Impact of a long photoperiod during lactation on immune status of piglets

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ABSTRACT: The effects of a long photoperiod treatment around parturition and throughout lactation on immune status of piglets were studied. Sows were assigned to 2 light regimens: i) standard short photoperiod (SP, n = 17), 8 h of daily light from d 112 of gestation until d 23 of lactation; and ii) long photoperiod (LP, n = 17), 23 h of daily light from d 112 of gestation to d 4 of lactation and 16 h thereafter. In front of the crates, under the side heat lamps and behind the sow, light intensities were 59 ± 5, 109 ± 6, and 44 ± 6 lx, respectively. On d 15 of lactation and at weaning (d 23), 2 piglets of similar BW per litter were selected and immunized intramuscularly with ovalbumin (OVA). Blood samples (5 mL serum and 10 mL whole blood) were taken at d 15 and d 23 of lactation, and at d 30, 37, and 44 of age after weaning to evaluate the antibody response to OVA and measure phagocytosis, lymphocyte proliferative response, and different circulating blood lymphocyte populations of piglets. Results showed that phagocytosis was increased in piglets submitted to LP ($P < 0.05$). A treatment × time interaction ($P < 0.001$) indicated that SP piglets developed a better IgG response to OVA than LP piglets. The percentage of B lymphocytes was also increased ($P = 0.02$) in SP piglets compared with piglets exposed to LP during lactation; the lymphocyte response to OVA tended to be enhanced ($P = 0.07$) over time in SP piglets. Different subpopulations of CD8+ lymphocytes were markedly increased in SP piglets at 23 d of age compared with piglets exposed to LP (treatment × time: $P < 0.05$). These results suggest that exposure of piglets to LP during lactation seems to reduce the capacity of piglets to develop a strong immune response to novel antigens. This may have important consequences on the ability of piglets to resist an infection after weaning.

Key words: immunity, lactation, photoperiod, piglet

INTRODUCTION

An extended photoperiod during farrowing and lactation has been used to increase sow milk production and piglet performance (Mabry et al., 1983; Gooneratne and Thacker, 1990; Lachance et al., 2010). Such a practice may have important consequences on the development of the immune system of a piglet, because there is clear evidence that both innate and adaptive immune responses of many animal species are affected by photoperiod and seasonal rhythms (Nelson, 2004; Wen and Prendergast, 2007; Haldar and Ahmad, 2010).

In swine, it was reported that a long photoperiod (16 h of light/d) compared with a short photoperiod (8 h of light/d) during the gestation and lactation periods increases neutrophil phagocytosis and circulating IgG of weaned piglets but reduces the lymphocyte proliferative response to concanavalin A (Con A) stimulation (Niekamp et al., 2006, 2007). These results contradict others that indicate that immune functions are generally enhanced by short days (Nelson, 2004; Haldar and Ahmad, 2010). In several rodent species, short days increase the number of circulating blood leukocytes and lymphocytes, as well as lymphocyte blastogenesis and cytolytic capacity of natural killer cells (Yellon et al., 1999; Bilbo et al., 2002; Nelson,
Animals and Treatments

All sows were transferred to another farrowing room on d 23 of gestation to determine whether exposure of lactating sows and their piglets to different photoperiod regimens influenced the development of the immune system of piglets. The objective of this study was to determine whether exposure of lactating sows and their piglets to different photoperiod regimens influenced the development of the immune system of piglets through the establishment of different lymphocyte populations and acquisition of immunological functional properties.

MATERIALS AND METHODS

Animals were cared for according to a recommended code of practice and procedures that were reviewed by an Institutional Animal Care Committee in accordance with the Canadian Council on Animal Care (2009).

