Title: Impact of early weaning and photoperiod manipulation on sow and piglet welfare- **NPB # 03-111**

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Abstract: These experiments were designed to determine the effect of (1) photoperiod manipulation pre- and post-gestation on sow and piglet performance and immune response and (2) photoperiod manipulation on early weaning success in piglets. In Exp 1, sows were exposed to either long day (LD; 16L:8D) or short day (SD; 8L:16D) photoperiod at d 90 of gestation. At farrowing, half of the sows remained on their gestation trt (LD:LD; SD:SD) and the other half were switched to the opposite trt (LD:SD or SD:LD). Blood samples were taken at d 0, 7, 14 and 21 post-trt. Piglet samples were taken at d 7 and 21. During gestation, there were significant trt×day interactions for several immune measures including N:L ratio and lymphocyte proliferation. On d 7 LD sows had higher N:L ratio ($P = 0.05$) and SD sows had higher ($P < 0.05$) T- and B-cell proliferation. On d 14 post-trt, LD sows had higher ($P < 0.05$) T- and B-cell proliferation. At 24h post-farrowing, sows on LD:SD had higher ($P < 0.05$) total WBC counts than all other groups. At weaning, sows on LD:LD had higher ($P < 0.05$) T-cell proliferation. There were significant linear trends for gestation trt on immune measures such that gestation trt significantly influenced immune responses in 7 d old piglets. Piglets from LD sows had higher ($P < 0.05$) CORT, WBC, and plasma IgG. At weaning, piglets from SD:SD sows had higher T-cell proliferation ($P < 0.001$) and CORT ($P = 0.054$), whereas, those from LD:LD had higher ($P < 0.005$) PHAG. There were significant sex effects on and trt×sex for CORT and immune measures. In addition, SD sows tended ($P = 0.07$) to have more piglets born alive, but LD sows had higher ($P = .002$) birth weights.

In Exp 2, all sows were subjected to SD from d 90 of gestation until the end of lactation. Piglets were weaned at 14, 21, and 28 d of age and assigned to either LD or SD until 10 wk of age. Blood samples were obtained from piglets at weaning and every 2 wk until 10 wk of age. There were significant trt effects on immune measures including NK, PHAG and proliferation. Piglets weaned at 21 and 28 d had higher NK cytotoxicity. 14 d weaned pigs had significantly higher B-cell proliferation and 21 d had higher ($P < 0.0001$) PHAG. There were significant trtxage interactions for total NE and LY, NK, and proliferation.

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Specifically, at 6 wk, piglets weaned at 14 and 21 d and kept on LD had significantly higher NK, whereas, 28 d weaned piglets kept on LD, regardless of age had the lowest NK. B-cell proliferation was higher ($P < 0.05$) among 28 d weaned piglets kept on SD. Piglets on LD and weaned at 28 d had significantly lower PHAG at 6 wk of age but higher at 8 wk. Also, there were significant trt effects on and trtxage for body weight. Overall, piglets weaned at 28 d and kept on LD were heavier throughout the experiment, whereas, 14 d weaned pigs kept on SD had the lowest body weight. It appears that photoperiod can manipulate sow physiology and productivity and may have an impact on piglet's immune response. More importantly, photoperiod influences immune responses in piglets that are weaned at different ages. In fact, one may be able to use photoperiod manipulation to counteract the negative impact of various stressors such as weaning stress. Further research is needed to determine the precise effects of photoperiod on gestational sows and their piglets.

Introduction: There is mounting concern among pork producers and customers on the impact of common production practices on animal welfare. Pig productivity and welfare have major impacts on the success and profitability of pork production. "Early weaning" is a common production practice that has welfare implications in the US and EU. Within the past few years, the EU adopted a provision that increased the minimum weaning age from 21 to 28 d of age. Not only does the production practice of early weaning raise a welfare concern, it has other drawbacks including higher medication costs and depressed sow productivity. Prophylactic antibiotic use raises another issue among public health officials and consumers. Once again the EU has banned another common practice, the use of antibiotics and the US is considering the use of antibiotics in animal agriculture and there are discussions on-going on the future of this practice. Regardless of the science, the perception exists among consumers that many of these practices benefit producers while negatively impacting consumers and compromising animal welfare.

