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Effect of maternal fish oil and seaweed extract supplementation on colostrum and milk composition, humoral immune response, and performance of suckled piglets

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ABSTRACT: An experiment with a 2 × 2 factorial arrangement of treatments (n = 10 sows/treatment) was conducted to investigate the effect of maternal dietary supplementation with seaweed extract (SWE: 0 vs. 10.0 g/d) and fish oil (FO) inclusion (0 vs. 100 g/d) from d 109 of gestation until weaning (d 26) on sow colostrum and milk composition, humoral immune response on d 5 and 12 of lactation, and suckling piglet performance. Furthermore, the influence of dietary treatment on the phagocytic activity of whole blood white cells at weaning was examined. The SWE (10 g) contained laminarin (1 g), fucoidan (0.8 g), and ash (8.2 g) and was extracted from a Laminaria spp. The FO contained approximately 40% eicosapentaenoic acid and 25% docosahexaenoic acid. The SWE-supplemented sows had greater colostrum IgG (P < 0.01) and milk protein (P < 0.05) concentrations on d 12 of lactation compared with non-SWE-supplemented sows. Piglets suckling SWE-supplemented sows had greater serum IgG (P < 0.01) and IgA (P < 0.05) concentrations on d 5 and 12 of lactation compared with non-SWE-supplemented sows. In contrast, FO supplementation exerted a suppressive effect on piglet serum IgA concentrations on d 5 of lactation (P < 0.05) compared with non-FO-supplemented sows. However, total leukocyte, lymphocyte, monocyte, and neutrophil numbers were not influenced by sow dietary treatment. Average piglet weaning weight and ADG between birth and weaning were not influenced by sow dietary treatment. In conclusion, the current study demonstrates that SWE supplementation from d 109 of gestation until weaning enhanced colostral IgG concentrations and circulatory IgG concentrations in suckled piglets on d 5 and 12 of lactation. Furthermore, the percentage of leukocytes and lymphocytes phagocytizing E. coli at weaning increased in piglets suckling FO-supplemented sows, indicating an enhancement of immune function against presenting pathogens. However, the combination of SWE and FO bestowed no positive effect on immune responses investigated in the current study.

Key words: fish oil, humoral immunity, piglet, seaweed extract

INTRODUCTION

The specialized porcine epitheliochorial placenta prevents in utero transfer of maternal immunoglobulins (Le Dividich et al., 2005), and neonatal survival is dependent on sufficient colostrum ingestion for humoral immune protection (Rooke and Bland, 2002) and energy provision (Pettigrew, 1981).

Previous studies indicated that maternal fish oil supplementation enriched the n-3 PUFA content of the milk of sows (Fritsche et al., 1993b; Lauridsen and Danielsen, 2004), reduced preweaning piglet mortality (Rooke et al., 2001b), enhanced piglet serum IgG concentrations at weaning (Rooke et al., 2003), and poten-
tiated an anti-inflammatory response in suckled piglets (Fritsche et al., 1993a).

The identification of novel biological active compounds from marine algae has extended their application to animal diets (Gardiner et al., 2008). Previous research has demonstrated that dietary provision of a Laminaria digitata-derived seaweed extract containing laminarin and fucoidan postweaning improved piglet performance (Gahan et al., 2009) and intestinal microbiota (Reilly et al., 2008). However, to our knowledge, no study has examined the effect of maternal Laminaria spp.-derived seaweed extract supplementation on performance and immune components in suckling piglets. Furthermore, investigations exploring the effect of maternal dietary supplementation on neonatal piglet growth and immune competence have received considerable attention in recent years (Ilsley et al., 2005; Corino et al., 2009).

Therefore, the aim of the present study was to evaluate the effects of maternal dietary supplementation with seaweed extract (SWE) and fish oil (FO) from d 109 of gestation until weaning (d 26) oncolostrum and milk composition, suckling piglet performance, humoral immune response on d 5 and 12 of lactation, and phagocytic capacity of piglet white blood cells at weaning. In addition, this study examined any beneficial response to the combination treatment of SWE and FO.