Animals and Treatments

Thirty-four Yorkshire x Landrace gilts were inseminated with pooled semen from Duroc boars. Gilts were subjected to 12 h of light daily (0600 to 1800 h) from mating to d 111 of gestation and were then randomly assigned to the following 2 light regimens from d 112 of gestation until d 23 of lactation: i) standard short photoperiod (SP, n = 17) consisting of 8 h of light (0730 to 1530 h) daily from d 112 of gestation until d 23 of lactation, and; ii) long photoperiod (LP, n = 17) consisting of 23 h of light (0000 to 2300 h) from d 112 of gestation until d 4 of lactation and 16 h (0730 to 2330 h) of daily light for the remainder of lactation (weaning on d 23). These primiparous sows were housed in individual stalls (0.6 m x 2.1 m) during gestation and were transferred to farrowing crates on d 112 of gestation. All sows were transferred to another farrowing room on d 4 of lactation, ~72 h after the end of farrowing, to ensure that all animals were subjected to the same stress. The LP sows and their litters were transferred and subjected to the change in photoperiod at this time. Farrowings took place from February to July 2008 and photoperiod treatments were equally distributed among each group of farrowing sows each month. Light conditions and quantification of light emissions in each farrowing room and each crate were described previously (Lachance et al., 2010). Briefly, there was an average of 59 ± 5 lx in front of the crates. Under the side heat lamps, light intensity was 109 ± 6 lx; behind the sow, it was 44 ± 6 lx.

Litter size was standardized to 10 ± 1 pigs on d 2 of lactation. No creep feed was distributed to piglets during lactation and they had no access to the sow feeder. Feeding conditions of sows and piglets after weaning were also previously described (Lachance et al., 2010). After weaning, each litter was housed in a pen (1.9 m x 1.9 m) and all piglets were subjected to 12 h of daily light (from 0600 to 1800 h).

Immunization and Blood Samplings

On d 15, 1 female and 1 male piglet of similar BW per litter were selected and immunized intramuscularly with 1 mL of a sterile solution of PBS containing 2 mg of ovalbumin (OVA; Sigma-Aldrich, ON, Canada) and 1 mg of Quil A (Bethyl Laboratories Inc., Cedarlane Laboratories Ltd., Burlington, ON, Canada), which was emulsified with an equal part of incomplete Freund adjuvant (IFA; Sigma-Aldrich). Intramuscular injections of OVA vaccine were administered on the 2 sides of the neck (0.5 mL/site). At weaning (d 23), a second immunization consisting of 1 mg of OVA, 2 mg of lysozyme (LYS), and 1 mg of Quil A, all mixed in a 50:50 IFA solution, was administered. The preparation and administration of the vaccine was the same as in the first immunization. Blood samples (5 mL) were taken from each piglet by jugular venipuncture between 0900 and 1000 h on d 15, 23, 30, 37, and 44, using tubes without anticoagulant for measuring primary and secondary antibody response to LYS and OVA in serum, respectively. On d 15, 23, 30, and 37, whole blood samples (10 mL) were also collected in tubes containing Na-heparin (for lymphoproliferative and phagocytosis assays) or K$_2$-EDTA (for identification of cell population by cytometry).

Tubes without anticoagulant were left at room temperature for 2 h and then centrifuged at 4°C for 15 min at 850 x g. Serum was stored at –20°C for subsequent assays.

Isolation of Peripheral Blood Mononuclear Cells and Neutrophils

Whole blood was diluted 1:2 with Hank’s balanced salt solution (HBSS), layered over a discontinuous gradient of Histopaque (density of 1.119 g/mL; Sigma-Aldrich) and Ficoll-Paque Plus (density 1.077 g/mL; GE Health Care Life Science, Quebec, Canada), and centrifuged at 750 x g for 40 min at room temperature. Peripheral blood mononuclear cells (PBMC) located over the Ficoll-Paque layer were collected and the contaminating red blood cells were lysed by hypotonic shock, using water and HBSS 10× to restore isotonicity. After 2 washes in sterile HBSS, the cells were resuspended in HBSS. Neutrophils were collected from the layer over the Histopaque and washed once in HBSS. After washing, red blood cells were also lysed by hypotonic shock. Neutrophils were centrifuged at 530 x g
for 8 min at room temperature, washed in HBSS, and the cell pellet resuspended in Roswell Park Memorial Institute (RPMI) 1640 containing 10% fetal bovine serum (FBS) and a solution of antibiotics (100 U of penicillin and 100 μg of streptomycin/mL). Both PBMC and neutrophils were counted using a hemacytometer and viability was assessed using trypan blue. Cell viability was always >95%.

