ABSTRACT: To examine the effects of exogenous porcine (p) ST on measures of stress and immune function in weaned pigs with or without transport, pigs (20 ± 1 d of age) received daily injections of pST (0.5 mg/kg; n = 16) or saline (n = 16) for 5 d. On d 5, a blood sample was collected immediately before injection. At 4 h postinjection, pigs were weighed, sampled for blood, injected with di-nitrophenyl-conjugated keyhole limpet hemocyanin, and weaned. One half of the pigs in each group were transported for 3 h before placement in the nursery. Pigs were weighed, and blood was collected on 1, 7, and 14 d postweaning. Statistical significance was set at P<0.05. Serum IGF-I concentrations were increased by pST and decreased by weaning, but not affected by transport. The free cortisol index was elevated in all pigs 1 d postweaning, although less in transported versus nontransported pigs. By 7 d postweaning, the free cortisol index returned to prewean values. Serum concentrations of immunoglobulin (Ig) G increased in all pigs by 14 d postweaning, but were not affected by pST or transport. Serum IgM concentrations were elevated at 7 and 14 d postweaning. Before weaning and again 1 d postweaning, pigs treated with pST had greater concentrations of IgM than did control animals. Circulating neutrophils increased in pST-treated pigs 4 h after the final pST injection. Improved immune function in weaned pigs by pST may lead to greater health and growth in a commercial setting.

Key words: growth, immune, pig, stress, weaning

INTRODUCTION

Maternal separation, relocation to new housing, introduction into new social groups, and changing to a dry diet can suppress growth in the recently weaned pig. The physiological mechanisms that underlie growth-related responses to these stressful situations, transport in particular, are poorly understood. Typical of the endocrine profile seen during undernutrition, weaning the pig increases concentrations of ST and decreases serum concentrations of IGF-I and IGF-II (Carroll et al., 1998; Matteri et al., 2000). Increased plasma and urinary cortisol concentrations and decreased plasma corticosteroid-binding globulin (CBG) have been reported after weaning (Le Dividich and Seve, 2002; Heo et al., 2003). The preweaning BW of a pig may affect its physiologic response to weaning as well (Matteri et al., 2000; Kojima et al., 2007).

Considering the immunosuppressed nature of the weaned and transported pig (McGlone et al., 1993; Hicks et al., 1998) and the elevated exposure of the pig to pathogens through feces during transport (Jones et al., 2001), the risk of being exposed to and succumbing to disease is greatly enhanced. Although the exact mechanisms are not yet clearly elucidated, there is evidence that treatment with exogenous ST has the potential to promote health in challenging situations such as weaning and transport. This experiment was designed to assess effects of exogenous porcine (p) ST on measures of immune function and growth (i.e., wellbeing) when administered to pigs immediately before weaning with or without subsequent transport.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the University of Tennessee Institutional Animal Care and Use Committee.

Animals and Diets

Crossbred pigs (of Landrace, Duroc, and Hampshire breeding), were farrowed in standard farrowing pens
and processed as per usual University of Tennessee Experiment Station practice between 4 and 7 d of age. Procedures included clipping needle teeth, tail docking, iron supplementation, ear tagging, and castration of males. Pigs were kept in farrowing pens with their dams until weaning, with creep feed (Tennessee Farmer’s Cooperative diet 554PE, LaVergne, TN) available at all times.

