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Shipping Stress and Social Status Effects on Pig Performance, Plasma Cortisol, Natural Killer Cell Activity, and Leukocyte Numbers[1]

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ABSTRACT: Crossbred pigs were used to evaluate the effects of shipping stress on natural killer (**NK**) cell activity, leukocyte numbers, plasma cortisol, and BW changes. In the first study, pigs were bled at a commercial farm and, after shipping, resident and shipped pigs were bled again. Plasma cortisol concentrations were not different ($P > .10$) because of large variation in cortisol concentrations. Furthermore, NK cytotoxicity was nondetectable among all pigs. A second study showed that plasma cortisol concentration rose by approximately 2.6 ng/mL ($P = .018$) for each minute after pigs were aroused. In the third, more controlled study, pigs were housed in pens of three pigs each. Video recordings were made during the first 24 h pigs were grouped to identify socially dominant, intermediate, and submissive pigs. At time zero (before shipping), resident pigs and those to be shipped had similar plasma cortisol concentrations. However, after the 4-h shipping experience, shipped pigs had elevated ($P < .05$) plasma cortisol compared with resident control pigs. Shipped pigs lost 5.1% of their BW ($P < .05$) compared with resident pigs, which gained .02% of their BW. Body weight change during shipping and plasma cortisol were negatively correlated ($r = -.34$, $P = .04$), indicating pigs that had greater adrenal response to shipping also lost more weight during shipping. Shipping reduced ($P < .05$) NK cytotoxicity among pigs of intermediate and submissive social status compared with shipped, dominant pigs. At the end of shipping or control treatments, the correlation between NK cytotoxicity and plasma cortisol was positive ($r = .35$, $P = .036$), indicating that pigs with greater cortisol response had greater NK cytotoxicity. In both shipping studies, numbers of blood neutrophils increased ($P < .01$), lymphocytes decreased ($P < .01$), and neutrophil: lymphocyte ratio increased ($P < .01$) after shipping. These data suggest that 1) social status and shipping stress interact in NK cytotoxicity response, 2) shipping has defined effects on numbers of circulating leukocytes, 3) controlling or measuring social status and handling procedures allow interpretation of certain shipping stress data, and 4) socially intermediate and submissive pigs are probably more likely to succumb to viral infections after shipping than are socially dominant pigs because their immune systems are suppressed.

Key Words: Stress, Behavior, Aggressive Behavior, Immunity, Natural Killer Cells

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Introduction

Stress represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects (Khansari et al., 1990). One response to acute stressors is activation of the hypothalamic-pituitary-adrenal (**HPA**) axis, resulting in elevated corticotropin releasing hormone (**CRH**). The CRH stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) and other peptides. Increased ACTH causes the release of glucocorticoids in the serum of stressed farm animals (reviewed by Dantzer and Mormède, 1983). Shipping (Nyberg et al., 1988; Parrott and Mission, 1989), social stress (Parrot and Mission, 1989), electrical stimulation and heat stress (Becker et al., 1985), and food and water deprivation (Houpt et al., 1983; Parrot and Mission, 1989) have been

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shown to increase plasma cortisol concentrations in pigs, presumably in response to increased CRH and ACTH levels.

During the production and marketing of pigs, a number of stressful situations lead to production losses and pathological disease. Pigs are exposed to many environmental stressors (i.e., heat, cold, mixing, weaning, noise, and shipping) that have been shown to increase disease susceptibility and impair immune function (Kelley, 1980, 1985).

Natural killer cells (**NK**) are large, granular lymphocytes that lyse viral-infected cells (and other cells) without prior sensitization (Trinchieri, 1989). Natural killer cells form a first line of defense against viral infections. Many types of environmental stressors influence NK cytotoxicity (Dunn and Berridge, 1990). Administration of CRH directly into the cerebral ventricles suppresses NK cytotoxicity in a manner similar to the stress-induced suppression in NK cytotoxicity (Irwin et al., 1990; Jain et al., 1991). Peripheral glucocorticoid administration also was shown to suppress NK cytotoxicity (Hochman and Cudkowicz, 1979; Aguila et al., 1988). However, peripheral ACTH administration was shown to enhance pig NK cytotoxicity (McGlone et al., 1991). Thus, stress affects NK cytotoxicity in a number of ways that are not predictable based on data from experiments in which single hormones are applied or inhibited.

The objectives of the study were to determine 1) the effects of a 4-h trip by truck (shipping) on plasma cortisol, circulating leukocyte numbers, and NK cytotoxicity, 2) the effects of handling time when obtaining blood by venipuncture on plasma cortisol, and 3) the effects of shipping, social status, and their interaction on pig performance, circulating leukocyte numbers, NK cytotoxicity, and blood cortisol concentrations.

Materials and Methods

General

Pigs were bled via jugular venipuncture using 20-mL heparinized syringes while inverted on a V-trough. The blood samples were centrifuged for 20 min to separate plasma. Plasma samples were stored frozen at −20°C in glass vials until they were assayed for plasma cortisol levels.

