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# Diverse production system and social status effects on pig immunity and behavior

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#### Abstract

Pig performance, immunity, and behavior may be influenced by production system and social status. A conventional indoor housing system was compared with an outdoor system to determine the effects of diverse production systems on pig performance, behavior, and immunity. At 21 days of age, weight matched pigs reared in a conventional indoor or an outdoor production system were weaned and randomly selected for use in the present study. Six replicate pens were evaluated per treatment, with two littermate female pigs in each pen. Social status was determined at 7 weeks of age by a feed competition test. Pig behavior was recorded for 24 h using a scan sample technique. At 9 weeks of age, blood samples were taken from indoor- and outdoor-reared pigs and immune assays performed. Both indoor- and outdoor-reared pigs were given a sheep red blood cell (SRBC) challenge at 6 and 8 weeks of age, and antibody titers to SRBC were measured at 6, 7, and 9 weeks of age. Performance data were recorded throughout the study period. Body weight and average daily gain were not influenced by the production system in which the pig was reared. The production system effected standing, lying, drinking, and oral/nasal/facial behaviors. The production system influenced the expression of certain behaviors depending on the time of day. Immune and blood measures were affected by production system. The percentage of phagocytosis was greater ( $P \le 0.05$ ) and antibody titers to SRBC challenge tended to be greater (P=0.066) among outdoor-reared pigs compared with indoor-reared pigs. Outdoor reared pigs had higher hemoglobin concentrations (P < 0.005), percentage of hematocrit (P < 0.005), mean corpuscular volume (P < 0.005), and mean corpuscular hemoglobin (P < 0.005) compared with indoor-reared pigs. Furthermore, dominance order influenced the immune system. Dominant pigs had greater phytohemagglutinin stimulated lymphocyte proliferation (P < 0.01) and baseline antibody titers to sheep red blood cells (P < 0.05) compared with submissive pigs. In conclusion, in the present study body weight and average daily gain were similar among diverse production systems. However, pig behavior and immune system were significantly influenced by the production system. Dominance order influenced the immune system regardless of the production system.

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## 1. Introduction

Pigs are reared in a wide variety of production systems. Currently, indoor systems are the most commonly used production system in the US.

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Alternative production systems include a variety of systems ranging from partial confinement to complete outdoor systems. Understanding how each production system impacts pig well-being is important. Production systems can have a major influence on pig immunity, growth, behavior, and meat quality; however, work to date has focused on sows and finishing pigs (Sather et al., 1997; Gentry et al., 2002a,b; McGlone and Fullwood, 2001; Dailey and McGlone, 1997).

Dominance order was shown to influence performance and the immune system in pigs (McGlone et al., 1993; Hicks et al., 1998; Sutherland et al., 2006). A relationship between a pig's dominance order and its immune response to a stressor has also been shown (McGlone et al., 1993; Morrow-Tesch et al., 1994; Hicks et al., 1998; Tuchscherer et al., 1998). Furthermore, in pigs subjected to an immune challenge with Aujesky's disease virus, submissive pigs showed more morbidity than dominant animals (Hessing et al., 1994). Therefore, it is of interest to determine the effect of dominance order on performance, behavior, and the immune system of pigs reared in diverse production systems.

The objective of this study was to determine if pig performance, behavior, or immunity differed among pigs reared from birth through weaning in diverse production systems and whether dominance order would have an impact on these measures. We utilized production systems in this work that represent two widely-different production systems similar to those found in commercial pork production.

# 2. Materials and methods

## 2.1. Experimental design

Pigs were farrowed indoors in conventional farrowing crates on slotted flooring or outdoors on pasture. Sows were Yorkshire×Landrace×Duroc cross-bred sows (Newsham Hybrid, Pueblo, CO). The indoor system included farrowing and nursery rooms with mechanical ventilation as well as supplemental heat. The outdoor system was located on a pasture with straw bedded huts for shelter. The production systems were described in more detail by Johnson et al. (2001).