Experimental Design

Thirty-two pigs (16 male and 16 female, age: 20 ± 1 d of age, BW: 4.89 ± 0.12 kg) from a pool of 6 litters were randomly assigned to treatment groups (n = 8 per group) while blocked on sex and BW and partially blocking on litter to minimize litter effects as much as possible. The experiment was a randomized block design with factorial treatments defined as receiving (YP) or not receiving (NP) pST, and transported (YT) or not transported (NT) following weaning, resulting in YPNT, YPYT, NPNT, and NPYT treatment groups. Upon allocation, pigs received daily i.m. injections containing pST (0.5 mg/kg; n = 16) or saline (n = 16) for 5 d and were weaned on the day of the last injection. The dose was chosen after considering the previous literature (Matteri et al., 1997; Wester et al., 1998; Carroll et al., 1999; Oliver et al., 2005) and consulting with F. Buonomo (Monsanto Company, St. Louis, MO, personal communication). The volume of all injections was less than or equal to 0.5 mL, and the injections were given in the neck, alternating sides daily. Immediately before the final pST injection, a blood sample was collected (time = 0). At 4 h after injection, all pigs were weighed, and blood was again sampled. At this time, all pigs were given an i.m. injection of di-nitrophenyl-conjugated keyhole limpet hemocyanin (DNP-KLH; 1 mg in 1 mL saline with 1 mL of incomplete Freund’s adjuvant, given in the neck contralateral to the last pST injection site) as an immune challenge. Pigs in the NT groups were then mixed and housed into 2 nursery pens, whereas pigs in YT groups were mixed, loaded onto a truck, and transported for 3 h before being brought back to the nursery and placed into the nursery pens. Each pen then contained 4 animals from each treatment group (16 pigs per pen). Pigs were 24 ± 1 d of age at weaning. Once in the nursery pens, pigs were fed a standard weaning diet ad libitum (Tennessee Farmer’s Cooperative diet 554PE). Pigs were weighed and bled again on d 1, 7, and 14 postweaning. Recombinant pST was kindly provided by Monsanto Company (St. Louis, MO).

Blood Collection and Analyses

All blood samples (7 mL) were collected via anterior vena cava puncture. For samples collected at 0, 4 h, and 1 d postweaning, the blood was allotted as follows: 1 mL placed into a tube spray-coated with 5.4 mg of K2 EDTA for differential white blood cell (WBC) analysis; 3 mL placed into a tube spray-coated with 86 units heparin for plasma collection; and 3 mL placed into a noncoated tube for serum collection. For samples collected on d 7 and 14, blood was aliquoted in approximately equal volumes into heparinized and noncoated tubes. The heparinized blood samples were centrifuged at 2,000 × g for 10 min and the plasma stored at -20°C until analyzed for cortisol and CBG concentrations. Blood samples in the noncoated tubes were allowed to clot overnight at 4°C and were then centrifuged, as described above, for serum collection. The serum was stored at -20°C until analyzed for immunoglobulin (Ig) G, IgM, and IGF-I.

Plasma total cortisol concentration was determined by radioimmunoassay as previously reported (Scroggs et al., 2002). Cortisol concentration was expressed as nanomoles per liter. Intra- and inter-assay CV was 2.4 and 3.8% for low (110 nmol/L), 4.0 and 5.5% for medium (325 nmol/L), and 4.8 and 3.6% for high (775 nmol/L) cortisol standards. The concentration of CBG (mg/L) was measured by a direct ELISA as described previously (Roberts et al., 2003). Intra- and inter-assay CV of a pooled pig plasma sample were 6.9 and 16.1%, respectively. The free cortisol index (FCI) was calculated using the ratio of plasma total cortisol to CBG concentration and reported as nanomoles per milligram.

A commercial ELISA kit (DSL-10-2800; Diagnostic Systems Laboratories, Webster, TX) was used to measure serum concentrations of IGF-I. A set of human IGF-I standards was used to create a standard curve with which porcine samples were compared. For validation in our laboratory, dilutions of pooled porcine serum from this experiment were used to confirm parallelism. The slopes of the 2 lines were compared by multisource regression and did not differ. High and low concentration samples were identified to use as porcine intra-assay controls. The assay was run in duplicates, and the standards, porcine pool dilutions and control samples were included in every plate. The intra- and inter-assay CV were 3.6 and 5.9%, respectively.

Serum concentrations of porcine IgG and IgM were quantified using commercially available ELISA kits (E100-104 and E100-100, respectively; Bethyl Laboratories, Montgomery, TX). The standard curve for each assay was derived from serial dilutions of a known porcine serum standard, and ranged from 0 to 500 ng/mL. For validation purposes, pooled porcine serum from this experiment was serially diluted (dilutions measured from 1:1,000 to 1:128,000) and measured against the standard curve. The slopes of the 2 lines were compared by multisource regression and did not differ. The procedures outlined by the manufacturer were followed with the following modifications: samples assayed for IgG were diluted 1:100,000, and those assayed for IgM were diluted 1:10,000. The horseradish peroxidase-conjugated IgG antibody was diluted 1:100,000, and that for the IgM antibody 1:50,000. The enzyme substrate reaction was stopped at 15 min in both assays. Intra- and inter-assay CV were 4.7 and
7.4%, and 5.9 and 7.3% for the IgG and IgM assays, respectively.