White Blood Cell Differentials. Blood smears were made from whole blood. The smears were fixed in methanol and stained with LeukoStat Solution I and II (Fischer Scientific, Houston, TX) for differential white cell counts. At a 1:500 dilution, white blood cell (**WBC**) numbers were counted using a Model Hemo-W Coulter cell counter (Coulter Electronics, Hialeah, FL).

Natural Killer Cell Assay. The NK cell assay was performed according to standard techniques for porcine NK assay (Yang et al., 1987; Pinto and Ferguson, 1988; Purswell et al., 1989; Lumpkin and McGlone, 1992). Twenty milliliters of blood was centrifuged for 20 min at 850 × *g*. The buffy coat, containing the peripheral blood mononuclear cells (**PBMC**), was removed and mixed with Roswell Park Memorial Institute (**RPMI**) 1640 (Sigma Chemical, St. Louis, MO) (with 2.0 g/L of NaHCO and 100 U/mL of gentamicin sulfate). The buffy coat-RPMI mixture was layered onto 4 mL of histopaque 1077 (Sigma Chemical) and centrifuged at 400 × *g* for 30 min at 25°C. The PBMC were collected and washed once in RPMI at 850 × *g* for 15 min. Adherent monocytes were removed by adherence to sterile plastic Petri dishes for 2 h in a 5% CO_2 humidified chamber. Nonadherent PBMC were collected by gently rinsing Petri dishes with RPMI then centrifuging recovered cells for 15 min at 850 × *g*. The recovered PBMC were resuspended in 1 mL of RPMI and were counted on a Coulter Counter. The samples were diluted in RPMI supplemented with 10% fetal bovine serum (**FBS**) (Sigma Chemical) and adjusted to a cell concentration of 1×10^7 cells/mL.

The targets for the assays were K-562 cells from a human chronic myelogenous leukemia cell line (American Type Culture Collection, Rockville Pike, MD). The targets were maintained in log growth in RPMI-10% FBS. Target cells were labeled with ^{51}Cr by incubating 5×10^6 cells in 1 mL of RPMI-10% FBS with 100 μCi of ^{51}Cr for 1 h in 5% CO_2. After 1 h, 10 mL of RPMI-10% FBS was added and targets were incubated an additional 1 h. The target cells were pelleted and washed twice and resuspended in RPMI-10% FBS to a final concentration of 1×10^5 cells/mL.

Samples were run in triplicate at effector:target ratios (**E:T**) of 12.5, 25, 50, and 100 to 1; 10^4 target cells were added to each well. Total volume per well was 250 μL. Maximum ^{51}Cr release was determined by adding 150 μL of 7.5% Triton-X detergent (Sigma Chemical) to lyse all targets. Spontaneous ^{51}Cr release was determined by adding 150 μL of RPMI-10% FBS to target cells and counting radioactive label in the supernatant.

Plates were incubated in a 5% CO_2 humidified chamber for 18 h. Using the Skatron semiautomatic collection system (Skatron, Sterling, VA), supernatants were collected in cotton plugs. Plugs were transferred to a plastic tube and counted for 1 min on a gamma counter.

Percentage of cytotoxicity was calculated using the following formula: [(experimental release cpm − spontaneous release cpm)/(maximum release cpm − spontaneous release cpm)] × 100.

Cortisol Radioimmunoassay. Plasma levels of cortisol were measured in nonchromatographed samples by an established RIA (Krey et al., 1975). Duplicate 5- or 10-μL aliquots diluted in 250 μL of PBS were added and extracted with 5 mL of ether to separate the steroid from corticosteroid-binding globulin before

the samples were assayed. The antibody (F3-314) was purchased from Endocrine Sciences, Tarzana, CA; the only physiological steroids it shows significant cross-reactivity with are cortisone (30%) and corticosterone (2.9%). The final dilution of the antibody used in the assay was 1:10,000. At this dilution, the standard curve ranged from 10 to 1,000 pg of cortisol and the sensitivity of the assay was typically 10 to 15 pg/tube. The plasma concentrations we observed in pigs ranged from 1 to 75 ng/mL (50 to 375 pg/5 μL) and were easily within detectable range. Recovery of [^{3}H]cortisol after ether extraction was 88% and intra- and interassay CV were 6 and 9.8%, respectively.

Flow Cytometry Analysis of Lymphocyte Populations. Aliquots of 10^6 PBMC (separated as described previously) from each sample were incubated for 30 min at 4°C with 50 μL of either mouse anti-pig CD4 (T-helper lymphocytes), mouse anti-pig CD8 (cytotoxic T lymphocytes), mouse anti-pig immunoglobulin M (for B-lymphocytes), or PBS (auto sample). Total lymphocytes were defined as the sum of CD4 and CD8 when T:B ratio was calculated (many T-lymphocytes are not detected by just CD4 or CD8 and some cells are positive for both CD4 and CD8 antigens). Specificity of these monoclonal antibodies has been previously described (Davis et al., 1987). All monoclonal antibodies (VMRD, Pullman, WA) were diluted to 15 μg/mL in PBS. Total volume for each sample was 450 μL. After labeling with the first antibody, cells were washed once in 1 mL of PBS then labeled with 1 mL (1:64) of goat anti-mouse fluorescein isothiocyanate (Sigma Chemical) for 30 min at 4°C. Samples were washed twice in 1 mL of PBS then resuspended in 1 mL of PBS. Control samples were incubated only with the second fluorescein-labeled antibody to determine background fluorescence and autofluorescence. Samples were analyzed for fluorescence using Coulter Epics C Flow Cytometer (5 W Argon laser, 488 nm). Windows for each animal were set using its own sample to exclude autofluorescence. Autofluorescent cells were excluded from analysis by gating. Less than 2% of cells showed autofluorescence.