**MATERIALS AND METHODS**

All experimental procedures described in this experiment were conducted under experimental license from the Irish Department of Health in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendments of the Cruelty to Animals Act, 1876) Regulations (1994).

**Experimental Design and Animal Management**

The experiment was designed as a 2 × 2 factorial arrangement, comprising 4 dietary treatments. Forty crossbred pregnant sows (Large White × Landrace genetic lines) were randomly assigned, accounting for parity (mean parity 3.4 ± 1.4) and expected farrowing date, to 1 of the 4 dietary treatments (n = 10 sows/treatment): 1) basal lactation diet, 2) basal lactation diet and 100 g of FO/d, 3) basal lactation diet and 10.0 g of SWE/d, and 4) basal lactation diet and 100 g of FO/d and 10.0 g of SWE/d from d 109 of gestation until weaning (d 26). The quantity of SWE used in the current study was based on previous work by Lynch et al. (2009) and McDonnell et al. (2010).

The SWE supplement (10 g) contained laminarin (1 g), fucoidan (0.8 g), and ash (8.2 g) and was extracted from a Laminaria spp. according to the procedure described by Lynch et al. (2009). The seaweed extract was obtained from a commercial company (Bioatlantis Ltd., Tralee, Co. Kerry, Ireland). The FO was obtained from a commercial company (Trouw Nutrition, Belfast, UK), and the analyzed fatty acid (FA) composition is presented in Table 1. The FO was derived from anchovy, sardine, and salmon oil; however, the oil was distilled to concentrate the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content.

The standard lactation diet (as-fed basis) was formulated using wheat (288 g/kg), barley (300 g/kg), soybean meal (242 g/kg), beet pulp (100 g/kg), soybean oil (45 g/kg), and minerals and vitamins (25 g/kg). Diets were formulated to contain similar concentrations of CP (190 g/kg), DE (14 MJ/kg), and ileal digestible Lys (9.0 g/kg). The AA requirements were met relative to Lys (NRC, 1998). Sows received specific amounts of feed in the following quantities: 2 kg/d of diet until the day of farrowing (d 0) and then the feed supply was increased by 1 kg/d until d 3 and then by 0.5 kg/d until d 6. Afterward, they were allowed ad libitum consumption of the standard lactation diet, which was adjusted for each sow depending on daily intake. The sows were fed in 2 equal meals provided at 0900 and 1500 h. The standard lactation diet was top-dressed each morning (0900 h) with experimental supplements to ensure consumption. The non-FO treatment group received 100 g of soybean oil/d to balance energy intake.

The experiment was initiated on d 109 of gestation when sows were moved into the farrowing house, and sows were offered experimental supplements until weaning at d 26. The sows and piglets were individually housed in farrowing pens (2.2 × 2.4 m) with crates, slatted floors, and heat pads for piglets. The sows were

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.14 ± 0.09</td>
</tr>
<tr>
<td>C16:0</td>
<td>2.27 ± 0.35</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.19 ± 0.07</td>
</tr>
<tr>
<td>C17:1</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.64 ± 0.21</td>
</tr>
<tr>
<td>C18:1n-9 cis</td>
<td>5.70 ± 0.45</td>
</tr>
<tr>
<td>C18:1n-9 trans</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>1.54 ± 0.02</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>0.89 ± 0.04</td>
</tr>
<tr>
<td>C18:3n-6</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>C20:1</td>
<td>3.36 ± 0.10</td>
</tr>
<tr>
<td>C20:2</td>
<td>3.00 ± 0.22</td>
</tr>
<tr>
<td>C20:3n-3</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>C20:3n-6</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>C20:4n-6</td>
<td>1.98 ± 0.09</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td>39.05 ± 0.54</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.50 ± 0.04</td>
</tr>
<tr>
<td>C22:1n-9</td>
<td>1.31 ± 0.32</td>
</tr>
<tr>
<td>C22:2</td>
<td>2.37 ± 0.04</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>24.25 ± 0.14</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.47 ± 0.09</td>
</tr>
<tr>
<td>C24:1</td>
<td>0.28 ± 0.02</td>
</tr>
</tbody>
</table>

fn = 4 fish oil samples.
individually fed and had ad libitum access to drinking water throughout the experimental period. No creep feed was offered to piglets throughout the experimental period.

