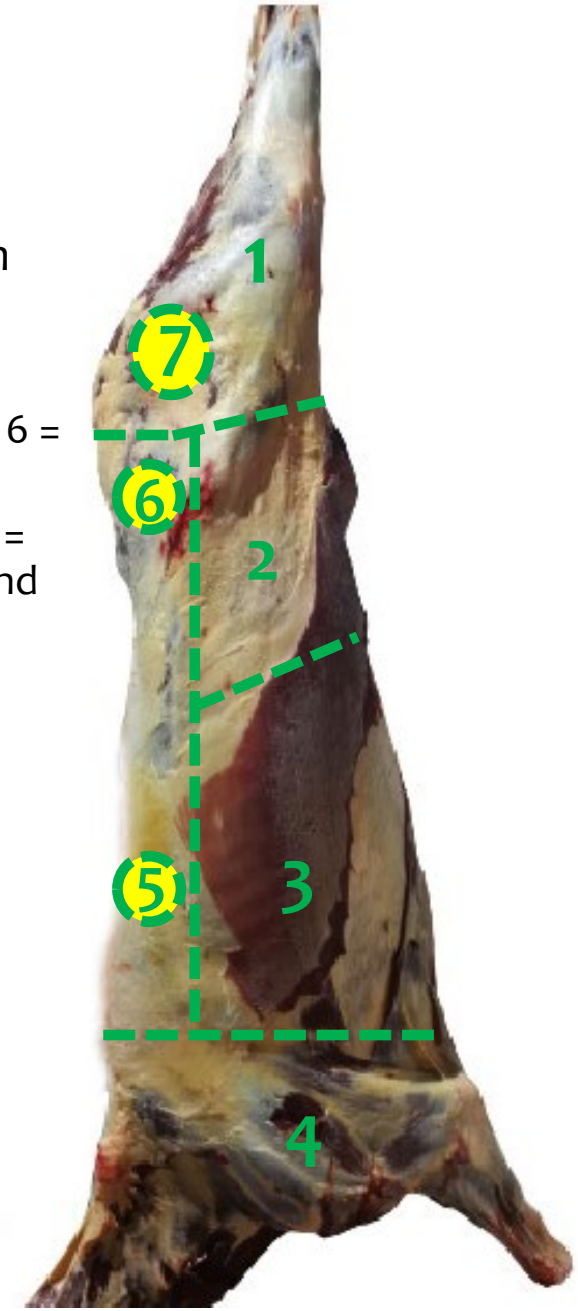
Acute phase proteins
- Ceruloplasmin (*Hussein et al., 2019*)
- Haptoglobin (*Jones et al., 1984*)

Oxidative stress biomarkers
- Reduced glutathione (GSH) (*Jollow et al. 1974*)
- Advanced oxidation protein products (AOPP)
- Total antioxidant capacity (TAC) (*Erel, 2004*)
- Total oxidative stress (TOS) (*Erel, 2005*)
- Oxidative Stress Index (OSI) = TOS/TAC

MATERIALS AND METHODS

- **Carcass bruise evaluation**
  - Both sides of the carcasses were assessed 45 minutes postmortem in the cooling room using a visual scoring system (*Miranda-de la Lama et al., 2021*)
    - **Total number of bruises per carcass**
    - **Anatomical region** (1 = rear limb, 2 = abdominal wall, 3 = thoracic wall, 4 = front leg, 5 = loin, 6 = *tuber coxae* and its muscular insertions, 7 = *tuber isquiadicum* and its muscular insertions)
    - **Bruise severity** (grade 0 = no visible bruises; grade = affecting subcutaneous tissue; grade 2 = includes subcutaneous tissue and muscle; grade 3 = involves subcutaneous tissue, muscle and bone)
    - **Bruise shape** (circular, linear, tram-line, mottled, and irregular)
    - **Bruise size** (small = ≥5 cm in diameter; medium = 6–10 cm; and large = ≥10 cm)





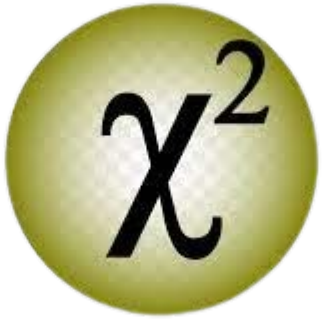
MATERIALS AND METHODS

- **Statistical analysis**

Statistical programme: SPSS software (Version 23.0, IBM Corporation, Armonk, NY, USA) (SPSS, 2015)
Statistical tests:

1. Two-way ANOVA (2 × 3) with Tukey's multiple comparison test to test the effect of loading density (high and low) and gender (mares, geldings and stallions), and their interaction
2. Chi-squared test to examine significant differences in carcass bruises between groups

Presentation of the results: means with standard deviations
Statistical significance : $P < 0.05$



RESULTS AND DISCUSSION

Table 1. Effects of loading density and gender on the selected blood welfare indicators (mean value ± standard deviation) of slaughter horses (n = 89).