Experiment 1

Animals. Nineteen growing pigs (average starting weight = 27.4 kg) derived from a three-breed rotational cross of Duroc, Hampshire, and Landrace breeds were used in the study. The pigs were housed on a commercial production unit in Mt. Vernon, TX. Pigs were randomly assigned either to travel (shipped) or to remain in Mt. Vernon (nonshipped or resident).

All pigs were bled via venipuncture using 20-mL heparinized syringes at approximately 0600. Blood samples were immediately placed on ice and transported to Texas Tech University. Nine pigs were loaded onto a truck and traveled 324 km (approximately a 4-h drive) from Mt. Vernon to New Deal, TX. Ten pigs remained (nonshipped) housed in Mt. Vernon. At the end of the 4-h travel period (1200) blood samples were obtained simultaneously at both locations. Immune and cortisol assays were performed on samples taken at 0600 and 1200.

Experiment 2

Animals. Twenty-one 9-wk-old crossbred nursery pigs derived from a four-breed rotational cross of Yorkshire, Hampshire, Duroc, and Landrace were bled over a 24-min period via venipuncture. Pigs used in the study were housed in three separate nursery pens at the time the blood samples were obtained. The time was recorded in seconds a) from when technicians entered the room until each pig was bled and b) from when pen was disturbed until blood was obtained for each pig. Blood samples were analyzed for plasma cortisol concentration. Regression analyses were used to examine the slope of cortisol over time. Independent variables were time from entering room until blood collection ended (time to enter room) and time from first arriving at the pen until blood collection was complete (time to enter pen).

Experiment 3

Animals. Within each block, four pens consisting of three gilts each were established 1 wk before the start of the study. Three blocks were conducted over a 6-mo period (total n =). In each block, 12 pigs of similar weight from ree different litters were randomly assigned to a 1.2-m × 1.2-m pen in the nursery (three gilts per pen). One five-hole feeder and one nipple waterer was provided in each pen. Pigs had ad libitum access to a 19% CP, nutritionally balanced (NRC, 1988), sorghum-soybean meal-based diet and water. One week later, pigs were subjected to either a 4-h trip by truck (shipped) or remained in the nursery (nonshipped). Blood samples were obtained from shipped and nonshipped pigs, at time 0 (before shipping) and 4 h (after shipping). Shipped pigs were returned to the same nursery pens after shipping.

Performance. All pigs were weighed at the start of the experiment, immediately before shipping, after shipping, and 1 wk after shipping. Feed weights were taken each time pigs were weighed. Feed intake and weight gain or loss were calculated.

Behavior. Each pen containing three unfamiliar pigs was videotaped in time lapse for 24 h, 1 wk before shipping. The tapes were viewed and each pig's social status was determined based on the outcome of aggressive interactions (McGlone, 1986). Pigs were identified as socially dominant, intermediate, or submissive. Dominant pigs defeated two penmates, and submissive pigs were defeated by both penmates. Pigs of intermediate status defeated one penmate and were defeated by one penmate.

Table 1. Time effects (averaged over shipped and control pigs) in Experiment 1

Measure	Time of study[a] Zero	6 h later	CV, %
B cells, %	14.0 ± 1.0**	9.7 ± 1.1	36.8
CD4, %	16.7 ± 1.7	16.9 ± 1.8	39.3
CD8, %	20.0 ± 1.3	18.8 ± 1.4	27.5
T:B ratio	3.22 ± .48*	4.50 ± .52	48.6
CD4:CD8 ratio	.79 ± .21	1.06 ± .23	90.3
White blood cells, No./μL × 10^3	18.0 ± .83	18.1 ± .88	20.1
Neutrophils, %	31.9 ± 3.0*	47.2 ± 3.1	32.8
No./μL	5,754 ± 770*	8,796 ± 786	45.2
Lymphocytes, %	60.5 ± 3.1**	46.6 ± 3.2	24.4
No./μL	10,924 ± 625**	8,243 ± 641	27.3
Neutrophil:lymphocyte	.56 ± .13**	1.13 ± .13	62.0
Monocytes, %	3.89 ± .45	4.34 ± .46	45.8
No./μL	708 ± 88	782 ± 90	50.1
Eosinophils, %	3.55 ± .40	2.74 ± .86	40.3
No./μL	603 ± 54	433 ± 117	32.2
Cortisol, ng/mL	70.7 ± 5.6	76.6 ± 5.6	32.7

[a]Time zero is approximately 0600.
*$P < .05$.
**$P < .01$.