The whole blood samples were sent to a commercial clinical laboratory (Antech, Southaven, MS) for determination.

Figure 1. A.) Changes in BW due to time and transport (TSP). Regardless of treatment, pigs weighed less 1 d after weaning compared with preweaning, surpassed their weaning BW by d 7, and continued to gain BW up to 14 d postweaning. *Transported pigs (YT) weighed less (P < 0.05) than their nontransported counterparts (NT) 14 d postweaning. B.) Changes in circulating concentrations of serum IGF-I due to time and porcine (p) ST. *Pigs treated with pST (YP) had greater concentrations of IGF-I (P < 0.05) than did their nontreated counterparts (NP) 4 h after the last pST injection and at 1 d postweaning. A–D For all graphs, letters represent least squares means across time; time groups with nonidentical letters differ (P < 0.05). Pigs were 24 ± 1 d-of-age at weaning.

Figure 2. A.) Changes in circulating concentrations of plasma cortisol. B.) Changes in circulating concentrations of plasma corticosteroid binding globulin (CBG). Weaning resulted in decreased CBG concentrations observable up to 7 d postweaning; by 14 d postweaning, CBG concentrations reverted to preweaning values. C.) Changes in the free cortisol index (FCI). Weaning resulted in increased FCI concentrations at 1 d postweaning; by 7 d postweaning, the FCI returned to preweaning values. *Decreased FCI was observed in YT relative to NT pigs at 1 d postweaning (P < 0.05). A–C For graphs B and C, letters represent least squares means across time; time groups with nonidentical letters differ (P < 0.05); for all graphs, TSP = transport; YT = transported pigs; YP = treated with porcine ST; NT = nontransported pigs; NP = not receiving pST. Pigs were 24 ± 1 d-of-age at weaning.
mination of total WBC concentration (WBC/µL), the concentration of neutrophils and lymphocytes, and the percentage of neutrophils and lymphocytes relative to total WBC concentration.

**Statistical Analyses**

Variables were analyzed with mixed model ANOVA, using a model for a randomized block design with factorial arrangement of treatments. Pig was the experimental unit, represented by block × treatment interactions. The statistical model included pST and transport as main effects with repeated measures and pretrial BW as a covariate, as we have shown previously that the preweaning BW of a pig may affect its physiologic response to weaning (Kojima et al., 2007). In a preliminary analysis, no effect of sex on any variable was observed and so sex was not included in this model. Least squares means were compared using Fisher's protected least significant difference. A significance level of \( P < 0.05 \) was used for all testing; trends where \( P < 1.0 \) were also reported. All graphical and textual descriptions of results are reported as least squares means. Graphical representations are of simplified models with nonsignificant effects \( (P > 0.05) \) removed for ease of viewing.

**RESULTS**

**BW and IGF-I**

Regardless of treatment, pig BW were less \((P < 0.05)\) 1 d after weaning compared with preweaning BW (Figure 1A). All pigs gained BW and surpassed their weaning BW by d 7, and continued to gain BW up to 14 d postweaning. Transported pigs weighed less than NT pigs at 14 d postweaning \((P < 0.05)\). Serum concentrations of IGF-I were affected by administration of pST and by the weaning process, such that YP pigs had greater concentrations of IGF-I at 4 h after the last pST injection and again at 24-h postweaning compared with NP pigs \((P < 0.05)\). All pigs had sharply decreased IGF-I concentrations on d 1 and 7 compared with preweaning concentrations \((P < 0.05); Figure 1B). Transport did not alter serum IGF-I concentrations.

**Plasma Cortisol, Corticosteroid Binding Globulin, and Free Cortisol Index**

A sharp increase in plasma cortisol concentration was observed in all pigs on d 1 postweaning (Figure 2A). A pST × transport × time interaction was observed \((P = 0.003)\) such that cortisol concentrations on d 1 were least in NPNT and YPYT animals. Plasma concentration of CBG decreased \((P < 0.05)\) by d 1 and remained low on d 7 postweaning (Figure 2B). By d 14, CBG concentration was similar to that observed before weaning. Plasma CBG was unaffected by pST or transport. The FCI was elevated \((P < 0.05)\) in all pigs on d 1 compared with preweaning values (Figure 2C). The FCI was less \((P < 0.05)\) in YT pigs compared with NT pigs. By d 7, the FCI in all groups returned to preweaning values.