**Farrowing and Piglets**

Farrowings were not induced and were attended. At parturition, each piglet was individually weighed and the number of live born piglets recorded. Three piglets of average birth weight were selected from each litter and tagged shortly after birth. Afterward, litter size was adjusted by cross-fostering piglets (not tagged) between treatments to ensure that sows nursed a similar number of piglets (10 piglets/sow), and this was maintained throughout the suckling period. All performance measurements were conducted on these same 3 piglets per litter throughout the experimental period. The selected piglets were weighed once a week from parturition until weaning to calculate ADG per litter. Piglets received an intramuscular injection of Fe-dextran (Ferdex 100, Medion Farma Jaya, Indonesia) on d 7 after birth.

**Sample Collection**

Colostrum samples (30 mL) were collected 1 h after the birth of the first piglet, and a milk (30 mL) sample was collected from each sow on d 12 of lactation after hand milking each functional gland. Piglets were removed from the sow until completion of farrowing to prevent suckling and the initiation of gut closure. To facilitate milk sampling, piglets were removed from the sow 1 h before and milk ejection was induced after intramuscular administration of 2 mL of oxytocin (Pitocina, Watson Laboratories Inc., Corona, CA). The colostrum and milk samples were immediately frozen at −20°C before analysis for FA, CP, total solids, and immunoglobulin composition.

During the suckling period, blood samples (5 mL) from the tagged piglets were collected from vena jugularis by puncture into Vacutainers (Becton, Dickinson, Drogheda, Ireland) on d 5 and 12 of lactation to facilitate immunoglobulin quantification. At weaning, a further blood sample was collected for FA analysis and to determine the phagocytic activity of whole blood cells. Blood samples were allowed to clot at −4°C and serum was collected after centrifugation (1,500 × g for 15 min at −4°C). Serum samples were stored at −80°C until analysis.

**Chemical Analysis of Colostrum and Milk**

The content of total solids in sow colostrum and milk was determined according to the AOAC (1995) procedures (16.032). Total lipids were measured according to the Roese-Gottlieb method (16.052; AOAC, 1995). Nitrogen was determined according to the method of Dumas using an automated N analyzer (Leco FP 528 instrument, Leco Instruments, UK Ltd., Stockport, UK). Crude proteins were estimated to be Kjeldahl N × 6.28 (Gordon and Whittier, 1965). The total laminarin content of the seaweed extract was determined using a commercial assay kit (Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland). Fucoidan concentrations were determined using the method of Usov et al. (2001). The FA composition of the FO was analyzed by gas chromatography using the method of Christie (1982).

**Quantification of Immunoglobulins**

Serum, colostrum, and milk concentrations of IgG, IgA, and IgM were assayed using specific pig-ELISA kits (Bethyl Laboratories Inc., Montgomery, TX). The IgG, IgA, and IgM were quantified according to the manufacturer’s instructions.

**Phagocytosis Estimation of Blood Cells by Flow Cytometry**

A commercial kit (Phagotest, Orpegen Pharma, Heidelberg, Germany; Thies et al., 1999), measuring the uptake of un-opsonized, fluorescein isothiocyanate (FITC)-labeled *E. coli*, was used according to the manufacturer’s instructions to measure the phagocytic activity in piglet whole blood white cells at weaning. The assay is based on the ingestion of fluorescent-labeled bacteria and detection by flow cytometry. The kit allows the quantitative determination of leukocyte phagocytosis. The samples were analyzed by a flow cytometer (Cyan-ADP, Dako, Glostrup, Denmark). The FITC was excited by a 488-nm laser, and its emission was collected using a 530/40 band pass filter. Subsequent analysis of the samples was performed using Summit software (Dako). Data were collected by flow cytometry to identify total leukocyte, lymphocyte, monocyte, neutrophil, and eosinophil numbers. To warrant a representative number of cells in the different cell types, a minimum of 2,000 monocytes per sample was analyzed because monocytes have been shown to be the least common cell population. The results of the Phagotest assay were expressed as the mean percentage of phagocytosing positive cells.

