The influence of pen density, weaning age, and feeder space on serum haptoglobin concentration in young growing swine

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Summary: *This study was conducted to evaluate the effect of production stressors (weaning age, pen density, and feeder space limitation) on serum haptoglobin (HPT) and average daily gain (ADG) in healthy growing pigs. Pen stressors were created by varying pen size (0.1 m^2 per pig or 0.3 m^2 per pig) and limiting feeder space (1 or 2 pigs per feeder space) during nursery production. Two groups of pigs, 32 pigs per group, were weaned at 2 or 3 weeks of age. Each group was randomly divided and placed into one of four pens: 1) high density, feeder space limited, 2) high density, feeder space unlimited, 3) low density, feeder space limited, or 4) low density, feeder space unlimited. Two replications were conducted. A repeated measures multiple linear regression model was used to analyze the data. Our serum HPT and ADG results suggest 2-week-old pigs may adapt to the production stressors (weaning, pen density, feeder space) better than 3-week-old pigs.*

In swine production medicine, increased attention is being focused on management practices to aid in preventing disease and optimizing growth potential. By maintaining high standards of husbandry, swine populations are better prepared for disease challenge or other adverse factors that may lead to decreased production. These other factors may include: age of weaning, mixing of litters, handling, density within pens, nutrition, air quality, temperature, water quality, noise, or other components of the swine environment. All of these factors influence swine health and may serve as an unrelenting form of production stress.[1-4]

Several approaches have been used to assess the relationship of environmental stress factors and physiologic response. In veterinary medicine, two common indices used are serum cortisol and neutrophil:lymphocyte (N:L) ratios.[5] Stress increases endogenous serum cortisol mediated through the hypothalamic-adrenocortical axis. This may lead to elevated N:L ratios in some animal species. Nonspecific elevations other than stress (circadian influence) as well as species tolerance (adaptation) may lead to misinterpretation of what may be just basal cortisol values.

Haptoglobin (HPT), an acute-phase protein, has been reported to be influenced by glucocorticoid.[6,7] Elevations in swine serum HPT concentrations were found after injections of corticotrophin and prednisolone (8 hours). Our goal in this experiment was to examine serum HPT concentrations in weaned pigs that were exposed to typical production stressors and then evaluate how HPT could be used as an indicator of stress in swine. Serum HPT concentrations were monitored weekly in 2- and 3-week-old pigs and the stressors examined were weaning age, pen density, and feeder space. Average daily gains (ADG) were also evaluated in each group for comparison.

Materials and methods

Experimental population

A total confinement swine farm served as our experimental study facility. This farm was a high-health herd that used a Landrace × Large White rotational crossing system. The high-health status of this herd had been maintained for a 9-year period. Sporadic cases of regional ileitis had been reported in finishing (75 kg) swine. No medications or biologicals were used on animals within this farm. Twelve litters on average were farrowed monthly. Each weaning group was managed on an all-in–all-out (AIAO) basis until reaching market weight.

Experimental design

Two replicates of this experiment were conducted; the experiments followed a 2 × 2 × 2 factorial design (Table 1). The stressors designed for this study were based upon common alterations to recommended guidelines for appropriate husbandry practices and on previous experiments designed to investigate the effects of stress in swine.[8-13] Animals in the low-density, feeder-space-unlimited pens were managed to follow swine care standards and served as a comparison group.

In each replicate, 64 pigs, 14 days of age, were randomly selected from 12 litters and divided into two groups. All pigs were ear tagged for identification. Group 1 (n=32) pigs were weaned from the sows at 14 days of age and then randomly divided into four nursery pens (eight pigs per pen). Group 2 (n=32) pigs remained with the sows and when weaned at 21 days of age were randomly divided into eight pigs each and isolated from Group 1. Remaining littermates of both weaning groups were weaned and placed in nurseries outside of the

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The authors thank the National Pork Producers Council for partial funding for this project. The authors also thank Dr. Thomas E. Eurell for the use of laboratory space and reagents.