At 21 days of age, 24 weight-matched female pigs were randomly selected from indoor (n=12) and outdoor (n=12) production systems and weaned. Two female littermates were chosen from each of 12 litters and littermates were penned together in pens of two. Six replicate pens were evaluated per treatment, with two littermate female pigs in each pen.

Pigs housed in the indoor system were kept in pens with metal woven wire flooring and had an average space allowance of  $1.2 \text{ m}^2$  per pig. Mechanical ventilation and fluorescent lights (12:12) were provided. Pigs housed in the outdoor system were kept on alfalfa pastures with metal welded wire fencing and had 125 m<sup>2</sup> per pig space allowance. Pigs were given water and feed ad libitum. Pig weights were measured each week after weaning throughout the course of the trial. The Texas Tech University Institutional Animal Care and Use Committee approved the experimental procedures.

# 2.2. Behavior

Behavior data were collected over a 24 h period during week 8 of age. Data were collected from indoorreared pigs using a time-lapse video recording system. Tapes were viewed using the same behavioral sampling technique as for outdoor-reared pigs. In the indoor system, behavior data were lost for one block due to technical problems. While pigs were being filmed, outdoor-reared pig behavior data were collected simultaneously from observers within a blind, using a 10 min scan sample of each pig, with 12 periods of 2 h each per 24 h period. At night, the outdoor pigs were observed with night vision scopes. The same scan sampling method was used when the indoor-reared pig video tapes were reviewed. The following mutually exclusive behaviors were recorded: walking, standing, lying, drinking, feeding, rooting, oral/nasal/facial (ONF) behaviors, pen mate contact, neighbor contact as well as total inactive behavior, total active behavior, and an in-hut behavior for only the outdoor pigs. Behaviors are defined in Table 1.

## 2.3. Social status

Social status was determined during week 7 using a feed competition test (Syme, 1974; McGlone, 1986). Briefly, a feeder that allowed only one pig to feed at a time was used daily. During week 7, the feeder was completely removed from the pen for a period of 24 h. The following day, the feeder was put back in the pen and data were collected on feeding behavior of the pigs to determine feeding dominance order. Each pen of pigs was observed for a total of 30 min, with 6 consecutive 5-minute observations. The time that each pig spent with its head in the single-hole feeder eating was recorded. The dominant pig was determined using two separately calculated values. A percentage was generated of the percent of time that each pig spent eating for the first 5-

Table 1 Description of behaviors

Behavior	Description						
Walking <sup>a</sup>	Relatively low speed locomotion in which propulsive force derives from the action of legs						
Standing <sup>a</sup>	Assuming or maintaining an upright position on extended legs						
Sitting <sup>a</sup>	Resting on the caudal part of the body						
Feeding <sup>a</sup>	The pig's head within the feeder and the head moving like it was chewing						
Drinking <sup>a</sup>	Pig's mouth/snout touching waterer and neck moving in a gulping movement as with oral ingestion of liquids						
Rooting <sup>a</sup>	Digging the ground with the snout						
Oral/nasal/ facial <sup>b</sup>	Licking/biting/nosing						
Lying <sup>a</sup>	Maintaining a recumbent position						
Contact (pen mate)	Exhibited when pigs from the same pen are engaged in physical contact						
Contact (neighbor)	Exhibited when a pig from one pen was engaged in physical contact with a pig from another pen						
In-hut (outdoors)	The pig(s) was/were located within the shelter hut						
Total active (indoors)	All behaviors combined, with the exception of the lying behavior						
Total active (outdoors)	All behaviors combined with exception of lying, contact, and in-hut behavior. (Total active=walk+ stand+sit+drink+feed+root+ONF).						
Total inactive (indoors)	Lying behavior						
Total inactive (outdoors)	Lying behavior and in-hut behavior						
ONF = oral/na	sal/facial.						

<sup>a</sup> Hurnik et al. (1995).

