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Effects of different positions during transport on physiological and behavioral changes of horses

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KEYWORDS:
transport position;
cortisol;
behavior;
hematological parameters;
horses

Abstract The aim of this study was to evaluate the effect of different transport positions on some physiological parameters in racehorses and their behavior patterns during and after the journey. Twelve horses made 3-hour journeys of 200 km on the same route, with the same driver, and in 3 different positions: facing forward, backward, and sideways in relation to the direction of travel. Physiological and behavioral parameters were registered before, during, and after the journey. Horses were checked at 5 different times: at rest (T0), at loading (T1), at unloading (T2), and at 2 (T3) and 4 (T4) hours after return from the journey. At each check, heart rate, respiratory rate, and rectal temperature were measured and blood samples were collected by jugular vein puncture to assess cortisol, packed cell volume, total protein, albumin, glucose, creatinine, triglycerides, cholesterol, urea, creatine kinase, lactate dehydrogenase, alanine transaminase, aspartate transaminase, alkaline phosphatase, calcium, phosphorus, and chlorine. Loading and unloading were filmed. Behavioral patterns were recorded by direct observation, during the travel, 2 and 4 hours after arrival in a new stall. The same parameters were recorded at the same times (excluding loading and unloading) in a control group that did not travel. All data were analyzed using a repeated-measures analysis (analysis of variance). Loading produced an increase of heart rate and packed cell volume in comparison with rest values. Horses facing in the direction of travel during journey made fewer forward, backward, and sideways movements than others, whereas horses traveling sideways lost their balance and touched the stall rails less frequently. Highest serum cortisol concentration value was recorded soon after unloading horses that had faced in the direction of travel ($P < 0.01$). Two hours after return, horses that had traveled sideways revealed an increase of creatine kinase ($P < 0.01$). The traveling position in the vehicle did not appear to affect postjourney behavior. In comparison with the control group, the horses that had traveled consumed concentrate faster, spent more time eating hay, and drank more frequently in the first 2 hours after return from the journey. Front-facing position led to an increase in serum cortisol concentration, whereas the sideways position caused some muscular tension, which disappeared 4 hours after the journey. Although facing backward was the travel position that provoked the greatest number of horses' movements, it did not have a negative effect on physiological and behavioral parameters during and after the journey. We concluded that for Standardbred trotters accustomed to travel, the latter may be the less stressful position during a 200-km transport.