Table 1

Experimental factors (weaning age, pen density, limited feed space) used in stress evaluation study of 14- and 21-day-old healthy pigs

	Pen			
Weaning age	1	2	3	4
14 days	0.1 m^2/pig 2 pigs/feeder	0.1 m^2/pig 1 pig/feeder	0.3 m^2/pig 2 pigs/feeder	0.3 m^2/pig 1 pig/feeder
21 days	0.1 m^2/pig 2 pigs/feeder	0.1 m^2/pig 1 pig/feeder	0.3 m^2/pig 2 pigs/feeder	0.3 m^2/pig 1 pig/feeder

study area. All pigs were fed ad libitum. The diet consisted of a least-cost single pelletized nonmedicated starter diet formulated at the University of Illinois.

Blood was collected by jugular venipuncture from each pig on days 0, 7, 14, 21, and 28 days postweaning. All pigs were place in dorsal recumbency in a padded trough to obtain the blood samples. The day 0 blood sample was obtained immediately prior to the time of weaning. Sera were harvested and stored at -70°C until assayed for HPT concentration.

Performance data (individual weaning weight, individual final weight, average daily gain, and pen feed:gain [F:G]) were recorded in each replicate.

Haptoglobin assay

To quantify serum HPT, a modification of a described method of cyanmethemoglobin binding capacity (CHBC mg per dL) was used.[14,15] Whole blood samples were collected in EDTA from a donor sow (7–10 mL). After centrifugation and saline wash, conserved red cells were diluted with Drabkin's (Sigma Chemical Company, St. Louis, Missouri) solution to prepare working cyanmethemoglobin reagent. Using a spectrophotometer (Milton Roy, formerly Bausch and Lomb, Rochester, New York), the final dilution was obtained when cyanmethemoglobin solution reached 0.409 optical density at 540 nm, resulting in a final concentration of 60 mg per mL. Cyanmethemoglobin reagent was filtered (0.45 µm) and stored at 4°C in amber glass. The reagent was freshly prepared every 7 days. Duplicate test and reference solutions were prepared for each porcine serum sample. Differential absorbance of each test and reference solution was quantified using a spectrophotometer blanked with distilled water at wavelengths 380 and 405 nm. Internal control was maintained during CHBC assays using a pooled porcine serum sample included with all 32 samples analyzed. The HPT calculation was: [(test 405 - reference 405) - (test 380 - reference 380)] × 455 = cyanmethemoglobin binding capacity (CHBC mg per dL). Mean CHBC were calculated from the duplicate values.

Statistical analysis

The effects of weaning age, pen density, and amount of feeder space on serum HPT in weaned pigs were examined using a repeated measures multiple linear regression model.[16,17] The independent variables were divided into between-subject and within-subject variance components. The between-subject factors were: weaning age, pen density, feeder space, and replicate. Mean serum HPT over post-treatment time for each subject was included as a between-subject factor as an alternative to subject coding (i.e., value of subject variable = subject mean of all treatments). The within-subjects factor was the linear component of four levels of time (7,14, 21, and 28 days post-treatment). The model-dependent variable was serum HPT concentration (mg per dL). Interaction terms between subjects and within subjects were included in the analysis of the statistical model. A hierarchical method of analyzing the independent variables by backward elimination of nonsignificant independent variables was used.

The validity of regression model assumptions was analyzed using residual analysis using the full model. Normality assumptions were valid without transformation.

To analyze the effects of production stressors on average daily gain (ADG), multiple linear regression was used.[18] The independent variables and interaction terms that were described for HPT analysis were also used in this model. The model-dependent variable was ADG (kg per day) from weaning through the end of nursery production (4 or 5 weeks depending on weaning age). All assumptions for the regression models were valid without transformation. In all statistical tests, an alpha level of 0.05 was used.