<sup>b</sup> Dailey and McGlone (1997).

minute period. This first dominance value (DV1) was calculated using the formula:

$$DV1 = \frac{\text{Time (s)}}{300 \text{ s}}$$

## 2.4. Immune assays

Pigs were sampled at the same time each morning (10:00 am) for each sampling period to prevent differences in blood measures due to circadian rhythm. Blood was taken from pigs at 9 weeks of age for immune and blood measures and serum was collected for cortisol and SRBC antibody determination. Blood was taken and serum collected from pigs at 6, 7, and 9 weeks of age for SRBC antibody determination.

Twenty seven milliliters of blood was taken from pigs by anterior vena cava puncture at 9 weeks of age. Twenty mL of blood was collected into vacutainers containing heparin (143 USP) and 7 mL into tubes without anti-coagulant for the collection of serum. Blood collected over heparin was used for whole blood leukocyte counts, neutrophil chemotaxis and chemokinesis, phagocytosis, and mitogen-induced lymphocyte proliferation assays using previously validated techniques. Serum was collected for the analysis of antibody titers to sheep red blood cells and cortisol concentrations. Differential leukocyte counts and percentages were obtained using a cell counter (Cell-Dyn<sup>®</sup>, Abbott laboratories, Abbott Park, IL) and the neutrophil to lymphocyte ratio was calculated by dividing the percent of neutrophils by the percent of lymphocytes.

## 2.4.1. Cell isolation

Porcine lymphocytes and neutrophils were isolated from 20 mL of whole blood by density gradient centrifugation using Histopaque-1077 (density=1.077 g/mL; Sigma, St. Louis, MO) and Histopaque-1119 (density=1.119 g/mL; Sigma). Whole blood was diluted with Roswell Park Memorial Institute (RPMI) media and layered over Histopaque-1077 and -1119 (Sigma), then centrifuged at 700  $\times g$  for 30 min at room temperature. Lymphocytes were collected from the 1077 layer, washed twice in RPMI, resuspended, and counted. Neutrophils and RBC were removed from the 1119 layer and washed once in RPMI. Red blood cells were lysed using cold endotoxin-free water and isotonicity was restored using 10× PBS. Neutrophils were centrifuged for 10 min at 475  $\times g$ , supernatant was decanted, pellet washed twice, and resuspended in RPMI.

## 2.4.2. Chemotaxis

The neutrophil chemotaxis and chemokinesis assays were performed according to published methods (Salak et al., 1993; Hulbert and McGlone, 2006). Briefly, a modified Boyden chamber (Neuro Probe, Cabin John, MD) was used to measure the migration of neutrophils across a polyvinylpyrrolidone-free filter (pore size 5  $\mu$ m; Neuro Probe) towards media (chemokinesis) or towards recombinant human complement-C5a (C5a; chemotaxis). The media or C5a (10<sup>-8</sup> M) were added in duplicate to the bottom wells of the chamber and the neutrophils, adjusted to 1 × 10<sup>6</sup> cells/mL, were added to the top wells of the chamber. The filter was fixed and stained using Lekostat I and II solution (Fisher Scientific, Houston, TX). Five fields per well of the cells were counted in a blind fashion at 1000× magnification.

## 2.4.3. Lymphocyte proliferation

The lymphocyte proliferation assay was used to determine proliferation of lymphocytes in response to

the mitogen phytohemagglutinin, according to an established method (Mosmann, 1983; Morrow-Tesch et al., 1994). Briefly, lymphocytes were adjusted to  $5 \times 10^6$  cells/mL and placed in triplicate in a sterile 96-well plate containing the mitogen phytohemagglutinin (Sigma). The mitogen was added at concentrations of 0, 0.2, 2.0, and 20.0 µg/mL. Radiolabeled thymidine was added to all wells after 48 h of incubation at 37 °C and then cells were harvested at 72 h. The uptake of the nucleic acid was proportional to activity of the cultured lymphocytes.