It is imperative to develop management approaches to counteract the negative impacts of early weaning on pigs as well as reduce the reliance on antibiotics. The effects of early weaning are not all negative, but there is significant evidence that sow and piglet well-being is compromised by this practice. Animals use cues from the physical environment to alter physiologic endpoints so that homeostasis is maintained across variable temperatures, humidity and food supplies. In most species, photoperiod is the most widely adapted signal for long-term physiologic adjustments. The predictive nature of photoperiod as a signal is superior to that of temperature and any other environmental cues. It seems possible that lighting programs could be implemented to have effects on production and health. In addition to the metabolic function of these hormones, they are also involved in modulation of immune function of many species. One possible approach is to enhance the immune status of animals through environmental manipulation. Limited evidence exists to support the concept that light exposure factors may influence sow and piglet health, performance, and well-being. Photoperiod manipulation provides a non-invasive, easily implemented, effective, method to improve immune status while enhancing productive efficiency.

Objectives: To determine the effect of photoperiod manipulation pre- and post-gestation on sow and piglet performance and health, and on early weaning success in the piglet.

Materials and Methods: At d 90 of gestation, 24 multiparous white cross sows were randomly assigned to either 16L:8D (long days (LD); lights on: 7:00 am; lights off: 11:00 pm) or 8L:16D (short days (SD); lights on: 7:00 am; lights off: 3:00 pm). Light intensity during the light phase was ~ 250 lux, whereas during the dark phase one could only see their hand several inches in front of their face. The gestation room was an experimental research room in which the sows were kept on solid concrete floors in standard gestation crates. Sows were maintained on their assigned light trt until farrowing. At farrowing, half of the sows remained on their original photoperiod (LD:LD or SD:SD) and the other half were switched to the opposite trt (LD:SD or SD:LD). Sows were moved to adjacent farrowing rooms in which they were kept in standard farrowing crates on tenderfoot. The same light intensity was achieved in the farrowing. Both rooms were in the same research building. Sows remained on post-parturition photoperiod trt until the end of lactation.

Blood samples were collected by jugular vein puncture on d 90 of gestation (d 0 of experimental period), d 7, d 14, and d 21 post-trt for immune and endocrine measures of the sows. Additionally, blood samples were taken 24 h after farrowing and at the end of lactation. On d 21 post-trt, non-surgical indwelling jugular catheters were inserted for repeated blood samples for prolactin measures. Circulating IGF-1, prolactin and cortisol (CORT) were measured.

A flow-cytometry-based assay was used to measure the % engulfment of fluorescent beads by neutrophils (phagocytosis; PHAG). The ability of neutrophils to migrate in response to chemoattractants (C5a and IL-8) was assessed using an *in vitro* chemotaxis (CHTX) assay. Metabolic activity of lymphocytes was measured by stimulating cells with mitogens, ConA and LPS. The ability of a sub-population of lymphocytes to kill tumor cells (natural killer cell cytotoxicity; NK) was measured using a non-radioactive cytotoxicity assay. Finally, plasma antibody levels will be measured using an enzyme-linked immunosorbent assay (IgG). Using the same methodology used for sows, immune and endocrine measures were taken at 7 d of age and at weaning from 4 to 6 pigs per litter.

Sow performance and productivity was measured. Milk intake of piglets was measured using the isotope (deuterium) dilution technique (D_2O), which is directly correlated to sow milk production. Birth and weaning weights of piglets were recorded.

Objective 2: Sows were maintained on SD during gestation, farrowing and lactation. This lighting trt was determined based on data from obj. 1. Piglets were randomly assigned a weaning trt (14, 21, or 28 d) and a lighting trt (SD or LD). At weaning pigs were moved to the nursery were they were housed 6 pigs per pen (2 m x 2 m) and maintained on their assigned lighting trt until ~ 10 wk of age. Pigs had *ad libitum* access to feed and water. Ambient temperature of the nursery was kept at ~ 78±2ºF. Blood samples and body weights were taken at weaning, and every 2 wks until 10 wks of age for endocrine and immune profiles.

Statistical analysis -- Data were analyzed using SAS v.8 (SAS Inst. Inc., Cary, NC). All traits were tested for departures from normality and when necessary natural logarithmic transformation was applied. For several measures, minimum values for were zero thus a value smaller than the lowest non-zero number was added to all the observations to allow the logarithmic transformation. A linear mixed effects model was used to analyze sow variables. Main fixed effects were treatment and day and random were pig and block. The model had a repeated structure on day allowing the incorporation of heterogeneity of variances across days. A general linear model was used to analyze piglet variables. The main fixed effects included treatment and sex and variance among litters was used as the error term. Linear and quadratic contrasts were conducted to evaluate the influence of the maternal lighting treatment on the piglets.

