Exhibit 22

32008L0097

Directive 2008/97/EC of the European Parliament and of the Council of 19 November 2008 amending Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists (Text with EEA relevance)

OJ L 318, 28.11.2008, p. 9–11 (BG, ES, CS, DA, DE, ET, EL, EN, FR, IT, LV, LT, HU, MT, NL, PL, PT, RO, SK, SL, FI, SV)

BG	ES	CS	DA	DE	ET	EL	EN	FR	GA	IT	LV	LT	HU	MT	NL	PL	PT	RO
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Directive 2008/97/EC of the European Parliament and of the Council

of 19 November 2008

amending Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists

(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 152(4)(b) thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Economic and Social Committee [1],

After consulting the Committee of the Regions,

Acting in accordance with the procedure laid down in Article 251 of the Treaty [2],

Whereas:

(1) Article 2 of Directive 96/22/EC [3] prohibits, inter alia, the placing on the market of stilbenes, stilbene derivatives, their salts and esters and thyrostatic substances for administering to animals of all species.

(2) The reason for that absolute prohibition was that potential abuse or misuse would be more difficult if there were no product authorised for any animal species whatsoever on the market.

(3) However, experience gained in particular with national residue plans submitted under Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products [4] has shown that the misuse of product presentations intended for pet animals does not play a role as a source of abuse or misuse. That is partly because it is economically unattractive to use presentations intended for pet animals for growth promotion in food-producing animals.

(4) Moreover, the prohibition of thyrostatic substances has harmful consequences for the welfare of pet animals (dogs and cats) due to the lack of an alternative treatment for hyperthyroidism in those animals.

(5) The Protocol on protection and welfare of animals annexed to the Treaty provides that the Community and the Member States are to pay full regard to the welfare requirements of animals in the implementation of Community policies, in particular with regard to the internal market.

(6) It is therefore appropriate to limit the scope of Directive 96/22/EC only to food-producing animals and withdraw the prohibition for pet animals, as well as to adjust the definition of therapeutic treatment.

(7) The Opinion of the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) of 30 April 1999 on the potential risks to human health from hormone residues in bovine meat and meat products (which was reviewed on 3 May 2000 and confirmed on 10 April 2002) concluded that there is a substantial body of recent evidence suggesting that oestradiol 17β has to be considered as a complete carcinogen, as it exerts both tumour-initiating and tumour-promoting effects, and that the data currently available do not make it possible to give a quantitative estimate of the risk to human health. As a result, Directive 96/22/EC was amended by Directive 2003/74/EC so as to, inter alia, prohibit permanently the use of oestradiol 17β as a growth promoter and reduce substantively all other circumstances in which it can be administered to all farm animals for therapeutic or zootechnical purposes pending further examination of the factual and scientific situation and the veterinary practices in the Member States.

(8) Article 11a of Directive 96/22/EC required the Commission to present a report by 14 October 2005 concerning the availability of alternative veterinary medicinal products to those containing oestradiol 17β for food-producing animals for therapeutic purposes. The Commission sought expert advice and established the relevant scientific report, which was forwarded to the European Parliament and the Council on 11 October 2005. That Report concludes that oestradiol 17β is not essential in the production of food-producing animals because the use of the available alternatives (especially prostaglandins) by practising veterinarians is already quite common in the Member States and that the complete prohibition of the use of oestradiol 17β for food-producing animals would have no, or only a negligible, impact on farming and animal welfare.

(9) Proper compliance with the relevant legislation and the elimination of inappropriate use of unauthorised substances can be enhanced by means of objective information and awareness campaigns.

(10) A temporary exemption was provided for the use of oestradiol 17β for oestrus induction in cattle, horses, sheep or goats until 14 October 2006. Since effective alternative products exist and are already used, and in order to ensure the high level of health protection chosen in the Community, that exemption should not be renewed.

(11) Directive 96/22/EC should therefore be amended accordingly,

HAVE ADOPTED THIS DIRECTIVE:

Article 1

Directive 96/22/EC is hereby amended as follows:

1. In Article 1(2), point (b) shall be replaced by the following:

"(b) "therapeutic treatment" shall mean the administering — under Article 4 of this Directive — to an individual farm animal of an authorised substance to treat, after examination by a veterinarian, a fertility problem — including the termination of unwanted gestation — and, in the case of beta-agonists, to induce tocolysis in cows when calving as well as to treat respiratory problems, navicular disease and laminitis and to induce tocolysis in equidae;".

2. Article 2 shall be replaced by the following:

"Article 2

Member States shall prohibit the placing on the market of the substances listed in Annex II for administering to any animals, the meat and products of which are intended for human consumption, for purposes other than those provided for in point 2 of Article 4.".

3. In Article 4, point 2(i) shall be replaced by the following:

"(i) allyl trenbolone, administered orally, or beta-agonists to equidae, provided they are used in accordance with the manufacturer's instructions;".

4. Article 5a shall be deleted.

5. In Articles 3, 6, 7, 8, 11 and 14 a, the references to Article 5a shall be deleted.

6. In Article 11, paragraph 1 shall be replaced by the following:

"1. Third countries whose legislation authorises the placing on the market and administration of stilbenes, stilbene derivatives, their salts and esters, or of thyrostatic substances for administering to all species of animals the meat and products of which are intended for human consumption may not appear on any of the lists of countries provided for under Community legislation from which Member States are authorised to import farm or aquaculture animals or meat or products obtained from such animals.".

7. Article 11a shall be replaced by the following:

"Article 11a

With regard to the substances listed in Annex III, the Commission shall seek additional information, taking into account recent scientific data from all possible sources, and keep the measures applied under regular review with a view to the timely presentation to the European Parliament and to the Council of any necessary proposals.".

8. The following Article shall be inserted:

"Article 11b

The Commission, in collaboration with the Member States, shall set up an information and awareness campaign on the complete ban on the use of oestradiol 17β in food-producing animals, aimed at farmers and veterinary organisations in the EU as well as the relevant organisations outside the EU which are directly or indirectly involved in the export to the EU of food of animal origin falling within the scope of this Directive.".

9. Annex II shall be replaced by the text appearing in the Annex to this Directive.

Article 2

1. Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 1 January 2009. They shall forthwith communicate to the Commission the text of such laws, regulations and administrative provisions together with a table showing the correlation between them and this Directive.

When they are adopted by Member States, these measures shall contain a reference to this Directive or shall be accompanied by such reference on the occasion of their official publication. The methods of making such reference shall be laid down by Member States.

2. Member States shall communicate to the Commission the text of the provisions of national law which they adopt in the field covered by this Directive.

Article 3

This Directive shall enter into force on the 20th day following its publication in the Official Journal of the European Union.

Article 4

This Directive is addressed to the Member States.

Done at Strasbourg, 19 November 2008.

For the European Parliament

The President

H.-G. Pöttering

For the Council

The President

J.-P. Jouyet

[1] OJ C 10, 15.1.2008, p. 57.

[2] Opinion of the European Parliament of 5 June 2008 (not yet published in the Official Journal) and Council Decision of 20 October 2008.

[3] OJ L 125, 23.5.1996, p. 3.

[4] OJ L 125, 23.5.1996, p. 10.

ANNEX

"ANNEX II

List of prohibited substances:

List A: prohibited substances

- Thyrostatic substances,
- Stilbenes, stilbene derivatives, their salts and esters,
- Oestradiol 17β and its ester-like derivatives.

List B: prohibited substances with derogations

- Beta-agonists"

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Exhibit 23

JOURNAL OF ANIMAL SCIENCE
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Responses of horses to trailer design, duration, and floor area during commercial transportation to slaughter

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Responses of Horses to Trailer Design, Duration, and Floor Area During Commercial Transportation to Slaughter[1]

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ABSTRACT: Nine trailer loads of horses (n = 306) transported to slaughter facilities with distances ranging 596 to 2,496 km were studied to characterize the type of horses used in commercial markets and the physiological responses and number of injuries due to transportation under summer environmental conditions. Slaughter horse candidates were middle-aged (11.4 ± .4 yr), possessed moderately fleshy body condition, weighed 432 ± 3.3 kg, and were of Quarter Horse or Thoroughbred breeding. The mean weight loss during commercial transport was 4%. The percentage of injured horses was greater ($P < .05$) for two-tiered "pot-belly" (29.2%) compared with straight-deck (8.0%) trailers; however, the stress indicators of cortisol and neutrophil:lymphocyte ratio and rectal temperature showed greater ($P < .05$) responses following transport in straight-deck trailers. As trip duration increased from 5 h 45 min to 30 h, muscle fatigue (lactate concentration) and dehydration (hematocrit and total protein concentration) were the major physiological considerations, especially in durations over 27 h. The percentage of horses injured was less ($P < .05$) in trailers with 1.14 to 1.31 m^2 of floor area per horse than in trailers with 1.40 to 1.54 m^2 of floor area per horse. However, most physiological responses (white blood cell count, total protein concentration, and neutrophil:lymphocyte ratio) to transportation were less ($P < .05$) in horses provided with the greater floor area.

Key Words: Horses, Transport, Handling, Stress, Animal Welfare

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Introduction

Approximately 87,200 horses (USDA, 1998) were processed for human consumption at U.S. slaughter facilities in 1997 and the resultant meat products were marketed for export. Horses tend to travel longer distances to slaughter than other livestock, because there is a limited number of equine slaughterhouses. The 1996 Farm Bill (Sec. 901–905) authorized the Secretary of Agriculture to issue guidelines for the regulation of commercial transportation of equines for slaughter within the United States. However, there are limited scientific data available to support appropriate transportation regulations for slaughter horses.