Statistical Analyses

All analyses were performed using SAS (1985) software. Analysis of variance was performed using the GLM procedure and means were separated with the predicted difference test. Experiment 1 was a completely random, split-plot design. Main plots were treatments (shipped vs nonshipped) and subplots were time (before and after the 4-h shipping period). Experiment 3 was a randomized complete block split-plot design with blocks performed two at a given time. Main and subplots were the same as in Exp. 1, except main plot also included effects for pig social status, shipping, and status × shipping interaction. Each block contained littermate pigs exposed to each treatment (shipped or nonshipped and pre- and postshipping data collection). Behavior, feed intake, and weight gain data were not a split plot; otherwise, the analyses were similar. The immune assay for NK cytotoxicity was a split-split-plot design. Different E:T ratios represented the second split (that is, each sample was assayed at four E:T ratios). Simple correlations and linear regression equations were calculated on certain data to describe relationships between time, behavior, NK cytotoxicity, and plasma cortisol concentrations.

Results

Experiment 1. There was no significant main effect of shipping for immune measures or cortisol concentrations. The effects of time, averaged over shipped and nonshipped pigs, on various immune measures and cortisol are shown in Table 1. The percentage of B cells decreased from 14 ± 1.0% to 9.7 ± 1.1% ($P < .01$) over time. The T:B cell ratio increased ($P < .05$) from 3.22 ± .48% at time zero to 4.50 ± .52% 6 h later. The number of neutrophils per microliter (5,754 ± 770 to 8,796 ± 786) and percentage of neutrophils (31.9 ± 3.0% to 47.2 ± 3.1%) increased ($P < .05$), whereas percentage of lymphocytes decreased (60.5 ± 3.1% to 46.6 ± 3.2%, $P < .01$) over time. The increase in percentage of monocytes over time (3.89 ± .45% to 4.34 ± .46%) was not significant. The N:L ratio significantly ($P < .01$) increased over time. Cortisol concentrations were not affected over time. The cortisol values we report here are much higher than those we reported for pigs with indwelling catheters (McGlone et al., 1991), and thus subsequent studies were planned.

Experiment 2. Plasma cortisol increased (b = .043 ± .017; $P = .018$) as time to obtain blood sample within each pen increased (Figure 1a). Plasma cortisol increased by 2.6 ng/mL every minute after the pen of pigs was aroused (from catching and bleeding pigs). The time from when the technicians entered the room until blood was obtained did not affect plasma cortisol ($P > .20$, Figure 1b). Time from when pigs were caught until blood collection was consistent and brief (< 1 min).

Experiment 3. The shipping effect was significant for BW change (Table 2). Shipped pigs lost 5.1% of their BW during shipping. Nonshipped pigs gained .02% of their BW during the 4-h period.

Change in BW and feed intake means from time 0 and 4 h are shown in Table 2. Shipped pigs lost .90 kg of their BW due to shipping. Nonshipped pigs gained .002 kg during the 4-h period in which shipped pigs were traveling. Three days later (72 h postshipping),

Table 2. Effects of shipping on weights of pigs and feed intake in Experiment 3

Measure	Nonshipped	Shipped	*P*-values SE	*P*-values S[a]
Weights, kg				
Time 0	17.3	17.5	1.0	.858
4 h	17.3	16.6	.93	.627
Weight change[b]	.002[y]	−.90[z]	.11	.001
72 h	19.0	18.6	1.04	.799
Weight change[c]	1.74[y]	1.11[z]	.18	.022
Feed intake, kg				
During shipping	.14	—	.06	—
4 to 72 h	1.66[y]	.77[z]	.63	.350

[a]S = Shipping effect.
[b]From time 0 to 4 h later.
[c]From time 0 to 72 h later.
[y,z]Means within a time group with different superscripts differ ($P < .05$).