Results: EXP 1: Photoperiod on immune measures on sows and piglets -- There were significant trt×day interactions for immune measures during gestation. On d 7 post-trt, sows on LD tended to have higher ($P = 0.098$) neutrophils (NE, %) and lower (P

= 0.10) lymphocytes (LY, %) compared to those on SD photoperiod. Increased NE %, consequently led to a decrease in LY which resulted in an increase (P = 0.05) in N:L ratio among LD sows. Also, on d 7 post-trt, sows on SD had higher T-cell (P = 0.003) and B-cell (P = 0.023) proliferation response, whereas 14 d post-trt LD sows had higher T- and B-cell proliferation (Fig 1). On d 21 post-trt, sows on LD tended to have higher (P = 0.07) total NE counts as well as higher (P = 0.09) PHAG than SD sows.

Sows maintained on LD:SD (gestation:lactation) had higher (P = 0.049) total WBC 24 h post-farrowing compared to all other trt groups. At the end of lactation, T-cell proliferation was higher (P = 0.045) among sows on LD:LD than those sows on LD:SD or SD:SD (**Fig. 2**). Photoperiod manipulation did not influence plasma CORT, IGF-1, IgG or NK. *Data are incomplete for neutrophil chemotaxis and prolactin.

Among 7 d of age, there were significant trt effects on and trt×sex for CORT and various immune measures. CORT was higher (P = 0.002) in piglets from sows on LD:SD compared to all other treatment groups. Piglets from sows that were on LD:SD had higher (P = 0.0014) total WBC and higher (P = 0.013) plasma IgG (**Fig 3**). Also, there were significant contrast effects on total WBC, plasma cortisol and IgG levels in which light trt during gestation influenced these responses. Piglets from sows that were on LD during gestation had higher plasma CORT (P = 0.02), IgG (P = 0.004), and total WBC (P = 0.004) than piglets from sows on SD during gestation.

Significant trt×sex interactions for percentage of LY (P = 0.022) and NE (P = 0.05; **Fig 4**) and N:L ratio (P = 0.047). Female piglets from LD:LD and LD:SD sows had significantly higher LY (%) than male piglets. Conversely, male piglets from sows on LD:LD and LD:SD had significantly higher (P < 0.01) NE (%) than females from sows on the same lighting treatment. Female piglets from sows on SD:LD had significantly lower (P < .05) LY (%) and higher NE (%) than male piglets whose sows were on the same lighting treatment. The N:L ratio was significantly higher among male piglets whose dams were on LD:LD and LD:SD. On the other hand, N:L was significantly higher among female piglets from sows on SD:LD trt. There were no sex differences for piglets from sows that were kept on SD:SD.

At weaning (~21 d of age) there were significant trt and sex effects on CORT and several immune measures. Plasma CORT (P = 0.053) was higher among piglets from SD:SD sows (**Fig. 5**) and those piglets had higher (P = 0.0006) T-cell proliferation. This T-cell response was partially influenced by gestation treatment. Piglets from sows on LD:LD had higher (P = 0.0035) PHAG but tended (P = 0.08) to have lower WBC. In fact, gestation trt significantly influenced PHAG with piglets from LD sows having higher PHAG. Piglets from sows on LD:SD had higher (P = 0.009) monocytes.

In addition, males had higher total WBC (P = 0.033) and plasma IgG levels (P = 0.020), regardless of trt. Male piglets from LD:SD and SD:SD sows had higher total WBC compared to female piglets. Whereas, male and female piglets from sows on LD:LD and SD:LD did not differ in their total WBC.

Photoperiod effects on performance and productivity -- Photoperiod trt had an effect on birth weight and number of piglets born alive. Sows on SD during gestation tended to have more (P = 0.070) born alive than sows on LD (**Fig.6**). However, sows on LD during gestation had heavier (P = 0.002) piglets at birth than sows on SD. There were no significant trt effects on number of piglets weaned, piglet weaning weight, sow weights, or number of stills, deaths, or mummified piglets. *Milk production as measured by D_2O is incomplete.

EXP 2: Effects of early weaning on various immune measures – During gestation and until weaning, all piglets were on the same photoperiod (SD:SD). Age at weaning influenced NK (all effector:target ratios; P < .05) , PHAG (P < 0.0001), B-cell proliferation (P = 0.023) and total NE (P < 0.001) and LY (P = .075) numbers. Piglets weaned at 28 d of age had lower NE counts compared to those weaned at 14 and 21.