Previous equine transport studies have examined behavioral and autonomic responses of orientation during travel (Smith et al., 1994; Waran et al., 1996), neuroendocrine responses (Baucus et al., 1990; Ferlazzo et al., 1993), or immunological changes (Anderson et al., 1985; Crisman et al., 1992) primarily in race or show horses. One recent study examined water deprivation during long-term transportation in university-owned, tame horses traveling in an open-type trailer (Friend et al., 1998). Horses may respond differently to transportation stress depending on their previous experience, health status, social interactions, environmental conditions, breed, age, and gender.

Although it is difficult to obtain knowledge about the previous handling and management of individual horses procured for slaughter, an applied study under commercial conditions will provide information to characterize the type of horses used in the system. Additionally, data collected on selected physiological responses and number of injuries incurred during transport for comparison of trailer designs, floor space per horse, and trip duration will assist in drafting science-based guidelines. Thus, our objectives for the study were to characterize slaughter horse candidates and to examine physiological responses and number of injuries due to commercial transport under summer environmental conditions.

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Materials and Methods

Horses and Basic Study Design

All phases of the study were approved by the University of California Animal Care and Use Committee. Horses (n = 306) were identified as slaughter horses by cooperating brokers and transport drivers. A total of nine tractor-trailer loads of horses were assembled in the states of Pennsylvania, California, Kentucky, and Texas. All loads were transported to a USDA inspected processing plant in Fort Worth, Texas. Horses were assembled in groups prior to loading for a minimum of 1 h, but the interval varied up to several days depending on the procurement procedure. The commercial trailers used were of two basic designs, either a single, straight-deck (**SD**, n = 5) or two-tiered "pot-belly" livestock trailer (**PB**, n = 4). The length and width of the SD trailers was approximately 15.2 m and 2.6 m, respectively, with ceiling heights between 2.1 and 2.7 m. Three or four compartments with swinging gates were available to segregate groups of horses. Rubber padding lined the sides of the trailers from the floor to an approximate height of 1.2 m. The length and width of PB trailers was 14.4 and 2.5 m, respectively. The PB trailers were divided into four compartments with the two middle sections stacked to form two tiers, each with a ceiling height of 1.7 to 1.8 m. The front and back sections were placed over the two front and two back wheel axles and the ceiling height was 2.1 m. There was an attempt to match the two trailer designs over routes of similar duration. The number of horses per load, the available floor area, distance traveled, and time between loading and unloading were recorded. No feed or water was available during transit, but they were available before loading.

Temperature and relative humidity inside the trailer during transport were monitored continually with microcomputer data logger units (Model WTA32 and SRHA32, respectively; Onset Instruments, Pocasset, ME) placed near the center of the trailer. The concentrations of ammonia and carbon monoxide gas were quantified using dosimeter gas detection tubes (Gastec, Yokohama, Japan) placed near the center of the trailer during transit.

Prior to loading into trailers, numbered tags were adhered to the hip of each horse as identification. The breed, gender, height at withers, and age as estimated by examination of teeth were documented. Body condition scores (**BCS**) were assigned using a scale (1 to 9) with maximum score of 9 (obese) (Henneke et al., 1983). Prior to loading, the location of any abrasions or lacerations were documented on a diagram profiling an outline of a horse. Upon arrival, any additional injuries were documented; thus, injuries sustained during loading, transportation, and unloading were deduced.

Collection of Physiological Data

Similar data collection procedures were used to collect data within 4 h (pre-samples) before transport and after arrival (post-samples) at the slaughter facility. Horses were either gently restrained with a halter or slowly processed through a chute to collect samples prior to transport. All horses were processed through a chute at the slaughter facility. Rectal temperature and body weight (if scales were available) were recorded. Blood samples (20 mL) were collected in evacuated glass tubes via needle puncture of the jugular vein. Blood samples to be analyzed for concentrations of cortisol, lactate, and total protein were immediately placed on ice and allowed to clot. Serum was obtained and frozen at −70°C. Samples intended for complete blood cell counts were collected in tubes containing EDTA.

Laboratory Analyses. Cortisol was determined in duplication with the microplate enzyme immunoassay technique (Munro and Stabenfedlt, 1984). Intra- and interassay CV were less than 10 and 15%, respectively. Lactate concentrations were determined using an autoanalyzer (YSI 2300 STAT Plus, Yellow Springs, OH). White blood cell counts (**WBC**) and hematocrit (**hct**) were determined using an automated cell counter (System 9000, Serono-Baker Diagnostics, Allentown, PA). Whole blood was used to determine WBC differential counts using standard laboratory staining techniques (Heckner et al., 1988); the neutrophil:lymphocyte ratio (**N:L**) was calculated. Total serum protein concentration was determined with commercially available enzymatic colorimetric assays (Procedure 541, Sigma Diagnostics, St. Louis, MO).

Statistical Analyses

Data were analyzed with ANOVA and analysis of covariance (ANOCOVA) methods using the GLM procedure (SAS, 1989). The factors considered in these analyses were a horse's age, gender, and breed, and the load in which it was transported. The analyses were run alternately as main effects models and as factorial models, to examine possible interactions among these factors. Whenever load, or an interaction involving load, was significant, contrast statistics were constructed to compare loads with respect to the trailer type (SD or PB), the duration of the trips (short, < 6 h; medium, 16 to 23 h; or long, 27 to 30 h), and the loading density represented by the available floor area per horse (high, 1.40 to 1.54 m^2/horse [**HFA**], or low, 1.14 to 1.31 m^2/horse [**LFA**]. Data were analyzed in raw form or following log transformation, as appropriate to satisfy the model assumptions of normality and constant residual variance. Significance is claimed whenever $P < .05$.

Results

The specific characteristics of each of the loads are given in Table 1. Distances from the point of loading to the slaughter plant ranged from 596 to 2,496 km, and duration of the trips ranged from 5 h 45 min to 30 h. The floor space per horse ranged from 1.14 to 1.54 m^2/horse. All trips to the slaughter plant occurred dur-

Table 1. Load characteristics including trailer design, length of travel, duration of trip, number of horses per load, floor area per horse, minimum and maximum temperature, minimum and maximum relative humidity inside the trailer, and time of the initiation of blood collection for the pre- and post-transport samples

Load	Trailer type[a]	km	Duration h:min	No. of horses per load	Floor area, m^2/horse	Temperature, °C		Humidity, %		Sampling time[b]	
						Min.	Max.	Min.	Max.	Pre	Post
1	SD	596	5:45	38	1.14	23	34	40	74	0500	1200
2	PB	596	5:45	39	1.54	22	31	32	85	1800	0300
3	SD	1,433	16:15	29	1.40	19	29	38	83	1000	0600
4	SD	1,433	17:30	31	1.31	20	25	NR[d]	NR	1300	0900
5	PB	2,496	28:00	44	1.23	3	31	14	71	1900	0100
6	PB	2,496	27:00	44	1.31	−2	33	8	88	1200	1800
7[c]	SD	2,335	30:00	15	1.24	13	39	21	100	0100	1000
8	SD	2,225	23:00	25	1.44	21	41	34	83	1800	2200
9	PB	2,415	30:00	41	1.43	15	38	31	100	1800	0300

[a]SD = straight-deck trailer; PB = two-tiered "pot-belly" trailer.
[b]Central Standard Time.
[c]Study horses constituted only a portion of the entire load.
[d]Data were not recorded due to equipment failure.

ing the months of July, August, and September. The maximum environmental temperature range for all loads was 25 to 41°C, and minimum temperatures ranged between −2 and 22°C. The range of minimum and maximum relative humidity inside all trailers was 8 to 40% and 71 to 100%, respectively. Eight of the nine loads were successful in recording readings on the gas diffusion sticks. The ammonia and carbon monoxide gas concentrations inside the trailer were 3.1 ± 2.0 and 2.4 ± 1.5 ppm, respectively. The ammonia gas concentration (5.2 ± 1.5 ppm) recorded in the three trailers bedded with either sawdust or straw on the floor was higher ($P < .05$) than that in the five trailers without bedding (1.8 ± .9 ppm).

Characteristics of Slaughter Horses

The mean age recorded (n = 285) was 11.4 ± .4 yr, with a range of 1 to 30 yr (Table 2). The percentage of horses with ages from 1 to 5, 6 to 15, 16 to 20, and 20 to 30 yr was 28, 39, 23, and 10%, respectively. Only 8% of the horses were yearlings or 2-yr of age. The breed of horse was recorded for 279 horses; 48% were classified as Quarter Horses, 18% as Thoroughbreds, 14% as having Morgan lineage, which included the Saddlebred and Standardbred breeds, 9% as Arabians, 6% as Appaloosas, and the remaining 5% as pony or draft breeds, mustangs, or mules. Mares constituted 50% of the slaughter horses, and geldings 47%. Fewer than 3% of the horses (n = 9) were stallions.

Table 2. Characteristics of slaughter horse candidates

Characteristic	n	Mean	SE	Range
Age, yr	285	11.4	.4	1–30
Weight, kg	301	432	3.3	284–807
BCS[a]	295	5.6	.08	2.0–9.0
Height, m	286	1.50	.01	1.12–1.68

[a]Body condition score (Henneke et al., 1983).