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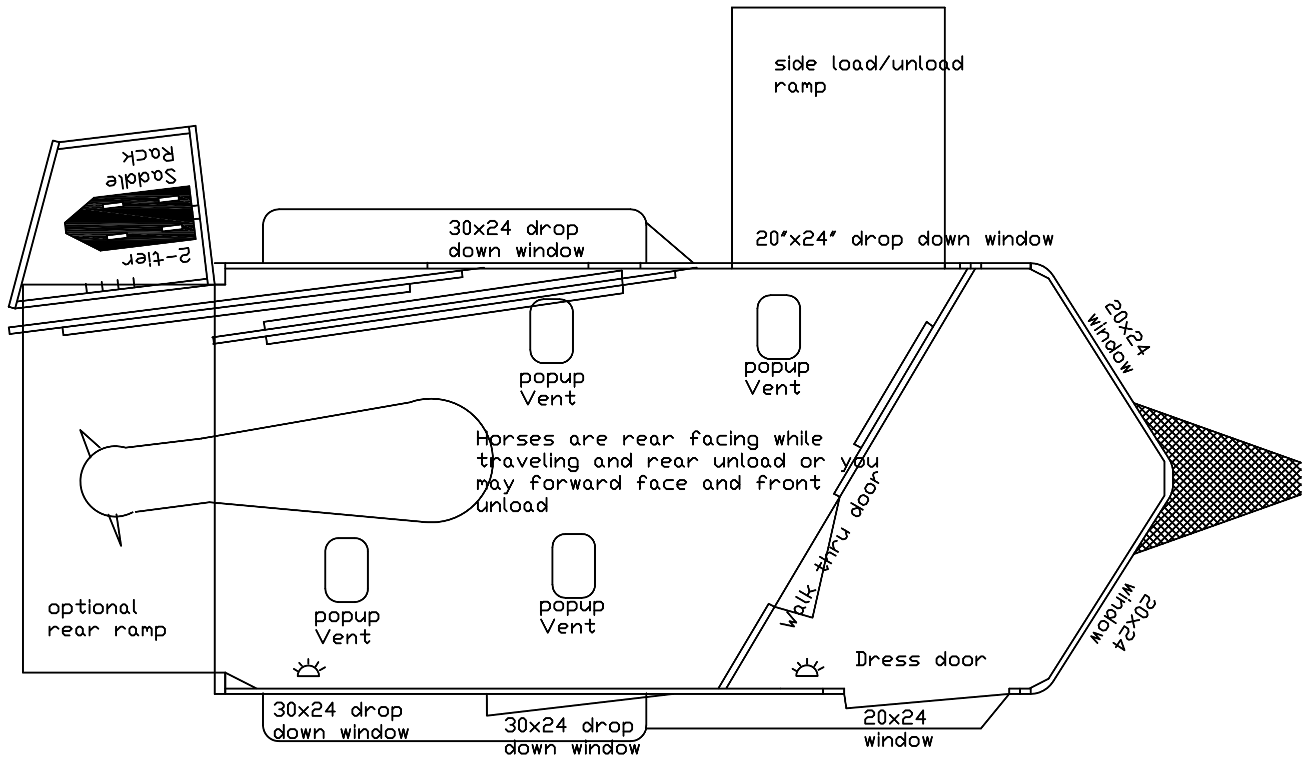
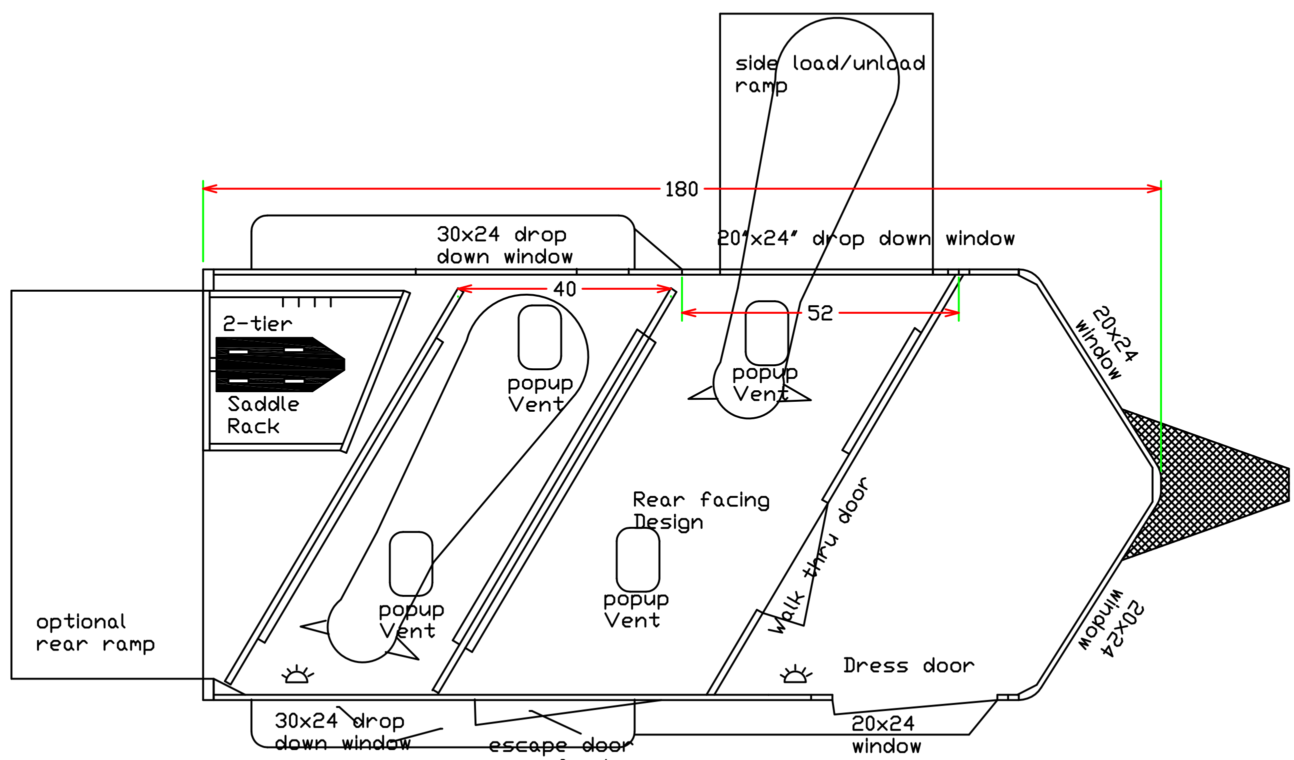
Introduction