NK was higher (P < 0.001) among piglets weaned at 21 and 28 d of age compared to those weaned at 14 d (Fig 7). Piglets weaned at 14 d of age had higher (P = 0.023) B-cell proliferation than those weaned at 21 or 28 d of age. PHAG was higher (P < 0.0001) in pigs weaned at 21 d of age compared to those weaned at 14 and 28 d of age (Fig 8). In fact, pigs weaned at 28 d of age had the lowest PHAG response compared to all other treatment groups. In addition, there was a significant sex effect on NK cytotoxicity. Male piglets had significantly higher NK than females, regardless of age or trt.

Effects of photoperiod manipulation on early weaning success: *Endocrine and immune* -- At weaning, pigs were allotted to either SD or LD until 10 wks of age. There were significant trt effects on number of lymphocytes and T- and B-cell mitogen-induced proliferation. Overall, lymphocyte numbers were highest in piglets weaned at 28 d and kept on LD (P = .01). Piglets weaned at 21 d of age and maintained on SD had higher (P = 0.07) T-cell proliferation, whereas, those weaned at 28 d and kept on SD had higher (P = 0.03) B-cell proliferation (**Fig. 9**).

There were significant trt×age for number of neutrophils and lymphocytes. At 4 wks of age, piglets weaned 21 d of age and kept on SD had higher (P = .033) numbers of neutrophils than those kept on LD. Conversely at 6 wk of age, piglets weaned at 14 d of age and kept on SD had significantly higher counts than LD 14 d old weaned pigs. In additions, 21 and 28 d weaned pigs kept on SD had lower counts than their LD counterparts. No differences were apparent at 8 or 10 wks among trt groups. Number of LY tended (P = 0.061) to be influenced by trt and age. There were no differences at 4 wk of age, but at 6 wk SD piglets tended to have higher LY counts than LD, regardless of weaning age. At 8 wk of age, pigs weaned at 28 d of age and kept on LD had significantly higher counts than all other treatment groups.

There was significant trt×age interaction for NK at all E:T ratios (50:1 reported). At 50:1, NK was higher (P = .002) at 6 wks of age among LD piglets (**Fig. 10**). In LD piglets, NK initially increased at 6 wk of age among 14 and 21 d weaned pigs but not 28 d weaned pigs. However, at 8 wk, regardless of weaning or photoperiod, NK significantly decreased. Among 14 and 28 d weaned pigs on LD, NK was lowest at 8 wk of age. Overall, 28 d weaned piglets kept on SD had higher NK than their LD counterparts (at all ages), except at 10 wk of age.

Within weaning trt, 4 wk old pigs, weaned at 14 d of age and kept on SD had significantly higher NK than LD. At 6 wks of age, NK was higher among LD piglets weaned at 14 d but lower at 8 wk. At 6 and 8 wk of age, 21 d weaned piglets on LD had higher NK cytotoxicity than SD. By 10 wk there were no differences. At all ages, SD piglets weaned at 28 d had higher NK than LD piglets. Piglets weaned at 28 d of age and kept on SD had higher (P = 0.038) B-cell proliferation in response to LPS.

There was a significant trt×age interaction for B-cell proliferation. Essentially, at 6 wk of age, piglets weaned at 28 d and kept on LD had lower B-cell proliferation and by 10 wk of age, SD had the highest compared to all other treatment groups. There was a significant sex effect on B-cell proliferation. Males had higher (P = 0.018) B-cell proliferation than females. In addition, barrows on SD had significantly higher B-cell proliferation than gilts. Weaning age and photoperiod trt influenced PHAG at 6, 8 and 10 wks of age. Piglets weaned at 28 d of age and kept on LD had the lowest PHAG at 6 wk of age but the highest at 8 wk of age. However, at 10 wk, those weaned at 28 d kept on SD had the lowest PHAG. *Neutrophil chemotaxis, plasma IgG, differentials and prolactin are incomplete.

Performance – There were significant trt effects on and trt×age for body weight. Overall, piglets that were weaned at 28 d of age and kept on LD were heavier (P = 0.008; **Fig 11**). At 8 wk of age, LD pigs that were weaned at 28 d were significantly heavier than pigs weaned at 14 d, regardless of light trt. However, LD pigs weaned at

28 d were also heavier than SD pigs weaned at 21 d of age. At 10 wk of age, piglets weaned at 14 d of age kept on SD, had significantly lower body weight compared to all other treatment groups. In fact, piglets weaned at 14 d and maintained on SD weighed significantly less than all other pigs throughout the duration of the study. LD piglets were heavier than those kept on SD, regardless of weaning treatment.