The BCS ranged from 2 to 9 with a mean score of 5.6 ± .08 for all horses. The mean BCS of the horses in the nine loads ranged from 5.3 to 6.4; horses with higher mean BCS originated from an equine feedlot. No difference ($P > .05$) in BCS was found between the trailer designs, trip duration, or floor area. Body weight (n = 301) documented at the slaughter facility following transport was 432 ± 3.3 kg, with a range of 284 to 806 kg. Height at the horse's withers ranged from 1.11 to 1.68 m, with a mean of 1.50 ± .01 m.

Physiological Comparisons

Age effects were not shown for any of the physiological variables evaluated, so this effect was suppressed in additional analyses. Breed effects were shown for body weight; breed and gender effects were shown for the hct values as described below in detail. Tables 3, 4, and 5 present pre- and post-transport data of the physiological indices for the comparisons of trailer design, trip duration, and floor area per horse, respectively.

Body Weight. Scales were only available at six (Loads 1, 2, 5, 6, 7, and 8) of the nine loading sites to obtain preshipment body weights. All horses were weighed upon arrival at the slaughter facility. The mean body weight for horses (n = 205) weighed prior to loading and at arrival was 441 ± 4 and 424 ± 4 kg, respectively, showing a 4% loss in body weight. The largest weight loss of 8% was recorded for Load 7, which was transported the longest (30 h). Breed effects ($P = .0003$) were shown for body weight prior to loading; Arabian horses (395 ± 10 kg) had lower body weights than Thoroughbreds (462 ± 8 kg), Quarter Horses (448 ± 5 kg), and Standardbreds (453 ± 7 kg). No difference ($P < .05$) in weight loss was demonstrated between SD and PB trailers, or LFA and HFA. Weight loss during short duration trips (12 ± 6 kg) was less ($P < .05$) than that during medium (24 ± 10 kg) or long (20 ± 6 kg) duration trips.

Table 3. Comparison of pre- and post-transit physiological data collected from horses transported on straight-deck or two-tiered "pot-belly" trailers; response values are the difference between the pre- and post-transit values

Variable	Straight-deck trailer				Pot-belly trailer			
	Pre	Post	Response	SE	Pre	Post	Response	SE
Temperature, °C	37.4	38.5	1.1[a]	.1	37.8	38.1	.3[b]	.1
WBC, × $10^3/\mu L$	11.2	13.4	2.1[a]	.3	11.5	12.4	.9[b]	.3
Neutrophil:lymphocyte ratio	2.9	4.7	1.8[a]	.3	3.2	4.2	.9[b]	.3
Cortisol, ng/mL	48	67	18[a]	2	44	50	6[b]	2
Hematocrit, %	43	45	2[a]	.6	43	47	4[a]	.5
Total protein, g/dL	8.5	8.9	.4[a]	.1	8.4	8.8	.4[a]	.1
Lactate, mg/dL	37	53	16[a]	2	33	49	16[a]	1
Weight, kg	447	425	22[a]	6	438	422	16[a]	5

[a,b]Means within a row with different superscripts differ ($P < .05$).

Rectal Temperature. Rectal temperature of 282 of the 306 horses shipped was measured prior to loading and upon arrival. Prior to shipping, rectal temperature was 37.7 ± .1°C, it increased to 38.2 ± .1°C following transport, but it remained in the normal range of 37.5 to 38.5°C for resting horses (Fraser, 1986). This increase was greater ($P < .05$) in the SD (.5 ± .2°C) than in the PB (.25 ± .05°C) group. Rectal temperature of horses shipped an intermediate duration (1.1 ± .1°C) increased ($P < .05$) more than rectal temperature of horses shipped for a short (.5 ± .1°C) or long (.3 ± .1°C) duration. There was no difference ($P > .05$) in rectal temperature response due to floor area per horse.

White Blood Cell Counts. The WBC counts increased slightly (11.3 ± .2 to 14.3 ± .3 × $10^3/\mu L$) with transportation, but these remained within the reference range (5.5 to 14.3 × $10^3/\mu L$) for healthy horses (Jain, 1993). The WBC counts of horses traveling in SD trailers were greater ($P < .05$) than those of horses shipped in PB trailers (2.1 ± .3 and .9 ± .3 × $10^3/\mu L$, respectively). Horses traveling longer than 27 h had greater ($P < .05$) elevations in WBC (1.8 ± .3 × $10^3/\mu L$) than those shipped for short (.7 ± .4 × $10^3/\mu L$) durations. White blood cell count elevations were greater ($P < .05$) in horses in trailers with LFA (2.0 ± .3 × $10^3/\mu L$) than in those in trailers with HFA (.6 ± .3 × $10^3/\mu L$).

Neutrophil:Lymphocyte Ratio. Transportation increased the mean N:L (n = 296) from 3.1 ± .1 to 4.4 ± .2, which was elevated over the normal range established for healthy horses of .8 to 2.8 (Morris and Large, 1990). Following transportation, horses traveling in SD (1.8 ± .3) showed ($P < .05$) a greater increase in N:L than horses shipped in PB (.9 ± .3). Horses shipped for a medium duration had a smaller increase ($P < .05$) in N:L (.6 ± .3) than horses shipped for short (1.6 ± .4) or long (1.6 ± .3) durations. Following transport, horses in trailers with LFA showed greater increases ($P < .05$) in N:L values than horses in trailers with HFA (1.9 ± .2 and .6 ± .3, respectively).

Cortisol. Using venous blood samples collected from indwelling jugular catheters, cortisol concentration in resting horses has been documented at 45 ± 1 ng/mL, with a circadian rhythm exhibiting peak concentrations (65 ± 2 ng/mL) in the morning hours (0600 to 1200) and low concentrations (25 ± 2 ng/mL) in the evening (1300 to 1600) (Stull and Rodiek, 1988). Transportation elevated cortisol concentrations in all horses from 46 ± 1 to 58 ± 2 ng/mL. Horses traveling in SD trailers showed greater elevation ($P < .05$) in cortisol concentration than horses in PB trailers (18 ± 2 and 6 ± 2 ng/mL, respectively). No difference ($P > .05$) in cortisol response was

Table 4. Comparison of pre- and post-transit physiological data collected from horses in trailers with trips of short, medium, or long duration; response values are the difference between the pre- and post-transit values

Variable	Short, < 6 h				Medium, 16–23 h				Long, 27–30 h			
	Pre	Post	Response	SE	Pre	Post	Response	SE	Pre	Post	Response	SE
Temperature, °C	38.0	38.6	.6[a]	.1	37.3	38.4	1.1[b]	.1	37.7	38.1	.3[a]	.1
White blood cells, × $10^3/\mu L$	12.7	13.5	.8[a]	.4	10.5	11.9	1.5[ab]	.4	11.1	13.0	1.8[b]	.3
Neutrophil:lymphocyte ratio	2.4	4.0	1.6[a]	.4	3.3	3.8	.6[b]	.3	3.3	4.9	1.6[a]	.3
Cortisol, ng/mL	51	65	14[a]	3	45	62	17[a]	3	44	51	7[a]	2
Hematocrit, %	44	45	3[a]	.5	42	45	4[b]	.8	43	47	5[b]	.6
Total protein, g/dL	8.4	8.1	−.3[a]	.1	8.9	9.4	.5[b]	.1	8.2	8.9	.7[c]	.1
Lactate, mg/dL	42	49	6[a]	2	36	54	18[b]	2	30	49	18[b]	1
Weight, kg	434	422	12[a]	6	455	431	24[b]	10	443	423	20[b]	6

[a,b,c]Means within a row with different superscripts differ ($P < .05$).

Table 5. Comparison of pre- and post-transit physiological data collected from horses in trailers with low (1.14 to 1.31 m^2/horse) and high floor area (1.40 to 1.54 m^2/horse); response values are the difference between the pre- and post-transit values

Variable	Low floor area				High floor area			
	Pre	Post	Response	SE	Pre	Post	Response	SE
Temperature, °C	37.8	38.4	.6[a]	.1	37.5	38.1	.6[a]	.1
White blood cells, × $10^3/\mu L$	11.4	13.6	2.1[a]	.3	11.2	11.8	.6[b]	.3
Neutrophil:lymphocyte ratio	2.8	4.7	1.9[a]	.2	3.4	3.9	.6[b]	.3
Cortisol, ng/mL	50	62	12[a]	2	40	51	11[a]	2
Hematocrit, %	44	46	2[a]	.5	42	46	4[b]	.6
Total protein, g/dL	8.2	8.7	.6[a]	.1	8.8	9.1	.2[b]	.1
Lactate, mg/dL	34	49	15[a]	1	36	52	16[a]	2
Weight, kg	441	423	18[a]	5	440	424	16[a]	8

[a,b]Means within a row with different superscripts differ ($P < .05$).

shown for duration of trip or the floor area allowed per horse.

Hematocrit. Hematocrit values in all horses prior to loading and upon arrival at the slaughter facility were 43.1 ± .4 and 46.2 ± .4%, respectively, which were within the normal range of 32 to 53% (Jain, 1993). Breed effects ($P = .0003$) were shown for the pre-transport hct values; Appaloosa (38 ± 2%) exhibited lower values than the Thoroughbred (46 ± 1%), Quarter Horse (43 ± 1%), Standardbred (41 ± 1%), or Arabian breeds (46 ± 1%). A gender effect ($P = .0001$) was shown in hct values in samples collected prior to loading. The hct values in mares (45 ± 1%) and stallions (45 ± 2%) were greater than in geldings (41 ± 1%), but all three gender categories were within the normal reference range. No difference ($P > .05$) was shown between SD and PB trailers. The increase in hct values of horses experiencing the trips of shorter duration (3 ± .5%) were smaller ($P < .05$) than those in horses that were shipped for medium (3.9 ± .8%) or long (4.5 ± .6%) durations. The increase during transportation in hct of 2.5 ± .5% in LFA horses was less than ($P < .05$) that in HFA horses (4.2 ± .6%).