#### 2.4.4. Phagocytosis

The phagocytosis assay was performed to determine the percent of latex beads engulfed by neutrophils, including the average number of beads phagocytized per cell, as previously described (Hulbert and McGlone, 2006). Briefly, opsonized latex beads (0.807 µm diameter, Sigma) at a concentration of  $1 \times 10^7$  beads/ mL were added to neutrophils adjusted to  $1 \times 10^6$  cells/ mL. The neutrophil/bead mixture was then incubated for 10 min in a humidified CO<sub>2</sub> chamber at 37 °C. Tubes were spun at 40  $\times$ g for 7 min at 8 °C and the supernatant was removed. The neutrophil/bead mixture was washed twice and then 200  $\mu$ L of solution was obtained from the neutrophil/bead mixture and spun using a cytofuge microcentrifuge (Cytofuge 2, model M801-22, Stat-Spin, Inc., Norwood, MA) for 2 min at 9693  $\times g$ . The slides were fixed and stained using Lekostat I and II solution (Fisher Scientific). A total of 100 neutrophils were counted and for each cell, the number of beads phagocytized were counted as 0, 1, 2, 3, 4, 5, or 6 or more. The percentage of cells that phagocyotyzed at least one bead and the average numbers of beads phagocytosized were determined.

## 2.4.5. Cortisol

Serum was assayed for cortisol using a commercially available radioimmunoassay kit (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA) following the manufacturer's instructions. The intra-assay coefficient of variation was 18.65%. The minimal detectable levels were 2 ng/mL.

# 2.4.6. Sheep red blood cell hemagglutination

Pigs were injected with 1 mL of 40% SRBC in PBS during week 6 and 8 of age. Serum samples were obtained from pigs at 6, 7, and 9 weeks of age for SRBC antibody determination. The hemagglutination assay was performed in duplicate to determine antibody response of the pig to SRBC, according to methods of Blecha and Kelly (1981). Briefly, serum samples were Table 2 Least squares means for performance measures of indoor and outdoor reared gilts

	Producti					
Measure	Indoor	SE	Outdoor	SE	P-value	
n	12		12			
Body weight, kg						
Day 0 (Birth)	1.72	0.10	1.93	0.10	0.466	
Day 21	7.01	0.23	7.27	0.25	0.440	
Day 28	8.02	0.23	8.54	0.23	0.748	
Day 35	9.66	0.31	10.32	0.31	0.142	
Day 42	11.85	0.41	13.01	0.41	0.059	
Day 48	14.89	0.50	15.64	0.50	0.302	
Day 56	18.17	0.66	18.57	0.66	0.814	
Day 63	21.35	0.68	21.72	0.68	0.705	
ADG, kg/day	0.36	0.02	0.36	0.02	0.681	

thawed and heat-inactivated for 30 min in a 57 °C water bath. Heat-inactivated samples (200  $\mu$ L) were added to a 96 round-bottom plate, PBS was added, and samples were serially diluted. To each well, 100  $\mu$ L 1% SRBC was added, plates were covered, and then incubated for 24 h at room temperature. The SRBC titers were determine by sedimented cells forming a distinct pattern on the bottom of the wells. The highest dilution yielding a positive reaction was deemed the titer.

#### 2.5. Statistical analysis

All data were tested for departures from normal distribution using the Shapiro-Wilk's test. Data were subjected to analysis of variance using Proc GLM procedure of SAS version 9.1 (SAS Institute, Inc., 1990). All analyses were performed as two-tail tests. The pen was the experimental unit. The study was a completely random design with two treatments (production systems: indoor vs. outdoor). The main fixed effects were block (three levels), production system (indoor and outdoor), and social status (dominant and submissive). The interaction between production system and social status was included in the model (1 df). Random effects of pen and pig were included. A total of 6 pens (12 pigs) were examined per treatment (n=24). Behavior data were analyzed as completely random design and split plot over time with two production system treatments. The 24-h behavior observation period was divided into 12, 2-h periods. For behavioral measures, the main fixed effects were block (three levels), period (12 levels) and production system (indoor and outdoor). The interaction between production system and period was included in the model (11 df). A total of 6 pens (12 pigs) were examined per treatment (n=24). Social status was not

Table 3	
The percentage of time (least squares means), over a 24 h period, that indoor and outdoor raised pigs were engaged in sele	ected behaviors