Farmed animals are transported at some stage in their lives, sometimes to places where food is more readily available, sometimes to a different owner or to a different housing


side load/unload ramp
Saddle Rack
2-tier
30x24 drop down window
20"x24" drop down window
20x24 window
popup Vent
popup Vent
Horses are rear facing while traveling and rear unload or you may forward face and front unload
Walk thru door
popup Vent
popup Vent
20x24 window
optional rear ramp
Dress door
30x24 drop down window
30x24 drop down window
20x24 window


side load/unload ramp
180
30x24 drop down window
20"x24" drop down window
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52
20x24 window
2-tier
Saddle Rack
popup Vent
popup Vent
Rear facing Design
Walk thru door
popup Vent
popup Vent
20x24 window
optional rear ramp
Dress door
30x24 drop down window
escape door
20x24 window

location, and sometimes to be slaughtered (Broom, 2005). Physiological changes associated with transport that indicate stress responses have been reported for sheep (Baldock and Sibley, 1990) and cattle (Tarrant, 1990). The transport of horses by road vehicles is a relatively recent practice that is increasing rapidly both within and between countries. It is well known that transport of horses can produce various physiological and pathological changes, such as heart rate (HR) increase (Cross et al., 2008), hematological changes (Yamauti et al., 1993), and stress-induced pneumonia (Oikawa et al., 2004), and it can thus be concluded that horses are subjected to stress during and after road transport (Pilkington and Wilson, 1991). The transport of animals is a complex procedure involving several potential stressors, including handling, loading, unloading, separation from familiar physical and social environments, confinement, vibration, changes in temperature and humidity, inadequate ventilation, and often deprivation of food and water (Waran, 1993). Factors that induce stress during transport are mostly psychological (White et al., 1991), but physical factors also contribute, such as the motion of the trailer, noise, and road conditions (Jones, 2003). Confinement is stressful for horses (Mal et al., 1991), but confinement in a stationary vehicle than in a moving one is generally considered to be less stressful for farm animals (Tarrant, 1990). Indeed, during transport, horses are subjected to changing forces due to, for example, acceleration, deceleration, and turning movements of the vehicle (Waran and Cuddeford, 1995). The anatomical conformation of the horse is such that it carries 60% of its body weight over its forelegs, and the hindquarters are poorly designed for continual shifting of weight and direction (Cregier, 1982). It is probably for this reason that the most commonly observed body posture of horses during transit is that of standing with the front limb and hind limb apart and the forelegs stretched forward. This exaggerated limb position during transit perhaps helps the horse to retain its balance by exerting inclined thrusts with one leg or the other, as occasion demands (Robert, 1990). Inappropriate orientation, and consequent loss of balance, may be the cause of injuries during horse transport (Collins et al., 2000). Among experts, there are different opinions about travel position for minimizing transport stress and optimizing horses' post-transport performance (Gibbs and Friend, 1999). Several studies have been carried out to determine the effects of orientation on a horse's ability to maintain balance during transport, but results have often been conflicting due to differences in trailer design and lack of simultaneous comparisons.

Clark et al. (1993) found that when transported in a 2-horse trailer, backward-facing horses had fewer side and total impacts and losses of balance as compared with forward-facing horse. Waran et al. (1996) found that horses transported in a 2-horse lorry without a saddle compartment and facing backward had a significantly lower HR, moved less frequently, and showed a greater tendency to rest their rumps on a partition. Toscano and Friend (2001) concluded that some horses demonstrated a superior ability to maintain their balance in a particular orientation; therefore, individual characteristics and other factors, rather than travel orientation alone, may be responsible for the ability of horses to maintain their balance during transport.

The aim of this study was to evaluate the effects of different positions of the horses during road transport; it focused not only on the journey itself but also on the 4 hours after transport to observe whether a different position modified physiological and behavioral patterns of the animals after they are introduced to new stalls.

