

## Effects of Stocking Density and Transportation Time of Market Pigs on Their Behaviour, Plasma Concentrations of Glucose and Stress-associated Enzymes and Carcass Quality

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**ABSTRACT :** It is known that the transportation stress of market pigs can affect their carcass quality and that blood concentrations of glucose, creatine kinase (CK) and lactate dehydrogenase (LDH) are indicators of the transportation stress. Fifty-seven gilts and 57 barrows weighing approximately 110 kg were randomly assigned into six groups in a 3 [high (0.31 m<sup>2</sup>/100 kg BW)-, medium (0.35 m<sup>2</sup>)- and low (0.39 m<sup>2</sup>)-stocking densities]×2 [1 h vs. 3 h transportation time] arrangement of treatments. Blood samples were taken during transportation and after 2 h lairage. The percentage of “standing” animals during transportation was less in the low- than in the medium- or high-stocking density; the opposite was true for the “sitting” posture. Plasma concentrations of glucose, CK and LDH increased after loading and declined to the resting levels after lairage. Concentrations of CK and LDH were greater in the 3 h vs. 1 h transportation group. Moreover, the LDH concentration was less in the low- than in the medium- or high-density group. Also detected was a significant interaction between the stocking density and transportation time in all of these blood variables. The incidence of pale, soft and exudative (PSE) carcass was greatest in the high-stocking density group. Interestingly, the PSE incidence increased following the 3 h vs. 1 h transportation at the low-density, but not at the medium-density. Results suggest that the medium-density may be preferable to the low-density in the long-distance transportation. (*Asian-Aust. J. Anim. Sci.* 2004. Vol 17, No. 1 : 116-121)

**Key Words :** Pig, Transportation, Stress, Behaviour, Carcass

### INTRODUCTION

It is now well recognized worldwide that transportation conditions of market pigs influence the carcass quality as well as the behaviour and welfare of the animals. It is also a domestic reality, however, that the importance of trucking density of market pigs is overlooked and accordingly the pigs are frequently overloaded. Moreover, most domestic producers transport their market pigs over one hour, the upper-limit transportation time recommended by NLRI (2002), to reach a nearest local abattoir. Both over-stocking and long-distance transportation of slaughter pigs are known to increase the stress of the animals (Payne and Payne, 1987; Becker et al., 1989; Barton-Gade and Christensen, 1998) which causes increased plasma concentrations of glucose, creatine kinase (CK) and lactate dehydrogenase (LDH) (Martoccia et al., 1995; Warriss et al., 1995; Warriss et al., 1998a). Plasma concentrations of glucose, CK and LDH, which increase following an increased secretion of adrenal stress hormones, muscle damage and general tissue damage, respectively (Knowles and Warriss, 2000), are thus used as indicators of transportation stress. Moreover, the long-distance transportation has also been reported to cause a behavioural change of the animals on the truck (Lambooj et al., 1985;

Hunter et al., 1994) and also to increase the incidence of dark, firm and dry (DFD) or pale, soft and exudative (PSE) carcass (Lee and Choi, 1999; Gospert et al., 2000). In somewhat contrast, over-stocking has been reported to exert a minimal or variable effect on animal behaviour and carcass quality (Barton-Gade and Christensen, 1998; Warriss et al., 1998b).

The present study was undertaken to investigate the effects of the stocking density and transportation time on animal behaviour, plasma concentrations of glucose and stress-associated enzymes and carcass quality and thereby to find insights into optimal transportation conditions for domestic slaughter pigs.

### MATERIALS AND METHODS

#### Animals

A total of 114 Landrace×Yorkshire×Duroc cross-bred market gilts and barrows (equal numbers) weighing approximately 110 kg were randomly assigned into six groups under a 3 (stocking density) ×2 (transportation time) factorial arrangement of treatments. As indicated in Table 1, low-, high- and medium-stocking densities were 0.39 m<sup>2</sup> [close to European Committee (EC) recommendation (0.42 m<sup>2</sup>)], 0.31 m<sup>2</sup> (domestic maximum stocking) and 0.35 m<sup>2</sup>/100 kg BW (medium of the previous two), respectively; short- and long-distance transportation times were 1 h (EC standard) and 3 h (local long-distance). All the animals were