Measure	Indoor	SE	Outdoor	SE	<i>P</i> -value		
					System	Period	System×period
n	10		12				
Walk	2.53	1.14	2.66	1.05	0.886	0.514	0.139
Stand	0.42	0.52	4.63	0.48	0.05	0.448	0.852
Sit	0.28	0.14	0.06	0.13	0.400	0.499	0.314
Lying	34.10	13.76	8.85	12.67	0.452	0.803	0.010
Feed	4.27	1.21	4.28	1.12	0.980	0.158	0.883
Drink	47.26	13.25	12.09	12.20	0.196	0.058	0.021
Root	5.52	1.61	9.38	1.49	0.323	0.377	0.758
ONF	0.63	3.54	7.64	3.26	0.438	0.047	0.035
Total active	60.90	14.65	40.74	13.49	0.367	0.090	0.375

ONF = oral/nasal/facial.

Total active=walk+stand+sit+drink+feed+root+ONF.

included in the behavior model as pigs were not individually identifiable at all times of day.

# 3. Results

## 3.1. Performance

Body weight at birth, 21, 28, 35, 42, 48, 56, and 63 days, and average daily gain did not differ between indoor- and outdoor-reared pigs throughout the period of the study (Table 2). Dominant pigs did not grow at a different rate than submissive pigs (Dominant:  $0.34\pm 0.02 \text{ kg/day}$ ; Submissive:  $0.38\pm 0.02 \text{ kg/day}$ ). Pigs grew at a normal rate and were healthy throughout the study compared with commercial pig production.

## 3.2. Behavior

Indoor-reared pigs spent less (P < 0.05) time standing compared with outdoor-reared pigs, regardless of time

day (Table 3). Outdoor-reared pigs spent less (P < 0.01) time lying compared with indoor-reared pigs throughout the 24 h observation period, except between 1700–1900 and 2300–0100 h (Fig. 1). Drinking behavior was performed less (P < 0.05) by outdoor-reared pigs compared with indoor-reared pigs, throughout the 24 h observation period (Fig. 2). However, outdoor-reared pigs spent more (P < 0.05) time performing ONF behaviors between 1300 and 1700 h compared with indoor-reared pigs (Fig. 3). Production system did not influence any other recorded behaviors.

# 3.3. Immune and red blood cell measures

Outdoor-reared pigs had a greater ( $P \le 0.05$ ) percentage of neutrophils that phagocytozed latex beads compared with indoor pigs (Table 4). Outdoor-reared pigs tended to have higher (P=0.066) baseline antibodies titers to the SRBC challenge compared with indoor-reared pigs (Table 4). Furthermore, outdoor-

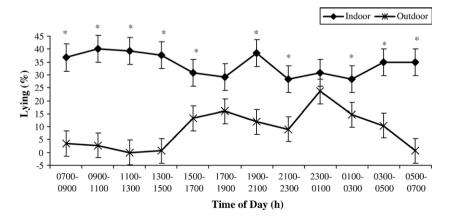


Fig. 1. The percentage of time spent lying by indoor reared (Indoor; n=10) and outdoor reared (Outdoor; n=12) pigs over a 24 h period. At each time point, least squares means accompanied by subscripts are different at P < 0.05.

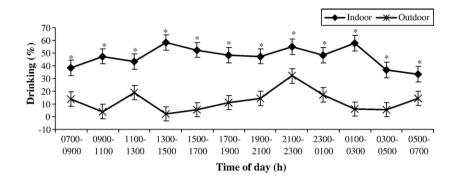


Fig. 2. The percentage of time spent drinking by indoor reared (Indoor; n=10) and outdoor reared (Outdoor; n=12) pigs over a 24 h period. At each time point, least squares means accompanied by subscripts are different at P < 0.05.

reared pigs had greater hemoglobin concentrations (P < 0.005), percentage of hematocrit (P < 0.005), mean corpuscular volume (P < 0.005), and mean corpuscular hematocrit (P < 0.05) compared with indoor-reared pigs (Table 4). Production system has no effect on chemotaxis in response to the mitogen C5a, chemokinesis in response to media, cortisol concentrations, or the neutrophil to lymphocyte ratio.