Materials and methods

Animals and route

Twelve Standardbred trotters (4 females, 4 males, and 4 geldings) aged 3-7 years were used. All animals had a mean body weight of 435 ± 35 kg (mean ± SD) and were qualified to race. As Standardbred trotters start travel at 18 months, all of them had the same traveling experience, were accustomed to traveling in different positions, and had last traveled 1 month before the test. A pre-experiment clinical examination (Reed et al., 2004) confirmed that the horses were clinically healthy. They were moved in 2, 6-horse trucks, one of which was arranged in "Italian-style" (3 horses facing forward and 3 backward in relation to the direction of travel) and the other in "French-style" (each horse traveled sideways at an angle of 90° to the direction of travel). Every animal traveled, tethered with a 50-cm rope on each side of the halter, in an individual tie stall (2.3 m [length] × 0.85 m [width]), giving a total space of about 2 m^2 and made 3 journeys of 200 km. Each journey was made 2 weeks after the previous one, in the winter, and on mild days (average temperature of approximately 8°C and relative humidity of approximately 85%), without rain or wind. During each journey, horses were transported in a different position: forward-facing, backward-facing, and sideways. Horses left their stables within the Sauri racecourse (Foggia, Apulia, Italy) at 12:30 AM and returned to the same racecourse 3 hours later. The driver and the route were always the same. The latter began at the racecourse with a 1-km road with gravel. Then, it was asphalted until the last kilometer to re-enter the racecourse. Of the 200 km, 40% was straight road and 60% was characterized by 128 left and 133 right bends, with a minimal road slope of 0% and a maximum of 4%. During travel, horses could not eat and drink, and after unloading, the horses were placed in a different transit stall (which generally houses the visiting horses that have to run) after each journey, whenever we changed boxes. After being placed in such boxes, animals received the same quantity of concentrate and hay and ad libitum water. Housing, management, and experimental procedures were carried out according to requirements of the Council for International Organizations of Medical Sciences.

Fifteen days before the trial, horses were moved to unfamiliar stalls and observed, according to the experimental protocol, after 3 hours of food and water deprivation. Observed data were used as control group parameters.

Experimental protocol and blood tests

Transported horses were examined 5 times: at rest in their stall at 12 AM (T0), soon after being loaded into the vehicle and taken to their individual tie stalls (T1), after being unloaded from the vehicle and housed in the transit stalls (T2), and 2 (T3) and 4 (T4) hours after return. These 5 intervals were chosen in agreement with Fazio et al. (2008) and Werner and Gallo (2008).

During the morning before 12 AM (T0), all horses were trained as part of their usual training program (30 minutes of trotting at 4 m/sec).

Each examination included recording of HR by auscultation, respiratory rate by chest wall movement observation, and measurement of rectal temperature (in °C) with an electronic thermometer (VedoDigit II; Pic Solution, Milano, Italy). A blood sample was collected with a needle and a syringe to determine the concentration of blood lactate with a lactic acid meter (Lactate Pro; Arkray, Kyoto, Japan); other samples were also taken in 2 Vacutainer tubes (Vacutainer; Becton Dickinson, Cockeysville, MD), with and without anticoagulant (ethylenediaminetetraacetic acid [EDTA]). Capillary tubes with EDTA were filled with the blood collected and centrifuged in loco at 12,000 rpm for 6 minutes to determine packed cell volume (PCV). Tubes without EDTA were centrifuged at 3,000 rpm for 15 minutes, and serum was divided into 2 parts and stored at −20°C.

The horses' serum cortisol concentrations were analyzed in duplicate using a commercially available Chemiluminescent Immunoassay System (Immulite 2000 System; Siemens, Deerfield, IL).

Blood serum was examined using a Beckman-Coulter DU 800 (Brea, California) spectrophotometer and commercial kits (SGM Italia; Farmalab, Lecce, Italy). Total protein, albumin, glucose, triglycerides, cholesterol, calcium, phosphorus, and chlorine levels were determined by means of colorimetric methods, whereas creatinine, blood urea nitrogen (BUN), creatine kinase (CK), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), and phosphates alkaline (SAP) were assessed with enzymatic methods.

Behavioral parameters

Horses' behaviors were recorded with a camera (HDR-Cx 350VE; SONY, Milano, Italy) for evaluating any reluctance or refusals during loading and unloading. During the journey, horses were observed with a closed-circuit camera, and 2 operators inside the vehicle observed and registered the horses' movements: forward, backward, lateral, and leaning on stable rails. After the journey, horses were observed for 4 hours, divided in 2-hour periods (I and II) by a 15-minute interval during which the horses were clinically examined (T3). Behavior observations were performed by 2 operators located outside each box, without disturbing the animals. One of them recorded the duration (minutes) of behavior patterns: standing on 3 legs, standing on 4 legs, hay feeding, concentrate feeding, walking, playing (solitary play: activities involving a sense of pleasure but apparently no immediate function), rubbing, and sniffing (smell with nostrils the new environment); the other recorded frequency of sniffing stall, movements of the head, movements of the ears, concentrate feeding, hay feeding, urination, defecation, drinking, snorting, autogrooming, licking the stall, pawing, kicking, yawning, playing, and rubbing. For each behavior pattern, the "latency time" was calculated from the time the horse was placed in the stall until first occurrence of behavior.