**FA Analysis**

To determine milk FA composition, samples were thawed and gently mixed in a water bath held at 40°C. Milk samples (10 mL) were centrifuged (978 × g for 20 min at 4°C), the cream extracted, flushed with N₂ gas for 10 to 15 s, and stored at −20°C overnight. The next morning the samples were heated to 40°C and centrifuged (978 × g for 10 min at 30°C). The extracted fat was then stored at −20°C until FA analysis. The FA composition was analyzed by gas chromatography.
according to the method of Christie (1982) with slight modifications. Approximately 1 mg of milk fat was dissolved in 95% dried n-hexane. Methyl acetate (20 μL) and 1 M sodium methoxide in anhydrous methanol (20 μL) were added after neutralization with a saturated solution of oxalic acid in methanol (30 μL). After centrifugation (1,500 × g for 5 min at 4°C), an aliquot of the upper phase was recovered for gas chromatography analysis. Fatty acid lipids were extracted from piglet serum according to the method of Folch et al. (1957), and the free FA were methylated according to the method of Doreau et al. (2007).

Samples were injected by an auto-sampler into a gas chromatograph (Varian GC 3800, Varian Inc., Palo Alto, CA) equipped with a flame-ionization detector. Fatty acid methyl esters (injected using a 10:1 split ratio) were separated on a fused silica capillary column (100 m × 0.25 mm i.d. × 0.39-μ film thickness; Varian Fame Select CP 7420, Varian Inc.). The injector temperature was held at 250°C, and the detector temperature at 260°C. The initial oven temperature was 140°C (held for 5 min), increased to 180°C at a rate of 4°C/min (held for 5 min), and then increased to 220°C (4°C/min; held for 20 min). Nitrogen was used as carrier gas. The pressure of the column was held at 275 kPa for 8 min and then increased to 448 kPa at a rate of 14 kPa/min and held for 24 min. The FA were identified by comparing the retention times of the peaks with those of known standards (Supelco 37 component FAME mix, Supelco Inc., Bellefonte, PA), and results are expressed as percentage of total FA.

### Statistical Analysis

The data were analyzed as a 2 × 2 factorial using the GLM procedure (SAS Inst. Inc., Cary, NC). The statistical model used included the main effects of dietary SWE supplementation, FO inclusion, and their associated 2-way interactions. The individual sow served as the experimental unit for all variables measured. Piglet BW (3 piglets/sow) and age were included as covariates in the model for serum immunoglobulin and phagocytic analysis. Sow parity was included as a covariate in the model for colostrum and milk analysis. The data were checked for normality using the PROC UNIVARIATE function of SAS. All data presented in the tables are expressed as least squares means ± SEM.

### RESULTS

#### Piglet Performance

The effect of maternal dietary treatment on piglet performance during the suckling period is presented in Table 2. Litter size, litter weight, and individual piglet birth weight were similar across treatments (P > 0.05). Piglets suckling SWE-supplemented sows had a reduced ADG at the end of wk 1 (P < 0.05) compared with piglets of non-SWE-supplemented sows. Furthermore, SWE supplementation resulted in a decrease in piglet BW at the end of wk 1 (P < 0.05) and wk 2 (P < 0.05) of lactation compared with non-SWE-supplemented diets. During wk 3 of lactation, ADG tended to be less in piglets from FO-supplemented sows (P = 0.053) compared with non-FO-supplemented sows. However, average piglet weaning weight and overall ADG were not influenced by sow dietary treatment (P > 0.05). No effect (P > 0.05) was observed for the combination treatment on piglet performance during the suckling period.