**Immune Assays**

Antibody responses to OVA and LYS were measured by indirect ELISA as described previously, with minor modifications (Lessard et al., 2005). Briefly, serum samples were diluted (dilution factors used were 1:250 and 1:500 on d 15 and 23; 1:4,000 and 1:8,000 on d 30; 1:8,000 and 1:16,000 on d 37; and 1:16,000 and 1:32,000 on d 44) and were added into wells of flat-bottomed, 96-well Nunc MaxiSorp plates (VWR International Ltd., Mississauga, ON, Canada) coated with 8 μg/mL of either OVA or LYS. They were then left overnight at room temperature. As a standard curve, serial 2-fold dilutions of an anti-OVA or anti-LYS pool of positive serum were included in each plate. Goat antipig IgG [fragment crystallisable (Fc) region] or antipig IgM (Fc) conjugated to horseradish peroxidase (Bethyl Laboratories Inc., Cedarlane Laboratories Ltd.) and KPL and tetramethylbenzidine (TMB) peroxidase substrate (Mandel, Guelph, ON, Canada.) were used to perform the assay. Absorbance values were measured and antibody titers of samples were calculated, using serial dilution values of positive serum in a linear regression analysis and dilution factors used when coating the plate. All samples and controls were tested in duplicate. Intra-assay and interassay CV were 5.37% and 19.6%, respectively, for IgM measurement to OVA, 3.6% and 16.6% for IgG to OVA, 6.6% and 11.1% for IgM to LYS, and 4.7% and 11.0% for IgG to Lys. Data were corrected with a logarithmic transformation before statistical analysis.

The lymphocyte proliferative response was measured using the CellTrace carboxyfluorescein succinimidyl ester (CFSE) Cell Proliferation Kit (Invitrogen Canada, Hamilton, Canada). Briefly, the pellet of PBMC was resuspended and labeled with 0.5 μM of CFSE diluted in HBSS. After the removal of unbound dye, the cells were resuspended in RPMI 1640 supplemented with 10% FBS and penicillin-streptomycin antibiotic mixture containing 5,000 U/mL of penicillin and 5,000 μg/mL of streptomycin (Wisent, St-Bruno, Canada). Then, 2.5 × 10^6 cells were added to each well of a 24-well plate (BD Biosciences, VWR International Ltd.) and incubated with Con A (0.5 μg/mL) or OVA (10 μg/mL), with or without a 30-min preincubation with 60 picogram (pg) mL of melatonin (MEL; Sigma-Aldrich) at 37°C in a 5% CO₂ incubator for 65 h or 6 d. At the end of the incubation period, PBMC were transferred to round-bottomed test tubes for cytometry (Becton Dickinson and Company, Mississauga, ON, Canada), washed, and fixed in Isoton II (Beckman Coulter, Mississauga, ON, Canada) containing 1% formaldehyde. The percentage of proliferating PBMC was determined using an EPICS-XL flow cytometer (Beckman Coulter).

Neutrophil phagocytosis was measured using the Vybrant Phagocytosis Assay Kit (Invivogen Canada). Briefly, 100 μL of neutrophil suspension, adjusted to 5 × 10^5 in RPMI supplemented with 10% FBS, were added to each experimental well of 96-well, round-bottomed microplates. Then, 50 μL of the prepared fluorescein isothiocyanate (FITC) labeled *Escherichia coli* K-12 bioparticles suspension were added to all samples at a bioparticle to cell ratio of 10:1. Control wells, which contained only polymorphonuclear neutrophils (PMN) and cell culture media, or only the beads, were also included to discriminate region of cells and unbounded beads by flow cytometry. After a 45-min incubation period at 37°C in a 5% CO₂ humidified incubator, cells were put on ice, centrifuged at 530 × g at 4°C for 5 min, and washed with cold HBSS to remove nonphagocytized bioparticles. Cells were resuspended in cold Isotonic II containing 0.5% BSA, transferred into tubes, and kept protected from light at 4°C. Negative controls were performed by incubating PMN and beads at 4°C; these controls were used to remove fluorescence from nonspecific-bound fluorescent bioparticles to the surface of PMN that were not phagocytized. Uptake of *E. coli* K12 bioparticles was determined using an EPICS-XL flow cytometer (Beckman Coulter). Results were expressed as the percentage of neutrophils engulfing 1 or more FITC-labeled *E. coli* bioparticles. The Overton algorithm was used to subtract values from controls kept at 4°C from samples incubated at 37°C (Overton, 1988).