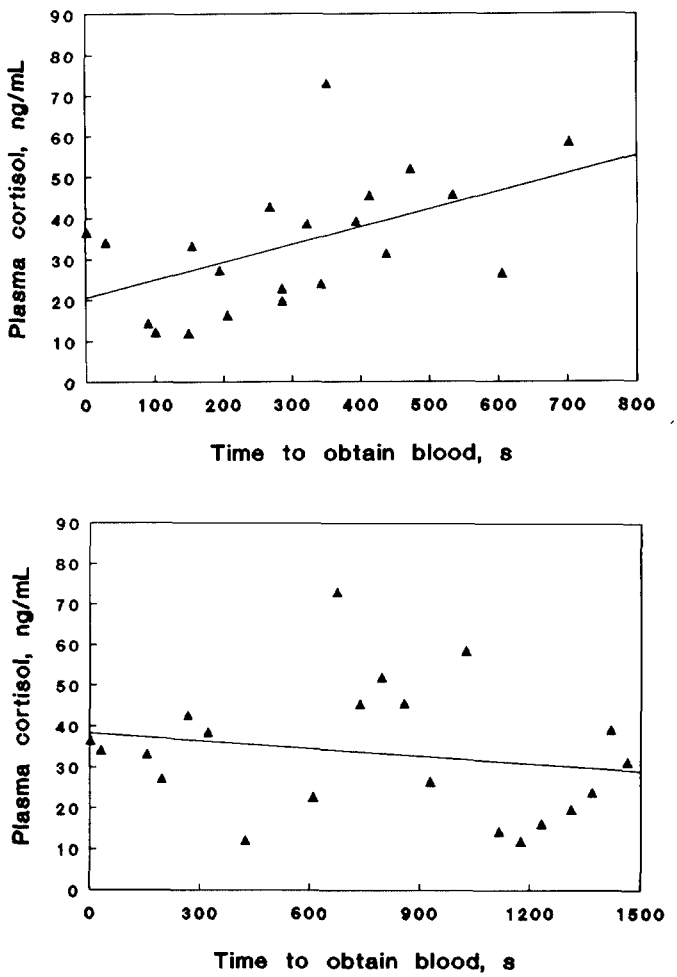

Figure 1. Relationship between plasma cortisol concentration and handling procedures. Figure 1a (top) shows that plasma cortisol increased ($P = .018$) as the time from rousing the pen of pigs until blood collection increased. In Figure 1b (bottom), the relationship between time to enter the room until blood collection did not show a significant relationship with plasma cortisol concentrations. n = 21 pigs.

shipped pigs had gained less ($P < .05$) weight (1.11 ± .18 kg) than nonshipped pigs (1.74 ± 18 kg). Feed intake was lower ($P < .05$) for shipped pigs (.77 ± .63 kg) than for nonshipped pigs (1.66 ± .63 kg) from 4 to 72 h postshipping.

The means for types of white blood cells and neutrophil:lymphocyte ratio at time 0 and 4 h are shown in Table 3. Neutrophils, lymphocytes, eosinophils, and neutrophils:lymphocyte ratio were affected by shipping and time ($P < .01$). Shipping × time interaction had an effect ($P < .05$) on eosinophils. Shipping × time interaction had a marginal effect ($P = .07$) on percentage of blood neutrophils. Percentage of neutrophils increased ($P < .05$) from time 0 to 4 h in shipped pigs. In nonshipped pigs the percentage of neutrophils also increased from time 0 to 4 h, but the increase in percentage of neutrophils was not as great among nonshipped pigs.

Plasma cortisol concentrations increased ($P < .05$) over time when pigs were shipped (Figure 2). Cortisol concentration was similar in nonshipped pigs at time 0 and 4 h. Among shipped pigs, the change in cortisol from time 0 to 4 h indicated that cortisol concentration increased in response to shipping.

There were no ship × time interactions or shipping effects on NK cytotoxicity (Figure 3), probably due to diurnal changes in NK cytotoxicity. Pig NK activity increased ($P < .001$) over time in shipped and nonshipped pigs.

Social status of the pig, shipping, and time had an interactive effect ($P < .05$) on pig NK activity (Figure 4). At 50:1 E:T ratio, nonshipped pigs (regardless of status) had similar NK activity. Among shipped pigs, at the end of the 4-h shipping experience, dominant pigs had higher ($P < .05$) NK activity than pigs of intermediate or submissive status. Intermediate and submissive pigs had similar NK activity.

Correlation analyses were performed to generate hypotheses that might be tested in future studies.

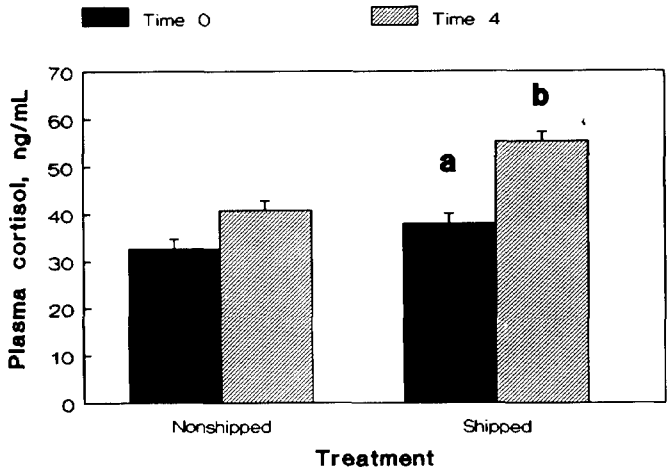

Figure 2. The shipping × time effect was significant (P = .03) for plasma cortisol concentration. For nonshipped pigs, cortisol concentrations were similar at time 0 and 4 h later. Shipped pigs had greater (P < .01, a,b) plasma cortisol concentrations after shipping than before shipping.