## 3.4. Social status

The effects of social status on behavior were not assessed because we did not have the pigs marked in such a way that we could identify the individual pigs throughout the entire 24 hour observation period, especially at night.

Lymphocyte proliferation was greater (P < 0.01) among dominant pigs than submissive pigs, regardless of the production system (Fig. 4). Furthermore, baseline antibodies titers to SRBC challenge were greater (P < 0.05) among dominant pigs compared with submissive pigs (Fig. 4). Dominance order did not influence cortisol concentrations or other blood or immune measures.

## 4. Discussion

The comparison made in this work is between two diverse production systems. Production systems differed in space allowance, floor/earth composition, photoperiod, light intensity, sunlight, effective environmental temperatures (EET), weather conditions and environmental diversity. We can not be sure which factor within each production system caused or did not cause the observed effects.

In the present study, body weight and average daily gain were similar for pigs reared in the indoor and outdoor production systems. One production system was not beneficial compared with the other as measured by body weight and average daily gain. Sather et al. (1997) found that outdoor (free-range) reared pigs took approximately 16 more days to reach market weight compared with indoor (confinement) reared pigs and indoor-reared pigs had a heavier commercial carcass weight than outdoor-reared pigs at finishing. Hence, other measures of performance including carcass composition may be necessary to get a thorough comparison of different production systems on performance in pigs. Additionally, average daily gain and

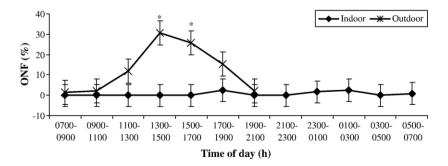


Fig. 3. The percentage of time spent performing oral/nasal/facial (ONF) behaviors by indoor reared (Indoor; n=10) and outdoor reared (Outdoor; n=12) pigs over a 24 h period. At each time point, least squares means accompanied by subscripts are different at P < 0.05.

Table 4 Immune and cortisol measures (least squares means) of indoor and outdoor reared gilts

	System			<i>P</i> -value			
Measure	Indoor	Outdoor	SE	System	Dominance order	System×dominance order	
N	12	12					
White blood cell $(10^3 \text{ cells}/\mu\text{L})$	16.65	18.03	1.09	0.380	0.457	0.765	
Red blood cells ( $10^6$ cells/ $\mu$ L)	6.47	6.50	0.12	0.858	0.236	0.279	
Hemoglobin (g/dL)	11.30	12.27	0.20	0.003	0.723	0.906	
Hematocrit (%)	32.21	35.13	0.60	0.003	0.700	0.985	
Mean corpuscular volume (fL)	49.90	54.10	0.80	0.002	0.325	0.239	
Mean corpuscular hemoglobin (pg)	17.49	18.89	0.28	0.002	0.345	0.253	
Phagocytosis (%)	74.40	88.27	4.03	0.050	0.862	0.566	
Chemotaxis (no./5 fields) <sup>a</sup>	181.16	111.23	65.21	0.506	0.278	0.415	
Chemokinesis (no./5 fields) <sup>b</sup>	6.06	19.05	44.02	0.195	0.312	0.536	
Lymphocyte proliferation <sup>c</sup>	9843.90	13031.23	1398.59	0.125	0.008	0.195	
SRBC antibody titers							
Baseline	3.13	3.42	0.11	0.066	0.022	0.412	
Day 7	4.33	4.08	0.18	0.342	0.342	0.749	
Day 21	4.50	4.50	0.23	1.00	0.156	1.00	
Cortisol (ng/mL)	42.29	52.76	4.74	0.136	0.555	0.116	

SRBC = Sheep red blood cell challenge.

<sup>a</sup> Chemotaxis using the chemoattractant human complement-5a.

<sup>b</sup> Chemokinesis using media.