Mononuclear cells isolated from whole blood were also labeled to characterize CD4^+ and CD8^+ T lymphocytes, as described previously (Lessard et al., 2009). The B lymphocytes were labeled with a mouse monoclonal antibody antipig CD21 (Clone BB6-11C9, VMRD, Cedarlane Laboratories Ltd., Burlington, ON, Canada), followed by a goat antimouse IgG1 conjugated to phycoerythrin (SouthernBiotech, Birmingham, AL). All samples were analyzed with the EPICS-XL flow cytometer (Beckman Coulter).

**Statistical Analyses**

Statistical analyses to evaluate the influence of different photoperiod schedules on antibody response, phagocytosis, and cell population during the periweaning
period were performed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Repeated-in-time analyses were done for data measured over successive days with the day × treatment interaction being included in the model. A statistical tendency was defined as $0.05 < P < 0.1$ and $P$-values ≤ 0.05 were considered significant. Results in tables and text are presented as least squares means ± SEM, except when mentioned otherwise.

**RESULTS**

*Peripheral Blood Mononuclear Cell Phagocytosis Activity*

A treatment × day interaction ($P = 0.001$) indicated that, on d 15, the percentage of PMN that phagocytized FITC- *E. coli* particles was less in SP piglets than in piglets maintained on LP. Values in SP piglets then increased to reach levels similar to those of LP piglets by d 23 (Figure 1A). A treatment × day interaction ($P = 0.008$) also showed that PMN phagocytosis capacity, which is estimated by the mean fluorescence index, declined from 32.7 to 23.0% between d 15 and 23 in SP piglets, whereas it slightly increased in LP piglets from 27.0 to 33.6% during the same period. These changes between d 15 and 23 resulted in a greater phagocytic capacity ($P < 0.05$) in piglets exposed to LP than SP on d 23 (Figure 1B). Similar results were obtained in culture media supplemented with 60 pg/mL of melatonin, a physiological concentration found in circulation (data not shown).

*Antibody Responses to Ovalbumin and Lysozyme*

Antibody responses to OVA and LYS are presented in Figures 2 and 3, respectively. Overall, a treatment × time interaction indicated that SP piglets developed a stronger primary and secondary IgG response to LYS and OVA than piglets exposed to LP ($P = 0.003$ and $P = 0.001$).
0.0001, respectively). Indeed, at 30 d of age (15 d after OVA injection), IgG titers were reduced in SP compared with LP piglets, but by 22 d and 29 d after OVA injection, IgG titers were greater in SP than LP piglets, indicating that the increase in IgG titers over time was greater in SP than LP piglets. Similar results were obtained for the response to LYS injection, also indicating that the increase in IgG titers was greater in SP than LP piglets. Production of IgM against OVA and LYS was also affected differently by photoperiod over time. In pigs exposed to LP, the increase in IgM titer to OVA and LYS was not as pronounced as in SP pigs in the weeks after injections, resulting in reduced IgM production directed against both antigens (treatment × time interaction with P-values of 0.10 for OVA and 0.004 for LYS).

**Lymphocyte Proliferative Response to Concanavalin A and Ovalbumin**

The lymphocyte proliferative response to Con A throughout the experimental period was not affected by photoperiod (P > 0.05; Figure 4A). However, on d 15, when lymphocyte cultures were supplemented with MEL, a treatment × MEL interaction (P = 0.02) indicated that addition of MEL to lymphocytes isolated from LP pigs augmented their response to Con A, whereas there was no effect on lymphocyte responses in SP pigs.

The lymphocyte proliferative response to OVA tended to be affected by photoperiod during the experimental period (P < 0.1; Figure 4B). The treatment × day interaction (P = 0.07) showed that the lymphocyte response to OVA tended to be lesser (P = 0.07) in SP pigs than LP pigs at the end of the photoperiod treatment (d 23), but thereafter there was a marked increase in proliferative response so that by d 37 it was greater in control than LP piglets. This time effect resulted in a significant increase (P = 0.02) of the lymphocyte response to OVA in SP piglets, whereas during the same period of time, the lymphocyte response in LP piglets remained the same. The presence of MEL did not modulate the lymphocyte proliferative response to OVA (results not presented).

**Figure 3.** Influence of photoperiod regimen during lactation on primary IgM (A) and IgG (B) responses of piglets immunized with lysozyme (LYS). Production and peak response of IgM to LYS were greater in short photoperiod (SP; 8 h of light/d) than long photoperiod (LP; 16 h of light/d) piglets [treatment (Trt) × d, P = 0.004]. Production of specific IgG to LYS increased over time in SP compared with LP piglets (Trt × d, P = 0.001).