#### Colostrum and Milk Composition

Colostrum IgG concentrations were greater in SWE-supplemented sows (P < 0.01) compared with non-SWE-supplemented sows (Table 3). However, SWE
supplementation had no effect on colostrum and milk IgA and IgM concentrations. Dietary FO supplementation had no effect on immunoglobulin concentrations in sow milk and colostrum. Furthermore, SWE supplementation increased the CP concentration in the milk of sows on d 12 of lactation ($P < 0.05$). There was no interaction ($P > 0.05$) between maternal SWE and FO supplementation on total solids, CP, crude fat, and immunoglobulin concentrations in sow colostrum and milk.

**Piglet Immunoglobulins**

Piglets suckling SWE-supplemented sows had greater serum IgG concentrations on d 5 ($P < 0.01$) and d 12 ($P < 0.05$) of lactation compared with non-SWE-supplemented sows (Table 4). In addition, piglets suckling SWE-supplemented sows had greater serum IgA on d 5 ($P < 0.05$) and serum IgM concentrations on d 12 ($P = 0.008$) of lactation compared with non-SWE-supplemented sows. Dietary FO supplementation had a suppressive effect on piglet serum IgA concentrations on d 5 of lactation ($P < 0.05$) compared with non-FO-supplemented diets. There was no interaction ($P > 0.05$) between SWE and FO on immunoglobulin concentrations in piglet serum.

**Effect of Treatment on Phagocytosis**

The effect of maternal dietary treatment on the phagocytic activity of piglet serum whole blood cells at weaning is presented in Table 5. Dietary treatment

| Table 3. Effect of dietary treatment on total solids, CP, crude fat, and immunoglobulin concentrations of sow colostrum and milk$^1$ |
|---------------------------------|---|---|---|---|---|
| Item                           | SWE No | Yes | SEM | FO No | Yes | SEM | P-value$^2$ |
| Colostrum                      |        |     |     |        |     |     |           |
| Total solids, %                | 25.24  | 25.05 | 1.11 | 24.99 | 25.29 | 1.11 | 0.897 0.834 |
| CP, %                          | 14.00  | 14.11 | 0.82 | 13.59 | 14.52 | 0.82 | 0.925 0.423 |
| Fat, %                         | 6.10   | 5.57  | 0.52 | 6.18  | 5.49  | 0.52 | 0.478 0.352 |
| IgG, mg/mL                     | 63.27  | 69.84 | 1.90 | 66.31 | 66.79 | 1.90 | 0.010 0.844 |
| IgA, mg/mL                     | 10.40  | 8.81  | 0.77 | 9.62  | 9.60  | 0.77 | 0.014 0.980 |
| IgM, mg/mL                     | 3.97   | 4.51  | 0.31 | 4.49  | 3.99  | 0.31 | 0.238 0.263 |
| Sow milk (d 12 of lactation)   |        |     |     |        |     |     |           |
| Total solids, %                | 19.79  | 19.99 | 0.35 | 20.17 | 19.62 | 0.35 | 0.687 0.273 |
| CP, %                          | 5.17   | 5.39  | 0.08 | 5.29  | 5.27  | 0.08 | 0.050 0.837 |
| Fat, %                         | 8.77   | 8.58  | 0.39 | 8.91  | 8.43  | 0.39 | 0.731 0.388 |
| IgG, mg/mL                     | 0.41   | 0.46  | 0.04 | 0.41  | 0.47  | 0.04 | 0.297 0.384 |
| IgA, mg/mL                     | 3.81   | 3.78  | 0.25 | 3.66  | 3.93  | 0.25 | 0.914 0.370 |
| IgM, mg/mL                     | 1.83   | 1.56  | 0.29 | 1.55  | 1.83  | 0.29 | 0.519 0.514 |

$^1$Ten sows per treatment; values are least squares means. SWE = maternal seaweed extract; FO = fish oil supplementation.