Results

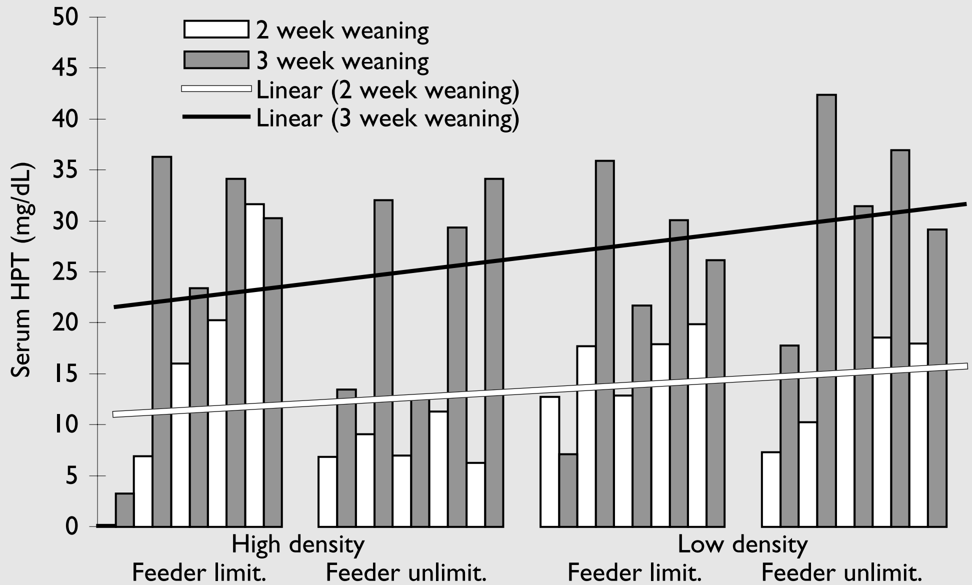
Haptoglobin (HPT)

Serum HPT did not significantly increase or decrease over time (within-subjects factors), although the trend was an increase (Figure 1). All within-subjects terms were deleted from the final statistical model, and average serum HPT was analyzed for effects of weaning age, pen density, and limited feeder space (Figure 2). A pen density × feeder space interaction was significant ($P < 0.05$), indicating that the effect of feeder space on serum HPT was dependent upon pen density. At high density, unlimited feeder space was associated with lower HPT concentration, whereas at low density, unlimited feeder space was associated with higher HPT concentration. Weaning age was highly significant ($P < 0.0001$). Pigs weaned at 2 weeks of age had lower serum HPT concentration. There was a significant replicate effect ($P < 0.05$). Mean serum HPT was greater during the second replicate.

Performance

Only ADG was analyzed to determine the effects of production stressors on performance (Figure 3). Feed efficiency (F:G) was not analyzed because there were only two pens per group for the experiment. When ADG was analyzed, a significant weaning age × pen density interaction was detected ($P < 0.05$) (Figure 4). The effect of pen density differed with weaning age. For weaning at 3 weeks, lower density was associated with higher ADG, whereas no effect of density was apparent

Figure 1



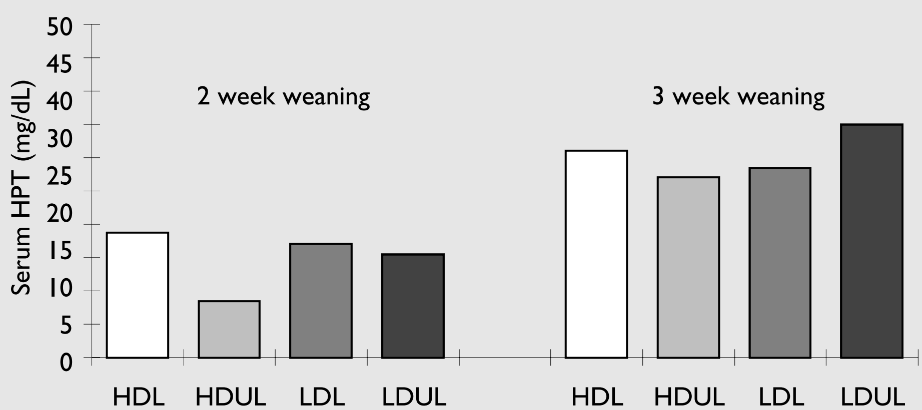
Mean serum haptoglobin (HPT mg/dL) concentration (0, 1, 2, 3, and 4 weeks postweaning in 14- and 21-day-old healthy pigs exposed to production stressors including weaning, pen density, or limited feeder space (n=128).

for pigs weaned at 2 weeks.