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**Table 1.** Transportation time and stocking density of the vehicle

	1 <sup>st</sup> transportation (1 h)		2 <sup>nd</sup> transportation (3 h)		3 <sup>rd</sup> transportation (1 h)	4 <sup>th</sup> transportation (3 h)
	Front <sup>a</sup> (5.4 m <sup>2</sup> )	Rear <sup>a</sup> (5.8 m <sup>2</sup> )	Front (5.4 m <sup>2</sup> )	Rear (5.8 m <sup>2</sup> )	Whole truck (11.2 m <sup>2</sup> )	Whole truck (11.2 m <sup>2</sup> )
Stocking density <sup>b</sup>	Medium	High	Medium	High	Low	Low
No. of animals	14	17	14	17	26	26

<sup>a</sup>The vehicle was divided by a barrier into the front and rear sections the areas of which are shown in parentheses.

<sup>b</sup>High-, medium- and low-stocking densities were 0.31, 0.35 and 0.39 m<sup>2</sup>/100 kg BW, respectively.

**Table 2.** Effect of the stocking density of market pigs on their posture during a 3 h transportation

Postures	Stocking density			Pooled SE	Significance
	High <sup>a</sup> (n=34)	Medium <sup>a</sup> (n=28)	Low <sup>a</sup> (n=52)		
	-----%-----				
Standing	88.1	84.2	60.1	6.1	**
Sitting	7.9	3.0	27.5	4.2	**
Lying	3.9	12.8	12.8	3.5	NS

<sup>a</sup>High-, medium- and low-stocking densities were 0.31, 0.35 and 0.39 m<sup>2</sup>/100 kg BW, respectively.

\*\* p<0.01, NS; non-significant.

transported on a same truck driven by a same driver to the abattoir of Pukyung Cooperative Swine Farms Association through the local road at an average speed of 68 km/h.

Jugular blood samples were taken into EDTA-vacutainers in the pen prior to loading, immediately after loading, after unloading and after 2 h lairage from five animals per each stocking density×transportation time combination. The animals were randomly selected at each sampling point.

#### Analysis of animal behaviour

Animal behaviour during transportation was observed through a window at a seat beside the driver and the numbers of “standing”, “sitting” and “lying” animals were recorded every 20 min beginning from five min after the start of transportation.

#### Determination of plasma concentrations of glucose and enzymes

Blood samples were carried on ice into the lab and plasma was harvested by centrifugation at 3,000 rpm for 10 min at 4°C. Plasma concentrations of glucose, creatine kinase and lactate dehydrogenase were determined using a blood analyzer Dri-Chem 3000 (Fuji Film, Tokyo, Japan) following manufacturer’s instructions (Lee et al., 2002).

#### Physicochemical analysis of the carcass

The animals were slaughtered after two hours of lairage. Following overnight chilling of the carcass at 4°C, the incidence of PSE, which was judged by the color, texture and the extents of moisture exudation and muscle separation of the carcass, was recorded by an expert of the abattoir. Longissimus muscle sections were prepared from

three randomly selected animals per each stocking density×transportation time combination as previously described (Lee et al., 2002). The color, pH at 24 h post-

mortem and 48 h drip loss of the muscle section were measured by the Commission Internationale de l’Eclairage (CIE; 1978) L\* (lightness), a\* (redness) and b\* (yellowness) standards, homogenization and suspension methods, respectively (Lee et al., 2002).

#### Statistical analysis

All the measurements were analyzed using the GLM procedure of SAS (1998). In the analysis of behavioural measurements of the pigs during three hours of transportation, i.e. percentages of “standing,” “sitting” and “lying” pigs, the model included only the main effects of stocking density and time after loading. For carcass measurements, the model included main effects of stocking density and transportation time and an interaction of them. Additionally included in the model for blood measurements were a main effect of blood sampling point, two-way interactions and a three-way interaction associated with the additional main effect. The experimental units were each blood measurement and the animal in the blood analysis and the rest, respectively.