Statistical analysis

All physiological and hematological parameters were subjected to a repeated-measures analysis of variance using the procedure of the Generalized Linear Model (SAS, 1999). Independent variables were the position during the journey, the time of observation (rest, loading, unloading, 2 and 4 hours after return), and the interaction between those variables. Data were normally distributed. Tukey's post hoc test was used to perform statistical multiple comparisons. P level was set at 0.05. All data were expressed as quadratic mean and mean standard error. For the ethological data, the minutes spent in each behavior studied were calculated, as was number of times the behavior was noted in the first 2 and the second 2 hours of observation in the transit stalls. The latency time values were subjected to variance analysis using the procedure of the Generalized Linear Model (SAS, 1999). Independent variables were the group to which the horses belonged (control, frontal position, sideway position, backward position), the observation period (I, II), and the interaction between those variables. The differences between the mean values were calculated using Student t test (SAS, 1999). All the data were expressed as quadratic mean and mean standard error. Behaviors recorded inside the vehicle during journey were subjected to analysis of variance between the 3 tests (frontal position, sideway position, backward position) (SAS, 1999).

Results

Figure 1 shows the physiological parameters recorded during the study. All parameters were within ranges previously reported by Reed et al. (2004).

Compared with values observed in T0, there was a significant increase in HR ($P < 0.05$) and PCV ($P < 0.05$) in all the horses in each position after loading (T1). Rectal

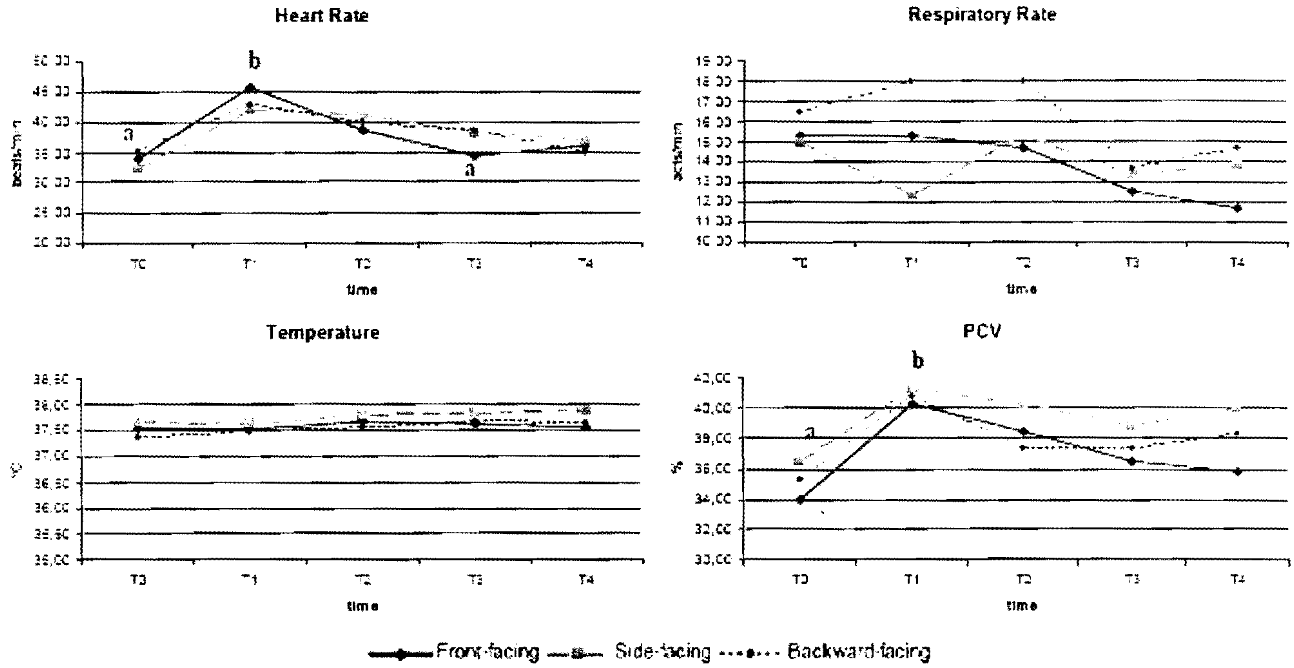

Figure 1 Physiological patterns (heart rate, respiratory rate, rectal temperature, and packed cell volume [PCV]) recorded at the 5 control stages: rest (T0), loading (T1), unloading (T2), 2 hours after return (T3), and 4 hours after return (T4). Different letters show statistical difference between the times only in all traveling positions (a, b: $P < 0.05$).