Total Protein. The total protein concentration (8.4 ± .1 g/dL) of all horses prior to transport was slightly elevated over reference range values of 5.2 to 7.9 g/dL (Kaneko et al., 1997). Following transportation, total protein concentration increased to 8.9 ± .1 g/dL. No difference ($P > .05$) was found between horses transported in different types of trailers. The difference between pre- and post-transportation total protein levels was significant ($P < .05$) for all three durations; horses transported for long or medium durations showed an increase in protein values (.7 ± .1 and .5 ± .1 g/dL, respectively), and those transported short durations showed a slight decrease (−.3 ± .1 g/dL) during transit. Floor area also affected total protein change during transportation, with a greater increase in LFA (.6 ± .1 g/dL) horses than in HFA ($P < .05$) horses (.2 ± .1 g/dL).

Lactate. The mean pre-transit concentration of lactate (34 ± 1 mg/dL) for all horses was elevated over established resting values of approximately 5 to 10 mg/dL (Stull and Rodiek, 1995). Transportation further elevated the concentration to 50.3 ± 1.0 mg/dL. There were no differences ($P > .05$) when trailer design or floor area per horse were compared. Trips of short duration increased lactate concentration (6 ± 2 mg/dL) less than ($P < .05$) trips of medium (18 ± 2 mg/dL) or long (18 ± 1 mg/dL) duration.

Injury Data

A total of 60 horses (19.6%, n = 306) sustained 81 injuries during transport (Table 6). The injuries consisted of either single or multiple abrasions and(or) lacerations. The percentage of horses traveling in PB (n = 168) that sustained injuries was 29.2%, as compared ($P < .05$) to 8.0% of the horses in SD (n = 138). The face/head was the most likely area for injury; 47 (58%) of the injuries reported occurred in this area. The other areas of the body with injuries were the legs, hips, and neck/shoulder/back, with 18 (22%), 12 (15%), and 4 (5%) injuries, respectively. Of the 47 face/head injuries, 38 occurred in 49 injured horses (78%) traveling in PB, and 9 occurred in 11 injured horses (77%) in SD. Of the 168 horses transported in PB trailers, the percentage of horses injured in the front, middle two tiers, or back compartments was 28.6, 31.0, and 16.7%, respectively. The percentage of horses injured in the HFA group (29%) was greater ($P < .05$) than in the LFA (12%) group. Additionally, 33% of the horses were injured in trips of long duration, and this was greater than ($P < .05$) the 9 and 8% of horses injured in trips of medium or short duration, respectively.

No horse died while in transit to the slaughter plant. However, one mare (Load 7) was removed from the load approximately 12 h after embarking. The 20-yr-old Paint mare died 2 d later. Prior to transport, she had a normal rectal temperature (37.9°C) and WBC count (8.8 × $10^3/\mu L$) and a BCS of 4, but an observable facial paralysis was noted prior to loading.

Discussion

Cold and heat stress can affect younger and sick animals much more severely than mature, healthy horses.

Table 6. The number of horses injured per load and the location of the injury

Body area	Load								
	1	2	3	4	5	6	7	8	9
Legs	—	1	—	1	2	—	1	1	12
Face/head	1	4	2	3	11	2	1	2	21
Hips/loin	—	—	—	5	—	—	—	—	7
Neck/shoulder/back	—	—	—	3	—	—	—	—	1
No. of horses injured[a]	1	5	2	3	13	2	2	3	29

[a]Injuries may have occurred in more than one area per horse.

The thermoneutral zone of horses is estimated to be between −1 and 24°C (Young and Coote, 1973). This optimal thermal environment promotes maximum performance and the least amount of stress in the animal (Curtis, 1983). As air temperature falls below −1°C, known as the lower critical temperature (**LCT**), horses must divert food energy from production and growth to produce additional metabolic heat and maintain body temperature. Several of the loads experienced temperatures near the LCT as the vehicles passed through desert conditions at night, but the duration was short and probably did not affect the physiology of the horses. The upper critical temperature (**UCT**) is approximately 24 to 32°C and is reached when horses cannot dissipate enough metabolic heat to the environment to maintain homeothermy (Curtis, 1983). This heat is then dissipated through sweating and respiration mechanisms. Relative humidity above 50% further exacerbates the ability of horses to dissipate heat. All loads exceeded the UCT, and humidity was recorded at values greater than 50%. Thus, horses experienced heat stress conditions while in transit, as would be expected under summer conditions.

Long-term exposure to toxic gas can predispose a horse to respiratory and eye irritations. No permissible levels of these gases have been established for horses. The permissible limit for an 8-h exposure for humans to ammonia and carbon monoxide is 25 and 50 ppm, respectively (ACGIH, 1989). The ammonia gas ranged from .7 to 6.7 ppm. Interestingly, the use of absorbent floor bedding with either sawdust or straw seems to increase the concentration of ammonia gas. This increase in ammonia gas concentration may partially be due to the accumulation of excreta resulting from the longer duration of the trip. None of the loads recorded gas levels for carbon monoxide near the permissible level (50 ppm) for humans, indicating that the exhaust fumes of the truck did not present a health hazard for the horses on board.

These slaughter horses reflected the general population of horses in the United States. Life expectancy in domestic horses is 20 yr or more, and maturity is considered to be attained between 3 and 4 yr. Over 90% of the study horses were mature and fewer than 10% were over 20 yr of age. The average horse was 11.4 yr old. Body condition scoring systems are used to evaluate the stored body fat in horses by visual appraisal and palpable fat cover in six areas of the body (Henneke et al., 1983). A BCS of 1 indicates extreme emaciation and 9 extreme fatness. The mean BCS of 5.6 ± .08 in this study indicated that the horses were neither emaciated nor extremely fat, but were considered moderately fleshy.

Two-thirds of the horses were either Quarter Horse or Thoroughbred breeds, and the mean weight of the horses was 432 ± 3.3 kg. The prevalence of these two breeds may represent the relative size of the breed registries, because the Quarter Horse and Thoroughbred breeds account for approximately 50% of the horses in the United States (NAHMS, 1998), or may be due to the buyers' selection of horses with similar conformation and weight to supply retail meat products of similar portions. No preference was shown for either mares (50%) or geldings (46%), and the study population contained fewer than 3% stallions. Stallions were managed during transportation by being tied by a halter and lead rope, placed unrestrained in compartments with only geldings, or integrated with a group of mares prior to loading and hauled with the same herdmates.

Physiological Responses to and Injuries from Transport

Rectal temperature and WBC were assessed as general indicators of morbidity and the ability of the horses to respond to heat stress during transit. The body weight, hct, and total protein indices were examined as dehydration measurements, becasue water (and feed) were not available during transit and environmental conditions were considered hot and humid. The stress variables of cortisol and N:L ratio have been previously documented as indicator of stress in other agricultural species (Friend et al., 1985; McFarlane et al., 1988; Stull and McDonough, 1994); N:L may be a more reliable indicator of stress than cortisol concentrations (Gross and Siegel, 1983). This may be partially attributed to the short half-life of cortisol of 1.5 h in resting horses that decreases during exercise to approximately 1 h (Lassourd et al., 1996). Additionally, cortisol concentrations in resting horses exhibit a daily circadian rhythm (Stull and Rodiek, 1988). This factor was not considered in the analyses because the distance of travel and time of departure and arrival was determined by the commercial transporters. Lactate produced during anaerobic metabolism was evaluated as an indicator of muscle fatigue.

Rectal temperature, WBC, and hct mean values were within reference range for a normal horse both prior to and following transport. The stress indicators of cortisol and N:L were slightly elevated above the reference range prior to transport and may have been due to the novel environment at the point of assembly, herd interactions, or other influences. The response to transportation further elevated stress variables, as expected. Total protein concentration prior to transportation showed a slight elevation over reference values, indicating mild dehydration, whereas hct was within normal range prior to and following transport. Elevated lactate concentration above established resting levels prior to transport may have been due to ambulatory activity at the point of assembly. Lactate concentration increased slightly following transport. However, compared to lactate concentrations greater than 200 mg/dL in horses following racing (Snow et al., 1983), this increase following transport is indicative of minimal anaerobic muscular fatigue.

Trailer Design. Two major types of trailers with capacity of over 15 horses are currently used to transport horses to slaughter. The PB trailers in this study carried up to 44 mature horses, and the SD hauled 38 horses, showing the economic advantage of increased capacity with the PB trailer. No difference was shown between PB and SD trailers for weight loss or increases in hct, total protein, or lactate responses to transportation. Thus, neither trailer design is advantageous in minimizing dehydration. The elevation in lactate was minimal, so muscle fatigue by anaerobic metabolism was not a limiting factor. Rectal temperature, WBC, cortisol, and N:L showed greater responses in horses shipped via SD trailers than in those shipped in PB trailers. Rectal temperature may reflect environmental heat stress, which ventilation assists in alleviating. The rubber padding lining the interior walls of the SD trailers limited the ventilation capacity and may have added to the heat stress. The WBC, cortisol, and N:L data indicate that the horses traveling in PB, as compared to SD, trailers experienced less stress and fewer immunological changes, supporting the use of PB trailers. However, there is the possibility, although it was not measured, that a specific design feature other than decking may have contributed to the difference.