**Serum IgG and IgM**

Serum concentrations of IgG increased \((P < 0.05)\) by d 14 in all pigs injected with DNP-KLH on day of weaning (Figure 3A). Neither pST nor transport altered IgG concentration at any of the times sampled. Serum IgM concentrations were elevated \((P < 0.05)\) on d 7 postweaning compared with preweaning concentrations, and continued to increase by d 14 (Figure 3B). Before the DNP-KLH injection, pigs treated with pST had a greater \((P < 0.05)\) concentration of IgM than did
NP animals; this difference was again observed on d 1 postweaning.

**White Blood Cells, Neutrophils, and Lymphocytes**

Overall mean (±SEM) WBC concentration tended ($P = 0.08$) to be greater in transported compared with nontransported pigs ($17.7 ± 1.1$ vs. $14.8 ± 1.1 \times 10^3$, respectively). The concentration of circulating neutrophils (Figure 4A) and the percentage of neutrophils (Figure 4B) were greater ($P < 0.05$) in YP pigs compared with NP pigs 4 h after the final pST injection. Plasma lymphocyte concentration declined over all treatment groups by 4 h postweaning (Figure 4C). The percentage of lymphocytes was less ($P < 0.05$) in pigs treated with pST relative to controls at 4 h postweaning but not at 24 h postweaning (Figure 4D).

**DISCUSSION**

Treatment of young pigs with 5 daily injections of pST resulted in increased circulating concentrations of IGF-I, IgM, and neutrophils at weaning. In regard to young pigs, most previous reports on effects of pST have focused on growth of the preweaned neonate. Although no effect on piglet growth was observed in 6-wk-old pigs implanted with sustained release pST implants (0.5 mg/d) at 3 d of age, the treatment did result in increased circulating concentrations of IGF-I and hepatic IGF-I mRNA expression (Matteri et al., 1997; Carroll et al., 1999). After 7 d of treatment with daily injections of pST (1 mg/kg), Wester et al. (1998) observed an increase in gain and food conversion efficiency in neonatal pigs. In a recent publication, Oliver et al. (2005) reported an increase in gain and circulating IGF-I in pigs receiving daily injections of pST (120 µg/kg) for 4 consecutive days following weaning. Pigs in that study were weaned at 10 d of age onto a liquid diet. In the present study, pST administration before weaning resulted in slight elevations in serum concentrations of IGF-I, but the effect was overcome by the sharp decrease in circulating IGF-I associated with the weaning process. Age at administration, timing of administration relative to weaning, and differences in weaning method (e.g., liquid vs. dry diet) may modu-
late the effects of pST. No effect of transportation was observed on serum IGF-I concentrations, although the sampling paradigm may not have allowed for observations of effects occurring between sampling periods.

In the present study, IgM concentrations were elevated at 7 and 14 d postweaning (relative to preweaning concentrations), likely due to the inoculation with DNP-KLH given the day of weaning. Likewise, serum concentrations of IgG were increased at 14 d postweaning. Neither pST nor transport altered IgG concentrations at any time. Interestingly, before inoculation with DNP-KLH, pigs treated with pST had greater concentrations of IgM than did control animals. This difference was again observed 1 d postweaning. This observation, although not previously reported in pigs, is similar to what has been found in other species. Ogueta et al. (2000) observed increased IgG and IgM concentrations in young mice transgenic for bovine ST relative to wild-type controls. Conversely, dwarf mice that lack ST showed a reduced ability to synthesize antibodies (Weigent and Blalock, 1995). Kimata and Yoshida (1994) observed that ST and IGF-I enhanced production of IgG subtypes 1–4, IgA subtypes 1 and 2, and IgM in human PCA-1+ plasma cells generated in vitro. There is a known association between ST deficiency and hypogammaglobulinemia in humans (Fleisher et al., 1980; Sitz et al., 1990).