 $^{c}\,$  Lypmphocyte proliferation using the mitogen phytohemagglutinin at a concentration of 2.0  $\mu g/mL.$ 

body weight were not affected by dominance order. In previous studies, dominant pigs were found to be heavier than submissive pigs (McGlone et al., 1993; Hicks et al., 1998), however, Sutherland et al. (2006) found no difference in body weight or average daily gain between dominant and submissive pigs. Conventional wisdom holds that dominant pigs grow faster and are heavier than submissive pigs; but this relationship may not hold for pigs with unlimited access to resources (feed, water, resting places).

Indoor- and outdoor-reared pigs spent a similar percent of time on total activity, regardless of time of day. However, pigs reared in the indoor production system displayed more drinking behavior compared with outdoor-reared pigs. The higher percentage of hematocrit and higher concentration of hemoglobin in outdoor-reared pigs in combination with a lower frequency of drinking behavior may suggest that outdoor- reared pigs were dehydrated compared with indoor-reared pigs. Furthermore, increased space allowance to exercise and a more enriched environment experienced by outdoor-reared pigs may have influenced hematological measures due to slight dehydration. The hematocrit and hemoglobin concentrations measured in our outdoor-reared pigs were still within normal range (Mersmann and Pond, 2001). A more likely conclusion is that outdoor-reared pigs consumed more iron since birth from rooting in the dirt and this caused the higher hemoglobin concentrations. Indoorreared pigs probably routinely consume more water than outdoor pigs which would cause the lower hematocrit among indoor-reared pigs compared with outdoorreared pigs. However, 10 min scan sampling is not effective at measuring infrequent, short term behaviors

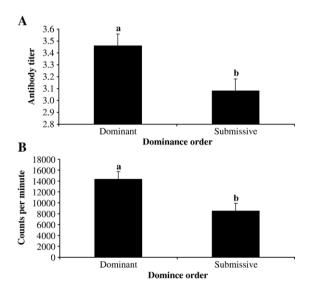


Fig. 4. The effect of dominance order on baseline antibody titers to a sheep red blood cell challenge (A) and phytohemagglutinin induced lymphocyte proliferation at a concentration of 2.0  $\mu$ g/mL (B) in indoor and outdoor reared pigs. Least squares means±S.E. accompanied by different subscripts are different at *P*<0.05. (Dominant: *n*=12; Submissive: *n*=12).

such as drinking behavior, which needs to be taken into account when interrupting these results. In future studies it is important to measure water intake more directly.

The percentage of time spent performing ONF behaviors was strikingly different between the two diverse production systems between 1300 and 1700 h, but similar between the production systems throughout the remaining observation period. Some ONF behaviors are a combination of normal oral behaviors while others are defined as stereotypic behaviors and are thought to be an indicator that an animal is experiencing stress (Dailey and McGlone, 1997 [for sows]). In the present study ONF activity increased among outdoor-reared pigs between 1300 and 1700 h, but ONF behaviors remained lower and constant among indoor-reared pigs throughout the 24 h observational period. The increase in ONF behaviors among outdoor-reared pigs during mid day hours may suggest a diurnal rhythm in ONF behavior rather than the performance of stereotypic behaviors in response to stress. The low level of ONF behaviors displayed among indoor-reared pigs may be due to a lack of suitable substrate and hence the waterer is a convenient device for the weanling pigs to manipulate. The drive to express ONF behaviors indoors may result in more time manipulating the waterer, while outdoor pigs, who may find rooting in soil more rewarding, spend more time rooting and chewing soil.