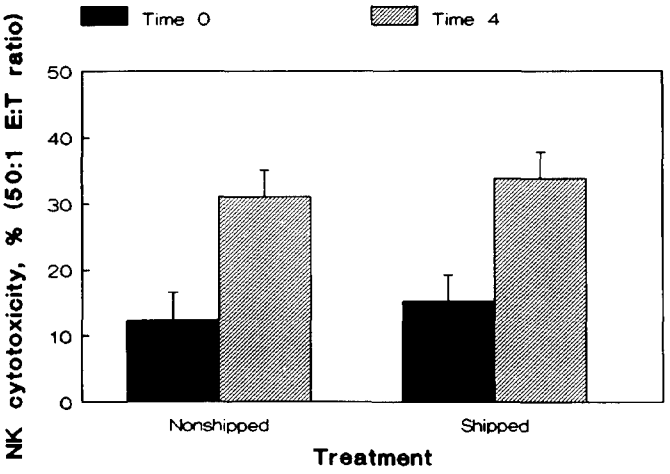

Figure 3. The shipping × time effect was not significant for natural killer cell (NK) cytotoxicity at any effector:target (E:T) ratio tested. Natural killer cell cytotoxicity increased (P < .001) over time, regardless of shipping treatment.

Data for correlation analyses were found to be homogeneous (and of similar magnitude and direction) for shipped and nonshipped pigs; therefore, data were pooled. The relationship between BW change during shipping and cortisol was negative (r = −.34, P = .04; Figure 5a). Pigs with higher plasma cortisol lost more weight than did pigs with lower plasma cortisol concentrations. The relationship between NK cytotoxicity and plasma cortisol was positive (r = .35, P = .036; Figure 5b). Pigs with higher plasma cortisol concentrations had higher NK cytotoxicity. Body weight change and NK cytotoxicity were negatively correlated (r = −.32, P = .058; Figure 5c). Pigs with higher NK cytotoxicity lost more weight than did pigs with lower NK cytotoxicity.

Discussion

Experiment 1 was designed to gather data in a field situation that reflects a commercial experience for pigs shipped from feeder pig producer to finisher. Pigs were housed and cared for on a commercial unit until the start of our study. Handling and human contact were minimal and, as expected, the pigs were difficult to handle for blood collection.

These commercial pigs had very high baseline plasma cortisol concentrations. In fact, plasma cortisol was more than twofold higher in pigs in Exp. 1 than in pigs used in our previous studies (McGlone et al., 1991). In addition, the NK cytotoxicity was zero. With no detectable NK cytotoxicity, we must conclude that all pigs, not just pigs that were shipped, experienced stress associated with handling. The stress experience associated with handling may have been greater than any potential treatment effects. Therefore, the use of commercial pigs to evaluate effects of shipping stress on NK cytotoxicity is likely to be an insensitive model system.

The second experiment was designed to measure potential increases in plasma cortisol associated with handling. The conclusion is that plasma cortisol concentration rises by 2.6 ng/mL per minute after the pen of pigs is aroused. These data argue against the use of many pigs in one pen because the more pigs there are in a pen, the greater cortisol will rise by the time the last pig is bled. Also, these data suggest to us that many pens of pigs can be bled, because bleeding one pen in a room did not cause elevated plasma cortisol among pigs housed in other pens.

One reason the group size of three was selected for the third experiment was that plasma cortisol concentration should not increase significantly by the time blood is obtained from the third pig in each pen. A second reason for using a group size of three pigs was that their social status can be determined. With larger group sizes, social relationships are more complex (triangles and other nonlinear relationships are possible). In the 18 pens of three pigs each used in Exp. 3, we found that each pen had a linear social order. Each pen had a clear dominant, intermediate, and submissive individual. When we studied group sizes of four or more in previous research (McGlone, 1986), some pens had nonlinear social relationships.

Experiment 3 was an attempt to control as many variables as possible, while studying shipping stress effects on immune function, with particular attention toward NK cytotoxicity. A previous study showed that mice experience a shipping-induced suppression in NK cytotoxicity (Aguila et al., 1988).