Discussion: These data provide potential support that photoperiod manipulation during gestation and lactation may directly and indirectly influence sow and piglet physiology and performance. Initially, 7 d following the exposure of sows to 16:8 photoperiod (light:dark) resulted in an increase in neutrophils (%) and N:L ratio compared to sows on SD, but by d 14 there were no differences in these responses. It is possible that this early increase in N:L ratio may be indicative of activation of the hypothalamic-pituitary-adrenal (HPA) axis among the sows on LD since an increase in neutrophils and N:L ratio is indicative of an acute stress response. In fact, LD treatment during gestation stimulated the immune response in these sows. This stimulation in lymphocyte proliferation and phagocytosis (21 d post-treatment) may be indicative of priming of the immune system in response to a perceived disruption in homeostasis. On the other hand, it could be the LD animal's immune system could be ready if an insult did occur. Sows that went from LD in gestation to SD at farrowing had higher total WBC 24h post-farrowing than all other trt groups. By the end of gestation, sows on LD:LD had higher proliferative responses compared to all other trt groups. In addition, there was a tendency for photoperiod manipulation to influence the number of piglets born alive and birth weights. Sows on SD tended to have more piglets born alive, but piglet birth weights were higher among LD sows. Since sows were only subjected to lighting treatment for ~21 d prior to farrowing the reason for this is unknown.

More importantly photoperiod manipulation during gestation and farrowing-lactation does appear to influence the sow's piglets. Plasma cortisol and IgG, and total WBC were higher in piglets from sows that were kept on LD:SD. This increase in IgG and total WBC may potentially be influenced by higher CORT in the piglets in conjunction with indirect influences of the dam. Since gestational trt had significant effects on immune responses in piglets. During gestation, LD photoperiod significantly influenced these responses, implying that photoperiod manipulation of the sow may have an impact on her offspring's physiological responses. At weaning, LD:LD piglets had the lowest total WBC, % monocytes and CORT but the highest PHAG. Conversely, piglets on SD:SD at weaning had higher T-cell proliferation and plasma CORT. Thus, implying that photoperiod manipulation can alter immune responses in pigs.

In addition, sex appears to be an important factor as well. Barrows subjected to LD during the gestational period had higher percentage of neutrophils thus leading to a higher N:L ratio than gilts, potentially indicating a disruption in homeostasis among these males. On the other hand, barrows from sows kept on SD during gestation had lower neutrophils and N:L ratio than females. Thus, it appears the effects of litter processing including castration could be potentially influenced by photoperiod. At weaning, barrows from sows on LD during gestation had higher percentage of monocytes than gilts. Gilts from sows kept on SD:SD had higher monocytes than barrows. Also at weaning, barrows had significantly higher IgG levels than gilts and this effect was partially due to gestational photoperiod the sows experienced.

Age at weaning had a significant impact on various immune measures. Piglets weaned at 21 and 28 d of age had higher NK cytotoxicity but lower B-cell proliferation. Also, piglets weaned at 28 d had lower neutrophils counts and PHAG compared to 14 and 21 d weaned pigs. Interestingly, neutrophils counts were not different between 14 and 21 d weaned pigs but phagocytosis was significantly higher in 21 d weaned pigs compared to 14 d. Importantly, photoperiod manipulation in conjunction with age at weaning influenced body weight and various immune measures throughout the study. Two wk post-weaning, 28 d weaned pigs on LD were heavier than all other groups including 28 d weaned pigs on SD. Overall, piglets weaned at 28 d were consistently heavier than all other weaning groups. At 10 wk, pigs weaned at 28 d and kept on LD were still heavier than all other piglets. In fact, pigs weaned at 14 d and kept on SD had the lowest body weight at all ages. B- and T-cell responses were stimulated by SD especially in those piglets weaned at 28 d of age, also this response was still higher in SD pigs weaned at 28 d at 10 wk of age.

It seems that photoperiod can be used to manipulate aspect of sows' physiology and potentially performance, however these effects may be short-term, but enough to have long-term effects on offspring. We speculate that these short-term or inconsistent effects could be due to sows becoming refractory or desensitized to photoperiod and that previous lighting experience (since we had multiparous sows) may be a contributing factor. These changes in the sow's immune response most likely are not due to shifts in circadian rhythm due to different lighting treatments. Whether or not a stimulated immune measures in response to photoperiod manipulation can be viewed as a positive or negative affect is unknown.

Photoperiod and weaning age can influence various aspects of the immune system and piglet body weight, these changes are apparent up to 10 wk of age. Taken together, these data provide support that photoperiod could be used as a potential management tool to not only enhance health and performance but well-being. Since photoperiod effects can influence not only immune and performance but may dampen the negative consequences of stress. For example, piglets weaned at 14 of age had lower body weights compared to all other piglets until 6 wks of age but by 8 and 10 wks of age those kept on LD were catching up to the other treatment groups whereas those weaned at 14 d and kept on SD had lower body weights at 10 wks of age.