**Figure 4.** Influence of photoperiod regimen during lactation on percentage of proliferative lymphocyte after concanavalin (Con A) or ovalbumin (OVA) stimulation: (A) on d 15, addition of melatonin (MEL) to lymphocytes isolated from long photoperiod (LP; 16 h of light/d) piglets augmented their response to Con A, whereas there was no effect on lymphocyte responses of short photoperiod (SP; 8 h of light/d) pigs [treatment (Trt) × MEL, P = 0.02]; (B) lymphocyte proliferation of SP compared with LP pigs (Trt × day, P = 0.07).
**Lymphocyte Populations**

The percentage of CD21\(^+\) B lymphocyte tended to be less \((P = 0.07)\) in LP than SP piglets (Figure 5). However, on d 15 of lactation, values were reduced in LP compared with SP pigs \((P = 0.02)\); 7 d after weaning (d 20), when all pigs were on the same photoperiod schedule, no difference between treatments was observed. Mean fluorescence intensity of B lymphocytes expressing CD21 cell surface marker was not modulated by photoperiod treatment (Table 1).

Among different subpopulations of T cells, the most affected by the prolonged photoperiod were the CD4\(^+\)CD8\(^+\) and CD4\(^-\)CD8\(^+\) T cells (Figure 6). The percentages of CD4\(^+\)CD8\(^+\), CD4\(^-\)CD8\(^+\)high, and CD4\(^-\)CD8\(^+\)low T cells, which are mainly associated with memory T cells, cytotoxic T cells, and NK T cells, were greater \((P < 0.001)\) in SP than LP pigs on d 23. Treatment × day interactions \((P = 0.06\) for CD4\(^+\)CD8\(^+\); \(P = 0.02\) for CD4\(^-\)CD8\(^+\)high; and \(P = 0.005\) for CD4\(^-\)CD8\(^+\)low T cells) indicated that the proportion of these T cells remained the same in LP piglets from d 15 to 23, whereas they were all enhanced in control piglets. A week after the end of photoperiod treatment (d 30), percentages of CD4\(^+\)CD8\(^+\), CD4\(^-\)CD8\(^+\)high, and CD4\(^-\)CD8\(^+\)low T cells increased in LP piglets compared with d 23 and reached percentages that were similar to the SP group. Photoperiod treatment had no effect on the percentage of circulating CD4\(^+\)CD8\(^-\) T helper cells. However, it is interesting to note that mean fluorescent intensity of CD4 on CD4\(^+\) T cells was increased in SP compared with LP pigs in both CD4\(^+\)CD8\(^-\) and CD4\(^+\)CD8\(^+\) T cells on d 15 and 30 (Table 1; \(P < 0.001\)). Similarly, the intensity of CD8 surface marker on CD4\(^+\)CD8\(^+\) T cells was also greater \((P < 0.001)\) in SP- than LP-treated piglets on d 15 and 30. On CD4\(^-\)CD8\(^-\) T cells, a treatment × day interaction \((P = 0.002)\) indicates that CD8 marker increased with time in control pigs, whereas it remained the same in LP piglets.

**DISCUSSION**

The present study indicates that the length of photoperiod during lactation in influences the establishment and functions of immune cells that are involved in both innate and adaptive immune responses of piglets. In piglets raised under standard conditions of an 8-h photoperiod daily, phagocytosis capacity of PMN, which was evaluated by measuring the number of fluorescent PMN and mean fluorescence index (an indicator of the number of phagocytized particles), appears to be reduced compared with that of piglets raised on LP. These results support previous results, indicating that exposure to SP vs. LP suppresses phagocytosis in hamsters (Yellon et al., 1999) and suckling piglets (Niekamp et al., 2006). However, they contrast with other data indicating that

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**Table 1. Influence of photoperiod regimen during lactation on the expression of different cell surface markers on circulating immune cells**