temperature and respiratory rate, instead, remained within the physiological range and did not show any variations.

Table 1 shows serum cortisol concentration and biochemical profile values recorded at the different stages.

At unloading (T2), horses that had traveled in front-facing position showed higher serum cortisol concentrations as compared with those that traveled in sideways position (240.92 nmol/L vs. 184.00 nmol/L; $P < 0.01$).

Table 1 Values of serum cortisol and the biochemical profile recorded at the 5 control stages between control group and the 3 different positions

Position	T0[1]	T1[2]	T2[3]	T3[4]	T4[5]	Mean standard error
Cortisol (nmol/L)						
Front-facing	182.83^{C}	210.50^{AC}	240.92^{A,X}	97.71^{B}	109.84^{B}	14.68
Side-facing	146.85^{AC}	177.83^{A}	184.00^{A,Y}	67.93^{B}	110.76^{BC}	
Backward-facing	189.17^{A}	199.67^{A}	207.33^{A}	88.85^{B}	126.87^{B}	
Creatine kinase (IU/L)						
Front-facing	194.83	190.33	184.33	184.67^{x}	200.67	8.23
Side-facing	203.83	206.67	198.63	225.33^{y}	207.50	
Backward-facing	200.33	193.50	186.67	183.17^{x}	197.83	
Creatinine (mg/dL)						
Front-facing	1.49	1.55^{a}	1.46	1.46	1.36^{b,X}	0.03
Side-facing	1.57	1.59	1.54	1.62	1.62^{Y}	
Backward-facing	1.48	1.49	1.42	1.48	1.39^{X}	
Glucose (mg/dL)						
Front-facing	102.20^{a}	95.01^{x}	99.48	104.58^{A}	90.00^{Bb}	2.27
Side-facing	95.65^{a}	83.62^{Bb,y}	98.23^{A}	96.28^{A}	98.65^{A}	
Backward-facing	98.82^{a}	86.87^{Ab}	97.00	104.25^{B}	90.28^{A}	

Note: Different letters in the same line show statistical differences (A, B, C: $P < 0.01$; a, b: $P < 0.05$).
Note: Different letters in the same row show statistical differences (X, Y: $P < 0.01$; x, y: $P < 0.05$).
[1]Rest.
[2]Loading.
[3]Unloading.
[4]2 hours after return.
[5]4 hours after return.

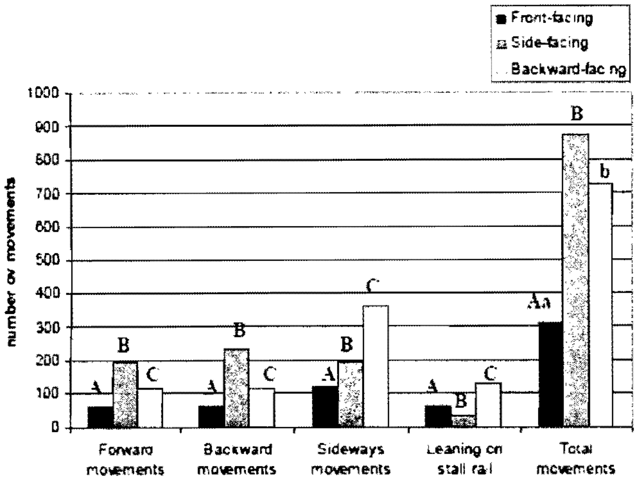

Figure 2 Number of movements during transport in different positions. Different letters show statistical difference between the traveling positions (A, B, C: $P < 0.01$; a, b: $P < 0.05$).

Moreover, in the horses that had traveled sideways, higher CK value at T3 ($P < 0.05$) and higher level of creatinine at T4 ($P < 0.01$), as compared with resting levels (T0), were noted.

Glucose levels were generally lower at loading (T2) than in other stages of the study.