## RESULTS AND DISCUSSION

#### Animal behaviour during transportation

Almost all the market hogs stood on the truck throughout the 3 h transportation when the animals were loaded at the high- (0.31 m<sup>2</sup>/100 kg BW) or medium-stocking density (0.35 m<sup>2</sup>/100 kg). At the low-stocking density (0.39 m<sup>2</sup>/100 kg), a substantial percentage of the animals which initially stood on the truck sat after 45 min (data not shown). However, the effect of time after loading on the percentage of “standing”, “sitting” or “lying” animals was not significant (p>0.05) and accordingly, this factor was not considered in subsequent analysis of the effect of stocking density on the posture of the animals

**Table 3.** Effects of stocking density and transportation time of market pigs on plasma concentrations of glucose, creatine kinase and lactate dehydrogenase

Blood sample point <sup>a</sup>	High-density <sup>b</sup>		Medium-density <sup>b</sup>		Low-density <sup>b</sup>		Pooled SE	Significance
	1 h <sup>c</sup>	3 h <sup>c</sup>	1 h	3 h	1 h	3 h		
Glucose (mg/dl)								
Before loading	58.0	50.0	57.8	73.2	66.0	58.6	4.8	density*, point**
After loading	77.2	66.2	67.4	74.0	63.0	70.6		density×transportation*
After unloading	65.0	69.2	62.8	69.6	60.4	55.4		
After lairage	59.2	66.8	57.4	71.8	57.4	53.2		
Creatine kinase (UI/L)								
Before loading	352	687	371	665	376	363	207	transportation**, point**
After loading	655	2,000	876	1,303	756	1,600		density×transportation**
After unloading	476	1,652	1,072	1,500	834	730		transportation×point*
After lairage	371	818	542	1,135	483	372		
Lactate dehydrogenase (UI/L)								
Before loading	146	261	161	447	242	198	60.1	density**, transportation**, point**
After loading	475	639	485	364	201	362		density×transportation**
After unloading	189	385	341	455	358	188		density×point*
After lairage	149	350	148	389	201	152		density×transportation×point**

<sup>a</sup> Blood samples were taken from five randomly selected animals at each stocking density×transportation time combination.

<sup>b</sup> High-, medium- and low- densities were 0.31, 0.35 and 0.39 m<sup>2</sup>/100 kg BW, respectively.

<sup>c</sup> Transportation time. \* p<0.05, \*\*p<0.01.

during the 3 h transportation (Table 2). The percentage of “standing” animals during the transportation was remarkably less at the low-stocking density than at the medium- or high-stocking density (p<0.01), whereas the opposite was true for the percentage of “sitting” animals (p<0.01). The proportion of “lying” animals, which was less than 13% at any stocking density, did not differ between the three groups.

Collins (1993) suggested that the ideal density was probably one which just allowed all pigs to lie down together, although more space than this would be needed in hot weather to enable the animals to regulate their body temperature. There is conflicting evidence, however, on whether pigs prefer to lie or stand during transport. Hunter et al. (1994) and Guise et al. (1996) found that the greater majority of pigs stood during transport for up to three and half hours. However, other works on the behaviour of pigs during short (40 min) transportation (Bradshaw et al., 1996) and on long-distance transportation (up to 1,300 km) (Lambooij et al., 1985) suggested that they preferred to lie down for most of the time.

#### Plasma glucose and stress-associated enzymes

Plasma glucose concentration was affected (p<0.05) by the stocking density (Table 3). The glucose concentration was less (p<0.01) at the low-stocking density (60.6 mg/dl; pooled SE=1.7 mg/dl) than at the medium-stocking density (66.8 mg/dl), but the difference between the high- (64.0 mg/dl) and low-densities was not significant. Plasma glucose concentration was not influenced by the transportation time. Instead, it was affected by an interaction of stocking density×transportation time (p<0.05).

At the medium-stocking density, glucose concentration was greater (p<0.01) in the 3 h transportation group than in the 1 h group (72.2 vs. 61.4 mg/dl; pooled SE=2.4 mg/dl), but such a difference between the two transportation times was not detected at the high- or low-stocking densities (63.1 vs. 64.9 and 59.5 vs. 61.7 mg/dl for the 3 h vs. 1 h in the high- and low-density groups, respectively). Plasma glucose concentration also changed during the transportation and lairage (p<0.01). It increased after loading and declined to the resting level after lairage (60.6, 69.7, 63.7 and 61.0 mg/dl before loading, after loading, after unloading and after lairage, respectively; 2.0 mg/dl).