Discussion

In healthy weaned pigs, the serum HPT response to different production stressors (weaning age, pen density, and feeder space limitation) was complex. Weaning age had dramatic effects, with reduced serum HPT concentration in early-weaned pigs (i.e., weaned at 2 versus 3 weeks). Inconsistent findings comparing early-weaned pigs and stress responses are reported in the literature.[19,20] Results from one experiment showed greater thymic weights and body weight:thymus gland ratios in early-weaned pigs (8–10 days) versus control pigs (17–21 days).[19] In comparison, thymic atrophy and immunosuppression were found in pigs associated with stressors including restraint or early weaning.[20,21] However, mixing nonlittermate pigs at weaning or 2 weeks later did not alter cell-mediated immunity.[22] Serum cortisol was significantly elevated in these studies. Haptoglobin concentrations can increase when glucocorticoid concentrations are elevated.[6,7] If early weaning increases basal cortisol concentration and serum HPT is influenced by glucocorticoid, one would expect higher concentrations of HPT in the early-weaned group. In our study, the levels of serum haptoglobin were dramatically lower in the early-weaned pigs. It is the authors' hypothesis that early weaning is associated with reduced aggressive behavior. Serum HPT may increase nonspecifically due to inflammatory conditions. Any source of inflammation, including trauma, may evoke an acute-phase response. If younger as well as smaller pigs are less aggressive, the degree of inflammation postweaning may be less. An observational study of aggressive behavior based on age, weaning, and mixing to determine correlation of behavior with serum HPT may support this hypothesis.

Figure 2



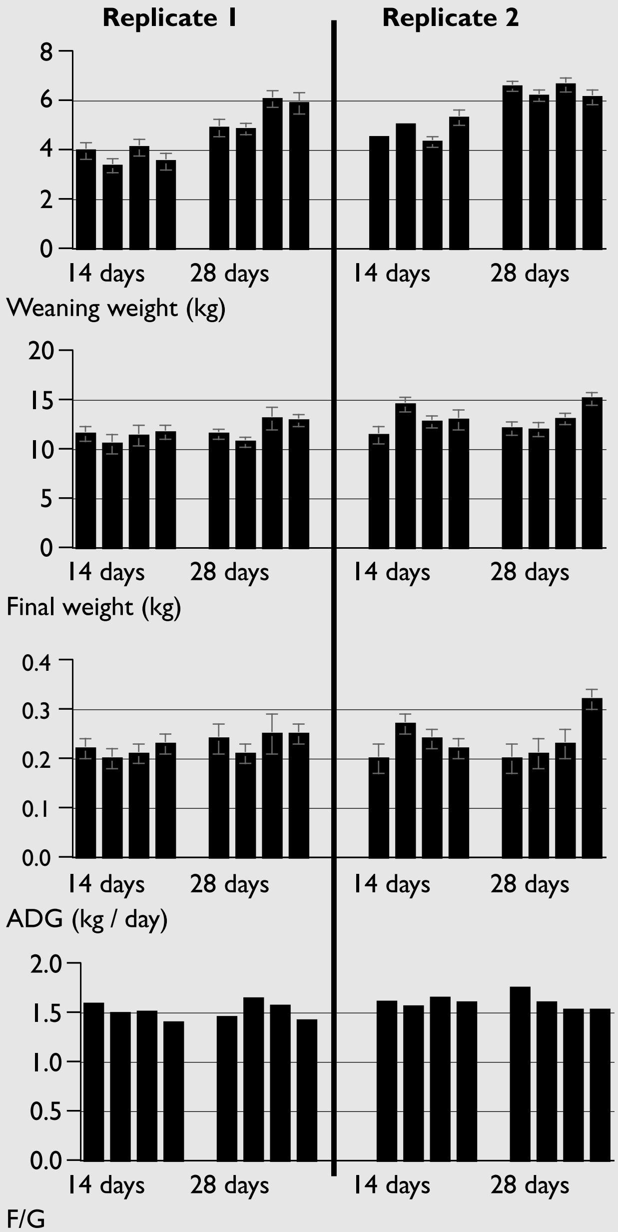
HDL: high density, limited feeder space
HDUL: high pen density, unlimited feeder space
LDL: low pen density, limited feeder space
LDUL: low pen density, unlimited feeder space