Sustained injures approximately 3.5 times greater in PB (29.2%) than in SD (8.0%) trailers may have occurred during loading, transport, or unloading. The door width of SD trailers is the same as entire width of the trailer, whereas doors in the PB trailer are only approximately one-third the width of the trailer. Because the head and face were the most prevalent area for injury, the width of the door opening may be a factor in the greater injury rate in the PB horses. Horses loading into PB trailers used ramps from the door located at the back of the trailer to enter the appropriate compartment, and this may have contributed to the increase in injury rate of horses in the front (28.6%) and middle (31.0%) as compared to the rear (16.7%) compartment. However, head injuries do not represent an economic loss to meat quality or quantity from the carcass.

Duration of Trip. The duration of travel ranged from 5 h 45 min to 30 h. The cooperating slaughter facility was located in Fort Worth, Texas, and most travel from any region of the United States to Texas can be completed in less than 30 h. Because horses were not fed or watered during the journeys, dehydration and muscle fatigue were considered probable. Lactate, an indicator of fatigue, showed greater elevation over pre-transit values in horses following the long (27 to 20 h) and medium (16 to 23 h) trips compared to short (< 6 h) trips. Greater weight loss in horses shipped for long and medium durations than in those shipped short durations may be due to dehydration and fecal and urine losses. Hematocrit and total protein concentration showed similar results, with incremental increases as duration increased. The mean post-transport values for hct of horses transported long, medium, or short distances did not exceed the normal reference range (Jain, 1993). However, the mean pre-transit concentration for total protein of 8.4 g/dL in all horses was slightly elevated above the reference range (Kaneko et al., 1997), and all three duration groups continued to exceed the reference range following transport, indicating that the horses were mildly dehydrated prior to loading and that this was exacerbated during transport. All horses had access to water in the interval between sample collection and loading, and this may account for the small decrease in total protein in the short duration group. These trends in dehydration indices support a previous study using tame horses undergoing road transport that showed hct values remained within reference range but total plasma protein concentration continued to rise during the 24-h transport period (Friend et al., 1998).

The mean WBC numbers were within normal reference range both before and after transit in horses shipped for long, medium, or short durations; however, the response for the long duration group was greater ($P < .05$) than that for the medium or short duration groups, indicating an immunological impact of transport over 27 h. The cortisol increase showed no difference due to the duration of transport, perhaps due to its short half-life of 1 to 1.5 h in horses (Lassourd et al., 1996). The response of the N:L ratio was greatest in long and short duration groups. This elevation may be due to the stress of loading in the horses shipped a short distance and the fatigue in those shipped the longest distance. Rectal temperature increased in the medium duration group compared to the short or long duration groups. However, the pre and post-transit rectal temperature for all three groups was within the normal range of 37.5 to 38.5°C.

Fatigue, dehydration, and stress may contribute to the threefold increase in injuries in horses traveling 27 to 30 h compared with those that traveled for a shorter duration. Additionally, the horses of the long duration group had an extended period of time to interact with other horses in the confined area, thus sustaining additional injuries.

Floor Area. The amount of space per animal has been studied for cattle (Eldridge et al., 1988; Tarrant et al., 1992) to identify the stocking density that provided maximum comfort and minimum injuries (Grandin, 1981, 1997). These data are limited for horses, because many recreational horses are transported individually in trailers or vans. Generally, an individual stall in a trailer or van is .76 to .91 m in width, 1.8 to 2.1 m in length, and provides 1.40 to 1.95 m^2/horse. The trailers with HFA (1.40 to 1.54 m^2/horse) provided floor areas similar to those in trailers with individual stalls, whereas the LFA (1.14 to 1.31 m^2/horse) trailers provided less floor area. No difference due to floor area was shown for weight loss or increase in rectal temperature, cortisol, or lactate following transport. The increase due to transport of WBC, N:L, and total protein values was less in the HFA than in the LFA group. The dehydration indicators, hct and total protein, showed conflicting responses. The LFA group showed less increase in hct change than the HFA, but all mean values were within the reference range (32 to 53%) (Jain, 1993). With the exception of the hct data, the physiologic indices exhibited significantly smaller responses in HFA than in LFA in pre- and post-transit samples. These data support the recommendation of providing not less than 1.40 to 1.54 m^2/horse during travel. This may depend on the weight, conformation, and size of the horse. However, there was a twofold increase in the percentage injured in the HFA compared to the LFA groups. Thus, providing more space per horse may increase the percentage of injured horses, but the physiological responses to transportation stress are generally less.

Implications

Slaughter candidates are middle-aged, mature horses, usually of Quarter Horse or Thoroughbred breeding, in moderately fleshy body condition. The design of trailers for transporting the horses unequivocally affects their physiological responses and frequency of injuries. Thus trailer designs should be improved. As the duration of the transport incrases, especially beyond 27 h in the summer conditions, muscle fatigue and dehydration become major physiological concerns, and the physiological responses should clearly be evaluated during each season of the year. Physiological data indicate the floor area should be approximately 1.40 to 1.54 m^2/horse), but the number of injuries seems considerably less in trailers with floor areas from 1.14 to 1.31 m^2/horse. This information is important for drafting regulations appropriate for transporting horses in commercial markets in the United States.

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Exhibit 24

JOURNAL OF ANIMAL SCIENCE
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A review of recent research on the transportation of horses

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A review of recent research on the transportation of horses[1]

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ABSTRACT: Horses are transported more frequently than any other type of livestock. Most of our understanding about horse transport has been based on custom and conjecture, but some recent studies have greatly increased our knowledge. Although some horses adapt to transport much better than others, transport is generally associated with lower reproductive rates, increased disease incidence, a temporary reduction in athletic performance, and the alteration of many other physiological traits that are indicative of stress. Horses show marked dehydration after 24 h and extreme dehydration after 28 h of transport in hot and humid conditions when there is little nighttime cooling. Watering horses onboard trailers alleviates dehydration, but fatigue can become extreme after 28 h of transport. Orientation either toward or away or diagonally from the direction of travel does not seem to significantly affect a horse's ability to maintain its balance. Allowing horses the ability to raise and lower their heads or hind quarters and to take at least one step in any direction seems to be the most important factor in their compensating for changes in inertial forces. Loose horses that are transported in groups at high densities (e.g., slaughter horses) do not hold each other up, but rather inhibit each other's attempts to compensate for changes in inertial forces. High density also increases injuries and inhibits the ability of horses to stand after they fall because other horses standover the downed horses. High density also prevents submissive horses from moving away from aggressive horses, resulting in repeated aggression. However, reducing density greatly increases transportation costs. Recent surveys have also shown that many of the worst injuries seen on horses arriving at slaughter plants originated before their transport to slaughter. Horse trailer design has undergone rapid change with the rise in popularity of slant-load aluminum trailers. A major challenge for the future is determining which of the myriad of trailer designs, suspension systems, and building materials available to horse owners and shippers are preferable.

Key Words: Behavior, Horses, Stress, Transport

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Introduction

Horses have been transported by boats for at least 3,500 yr (Cregier, 1982). There are many later accounts of horses being transported by horse-drawn wagons in many parts of the world. Transport by rail was very common during the middle to late 1800s but gave way to transport by trucks after World War II following the building of the interstate highway system and the development of more reliable trucks and trailers. The 1960s and 1970s became known as the "trailer age" for horse transport (Cregier, 1982), and the 1980s and 1990s are sometimes referred to as the "air age" of horse transport due to the frequent use of large cargo aircraft.

There are an estimated 6.9 million horses in the United States, and their owners spend an estimated $2.17 billion per year transporting those horses (American Horse Council, 1996). Although national estimates are not available, the estimated inventory value for all horse trailers in Texas is $2.16 billion (Gibbs et al., 1998). The typical Texas horse owner transports 2.5 horses (Gibbs et al., 1997) on 24 trips per year averaging 380 km per trip (Gibbs et al., 1998).

There were also 72,120 horses transported to slaughter in 1998 (USDA, 1999). Continuous transport of slaughter horses for 30 h is common (Stull, 1999), and some trips last 36 h or longer. Public concern over the transport of horses going to slaughter was the driving force behind the Safe Commercial Transportation of Equine to Slaughter Act (§ 901–905) that was passed as part of the 1996 U.S. Farm Bill. In an effort to provide a basis for formulating regulations, the

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Transport-Related Stress

Transported horses can be subjected to a wide range of potential stressors, including isolation from herdmates, forced close proximity to unfamiliar or aggressive horses, novel or threatening surroundings, exposure to new pathogens, restraint of normal activity patterns, forced adoption of an abnormal posture, extremes in temperature, water and feed deprivation, and blowing dust and particulate matter. Transport has long been known to be associated with morbidity in horses. The term "shipping fever" was originally used for the pneumonia that commonly developed in shipped horses during World War I (King, 1992). However, with proper training and experience, many horses readily adjust to being transported in a wide range of vehicles.