<table>
<thead>
<tr>
<th>Type of cells</th>
<th>Markers</th>
<th>Trt(^1)</th>
<th>Mean fluorescence intensity(^2)</th>
<th>SEM</th>
<th>(P)-value</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>d 15</td>
<td>d 23</td>
<td>d 30</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>CD21(^+)</td>
<td>SP(^1)</td>
<td>18.4</td>
<td>15.6</td>
<td>16.4</td>
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<tr>
<td></td>
<td></td>
<td>LP(^1)</td>
<td>17.7</td>
<td>15.5</td>
<td>16.1</td>
</tr>
<tr>
<td>T helper cells (CD4(^+)CD8(^-))</td>
<td>CD4(^+)</td>
<td>SP (\star)</td>
<td>5.5</td>
<td>4.1</td>
<td>3.9</td>
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<tr>
<td></td>
<td></td>
<td>LP (\star)</td>
<td>2.6</td>
<td>3.3</td>
<td>2.6**</td>
</tr>
<tr>
<td>T memory or effector cells (CD4(^+)CD8(^+))</td>
<td>CD4(^+)</td>
<td>SP (\star)</td>
<td>5.0</td>
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<td></td>
<td>LP (\star)</td>
<td>2.6**</td>
<td>3.2</td>
<td>2.7**</td>
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<tr>
<td></td>
<td>CD8(^+)</td>
<td>SP (\star)</td>
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<td>8.6</td>
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<td></td>
<td></td>
<td>LP (\star)</td>
<td>5.6</td>
<td>6.0*</td>
<td>6.0*</td>
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<tr>
<td>T cytotoxic (CD4(^+)CD8(^+)high)</td>
<td>CD8(^+)high</td>
<td>SP (\star)</td>
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<td>LP (\star)</td>
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<td>11.4</td>
<td>10.5</td>
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<td>NK T cells (CD4(^-)CD8(^+)low)</td>
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<td>SP (\star)</td>
<td>62.7</td>
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<td>LP (\star)</td>
<td>62.9</td>
<td>56.8**</td>
<td>61.3**</td>
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\(^1\)Trt = Treatment; SP = short photoperiod; LP = long photoperiod.

\(^2\)Data with * or ** indicate that LP differs from SP at \(P \leq 0.05\) and \(P \leq 0.01\), respectively.
neutrophil phagocytosis was increased in piglets submitted to standard photoperiod after weaning (Niekamp et al., 2007). These differences in phagocytic activity can be explained by the time of measurement. In the current study, measurements were performed during the lactation period, whereas in the study of Niekamp et al. (2006), piglets were assigned to light treatments after weaning and PMN phagocytic activity was measured after weaning. Therefore, it appears that photoperiod treatment during the lactation period or after weaning may have different effects on the uptake of antigens by PMN. However, at weaning, the percentages of phagocytic PMN were similar in both groups of piglets and only phagocytic capacity was increased in LP piglets compared with SP. These results suggest that length of photoperiod during lactation has minor effects on the number of circulating PMN but may have some impact on the maturation of phagocytic cells and their potential to process new antigens.

Figure 6. Influence of photoperiod regimen during lactation on CD4⁺CD8⁻ (A), CD4⁺CD8⁺ (B), CD4⁺CD8⁺high (C), and CD4⁺CD8⁺low (D) T lymphocytes. In short photoperiod (SP) piglets (8 h of light/d), the percentage of different subpopulations of T lymphocytes expressing CD8 cell surface marker was greater on d 23 compared with long photoperiod (LP; 16 h of light/d) piglets (treatment × d; P values of 0.06, 0.02, and 0.005, respectively, for CD4⁺CD8⁺, CD4⁺CD8⁺high, and CD4⁺CD8⁺low T cells).
In addition to the effect of photoperiod on PMN phagocytosis, primary and secondary antibody responses to LYS and OVA were respectively enhanced in piglets exposed to standard photoperiod compared with LP during lactation. This is consistent with previous studies in which humoral immunity was increased in Syrian hamsters exposed to short photoperiod compared with long photoperiod (Drazen et al., 2002). Interestingly, the percentage of B lymphocytes was also increased in piglets exposed to SP compared with LP during lactation. To the best of our knowledge, this is the first time that a photoperiod effect has been reported on B cells and specific antibody response in piglets. Such increases in lymphocyte counts were previously reported to be enhanced after exposure to short photoperiod in rodents (Haldar and Ahmad, 2010). In swine, it was reported that total IgG concentrations and total leukocyte numbers are enhanced in piglets weaned at 14 d and kept on a short-day photoperiodic regimen from birth through the nursery phase (Niekamp et al., 2007).