Loading density	High			Low			Main Effects		Interaction
Gender	Mares	Geldings	Stallions	Mares	Geldings	Stallions	Loading density	Gender	Loading density × Gender
Number of horses	21	17	12	14	14	11	*P*-value		
Stress metabolites									
Lactate (mmol/L)	8.54 ± 0.71^{a}	6.61 ± 0.50^{b}	14.44 ± 1.72^{c}	4.88 ± 0.43^{d}	4.23 ± 1.19^{d}	10.94 ± 0.97^{e}	<0.0001	<0.0001	0.021
Glucose (mmol/L)	6.24 ± 0.92^{a}	5.14 ± 0.27^{b}	8.33 ± 1.02^{b}	4.46 ± 0.14^{d}	4.40 ± 0.47^{d}	6.00 ± 0.60^{a}	<0.0001	<0.0001	<0.0001
Acute-phase proteins									
Haptoglobin (g/L)	2.39 ± 0.23	2.41 ± 0.18	2.33 ± 0.39	2.37 ± 0.14	2.32 ± 0.27	2.41 ± 0.08	0.864	0.952	0.462
Ceruloplasmin (mg/dL)	11.26 ± 0.84^{a}	7.99 ± 1.49^{b}	21.21 ± 3.44^{c}	7.24 ± 0.49^{b}	4.45 ± 1.20^{d}	13.93 ± 0.88^{e}	<0.0001	<0.0001	<0.0001
Oxidative stress biomarkers									
GSH (μM/L)	0.42 ± 0.11^{a}	0.65 ± 0.06^{b}	0.10 ± 0.05^{c}	0.89 ± 0.11^{d}	1.64 ± 0.49^{e}	0.31 ± 0.08^{a}	<0.0001	<0.0001	<0.0001
AOPP (μmol/L)	63.35 ± 2.25^{a}	56.10 ± 2.97^{b}	71.90 ± 3.83^{c}	57.49 ± 0.88^{b}	43.13 ± 5.20^{d}	64.33 ± 2.06^{a}	<0.0001	<0.0001	<0.0001
TAC (mmol/L)	0.83 ± 0.34	0.70 ± 0.41	0.83 ± 0.40	0.73 ± 0.51	0.77 ± 0.53	0.93 ± 0.54	0.821	0.507	0.655
TOS (μmol/L)	84.16 ± 22.29	76.42 ± 22.10	78.87 ± 25.34	75.59 ± 26.39	71.06 ± 26.68	75.77 ± 24.57	0.285	0.601	0.914
Oxidative stress index	0.12 ± 0.06	0.16 ± 0.14	0.16 ± 0.18	0.31 ± 0.41	0.19 ± 0.21	0.16 ± 0.19	0.112	0.656	0.222

Abbreviations: GSH—glutathione; AOPP—advanced oxidation protein products; TAC—total antioxidant capacity; TOS—total oxidative stress;
Note: different letters in the same row indicate a significant difference at $P < 0.05$ ($^{a–e}$).

RESULTS AND DISCUSSION

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Table 2. Effects of loading density and gender on the occurrence of carcass bruises in slaughter horses (n = 89).

Loading density	High			Low			Main Effects		Interaction
Gender	Mares	Geldings	Stallions	Mares	Geldings	Stallions	Loading density	Gender	Loading density × Gender
Number of horses	21	17	12	14	14	11	*P*-value		
Bruise severity (%)									
No carcass bruises (grade 0)	47.62^{a}	58.82^{a}	25.00^{b}	85.72^{c}	92.86^{c}	81.82^{c}	<0.0001	0.2447	0.0012
Mild carcass bruises (grade 1)	42.86^{a}	35.29^{a}	16.67^{ab}	7.14^{b}	7.14^{b}	9.09^{b}	0.0042	0.3827	0.0383
Severe carcass bruises (grade 2)	9.52^{a}	5.89^{a}	58.33^{b}	7.14^{a}	0.00^{a}	9.09^{a}	0.0599	0.0020	0.0002
Bruise size (%)									
Small (< 5 cm)	28.57	23.53	0.00	7.14	7.14	9.09	0.1353	0.2448	0.1596
Medium (6–10 cm)	23.81	17.65	16.67	7.14	0.00	9.09	0.0599	0.6735	0.4011
Large (≥10 cm)	0.00^{a}	0.00^{a}	41.67^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.0649	0.0005	<0.0001
Bruise shape (%)									
Circular	14.29^{a}	17.65^{a}	58.33^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.0004	0.0440	0.0001
Linear	9.52	5.88	0.00	7.14	7.14	9.09	>0.9999	0.8187	0.9400
Tram-line	9.52	5.88	0.00	7.14	0.00	9.09	>0.9999	0.6125	0.7817
Mottled	9.52	5.88	0.00	0.00	0.00	0.00	0.2531	0.4980	0.4733
Irregular	9.52	5.88	16.67	0.00	0.00	0.00	0.0649	0.6887	0.3375
Anatomical region (%)									
Rear limb	4.76	5.88	0.00	0.00	0.00	0.00	0.5020	0.6966	0.7308
Abdominal wall	4.76^{a}	5.88^{a}	33.33^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.0330	0.0609	0.0056
Thoracic wall	4.76^{a}	5.88^{a}	41.67^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.0167	0.0162	0.0004
Front leg	4.76	5.88	0.00	0.00	0.00	0.00	0.5020	0.6966	0.7308
Loin	9.52	5.88	0.00	7.14	0.00	0.00	0.6282	0.2789	0.6563
Hip	9.52	5.88	0.00	7.14	0.00	9.09	>0.9999	0.6125	0.7817
Pin	9.52	5.88	0.00	0.00	7.14	9.09	>0.9999	0.9459	0.7817

Note: different letters in the same row indicate a significant difference at $P < 0.05$ ($^{a–e}$).

RESULTS AND DISCUSSION

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CONCLUSIONS

- The results of this study showed that high loading density, regardless of gender, negatively affects horse welfare during transportation
- In addition, stallions were the most sensitive to poor transport conditions, while geldings were the most resistant
- In contrast, transport of slaughter horses at lower densities, irrespective of gender, resulted in improved animal welfare
- To define optimal travel density, additional research is necessary to determine the effects of different loading density on behaviour, physiology and carcass and meat quality of slaughter horses