Lay Interpretation: Animals adapt more readily to photoperiod cues than other environmental cues since photoperiod is more predictable and consistent over time. Previous work has shown limited (if any) affects of photoperiod on pig physiology and performance. However, these data begin to provide support that photoperiod may potentially be used to manipulate physiology and performance as well as counteract the negative effects of stress. Photoperiod manipulation used at critical points during development may actually influence physiological responses later in life which may ultimately have impact on pig well-being, health and performance. These data provide support that immune responses and performance/productivity of sows, neonates, and young pigs can be influenced by photoperiod. In addition, sex of the piglet also interacts with these factors. It appears that the photoperiod treatment a sow is subjected to may influence early responses of her piglets. More, importantly, photoperiod may potentially provide a management tool that could be used to counteract the negative consequences of weaning stress and other stressors. It is imperative that we understand whether immune stimulation (increased immune responsiveness) is beneficial or detrimental to the challenges that a sow or piglet may encounter throughout production. This question remains to be resolved.

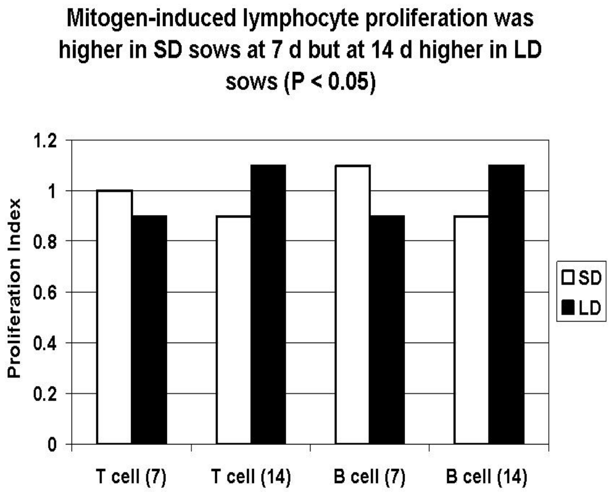
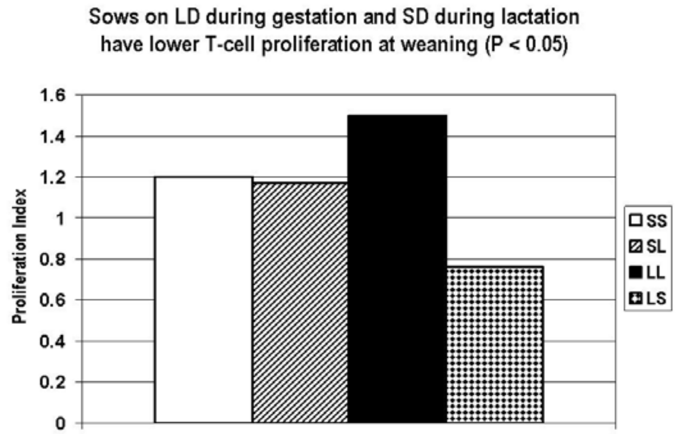


Fig 1



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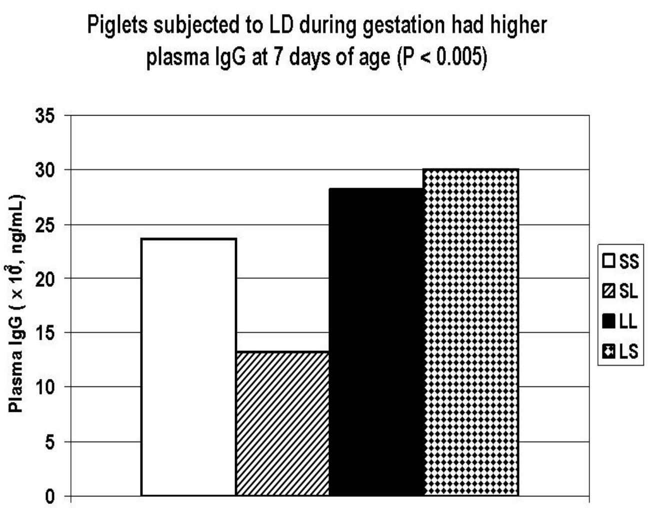