No variations were recorded in time and between the tests for AST, ALT, LDH, SAP, BUN, calcium, chlorine, phosphorus, total protein, albumin, cholesterol, and triglycerides. Blood lactate was always below the level of sensitivity of the apparatus (<0.7 mmol/L).

The video recordings showed that the horses displayed no reluctance or trouble in loading and unloading from truck.

Figure 2 shows horses' movements registered within the vehicle during the journey. Horses facing in the direction of travel made fewer forward, backward, and sideways movements; changes were significantly different from those measured in horses in the other 2 positions. Horses traveling sideways, instead, lost their balance and touched the stall rails less frequently.

Tables 2 and 3 summarize the data recorded on postjourney behavior. It was noted that horses that had traveled ate the concentrate in less time and with fewer eating bouts and spent more time eating hay. The control group sniffed their stalls sooner (latency time: control group, 2.5 minutes; front-facing, 18.5 minutes; side-facing, 29 minutes; backward-facing, 39 minutes; $P < 0.05$) and more frequently. In the first period of observation, the control group moved their heads more frequently, in comparison with side- and backward-facing groups, and defecated and performed self-grooming more often, compared with front-facing group. In addition, the transported horses ate and drank more frequently in period I than in period II.

Discussion

Even if horses used in this study were already accustomed to traveling, they perceived loading as a stressful experience that, regardless of the position, caused a rise in HR and PCV. These variations are due to catecholamine release by the adrenal glands in response to fear (Hodgson and Rose, 1994). In fact, there was a slight increase of cortisol concentration during loading compared with the basal level at T0, but it was not statistically significant. However, it should be noted that in this study, blood samples at T0 were collected at 12 AM, after the normal training plan; therefore, our cortisol values are probably higher than values that could be registered at 8 AM, as we found that our values were

Table 2 Behavior calculated (minutes) in the first and second 2 hours of direct observation after return to transit stalls

Behavior	Part	Control group	Front-facing	Side-facing	Backward-facing	Mean standard error
Resting on 3 legs	I	16.3	8.7	3.4^{X}	2.8	6.8
	II	32.5	24.8	31.4^{Y}	18.8	
Staying on 4 legs	I	15.0	16.5	14.0	12.4	5.9
	II	28.0	29.7	29.8	28.5	
Concentrate feeding	I	$41.0^{Aa,X}$	$27.7^{b,X}$	$26.4^{B,X}$	31.4^{X}	3.7
	II	$13.5^{a,Y}$	3.0^{Y}	$0.8^{b,Y}$	$2.0^{b,Y}$	
Hay feeding	I	44.0^{Aa}	62.5	$73.0^{B,x}$	67.8^{b}	7.2
	II	41.3^{a}	57.5	49.8^{y}	62.2^{b}	
Walking	I	0.7	0.3	1.4	0.8	1.1
	II	0.1	1.0	2.8	2.5	
Playing	I	0.5	2.2	0.4	0.5	1.0
	II	2.8	2.2	0.8	1.5	
Rubbing	I	0.3	0.5	0.5	0.8	0.8
	II	1.3	0.5	0.8	1.5	
Sniffing	I	1.0	1.7	1.1	4.0	1.4
	II	0.3	1.4	0.5	3.2	

Note: Different letters in the same line show statistical differences (A, B: $P < 0.01$; a, b: $P < 0.05$).

Note: Different letters in the same row show statistical differences between the 2 time parts considered (X, Y: $P < 0.01$; x, y: $P < 0.05$).

Table 3 Frequency of some behavioral patterns in the first and second 2 hours of observation (n/2 hours)