Plasma glucose concentration has been reported to be influenced by the stocking density, but the relative stocking density at which glucose concentration is elevated has not been consistent among published results. Lambooij et al. (1985) have reported that plasma glucose concentration was greatest at 0.44 m<sup>2</sup>/100 kg BW when market pigs were allowed 0.33, 0.44 or 0.66 m<sup>2</sup>/100 kg, whereas in the study of Warriss et al. (1998a), glucose concentration was greatest at 0.36 m<sup>2</sup>/100 kg among 0.31, 0.36, 0.42 and 0.50 m<sup>2</sup>/100 kg. Plasma glucose concentration has also been reported to increase with increasing transportation time (Becker et al., 1989), although such an effect of transportation time was not apparent in the present study. These results suggest that plasma glucose concentration is more likely to be influenced by an overall transportation condition, including the stocking density and transportation time, rather than by a single factor.

Plasma creatine kinase (CK) concentration was less (p<0.01) in the 1 h than in 3 h transportation group (597 and 1,069 UI/L for 1 h vs. 3 h transit; pooled SE=60 UI/L) and

**Table 4.** Effects of stocking density and transportation time of market hogs on physicochemical characteristics of the longissimus muscle and the incidence of PSE

Item	High-density <sup>a</sup>		Medium-density <sup>a</sup>		Low-density <sup>a</sup>		Pooled SE	Significance
	1 h <sup>b</sup>	3 h <sup>b</sup>	1 h	3 h	1 h	3 h		
Carcass wt.(kg)	81±4.7	84±4.5	83±5.2	81±5.8	85±4.0	82±3.9	-	
pH at 24 h <sup>c</sup>	5.16	5.26	5.20	5.03	5.38	5.28	0.05	density** density×transportation*
Color <sup>c, d</sup>								
CIE L <sup>*</sup>	54.5	49.5	52.2	55.8	51.6	54.4	0.9	density×transportation*
CIE a <sup>*</sup>	8.6	7.0	8.7	10.1	8.3	8.9	0.74	
Drip loss <sup>c</sup> (%)	3.6	1.7	1.1	4.0	2.0	3.7	0.98	
PSE <sup>c</sup> (%)	23.5	29.4	14.3	14.3	11.5	26.9	-	NA

<sup>a</sup>High-, medium- and low- densities were 0.31, 0.35 and 0.39m<sup>2</sup>/100 kg BW, respectively.

<sup>b</sup>Transportation time. <sup>c</sup>Data are means of three observations. <sup>d</sup>Greater L\* and a\* values indicate more light color and more red color, respectively.

<sup>e</sup>Corresponds to the lower no.1 or 2 grade of the 6-scale redness score or two or three cases of the lowest grade in the 3 scale grading for texture, moisture exudation and muscle separation by the domestic criteria; numbers of animals ranged from 14 to 26.

NA; not applicable. \* p<0.05, \*\* p<0.01.

also tended (p=0.053) to be less in the low-stocking density group (689 UI/L) than in the high- (877 UI/L) or medium-density (933 UI/L; pooled SE=73 UI/L). This blood variable also increased after loading (p<0.01) and declined to the resting level (p<0.01) after 2-h lairage (469, 1,198, 1,044 and 620 UI/L before loading, after loading, after unloading and after lairage, respectively; pooled SE=85 UI/L). These results, which are consistent with previous ones (Warriss et al., 1988a; Warriss et al., 1998b; Lee et al., 2001), indicate that plasma CK concentration, which, as an indicator of the transportation-associated stress, increases during transport, especially immediately after loading, also increases with increasing stocking density and transportation time. In addition, the CK concentration was also affected by the stocking density×transportation time interaction (p<0.01). This variable was greater (p<0.01) in the 3 h than in 1 h transportation group at the medium- or high-stocking density (1,151 vs. 715 UI/L at the medium-density and 1,289 vs. 464 UI/L at the high-density; pooled SE=104 UI/L), but not at the low-stocking density (612 and 766 UI/L for the 3 h vs. 1 h transportation group). This implicates that that when an enough truck space is provided to market pigs, the animals may be minimally stressed to maintain the normal plasma CK concentrations even following a long-distance transportation. The CK concentration was also influenced by the transportation time×blood sampling point (p<0.05), although biological significance of this is less than clear.