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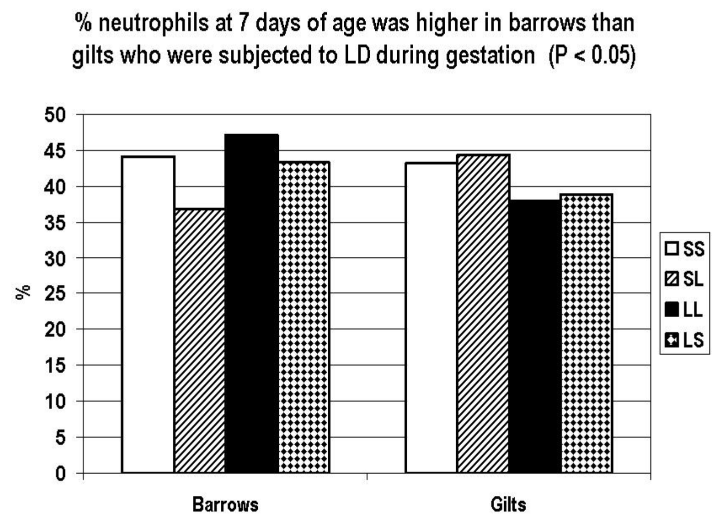

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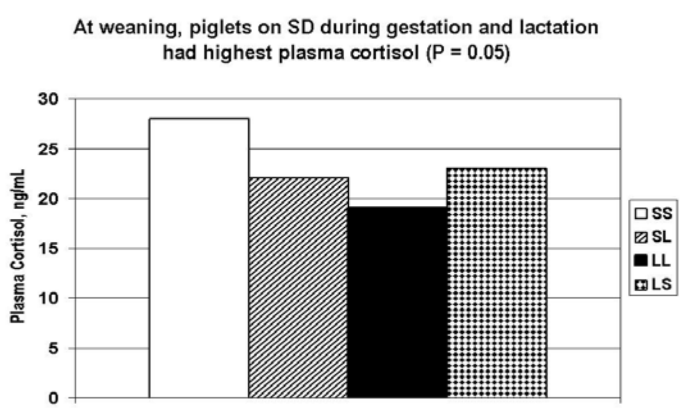

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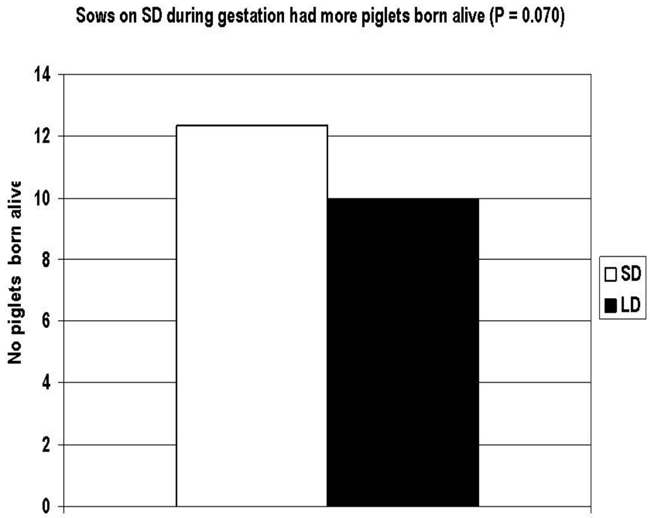

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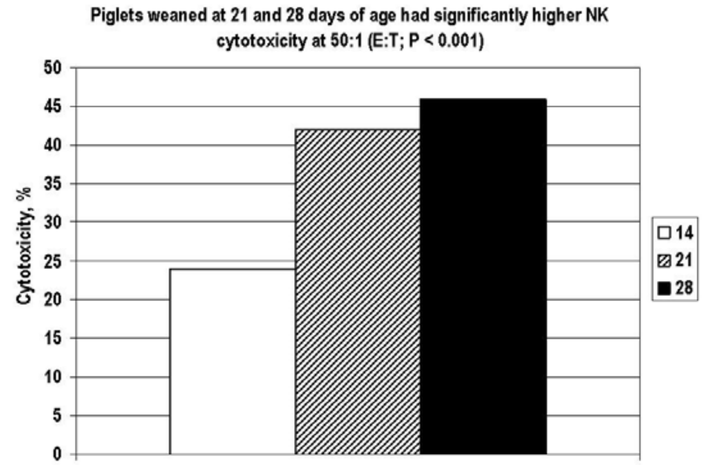