Numbers of circulating neutrophils and the neutrophil percentage were greater in pigs treated with pST when measured 4 h after the last pST injection. Conversely, over this same time period, the percentage of lymphocytes was less in pigs treated with pST relative to controls. Although this observation is novel in the pig, it is not altogether unexpected given the body of literature from rodent and human models. Ibanez et al. (2005) reported that treating children born small for gestation age (and with short stature) with exogenous ST raised neutrophil counts. Subsequent studies by the same laboratory confirmed this finding (Ibanez et al., 2006). Japanese adult deficient in ST also had increased neutrophil counts up to 50% within 2 mo of treatment with exogenous ST (Sohmiya et al., 2005). The mechanism(s) by which ST increases circulating neutrophils and stimulates lymphocyte activity is not yet fully elucidated, but appears to include IGF-I-dependent and IGF-I-independent pathways as reviewed by Weigent and Blalock (1995).

In the present study, transport had little effect on immune cell populations, except for a tendency for greater WBC concentration. This is in contrast to the findings of McGlone et al. (1993), who observed increased numbers of blood neutrophils and decreased numbers of lymphocytes in pigs transported for 4 h, indicating a potential decrease in the specific immune response. That study used pigs that were already weaned and much heavier; differences in physiological responses due to age cannot be ruled out. Another difference between the 2 studies is the timing of sampling. In the present study, the first posttransport blood sample was collected 24-h postweaning, whereas in the previous study the authors collected a sample immediately after transport. Salak-Johnson et al. (1997) also reported increases in circulating neutrophils and decreases in circulating lymphocytes in pigs 2 h after an intracerebroventricular injection of corticotropin-releasing hormone, but the authors did not attempt to observe past that time point. When Bilandzić et al. (2006) challenged boars with 3 daily injections of adrenocorticotropic hormone, they observed increased neutrophil and decreased lymphocyte concentrations during the challenge period, but those differences were resolved within 48 h of the last injection, suggesting that the effects of cortisol on circulating immune cell populations may be strong but short-lived. In the present study, the numerical increases in neutrophils and decreases in circulating lymphocytes noted across all treatment groups at 1 d postweaning may suggest that the weaning process overrode any effect of transport at the times these parameters were measured, but this speculation would need to be confirmed through additional work.

The sharp increase in plasma cortisol concentration in the pigs on d 1 postweaning has been reported previously (Le Dividich and Seve, 2002). Likewise, the corresponding decrease in CBG following weaning is similar to that which was observed in an earlier study (Heo et al., 2003). In general, glucocorticoids (endogenous and synthetic) have been shown to have an inhibitory effect on CBG production (Seralini, 1996). A reduction in CBG concentrations can result in an increase in the FCI (an indirect measure of biologically active cortisol) especially in the acute stress phase (Bright, 1995), and within 1 wk subsequent to the elimination of the stressor (Heo et al., 2005). Indeed, the calculated FCI for pigs in the present study was elevated on d 1 postweaning. The increase in FCI was less in the transported pigs, which may be attributed to reduced aggressive and increased exploratory behavior as noted previously (Dalin et al., 1993). Regardless of whether the pigs were given pST or subjected to transport, it would appear that based upon similar changes in cortisol, CBG, and FCI, weaning is perceived by the pig as a major stressor.

In the present study, treatment with 5 daily injections of pST before weaning did not alter growth before or after weaning. As expected, most pigs lost BW during the 24-h period following weaning. Pigs gained BW and surpassed their weaning BW by d 7, and continued to gain BW up to 14 d postweaning. However, by d 14, transported pigs weighed less than nontransported pigs. Hicks et al. (1998) reported an immediate 2.9% loss in BW in pigs transported for 4 h; these pigs had BW comparable with controls at 5 d posttransport. McGlone et al. (1993) also noted an immediate 5.1% decrease on BW in pigs transported for 4 h; transported pigs also ate less and gained less BW than controls in the 72-h period following transport. The present study utilized younger pigs than did both of these previous reports and incorporated weaning into the experimental design.
In summary, treatment with 5 daily doses of pST before weaning resulted in noticeable changes in immune-related endpoints, but did not result in differences in growth in weaned pigs. These data support the hypothesis that exogenous pST may abrogate some of the negative effects of weaning and transport, particularly through the effect of pST on immune cell and antibody concentrations in young pigs. Further elucidation of the mechanisms by which ST acts to modulate immune function during a time of immunosuppression such as weaning may lead to improved health and productivity of the pig.

**LITERATURE CITED**