Behavior	Part	Control group	Front-facing	Side-facing	Backward-facing	M.S.E
Sniffing box	I	24.7	7.8	11.7	13.8	6.9
	II	9.0	9.5	11.7	17.8	
Head movement	I	105.0^{a}	70.7	50.2^{b}	52.2^{b}	16.1
	II	68.5	50.3	25.2	43.2	
Ears movement	I	222.0	282.3	518.8	233.7	105.8
	II	173.7	266.3	303.0	218.5	
Hay feeding	I	25.3	31.7	20.3	28.3	5.9
	II	14.8	20.8	11.3	14.8	
Concentrate feeding	I	20.7$^{Aa;X}$	12.0$^{bc;X}$	11.3$^{Bb;X}$	18.7$^{ac;X}$	2.4
	II	3.7^{Y}	1.7^{Y}	0.5^{Y}	1.5^{Y}	
Urination	I	3.7	2.0	1.7	2.2	0.8
	II	2.7	1.3	0.8	0.7	
Defecation	I	1.2^{a}	0.2^{b}	0.3	0.8	0.4
	II	1.3	0.8	0.5	0.5	
Drinking	I	8.3	10.8^{X}	12.0^{X}	10.2^{x}	1.6
	II	5.3	3.2^{Y}	2.3^{Y}	4.7^{y}	
Blowing	I	22.2	24.7	19.3	33.2^{x}	5.6
	II	15.0	16.8	10.2	13.2^{y}	
Self-grooming	I	7.0^{a}	2.2^{b}	3.2	4.3	1.7
	II	2.5	1.8	0.7	1.8	
Licking	I	2.2	2.5	1.7	0.3^{x}	1.5
	II	2.7	1.8	1.7	4.7^{y}	
Pawing	I	26.0	27.7	7.3	5.7	8.8
	II	8.7	10.8	6.2	4.8	
Kicking	I	4.5	1.2	2.0	1.8	4.9
	II	0.3	0.0	13.5	0.3	
Yawning	I	0.0	0.7	0.0	0.0	0.4
	II	0.7	0.2	0.8	0.0	
Playing	I	1.3	2.6	1.2	0.8	2.1
	II	2.7	2.8	4.8	5.2	
Rubbing	I	1.8	1.3	4.3	2.7	2.2
	II	2.7	2.2	6.7	3.7	
Walking	I	29.4	43.3^{x}	22.3	19.7	9.1
	II	13.2	13.0^{y}	10.7	12.2	

Note: Different letters in the same line show statistical differences (A, B: $P < 0.01$; a, b, c: $P < 0.05$).
Note: Different letters in the same row show statistical differences between the 2 time parts considered (X, Y: $P < 0.01$; x, y: $P < 0.05$).

similar to post-training increased cortisol levels observed by Tateo et al. (2008) in Standardbred trotters.

Knowles and Warriss (2000) reported an increase of triglycerides in transport; however, in our study, neither travel nor position in the truck affected AST, ALT; LDH, SAP, BUN, calcium, phosphorus, chlorine, total protein, albumin, and triglycerides. These unchanged parameters could be explained by the habit of the study horses to travel and by the much more stability of the truck in comparison with trailers, used in other studies.

Restricted movements in tied horses during transport did not affect muscle fatigue, as was indicated by our lactate values, which were in agreement with resting levels reported by Stull and Rodiek (2000).

Tateo et al. (2011) concluded that in comparison with long transport (200 km), short transport (50 km) caused an increase of serum cortisol concentration. In our study, maximal values of serum cortisol were recorded at unloading in horses facing in the direction of travel. Probably, they were unable to adapt to truck displacement in this situation, as they could in the other positions. Giovagnoli (2008) reported that in the front-facing position, horses tend to fall forward more easily; therefore, in this position, fear of falling caused more stress in horses.

Gibbs and Friend (1999) noted that horses showed a slight preference for traveling at about 45° to the direction of travel; however, in our study, horses traveling sideways developed a slight muscular fatigue, confirmed by variations in the hematic concentrations of CK at T3.

Horses made much more movement within the vehicle in the backward-facing position, but at the end of the journey and in the subsequent 4 hours, they had no variation of physiological patterns. We concluded that such a position is less stressful for them, as horses were thought to absorb deceleration with their haunches and were better able to maintain stability (Cregier, 1981, 1982). In fact, backward position was voluntarily chosen for untethered horses during vehicle transport (Kusunose and Torikai, 1996).

Compared with control group, the journey positions did not affect the horses' postjourney behavior. After the journey, horses that had traveled showed greater interest in food. Although fasting hours and morning training programs were similar in all groups, transported horses were much more motivated to feed and finished their food more quickly, whereas the control group spent more time in explorative activities. Transported horses did not rest more than the control group, maybe due to their need to feed for recovering energy spent to maintain balance in the truck. This is in agreement with observation reported by Waran (1993). Further behavioral studies are required to help improve wellbeing of horses during and after travel.

Conclusion

After a journey of 200 km, horses in the sideways position needed 4 hours for restoring basal condition of CK and creatinine, and front-facing position caused an increase of serum cortisol concentration at unloading. Indeed, for Standardbred trotters accustomed to travel, the backward-facing direction has been suggested, as it is the less stressful position for a 200-km travel.

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