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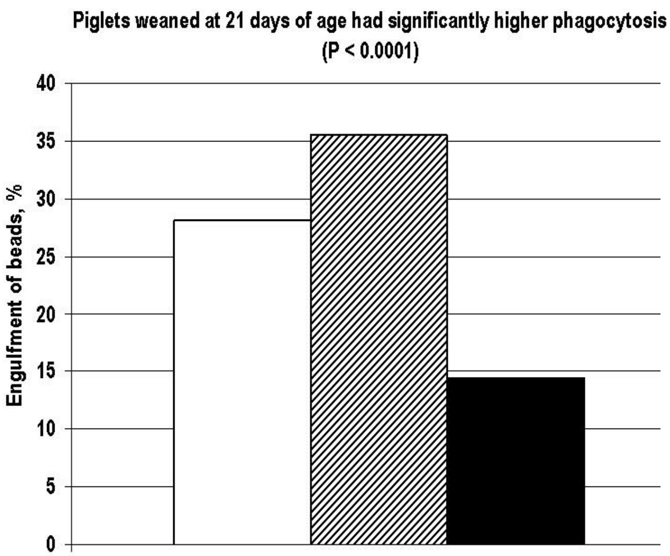

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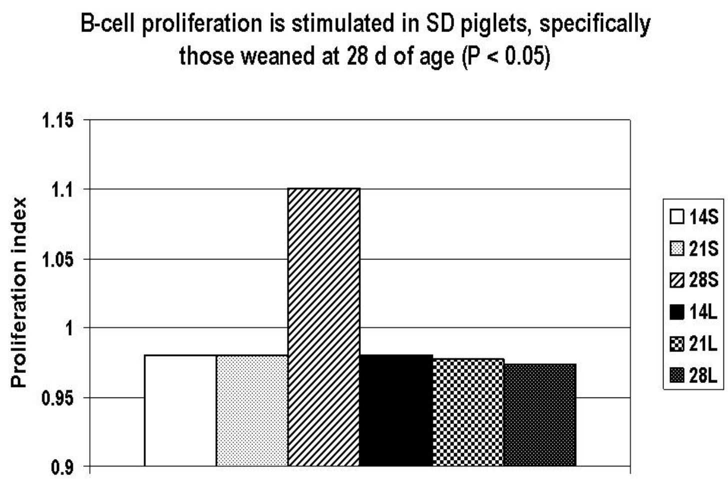

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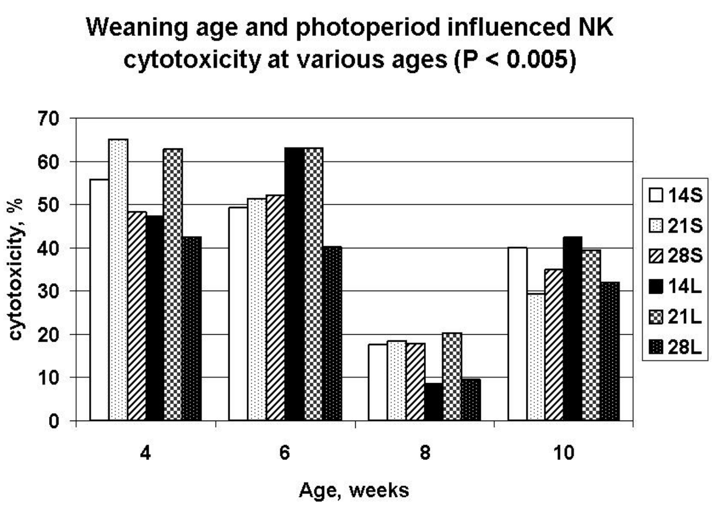

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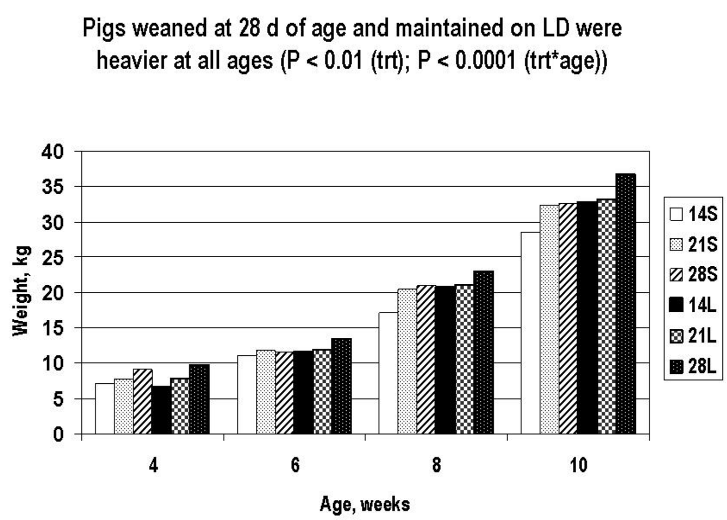

Fig 11.