$^2$There was no interaction between SWE and FO on total solids, CP, crude fat, and immunoglobulin concentrations in sow colostrum and milk ($P > 0.05$).

Table 4. Mean serum immunoglobulin concentrations in piglets suckling supplemented sows on d 5 and 12 of lactation$^1$

<table>
<thead>
<tr>
<th>Item</th>
<th>SWE</th>
<th>FO</th>
<th>P-value$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulin, mg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>19.31</td>
<td>22.91</td>
<td>0.89</td>
</tr>
<tr>
<td>IgA</td>
<td>2.51</td>
<td>3.13</td>
<td>0.19</td>
</tr>
<tr>
<td>IgM</td>
<td>1.47</td>
<td>1.50</td>
<td>0.16</td>
</tr>
<tr>
<td>d 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>9.98</td>
<td>12.04</td>
<td>0.63</td>
</tr>
<tr>
<td>IgA</td>
<td>0.38</td>
<td>0.33</td>
<td>0.06</td>
</tr>
<tr>
<td>IgM</td>
<td>0.54</td>
<td>0.63</td>
<td>0.04</td>
</tr>
</tbody>
</table>

$^1$Ten sows per treatment; values are least squares means. SWE = maternal seaweed extract; FO = fish oil supplementation.

$^2$There was no interaction between SWE and FO on immunoglobulin concentrations in piglet serum ($P > 0.05$).
Table 5. Effect of dietary treatment on the phagocytic activity (total number and % positive phagocytosis) of piglet whole blood cells at weaning

<table>
<thead>
<tr>
<th>Item</th>
<th>SWE</th>
<th>FO</th>
<th>P-value&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, No.</td>
<td>22,475</td>
<td>20,912</td>
<td>1,637</td>
</tr>
<tr>
<td>Positive, %</td>
<td>57.6</td>
<td>64</td>
<td>2.2</td>
</tr>
<tr>
<td>Lymphocytes, No.</td>
<td>6,650</td>
<td>5,127</td>
<td>672</td>
</tr>
<tr>
<td>Positive, %</td>
<td>13.3</td>
<td>10.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Monocytes, No.</td>
<td>2,641</td>
<td>2,578</td>
<td>83</td>
</tr>
<tr>
<td>Positive, %</td>
<td>74.1</td>
<td>77.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Neutrophils, No.</td>
<td>8,650</td>
<td>9,489</td>
<td>883</td>
</tr>
<tr>
<td>Positive, %</td>
<td>91.1</td>
<td>92.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Eosinophils, No.</td>
<td>512</td>
<td>338</td>
<td>61</td>
</tr>
<tr>
<td>Positive, %</td>
<td>26.0</td>
<td>21.8</td>
<td>2.1</td>
</tr>
</tbody>
</table>

<sup>1</sup>Ten sows per treatment. Immune cell subpopulations were identified by flow cytometry. SWE = maternal seaweed extract; FO = fish oil supplementation.

<sup>2</sup>There was no interaction between SWE and FO on phagocytic capacity of whole blood cells in suckled piglets at weaning (d 26) against *E. coli* (*P* > 0.05).

had no effect (*P* > 0.05) on total leukocyte, lymphocyte, monocyte, and neutrophil numbers. However, SWE supplementation exerted a suppressive effect on total eosinophil numbers (*P* < 0.01) in suckled piglets. Dietary SWE supplementation resulted in a greater percentage of *E. coli* phagocytizing leukocytes (*P* < 0.05) and a decreased percentage of *E. coli* phagocytizing lymphocytes (*P* < 0.01) compared with non-SWE-supplemented diets. Dietary FO supplementation resulted in a greater percentage of leukocytes (*P* < 0.05) and lymphocytes (*P* < 0.05) phagocytizing *E. coli* compared with non-FO-supplemented diets. There was no interaction (*P* > 0.05) between SWE and FO on phagocytic capacity of whole blood cells in suckled piglets at weaning.