Transport in general is a stressor in horses that can, for example, lead to the reactivation of salmonellosis (Owen et al., 1983), elevate heart rate (Clark et al., 1993; Smith et al., 1994b; Warren and Cuddeford, 1995), cause hormonal and plasma ascorbic acid (Baucus et al., 1990a) and cortisol (Clark et al., 1993; Friend et al., 1998; Friend, 2000; Stull and Rodiek, 2000) changes indicative of acute stress, and cause dehydration (Friend et al., 1998; Friend, 2000; Stull and Rodiek, 2000). However, short-term transport has been reported to affect neither the incidence of embryonal death (Baucus et al., 1990a) nor estrus behavior, ovulation, the estrous cycle, or estradiol and LH surges (Baucus et al., 1990b). McGee (1969), however, recommended against long-distance transport of mares in late gestation because transport could lead to the rupture of the uterine artery and the subsequent death of the mare. When interpreting these studies, it is important to realize that most researchers have used relatively tame horses that are experienced at being transported or readily accept being transported.

When comparing the percentage in change of some serum enzymes and hematochemical concentrations, Codazza et al. (1974) found that, in general, transportation (300 km) has a different effect on muscular function than exercise. However, they concluded that horses should still be transported at least 48 h before a race to permit the hematochemical values to return to normal. When horses were transported only 90 km, transport had no significant effect on serum concentrations of sodium, potassium, chloride, glucose, or total protein or on submaximal exercise following transport (Beaunoyer and Chapman, 1987).

Transport can also result in physical injury to horses. This can occur through collisions involving the transport vehicle, sudden stops, fatigue, loss of balance, or attacks from aggressive horses. However, Grandin et al. (1999) reported that the majority of severe welfare-threatening injuries observed at slaughter houses were sustained before transport.

Long-Distance Transport

Horse owners and shippers are very concerned about the maximum duration that horses may be safely transported. Provided that horses are healthy and in good physical condition, the maximum duration that horses may be transported is a function of dehydration (weight loss) and fatigue. Data are available from which useful inferences can be drawn regarding the progression of dehydration under different climatic conditions. The database from which to draw inferences regarding fatigue is much more limited.

Weight Loss and Dehydration

Recent research has shown that the rate of weight loss (dehydration) of horses in pens can approximate the dehydration rate of horses being transported under similar climatic conditions (Friend et al., 1998; Friend, 2000). There has been a relatively large number of studies that have deprived stalled horses of water for varying durations to establish the relationship of certain measurements to progressive dehydration. Three of the more notable studies (Tasker, 1967; Carlson, 1979; Sneddon et al., 1991) are summarized in Table 1. The relationship between rate of dehydration and temperature can be seen by comparing those three studies. Some of Tasker's horses went as long as 8 d without water and feed and lost only 10% of their body weight. Tasker described a "tucked-up" appearance of the abdomen on the 5th d of water deprivation and took horses off the study when it began to appear that they were severely dehydrated. It is useful to note that horses that were offered hay and other dry feed would have dehydrated at a much greater rate. All of the horses used in the studies reported in Table 1 recovered with little adverse effect. Horses have a remarkable capability to go for extended periods of time without water and feed during cool weather (e.g., Tasker, 1967). Dehydration rates greatly increase, however, when ambient temperature increases and when the low temperature for the period remains relatively high, as seen in the study by Sneddon et al. (1991). The same general relationship is evident in two relatively similar studies in which the major difference was temperature (Smith et al, 1996a; Stull and Rodiek, 2000). Although the horses in both studies had periodic access to water and continuous access to hay during transport, the horses that experienced the highest maximum and minimum temperatures (Stull and Rodiek, 2000) lost more than twice as much body weight during the same time period.

Recent studies on horses transported in large groups (i.e., "slaughter horses") can also give some useful insights into the relationship of temperature and dehydration rate. The dehydration rates (7 and 6%) of the

Table 1. Summary of recent studies characterizing weight loss during water deprivation and(or) transport of horses

Author(s)	Weight loss, %	Duration of water deprivation	Housing	Temperature range, °C	Mean temperature, °C
Not transported					
Tasker, 1967	10.0	6–8 d	Stalled	0–20	10
Carlson, 1979	10.7	72 h	Penned in sun	11.8–33.2	22.5
Sneddon , et al., 1991	12.0	72 h	Stalled	18.7–29.9	24.5
Transported in small trailers		Duration of transport			
Smith, et al., 1996	2.7	24 h	Covered trailer with hay and water	13–30	21.5
Stull and Rodiek, 2000	6.0	24 h	Covered trailer with hay and water	19–35.5	27.3
Transported in large trailers					
Friend, et al.,1998	9.0	24 h[a]	Penned in sun	26–36	31
	8.0	24 h	Transported in Open top trailer	26–36	31
	7.0	24 h	Penned in sun And watered	26–36	31
	6.0	24 h	Transported in open-top trailer and watered	26–36	31
Stull, 1999	8.0	30 h	Covered trailer with hay and water	13–39	26
Friend, 2000	12.8	30 h[a]	Penned in sun	24–37	30.5
	10.3	30 h	Transported in open-top trailer	24–37	30.5
	4.2	33 h	Transported in open-top trailer and watered	24–37	30.5
	3.8	33 h	Penned in sun and watered	24–37	30.5

[a]Horses were deprived of water for 6 h before transport but were held in shaded stalls, so the overall effect was minimal.

horses in the treatments of Friend et al. (1998) that received periodic water during transport were very similar to those of the horses in a study by Stull and Rodiek (2000) (Table 1). The maximum weight loss observed in the nine shipments of slaughter horses surveyed by Stull (1999) further supports the relationship between temperature and weight loss when compared with the 10.3 and 12.8% losses found in horses transported for 30 h at with a much higher minimum temperature (Friend et al., 2000). The higher the average temperature becomes over a period of time, the greater the rate of weight loss or dehydration in horses. Horses cool largely by sweating, and, under conditions of high sustained temperatures averaging 30°C or more, water loss due to dehydration can become extreme after 28 h (Figures 1 and 2).

Weight loss and dehydration can be greatly reduced or stabilized after an initial weight loss by providing periodic watering on board the transport vehicle (Figure 1). All of the watered horses in Figure 1 readily consumed water when it was offered, but that was water to which those horses were accustomed. Although the tendency for many performance horses to be reluctant to drink water during transport may create concern for their owners, most horses will readily drink on board trailers when they are dehydrated enough. Under relatively hot conditions, that usually occurs about 8 to 24 h after initiation of water deprivation (Friend et al, 1998; Friend 2000; Gibbs and Friend, 2000). Even though weight loss and dehydration may be limited by offering water on board transport vehicles, fatigue will likely become the next limiting factor regarding how long they may be transported.

Recovery from dehydration is extremely fast. Sodium, chloride, and osmolality approach normal ranges within hours of rehydration (Carlson et al., 1979; Friend, 2000), and blood proteins return to basal concentration by 3 (Figure 2) to 12 h, or longer (Tasker, 1967; Carlson, et al., 1979; Stull and Rodiek, 2000), depending on the duration and severity of dehydration.

There are no published studies that were specifically designed to provide a basis for recommended rehydration rates in dehydrated horses. Dehydrated horses may drink to excess and limiting water to 12 L at 30-min intervals is too rapid to avoid mild colic when healthy horses were extremely dehydrated (Friend, 2000). However, no signs of colic were reported when horses were moderately dehydrated and offered 1 h of access to water (Carlson et. al., 1979) or when offered 14 L of water at 30-min intervals. Dehydration rates

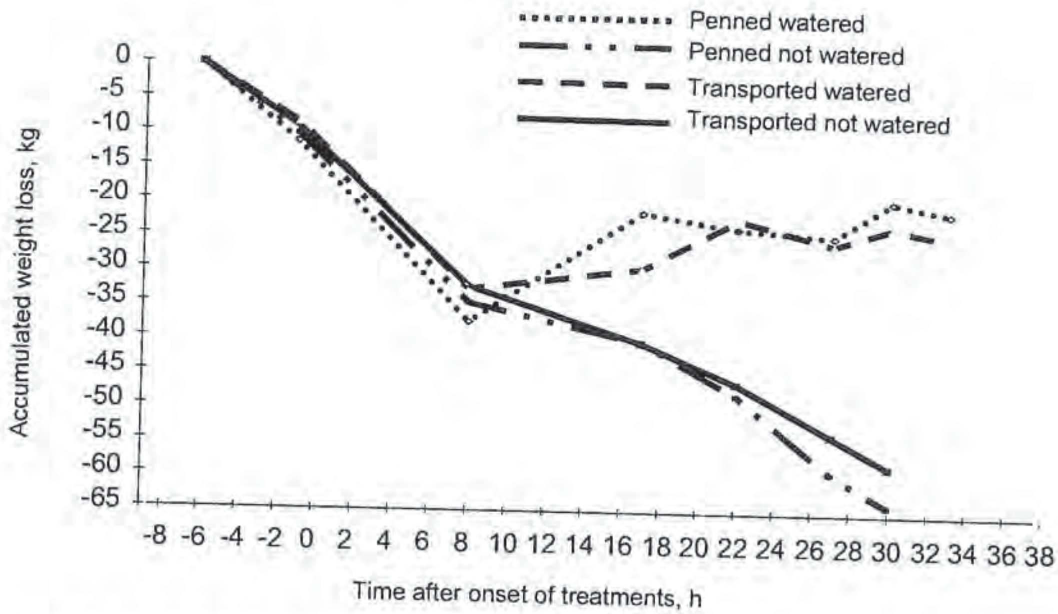

Figure 1. Means of accumulated weight loss before and during treatments and during recovery (Friend, 2000). Before and during treatments, n = 10 per treatment group for the transported horses and n = 5 per treatment group for the penned horses. During recovery, from 36 to 54 h, n = 5 for the transported, unwatered and n = 2 for the penned, unwatered horses.

and mild signs of colic from overly rapid rehydration were similar for horses that were transported or maintained in a pen in full sun (Friend, 2000). It is important to recognize that these rehydration rates are based on horses that had not been worked or exercised.