Grand mean of serum haptoglobin (HPT mg/dL) concentration (1–4 weeks postweaning) in 14- and 21-day-old healthy pigs exposed to production stressors including weaning, pen density, or limited feeder space (n=128).

Maternal antibodies may also influence the serum HPT concentration in the early-weaned pig. Early weaning may prevent transmission of infectious agents if lactogenic antibodies are present in the weaned pig. The result is presumably healthy pigs without increased acute-phase protein concentrations. The pigs in our experiment were all apparently healthy. However, if subclinical disease was present in the 3-week-weaned group, one may expect elevated serum HPT concentrations.

Previous studies have shown crowding pigs for 29 days had no effect on thymus, spleen, or adrenal weights, indi-

Figure 3



Performance data from two trials of 14- and 21-day-old exposed to weaning, pen density, and feeder space stressors.

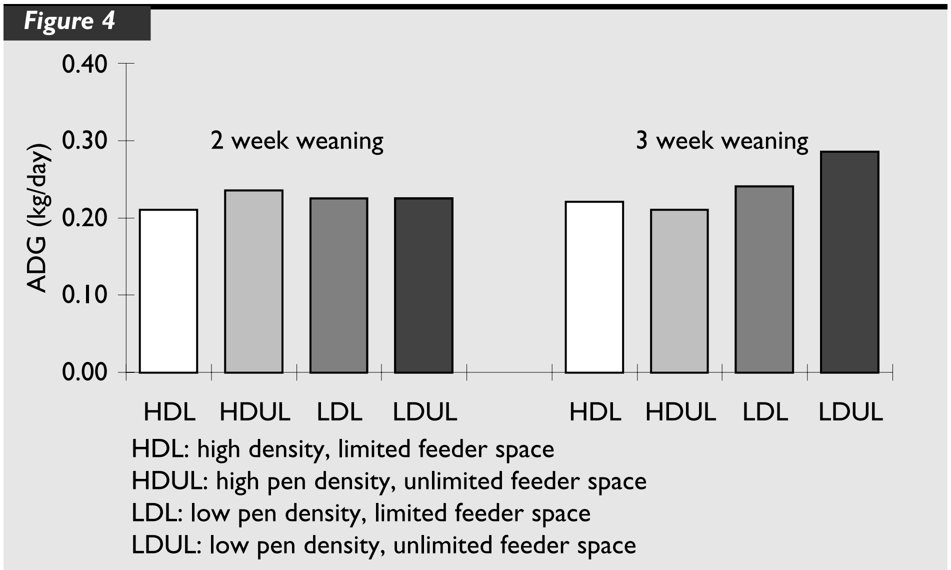
cating lower serum cortisol.[11] In the same study, N:L ratios were not affected by increased density. A previous study showed increased aggressive behavior in weaned pigs by crowding.[23] In our experiment, pen density and limited feeder space affected serum HPT. Our study indicated that a lower serum HPT concentration is expected in high-density, feeder-space-unlimited pens; whereas low-density, feeder-space-unlimited environments may increase serum HPT in healthy weaned pigs. This interaction may be related to weaning weight. Overall, pigs that were placed in high-density, feeder-space-unlimited pens were smaller than pigs in other groups, an unforeseen bias at the time the experiment started. This phenomenon may also be related to environmental temperature, especially since this experiment was conducted during December and January. The artificial plywood barriers created to increase pen density may have provided warmer conditions, and less chance of drafts and consequently chills in these pigs. The effect of weaning weight may also be responsible for the observed differences in serum HPT concentration between weaning groups. Pigs weaned at 2 weeks of age were much smaller than pigs weaned at 3 weeks. It is noted however, that serum HPT levels remained lower throughout the experimental period in the early-weaned group.