### Milk FA Composition

The FA composition of the milk of sows on d 12 of lactation is presented in Table 6. Supplementation of the maternal diet with SWE had no effect (*P* > 0.05) on milk FA composition. Dietary FO supplementation increased the proportion of 18:3n-3 (*P* < 0.001), 18:4n-3 (*P* < 0.001), 20:5n-3 (*P* < 0.001), 22:5n-3 (*P* < 0.001), and 22:6n-3 (*P* < 0.001) in the milk of sows compared with non-FO-supplemented diets. Furthermore, dietary FO supplementation decreased the n-6:n-3 PUFA ratio (*P* < 0.001) in sow milk on d 12 of lactation. There was no interaction (*P* > 0.05) between SWE and FO on milk FA composition on d 12 of lactation.

### Piglet Serum FA Composition

Piglets suckling FO-supplemented sows had greater proportions (% of total FA) of 20:5n-3 (8.45 vs. 1.60%; *P* < 0.001), 22:5n-3 (2.89 vs. 0.80%; *P* < 0.001), and 22:6n-3 (5.18 vs. 1.16%; *P* < 0.001) compared with non-FO-supplemented sows at weaning (Table 7). In addition, FO supplementation enhanced serum n-3 PUFA content (17.24 vs. 4.82%; *P* < 0.001) and decreased the n-6:n-3 ratio (2.41 vs. 9.1; *P* < 0.001). There was an interaction between FO and SWE on proportions of C22:5n-3 in piglet serum (*P* < 0.05). Dietary FO supplementation enhanced serum proportions of C22:5n-3; however, the combination of FO and SWE had no further effect (*P* > 0.05) on C22:5n-3 proportions.

### DISCUSSION

The aim of the current experiment was to examine the effect of maternal dietary supplementation with SWE (laminarin-fucoidan mix) and FO from d 109 of gestation until weaning (d 26) on colostrum and milk composition, humoral immunity on d 5 and 12 of lactation, leukocyte phagocytosis at weaning, and neonatal piglet growth.

The present data indicate that overall ADG during the suckling period and average piglet weaning weight were not affected by sow dietary treatment. Data available in the literature purporting to improve piglet growth during the suckling period when feeding marine oils to sows are inconsistent. Lauridsen and Danielsen (2004) observed that FO supplementation from d 108 of gestation favored no improvement on piglet daily BW gain. In contrast, Mitre et al. (2005) reported that piglets from sows fed diets supplemented with shark liver oil from d 80 of gestation had a greater weaning weight. Furthermore, Rooke et al. (2001a) reported that piglets born to dams fed supplemental tuna oil from d 92 of gestation were heavier than piglets from control sows. It is possible that a longer period of supplementation before farrowing is required to elicit an effect on neonatal piglet growth. Interestingly, our results indicate that FO supplementation tended to suppress ADG during wk 3 of lactation. Lauridsen and Danielsen (2004) suggested that FO supplementation improved piglet growth through an enhanced output of fat and energy into mammary secretions; however, no differences on
milk fat concentrations were observed on d 12 of lactation. It is noteworthy to mention that FO supplement offered in the current study contained considerably greater proportions of EPA and DHA in comparison with other marine oil investigations (Fritsche et al., 1993b; Mateo et al., 2009). This indicates that offering sows a rich EPA and DHA dietary fish oil source resulted in no improvement of piglet growth performance during the lactation period.

Dietary SWE supplementation actually impaired piglet growth during wk 1 of lactation and resulted in a decreased piglet BW at the end of wk 1 and 2 of lactation. To our knowledge, no study has examined the influence of maternal SWE (laminarin-fucoidan mix) supplementation on piglet growth during the suckling period. The Laminaria spp.-derived SWE used in the current study contained the bioactive compounds laminarin and fucoidan. Laminarin represents a group of low molecular weight, water soluble β-(1–3)-linked glucans with β-(1–6) linked side chains of varying distribution and length (Read et al., 1996, Brown and Gordon, 2005). Fucoidans represent a group of sulphated polysaccharides, containing L-fucose as one of the major monosaccharides extracted from the extracellular matrix of various species of brown seaweeds (Berteau and Mulloy, 2003). Chau et al. (2008) reported no improvement in the growth rate of neonatal piglets whose dams were supplemented with yeast-derived β-glucans. In addition, the uptake of these bioactive compounds (laminarin or fucoidan or both) into mammary secretions was not detected in the current study.