When the ambient temperature averaged 10°C, horses were deprived of water for up to 8 d without apparent ill effects (Tasker, 1967; Table 1). There are no scientific studies on the effect of transportation of horses during extreme cold weather, although trans-

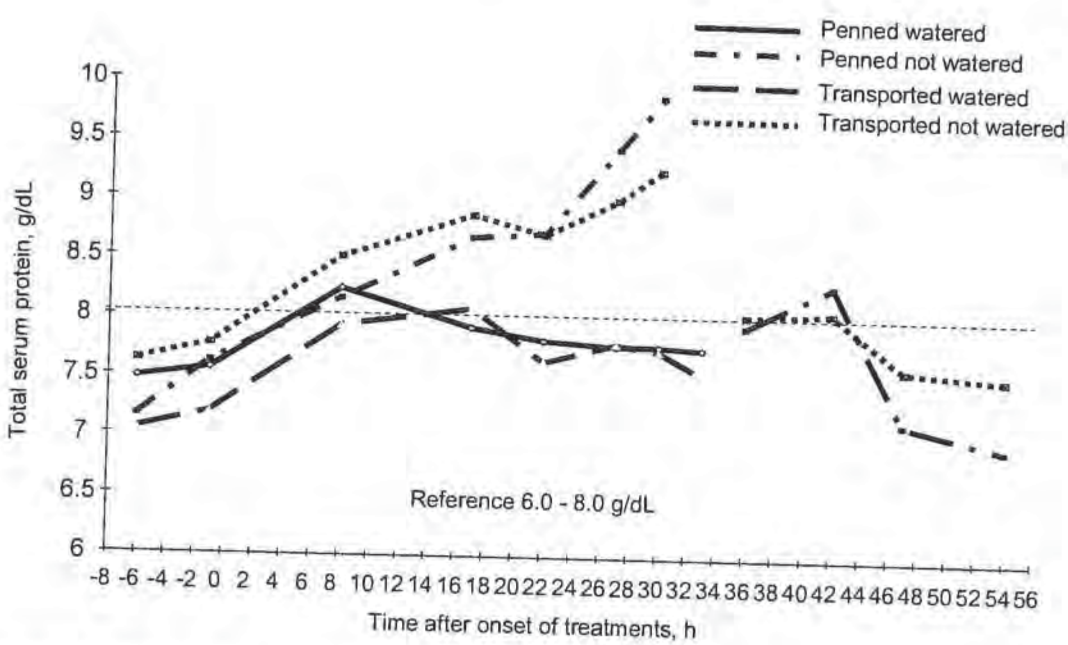

Figure 2. Means of total serum protein before and during treatments and during recovery (Friend, 2000). Before and during treatments, n = 10 per treatments group for the transported horses and n = 5 per treatment group for the penned horses. During recovery, from 36 to 54 h, n = 5 for the transported, unwatered and n = 2 for the penned, unwatered horses. Reference (normal) range is from Boon and Rebar (1998) and is represented by the dashed line.

porting horses under cold and wet conditions could lead to hypothermia and possibly to death. Helping transported animals conserve body heat by reducing ventilation and keeping them dry is easier to accomplish than reducing heat load when ambient temperatures are extremely high. Transporting horses during cool weather will greatly reduce dehydration rates, but fatigue will then become the next limiting factor.

Fatigue

Transportation requires effort on the part of the horse that can be similar to exercise (Codazza et al., 1974). Short trips (90 km) have relatively little effect on the performance of horses (Beaunoyer and Chapman, 1987), whereas longer transport (900 km) should be completed at least 48 h before a race to permit hematochemical values to return to normal (Codazza et al., 1974). There is only one study in the literature in which horses were transported until fatigue became apparent (Friend, 2000). Those horses were transported in two groups of five on a large semi-trailer but were individually watered at regular intervals. Those horses showed increasing signs of fatigue (closing their eyes, lower head carriage, less social interaction, and less response to stimuli) after 24 h. Although not quantified, there was a striking absence of social interactions, especially threats and nipping each other after 27 h of transport. Transport was terminated after 33 h based on the absence of social interactions, a greatly reduced response to stimuli, and the horses' generally depressed appearance.

Although advancing dehydration may be stopped by providing horses water in transport vehicles or by transporting them during cooler weather, fatigue will continue to increase to a threatening level after 28 h of transport of fit and healthy horses. I observed one seemingly healthy, but old, slaughter horse lying down in a crowded trailer after 8 h of transport. Lying down during transport is not necessarily a problem when horses are in individual stalls, but a prone horse will probably be trampled under crowded group conditions.

Orientation

Sharon Cregier, from Prince Edward Island, Canada, was very important in raising the consciousness of people in North America to the importance that orientation of horses may have on the ability of horses to maintain their balance in certain types of trailers. Her inspiration (Cregier, 1982) was the observations of Wentworth Tellington (Tellington and Tellington-Jones, 1979), a California engineer and horse trainer, and David Holmes's informal experiments that resulted in his developing a rear-facing "safety trailer" in New Zealand.

Cregier (1982) hypothesized that horses are instinctively aware of the vulnerability of their head and chest when facing forward during transport. The expectation of decelerations and stops results in the horse's carrying its head in an unnaturally high position that shifts weight to the hindquarters. Because the forequarters carry 60% of the horse's body weight and deal with direction changes, when the horse's weight is shifted to the hindquarters its balancing ability is assumed to be impaired (Cregier, 1982). She also believed that the fleshy rump better absorbs the shock of impacts due to rapid deceleration and stops, and thus transporting horses facing away from the direction of travel is a safer alternative than transporting them facing toward the direction of travel.

Subsequent research on orientation, however, has found inconsistent results, perhaps related to differences in the design of trailers or preferences of certain horses. For example, in two horse trailers, rear-facing horses could better maintain their balance (Clark et al., 1993) and had lower heart rates and less movement (Warren et al., 1996) than forward-facing horses. However, Smith et al. (1994b) were unable to detect a difference in heart rates between horses facing forward and those facing backward when they were transported in the back half of a four-horse stock trailer. In another experiment, horses spent significantly more time facing backward when the trailer was in motion (Smith et al., 1994b). However, some horses showed a very strong preference to face toward the direction of travel, and no differences in heart rates for either forward or backward orientations were evident. Kusunose and Torikai (1996) did find that yearling Thoroughbred horses increasingly oriented away from the direction of travel as the number of trials increased.

A series of experiments using a large semi-trailer to conduct simultaneous comparisons of different treatments (orientations) of horses (Gibbs and Friend, 1999) transported four times over a course containing bumps, high-speed turns, and sudden stops found that orientation had only a slight effect. Individual horses transported loose in pens showed a slight preference (57% of their time) for facing in the direction of travel, but it was not statistically significant. When individual horses were tied to the right side of the trailer, they spent 59% of the time facing the direction of travel. When tied on the left side of the trailer, the horses spent 52% of their time facing away from the direction of travel. The tendency to face forward when tied on the right and to face to the rear when tied on the left side of the trailer indicates a slight preference for the left side to be more exposed and the right side closer to the wall. The horses' ability to maintain balance when confined to stalls set at different orientations within the trailer was also determined in a series of trials. Horses facing backward did slip more than horses that were forward facing, facing forward at a 45° angel, and rear facing at 45° ($P = 0.03$), but leg movement and impacting or leaning on barriers or walls were not influenced by orientation ($P > 0.10$). There was no significant difference between unshod and shod horses. Overall, a slight preference for a 45° orientation was observed, but no preference for facing either toward or away from the direction of travel was detected. Balancing ability was not meaningfully affected by orientation (Gibbs and Friend, 1999). If facing forward is adverse, horses would be expected to spend little time in that orientation, but the research data show only a slight preference, at best, for facing away from the direction of travel.

The strong preference that some horse owners have observed for horses to voluntarily face away from the direction of travel may in part be due to an improved ability to maintain their balance by avoiding saddle compartments that restrict the movement of their head and neck and hence their ability to shift weight and maintain balance. There is also a strong preference to avoid the dark-cave effect associated with most smaller horse trailers and a strong desire to face the more open rear of a trailer that often has a large opening between the top of the rear doors and the roof of the trailer. Informal observations from a large commercial horse transport company that operates large vans in which horses face both toward and away from the direction of travel indicate that having horses tied in such a manner that they can readily raise and lower their heads is the most important factor in reducing transport stress (Robert Maxwell, personal communication).

There may be a slight advantage to slant-load trailers regarding the ability of horses to maintain their balance. Slade (1987) speculated that when turning, horses "in-line" (parallel) must lean diagonally, whereas slant-loaded horses are already oriented more perpendicular to the centrifugal forces of the turn and can therefore more easily maintain balance. Slade (1987) measured the velocities of horses during the last 137.2 m of a 365.8-m run immediately after transport facing forward or backward in a slant-load trailer. Each horse served as its own control; each faced slant-forward for one trip and slant-backward for another. Slade found that horses were only slightly faster after transport facing the rear than after transport facing the front. Although this was not a statistically significant difference, Slade suggested that it may be of importance in a close contest.