This early increase in B cells may be responsible for the improved antibody response to OVA and LYS in the current study and suggests that processing and presentation of antigens by antigen-presenting cells (macrophages and dendritic cells) to T cells were not affected. In fact, the lymphocyte proliferative response to OVA tended to be increased after the boost injection in control piglets exposed to 8 h of light daily compared with piglets on the LP regimen during lactation. This increased proliferative response to OVA may also stimulate production of anti-OVA through a greater activation of B-cell response by primed T-helper cells. These results are in agreement with data reported previously, where short days had a stimulatory effect on T cell-dependent B cell antibody production (Yellon et al., 1999; Yellon, 2007) and lymphocyte proliferative responses (Mann et al., 2000; Nelson, 2004). There was no effect of photoperiod on lymphocyte proliferative response after mitogenic stimulation in the present study, thereby corroborating previous data where the lymphocyte proliferative response to Con A was not affected in 21-d-old piglets exposed to SP during the lactation period only (Niekamp et al., 2006). However, when sows and their litters were subjected to SP during late gestation and lactation, piglets had an increased lymphocyte proliferative response compared with sows and piglets maintained on LP (Niekamp et al., 2006).

Current results show that different subpopulations of CD8+ lymphocytes were markedly increased in control piglets compared with LP piglets at 23 d of age. The cell surface expression of CD4 or CD8 markers on different subpopulations of T cells was also increased in control compared with LP piglets. These data support previous results indicating that the number of T cells (CD3+, CD8+, CD8+CD25+, CD4+CD25+) capable of immunosurveillance and regulation of inflammatory responses is enhanced in the blood of rats housed in SP as opposed to LP for 10 wk (Prendergast et al., 2007). It is interesting to note that the establishment of CD8+ T cells in mucosal tissues begins at ~15 to 21 d of age (Bailey and Haverson, 2006) and markedly increases in blood between 7- and 18-d-old piglets (Brown et al., 2006). Present results further suggest that during the neonatal period, which is a critical time for the development and maturation of the immune system of piglets, photoperiod may have an impact on the establishment of immunological cells and their functional properties.

Although the precise mechanisms mediating changes in immune function by photoperiod are not yet well understood, it is possible that modulation of immunity could be due to increased secretion of melatonin during SP. In the present study, the proliferative response of lymphocytes isolated from piglets on LP on d 15 of lactation was increased by the addition of melatonin compared with that of SP piglets, whereas melatonin did not affect proliferative response on d 23 and 37. As observed in other in vitro studies, the effect of melatonin on the lymphocyte response to mitogenic stimulation is not clear. In some studies, addition of melatonin into lymphocyte culture increases lymphocyte proliferative response to mitogens, whereas other in vitro studies have shown no effect of melatonin on resting or activated lymphocytes with phytohemoagglutinin or Con A (Carrillo-Vico et al., 2005). Yet, the main influence of in vitro addition of melatonin on immunological functions is its potential to modulate production of immune mediators, such as cytokines (Garcia-Maurino et al., 1997, 1999).

In in vivo studies, there is also clear evidence that during the dark period, the production of many cytokines involved in regulation of immune responses, such as IL-2, IL-6, IL-12, and interferon-γ, is modulated (Garcia-Maurino et al., 1997, 1999; Carrillo-Vico et al., 2005). It is interesting to note that colostrum and milk content of different cytokines are also regulated by the nocturnal melatonin surge (Pontes et al., 2007). In this study, photoperiod did not alter concentrations of melatonin in blood and milk from sows (Lachance et al., 2010). However, samples were collected after 1000 h, being late after the nocturnal surge of melatonin. Taking into account that melatonin and cytokines are secreted in colostrum and milk following a circadian rhythm and represent a fraction of the concentration in the blood (Rudloff et al., 1992), further experiments are necessary to better understand the impact of photoperiod on milk composition of sows and development and maturation of the immune system of piglets.

In conclusion, both innate and adaptive immune functions were modulated in piglets exposed to different
photoperiods during lactation. Exposure of piglets to a LP regimen during this period seems to reduce the capacity of piglets to develop a strong immune response to novel antigens. Consequently, a LP regimen during lactation should be considered unfavorable for piglet health and may have important consequences on their potential to cope with infections after weaning.

LITERATURE CITED