Serum HPT did not significantly change (overall linear increase or decrease) over time, i.e., from weaning till the end of nursery production. However, it was apparent from our data (Figure 1) that serum HPT increased immediately after weaning, and the trend for an increase occurred. Fluctuations in HPT levels may indicate normal physiological variation or animal adaptation. Furthermore, the half-life of porcine serum HPT is not known. Blood sampling occurred at weekly intervals. Changing the blood sampling time may provide more precise information about temporal change in this acute-phase protein. Carryover effects, such as the stress from restraint or inflammation caused by collecting the blood, must be accounted for if a shorter sampling time is considered.

The higher serum HPT levels in the second replicate may be related to weaning characteristics of the pigs. Average weaning weight was greater in the second replicate (5.0 kg versus 5.6 kg). Baseline serum HPT concentration (time 0) was greater (10.8 mg per dL versus 3.62 mg per dL) during the first replicate. Normal values of HPT concentration in 4-month-old specific-pathogen-free swine have been reported to be 5.79 mg per dL.[25] In the same study serum HPT was found to be as high as 18.63 mg per dL in pigs from a farm with a history of chronic *Actinobacillus pleuroneumoniae*. In our study, animals were clinically normal prior to and throughout the experiment in both replicates. It was assumed the differences in baseline values represented normal fluctuations in HPT concentration and not subclinical disease. Alternatively, blood sampling techniques improved with time, resulting in fewer hemolyzed samples. Hemolysis reduces HPT values.[15]

Weaning pigs at 2 weeks had no affect on ADG in crowded pens. Other experiments have shown early weaning age affects weight gain, reducing or increasing performance depending upon medication.[19,24] In our study, higher pen density reduced weight gain in healthy pigs weaned at 3 weeks of age. This finding is supported by other investigations in which pig performance (ADG, F:G) is reduced in high-density environments.[8-11] Smaller pigs would presumably have greater adaptation time in high density environments, i.e., "grow into their environment." Greater ADG and reduced HPT concentration in these early-weaned pigs verifies adaptation. Limiting feeder space may have the same influence if restricted more than what was used in this experiment.

A decrease in serum HPT may have positive effects on pig performance. Serum HPT in pigs 7 weeks of age was used to differentiate high versus low weight gain in feeder pigs, lower HPT concentrations at this age predicted higher performance.[26] In our experiment, early-



Average daily gain (kg/day) in 14- and 21-day-old healthy pigs exposed to production stressors including weaning, pen density, or limited feeder space (n=128).

weaned pigs with the greatest weight gain had the lowest serum HPT concentration. However, pigs weaned at 3 weeks and placed in low-density pens with unlimited feeder space had the greatest serum HPT and the best weight gain. Therefore, age or size may influence the predictability of this serum protein for pig performance or the reliability as an indicator of stress.

Based on serum HPT and ADG, 2-week-old pigs may adapt to production stressors (weaning, pen density, feeder space) better than 3-week-old pigs. Caution must be applied when considering early weaning as an alternative in conventional swine production. Increased management, feed, and labor costs must be considered.

Implications

- Serum haptoglobin is lower in pigs weaned at 2 weeks than at 3 weeks of age.
- Pen density may not affect average daily gain (ADG) in early-weaned pigs if feeder space is adequate.
- Further investigations are needed to determine whether serum HPT or other acute-phase proteins are reliable indicators of production stress in swine.

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