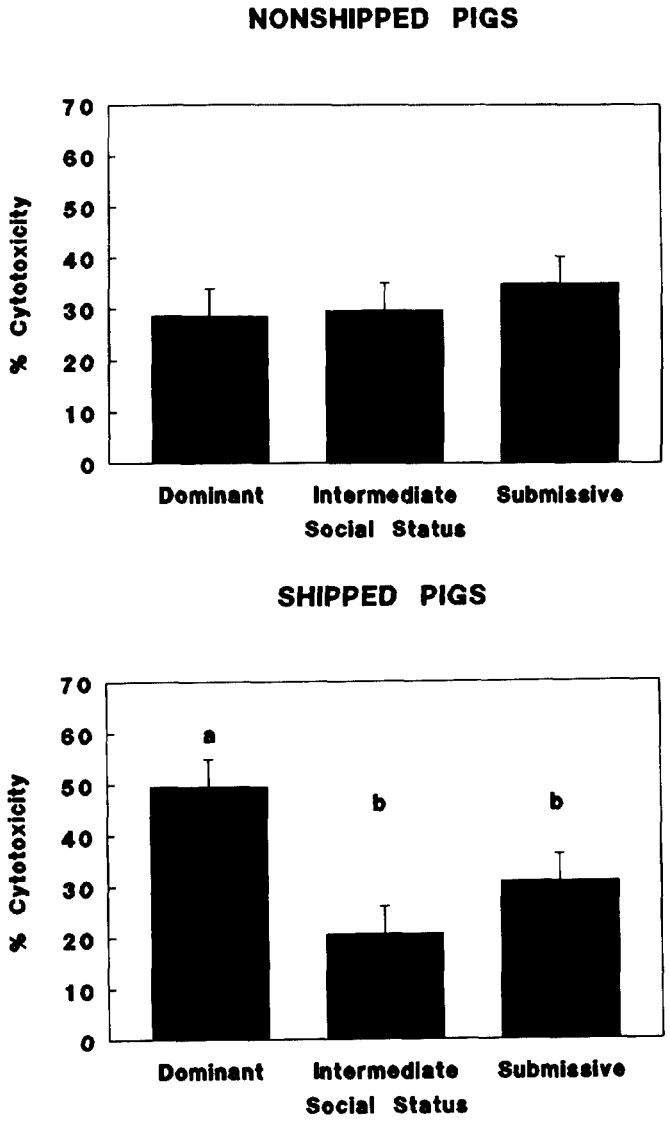

Figure 4. The interaction between shipping stress, social status, and time was significant ($P = .04$). At the preshipping time, pigs had similar natural killer cell cytotoxicity. At the end of a 4-h period, nonshipped pigs did not differ in natural killer cell cytotoxicity at each social status (Figure 4a, top). Among shipped pigs (Figure 4b, bottom), socially dominant pigs had greater ($P < .02$) natural killer cell cytotoxicity than did pigs of intermediate or submissive social status.

In Exp. 3, shipped pigs showed a decrease in BW (5%) that was greater than might be expected from simply a lack of feed and water during shipping. Besides the weight loss associated with shipping, shipped pigs had elevated plasma cortisol and an increased percentage of circulating neutrophils. Similar shipping-induced changes in neutrophil numbers are also well documented in cattle (Murata et al., 1987; Cole et al., 1988). The weight loss and increased numbers of circulating neutrophils were correlated with elevated cortisol. Thus, elevated HPA function