Plasma lactate dehydrogenase (LDH) concentration also was influenced (p<0.01) by the stocking density, transportation time and blood sampling point. The LDH concentration was less in the low-density group (238 UI/L) than in the medium- (349 UI/L) or high-density (324 UI/L; pooled SE=21 UI/L). As in the case of CK, LDH concentration was less in the 1 h transportation group than in the 3 h (258 and 349 UI/L for the 1 h vs. 3 h group; pooled SE=17 UI/L). The LDH concentration also increased after loading and declined to the resting level after lairage

(242, 421, 319 and 231 UI/L before loading, after loading, after unloading and after lairage, respectively; pooled SE=25 UI/L). This variable also was affected by the stocking density×transportation time interaction (p<0.01). The LDH concentration was greater in the 3 h transportation group than in the 1 h when the market hogs were transported at the high- [409 (3 h) vs. 240 UI/L (1 h)] or medium-density (414 vs. 284 UI/L; pooled SE=30 UI/L), but not when they were transported at the low-density (225 and 250 UI/L for the 3 h vs. 1 h transit). In addition, this variable was also affected by the stocking density×blood sampling point interaction. At the low-density, the LDH concentration did not differ between the blood sampling points (220, 282, 273 and 176 UI/L before loading, after loading, after unloading and after lairage, respectively; pooled SE=42 UI/L). At the medium- or high-density, by contrast, it increased after loading and declined to the resting level after unloading or after lairage. The interaction of stocking density×transportation time×blood sampling point interaction also was significant in this variable (p<0.01), although the complicated nature of this interaction precluded any detailed descriptions of it.

Effects of stocking density and transportation time on plasma LDL concentration are not consistent among published results including the present one. Barton-Gade and Christensen (1998) and Warriss et al. (1998a) have reported that plasma LDH concentration was not affected by the stocking density, which contrasts with the increased LDH concentration at higher stocking density in the present study. Likewise, Martoccia et al. (1995) and Perez et al. (2002) have reported an increased LDH concentration in response to an increased transportation time, whereas Warriss et al. (1998) have reported an opposite result. It thus appears that environmental factors including temperature, season and the overall transportation condition other than the stocking density and transportation time may also affect the plasma LDH concentration (Warriss et al., 1998a).

### Carcass analysis

The pH of longissimus dorsi muscle at 24 h after slaughter was affected by the stocking density and its interaction with transportation time (Table 4). However, these effects were more of a statistical significance rather than biochemical, on the ground that the pH was within a normal range for the reddish-pink, firm and non-exudative (RFN) carcass (Warner et al., 1997) across the treatments. Likewise, the density×transportation time interaction in the lightness ( $L^*$  value) also is considered to be more of a statistical significance. The redness ( $a^*$  value) and drip loss of the muscle were not affected by either stocking density or transportation time. These effects of the stocking density, transportation time and their interaction on the physicochemical characteristics are thus considered not to be significant to affect the carcass quality. In somewhat contrast, the incidence of PSE carcass was greater in the high-stocking density group than in the medium-density regardless of the transportation time. Of note, the incidence of PSE increased following the 3 h vs. 1 h transportation at the low-density, but not at the medium-density. It is thus tempting to speculate that the medium-density may have been more favorable than the low-density for the animals to balance themselves during the long-distance transportation. If this were the case, the incidence of stress-associated PSE could paradoxically increase more following a low-stocking density transportation than following transportation at a greater stocking density. Obviously, more studies are necessary to confirm a likelihood of this speculation.

### IMPLICATIONS

Following are the implications of the present results. Market pigs receive the most stress during loading and subsequently recover from the stress during lairage, on the basis that circulating concentrations of glucose, creatine kinase and lactate dehydrogenase are reflective of the transportation stress. However, pre-slaughter blood concentrations of these are not indicative of the carcass quality of market pigs. Over-loading and long-distance transportation are likely to increase the incidence of PSE. However, when market pigs have to be transported for a long time, the medium-stocking density ( $0.35 \text{ m}^2/100 \text{ kg}$ ) may be preferable to the low density ( $0.39 \text{ m}^2$ ).

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