Outdoor-reared pigs had a higher percentage of neutrophil phagocytosis compared with indoor-reared pigs, in the present study. Increased phagocytosis in outdoor-reared pigs suggests that pigs reared in this production system had increased neutrophil function. Neutrophils are primarily involved in the innate immune system in response to extracellular pathogens, such as bacteria. Therefore, outdoor-reared pigs may have an activated innate immune system and could possibly be better able to cope with a bacterial challenge which could consequently benefit the overall health of the animal. Outdoor-reared pigs also produced higher basal antibody titers to sheep red blood cells. As pigs were not exposed to SRBC challenge at this point, these higher antibody titers probably reflect higher antibody levels to foreign antigen in general due possibly to more frequent or diverse immune challenges experienced by pigs reared in the outdoor production system. The production of antibodies in response to an antigen is an aspect of the humoral immune response. Kleinbeck and McGlone (1999) found that indoor-reared pigs had greater natural killer activity compared with outdoor-reared pigs. Therefore, indoor-reared pigs may be better able to cope with an intracellular challenge (ex. Viruses) compared with outdoor-reared pigs. The combination of increased neutrophil function and antibody production in outdoor-reared pigs suggests that these pigs may have had an activated T-helper 2 or humoral immune status, whereas indoor-reared pigs may have a more cell mediated or T-helper 1 immune response due to the increased concentration of selected microbes. Therefore, the immune system of outdoor-reared pigs may be directed more towards fighting a bacterial challenge and conversely indoor-reared pigs may have an immune system activated towards a challenge from intracellular pathogens. Further research is required to determine if this is the case and to understand the consequences of differential immune system activation.

Cortisol concentrations or neutrophil to lymphocyte ratio did not differ between the two diverse production systems used in the present study. Cortisol concentrations in pigs have been shown to increase in response to acute transport stress (Hicks et al., 1998), castration (Prunier et al., 2005), and mixing (Morrow-Tesch et al., 1994). Conversely, cortisol concentrations have been shown to decrease in response to chronic heat stress (Heo et al., 2005; Sutherland et al., 2006). Furthermore, the neutrophil to lymphocyte ratio has been shown to shift in response to stress in pigs (McGlone et al., 1993). However, one sample period is not sufficient to measure stress accurately and cortisol and the neutrophil to lymphocyte ratio is more a measure of acute stress, hence future studies should include continuous sampling throughout the nursery period to get a better understanding of the effect of different production systems on of the stress response in pigs and include chronic measures of stress and the immune system, such as an immune challenge. Performance as measured by body weight and average daily gain was also similar between production systems. Therefore, the similar cortisol concentrations, neutrophil to lymphocyte ratio, and performance of pigs in both indoor and outdoor production systems suggests that neither production system caused more or less stress than the other system.

In the present study, dominance order influenced lymphocyte proliferation and baseline antibody titers to sheep red blood cells. Dominant pigs had greater lymphocyte proliferation and baseline antibody titers to sheep red blood cells irrespective of the production system. Morrow-Tesch et al. (1994) found that lymphocyte proliferation was greater among intermediate pigs than dominant and subordinate pigs when using a three pig model of dominance order. However, most studies showing an affect of dominance order on immune measures has been in relationship to a pig's response to a particular stressor (McGlone et al., 1993, Morrow-Tesch et al., 1994; Tuchscherer et al., 1998; Sutherland et al., 2006). The greater lymphocyte proliferation response and higher antibody titers in dominant pigs suggests that dominant pigs may have a more activated, hence stronger immune system than submissive animals. In pigs subjected to an immune challenge with Aujesky's disease virus, submissive pigs showed more morbidity than dominant animals (Hessing et al., 1994). However, more research is necessary to determine whether the influence of dominance order on the immune system affects a pig's ability to cope with a pathogen challenge.

# 5. Conclusion

Diverse production systems can affect the behavior as well as selected immune measures in pigs, without influencing body weight or average daily gain differently. The behavioral differences displayed by pigs in these diverse production systems appear to reflect the different environments they are housed in rather than differences in animal well-being. However, the differences in the immune system of pigs reared in these diverse production systems (from this work and our past work) suggest to us that outdoor-reared pigs may have an activated T-helper 2 immune response compared with indoor-reared pigs, suggesting that these animals could have a potential health advantage to extracellular pathogens. Furthermore, dominance order appears to influence the immune system of pigs regardless of production system. Therefore, more research needs to be carried out to determine the effect of diverse production systems on the immune system of pigs and the possible immunological advantages to an immune challenge. Dominance order also needs to be taken into account when designing studies researching the immune system and behavior of pigs.

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