From the research that is available, the reduction in transport-related problems that many people report when they switch to a slant-load trailer may be due to attributes other than being transported at a slant. That is, there is less of a "dark-cave" effect because most slant-load trailers have a large window in the front of each stall, the horses usually have an increased ability to raise and lower their heads because of the lack of a saddle compartment, and the stalls are usually longer than in conventional horse trailers.

Density

Because the vast majority of competition, performance, and companion horses are transported in individual stalls, the density of horses during transport is not an issue for many horse owners. However, density is a very important issue for horses being transported in loose groups, as when they are transported to slaughter. The driver in the cab of a tractor that is pulling a large trailer loaded with loose horses (or cattle) can easily feel and hear horses shifting and impacting the sides and deck of the trailer. There is noticeably less banging from the trailer when the horses are tightly loaded, giving rise to the old saying that horses (and other stock) should be loaded tightly so that they "hold each other up." However, recent studies with horses (Collins et al., 2000) and cattle (Eldridge and Winfield, 1988; Tarrant et al., 1988; 1992; Tarrant and Grandin, 1993) have found no basis for that claim and instead concluded that moderate density is preferable. Stull (1999) did report reduced injuries at higher density; however, most of the physiological responses indicative of stress obtained by Stull were less in horses provided with greater floor area. Transportation studies are particularly difficult to do because a large proportion of injuries within a shipment of horses may be caused by one aggressive horse (Grandin, 1999; Collins et al., 2000), making the experimental unit the group or shipment.

The distance that horses were displaced during hard stops was similar between high- and low-density treatments (Collins et al., 2000), indicating that horses in the high-density treatment did not "hold each other up" any more than those in the low-density treatment. In moderate-density situations, horses have more opportunity to move a hoof or change their posture to compensate for changes in speed, and hence there are more body and hoof impacts. This concurs with Tarrant and Grandin's (1993) conclusion that shifting within trailers is inhibited at high stocking density and that there is a corresponding increase in struggles and falls at high stocking density. In addition, the ability of horses to stand up after a fall was clearly hampered by high density (Collins et al., 2000; Table 2), which contributed to the greater number and greater severity of injuries observed in those horses (Collins et al., 2000).

When stocking density increases, horses may not be able to adopt improved balancing strategies because the high density does not allow them any freedom to change their behavior (Collins et al., 2000). Dominant or aggressive horses may repeatedly bite, kick, shove, and displace other horses when establishing their orientation, assuming that even the aggressive horses are successful in obtaining maneuvering room. When horses went down at a high stocking density, they were trapped on the floor by the remaining animals "closing over" and occupying the available standing space (Collins et al., 2000). This can be readily seen in the controlled studies conducted by Collins et al. (2000; Table 2) in which horses that fell in the high-density treatment spent much longer time down. Although those horses were transported over a standardized course for just 25 min, one horse in the third trial ceased attempting to get up and had to be rescued. Most horses that go down under high-density conditions during long

Table 2. Comparison of falls and duration of time down for horses transported at two densities (based on Collins et al., 2000)

Trial	Density	Number of horses	Area per horse, m^2	Number of falls	Horses that fell, %	Mean down time, s/fall
1	Low	8	2.23	1	13%	2
1	High	14	1.28	2	14%	8
2	Low	8	2.23	1	13%	1.5
2	High	14	1.28	8	57%	17.5
3	Low	8	2.23	2	25%	41.5
3	High	14	1.28	7	50%	93.9
Average	Low	8	2.23	1.3	17%[a]	15[b]
	High	14	1.28	5.7	40%[a]	39.8[b]

[a]$P = 0.046$.
[b]$P = 0.14$.

distance transport will likely be trampled if aid is not rendered quickly.

The increased incidence and severity of injuries in high-density conditions may be due to a number of factors. Experienced horses avoid contacting surfaces during transport (Gibbs and Friend, 2000) and prefer to maintain their balance independently of other horses. Horses in high-density conditions are crowded to the point that they can move only several centimeters and are forced to have contact with other horses and the sides of trucks (Collins et al., 2000). They are constantly repositioning their feet in an apparent attempt to maintain their balance and frequently stepped on the hooves and pasterns of other horses. Aggressive horses bit and kicked other horses, resulting in injuries, as they maneuvered for room. A few aggressive horses may inflict most of the injuries incurred in a trailer-load of animals (Grandin, 1999; Collins, et al., 2000). In addition, the attempts of horses being bitten to stop the biting by kicking with their hind feet also contributes to injuries. Horses that fall down in high-density conditions sustain more injuries and severe injuries due to the "closing over" effect. Table 3 illustrates the positive relationship that the number of injuries and severity of injuries has with high density. Injuries may also be influenced by the type of flooring and other factors affecting a horse's traction.

Moderate stocking density would likely reduce injury and bruising during transportation but would also increase transport costs. High stocking densities create a situation of constant struggle for the horses. Decreasing density would reduce the overall stressfulness of long-distance transport by allowing the horses some maneuvering room to avoid aggressive horses, to stand in a more comfortable position, and to adopt their preferred orientation, and perhaps it would allow them to rest during periods when the truck is stopped (Collins et al., 2000).

Engineering Considerations

Suspension

Vertical movement created by running over an artificially created series of bumps that were enough to jounce observers out of their chairs had little observable effect on horses in regard to balancing ability (Gibbs and Friend, 1999). The horses stood still with their feet apart and absorbed the shock for the approximately 10-s duration of the bumps. However, during longer periods of transport the suspension on a vehicle will have a major impact on the fatigue and the well-being of horses. Although there are several types of suspensions available for standard horse trailers, the only scientific

Table 3. Number and severity of injuries for horses transported at two densities (based on Collins et al., 2000)

Trial	Density	Number of horses	Area per horse, m^2	Number of injuries	Horses injured, %	Injuries per horse	Mean severity score
1	Low	8	2.23	0	0	0	0
1	High	14	1.28	11	35%	0.79	1.91
2	Low	8	2.23	6	25%	0.75	1.33
2	High	14	1.28	43	93%	3.07	1.77
3	Low	8	2.23	12	63%	1.50	1.2
3	High	14	1.28	42	64%	3.00	1.64
Average	Low	8	2.23	6	29%[a]	0.75[b]	0.92[c]
High	14	1.28	32	64%[a]	2.29[b]	1.77[c]	

[a]$P = 0.006$.
[b]$P = 0.11$.
[c]$P = 0.48$.

comparison was conducted by Barbara Smith at the University of California at Davis. Leaf-spring suspension with low-pressure radial tires was statistically the smoothest combination tested (Smith et al., 1996b). The torsion-bar suspension with normal-pressure radial tires, which is very popular on newer trailers, was among the roughest. When the effects of transport with leaf-spring suspension with bias-ply tires was compared to torsion-bar suspension with normal-pressure radial tires, the difference was not biologically significant. Although there was a difference in regard to smoothness of the ride, the presence of other stressors during transport probably masked any effect that may exist (Smith et al., 1996b). Unfortunately, Smith did not compare air suspension systems, which are widely accepted as offering the smoothest ride.

Saddle Compartments

Securing a horse's head over a saddle compartment or similar structure forces a horse to keep its head raised in an unnatural position. Not only does restricting the movement of the head increase fatigue and make it more difficult for horses to maintain their balance during transport (Cjrieger, 1982; Clark et al., 1993), but it may also directly lead to pathology. Keeping the heads of healthy horses raised leads to an increased bacterial burden in their tracheobronchial secretions (Racklyeft and Love, 1990). Those authors hypothesized that allowing the horse to lower its head may promote drainage and facilitate clearance of bacteria from the tracheobronchial tree.

Future Research Opportunities

The recent interest in the transport of slaughter horses has stimulated considerable research and greatly increased our knowledge about the transport of loose horses over long and short distances. There has not been a scientific evaluation of what is currently the most popular horse trailer, the aluminum slant load with rubber-torsion suspension. Although air suspensions offer the best rides for larger trailers, there are no scientific data determining the effect alternative suspensions have on reducing fatigue in horses transported long distances. Insulating trucks and trailer roofs and side walls has the potential to greatly reduce the heat load and dehydration rate in horses transported long distances under hot conditions; however, no comparisons have been made. Determining the possible benefit of constructing trailers of insulating materials for transport of horses during cold weather transport may also be useful. There is also a conspicuous lack of scientific data on the transport of horses during cold conditions and the transport of horses by aircraft.

The transportation of horses is certainly a fertile field for research and is justifiable considering the huge investment in horse transportation equipment. There are excellent means of relaying the results of research to horse owners. However, the diffuse nature of the horse industry and the relatively large number of trailer manufacturers and a general reluctance of the manufacturers to fund applied research is problematic.

Implications

Horses show extreme dehydration after 28 h of transport in hot and humid conditions. Watering horses on board trailers will alleviate dehydration, but fatigue can become extreme after 28 h of transport. Orientation (facing forward, back, or diagonal) does not seem to significantly affect a horse's ability to maintain its balance. Loose horses that are transported in groups at high densities (e.g., slaughter horses) do not hold each other up, but inhibit each other's attempts to compensate for changes in inertial forces and have increased injuries; the ability of horses to stand once they fall is also inhibited. High density also prevents submissive horses from moving away from aggressive horses, resulting in repeated aggression. Reducing density, however, greatly increases transportation costs. A major challenge is determining which of the myriad of trailer designs, suspension systems, and building materials available are preferable from the standpoint of the horse.

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