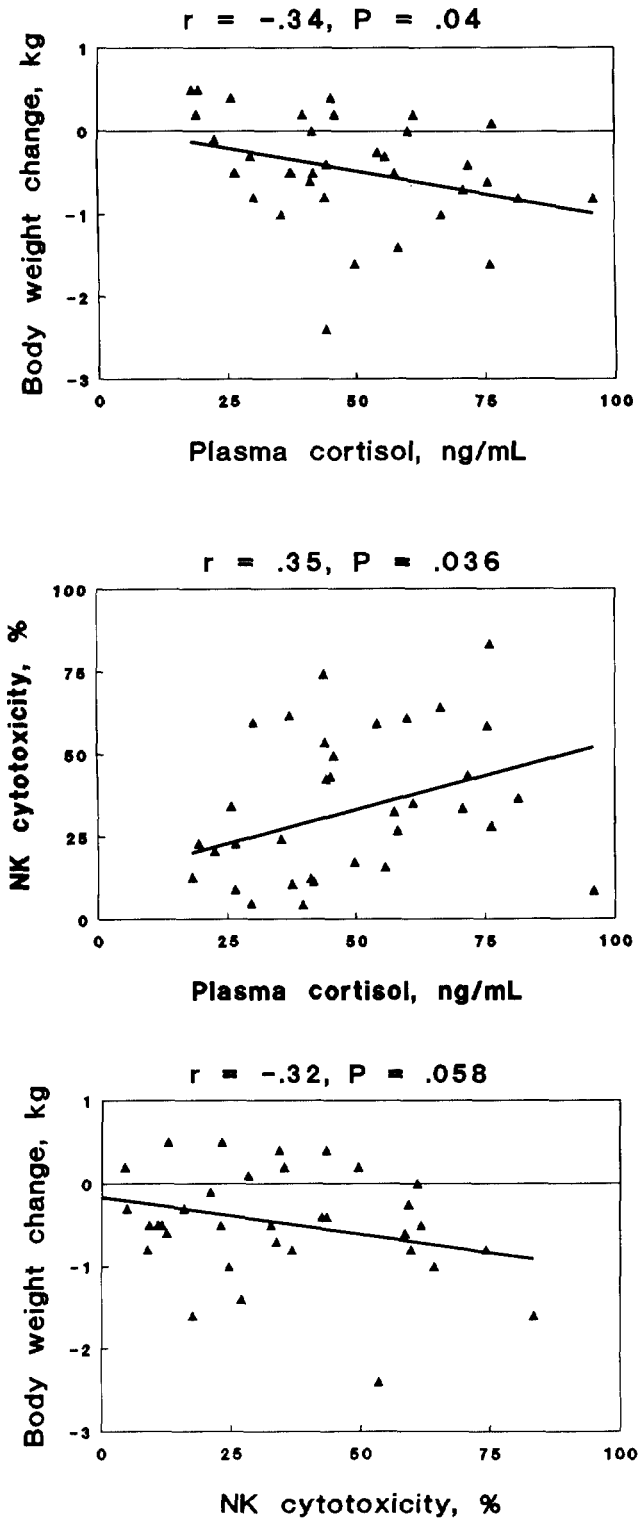

Figure 5. Correlation analyses for body weight change during shipping, plasma cortisol concentration, and natural killer cell (NK) cytotoxicity. Plasma cortisol increased among pigs that lost more weight during shipping (Figure 5a, top). Pigs with greater NK cytotoxicity at the end of the 4-h period had greater plasma cortisol concentrations (Figure 5b, middle). Pigs that lost more weight during shipping had greater NK cytotoxicity than pigs that lost less weight (Figure 5c, bottom). These relationships should be viewed with caution because they are based only on correlation analyses.

Table 3. Effects of shipping, time, and shipping × time interaction on differential white blood cells and performance in Experiment 3

	Time 0		Time 4 h		*P*-values				
Measure	Nonshipped	Shipped	Nonshipped	Shipped	SE[a]	S[b]	SE[c]	T[b]	S × T[b]
Total white blood cells, no./μL × 10^e	20.7	19.8	20.2	21.7	2.01	.877	1.54	.648	.428
Neutrophils, %[d]	32.7[y]	40.8[z]	38.0[y]	55.2[z]	2.67	.001	2.46	.001	.07
No./μL[d]	6,934	8,445	7,661[y]	12,179[z]	1320	.030	1000	.03	.14
Lymphocytes, %[d]	65.4[y]	57.1[z]	59.6[y]	44.3[z]	2.52	.001	2.47	.001	.17
No./μL[d]	13,353[y]	10,887[z]	12,179[y]	9,495[z]	1010	.019	887	.133	.964
Neutrophils: lymphocytes[d]	.52[y]	.83[z]	.68[y]	1.4[z]	.12	.001	.12	.005	.110
Monocytes, %	.61	.61	.72	.56	.25	.742	.20	.889	.581
No./μL	122	138	136	115	52	.955	44.8	.915	.675
Eosinophils, %[d]	1.2	1.6	1.2[y]	.22[z]	.35	.474	.28	.017	.016
No./μL[d]	238	326	237[y]	54[z]	70	.503	62.1	.031	.033

[a]Standard errors for main plot effect of shipping.
[b]S = shipping effect, T = time effect, S × T = shipping × time interaction.
[c]Standard errors for sub-plot effects of time and shipping × time.
[d]For shipping, time 0 data differ from time 4 h ($P < .05$).
[y,z]Means within a time group with different superscripts differ ($P < .05$).

may, at least in part, explain the effects of shipping on weight loss and circulating leukocyte numbers. Activation of the HPA does not, however, provide a clear explanation of observed treatment effects on NK cytotoxicity. Social status and shipping interacted for their effect on NK cytotoxicity. Control (nonshipped) pigs showed no relationship between social status and NK cytotoxicity. However, when pigs were shipped, the dominant pigs had greater NK cytotoxicity than pigs holding the intermediate or submissive social positions (Figure 4). Therefore, stressed pigs (with low NK cytotoxicity) of low social status may be more susceptible to pathogens. Another report indicated that transportation stress reduced interferon-alpha production from porcine mononuclear cells (Artursson et al., 1989). Taken together, shipped pigs may show two signs of immunosuppression (reduced NK and interferon) that would make them more susceptible to viral pathogens.

Plasma cortisol concentrations did not explain the observed treatment effects on NK cytotoxicity. In fact, pigs with higher plasma cortisol concentrations had elevated NK cytotoxicity (Figure 5b).

A previous study from our laboratory (McGlone et al., 1991) showed that pigs given a single bolus of ACTH had elevated NK cytotoxicity 4 h later. Our data in Exp. 3 support the notion that at some point after a stressor begins to act, NK cytotoxicity may actually be enhanced. Likewise, in spite of the fact that a wide range of stressors reduce NK cytotoxicity (Trinchieri, 1989), certain stressors, such as loud noise, enhance NK cytotoxicity (Irwin et al., 1989). Enhanced NK cytotoxicity among stressed animals could be mediated by catecholamines (Tennesen et al., 1984) or ACTH (McGlone et al., 1991) or by β-endorphin (Mathews et al., 1983).

The positive, but low, correlation between NK cytotoxicity and weight loss during shipping could have interesting implications. Pigs that lose more weight and have the associated greater HPA activation may actually be less susceptible to viral pathogens, because NK cells are a first line of defense against viral pathogens (Trinchieri, 1989). The relationships illustrated in Figure 5 could be examined in future studies.

Implications

These studies have implications for future shipping stress studies. Potential interactions between social status and shipping stress as well as the stress of handling may preclude finding treatment effects. Controlling for social status along with other factors (genotype and environment) will provide a more sensitive model system. Furthermore, only certain animals (such as socially submissive pigs) may be negatively affected by shipping stress.

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