Our results indicate that maternal SWE supplementation from d 109 of gestation increased colostral IgG concentrations. Furthermore, piglets suckling SWE-

Table 7. Effect of dietary treatment on piglet serum long-chain fatty acid composition at weaning (d 26)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>SWE</td>
</tr>
<tr>
<td>C18</td>
<td>13.70</td>
<td>10.90</td>
</tr>
<tr>
<td>C18:1</td>
<td>13.10</td>
<td>16.90</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>35.70</td>
<td>35.90</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>1.37</td>
<td>1.23</td>
</tr>
<tr>
<td>C20:4n-6</td>
<td>6.53</td>
<td>5.49</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td>1.93</td>
<td>1.27</td>
</tr>
<tr>
<td>C22:5n-3</td>
<td>0.75</td>
<td>0.77</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>1.20</td>
<td>1.13</td>
</tr>
<tr>
<td>Total n-6</td>
<td>42.30</td>
<td>41.40</td>
</tr>
<tr>
<td>Total n-3</td>
<td>5.25</td>
<td>4.40</td>
</tr>
<tr>
<td>Ratio n-6:n-3</td>
<td>8.70</td>
<td>9.50</td>
</tr>
</tbody>
</table>

1Ten sows per treatment; values are least squares means. SWE = maternal seaweed extract; FO = fish oil supplementation.
2There was no interaction between SWE and FO on piglet serum fatty acid composition at weaning (P > 0.05).
3Total n-6 is the sum of C18:2n-6 and C20:4n-6.
4Total n-3 is the sum of C18:3n-3, C20:5n-3, C22:5n-3, and C22:6n-3.
supplemented sows had greater serum IgG concentrations on d 5 and 12 of lactation. The mechanism responsible for increased colostral IgG concentrations in SWE-supplemented sows may relate to the immune modulating properties of laminarin, a novel source of β-(1–3)/(1–6) glucans (Read et al., 1996). These water soluble β-glucans are widely recognized to stimulate host immune function through activation of dectin-1 receptors normally expressed on the cell surface of monocytes, macrophages, and neutrophils (Brown and Gordon, 2005; Volman et al., 2008). Krakowski et al. (1999) previously reported that administration of yeast-derived β-glucans to pregnant mares increased colostral IgG concentrations, indicating a nonspecific immunomodulatory property of yeast-derived β-glucans. Therefore, based on these findings we speculate that laminarin potentiates a nonspecific immune response in supplemented sows responsible for increased colostral IgG concentrations. It has previously been reported that nonspecific immunostimulation of pregnant sows increased colostral IgG concentrations (Krakowski et al., 2002). Further studies are warranted to elucidate the exact mechanism as sow serum was not obtained in the current study.

In addition, piglets suckling SWE-supplemented sows had greater circulatory IgG concentrations on d 5 and 12 of lactation, indicating an enhanced humoral immune protection against presenting pathogens. Klobasa et al. (1981) reported that sucking piglets initiate de novo IgG synthesis after 7 d of age and IgG synthesis is positively correlated with the volume of passively acquired IgG (Rooke et al., 2003). Furthermore, Rooke et al. (2003) reported that serum IgG concentrations on d 7 positively correlated with IgG concentrations at weaning. However, the majority of research examining the influence of dietary β-glucans on humoral immune function in pigs has focused on manipulation of the weaning diet (Wang et al., 2008). Similar observations have been reported in broiler chicks supplemented with yeast-derived β-glucans (Zhang et al., 2008).

In the current study, dietary FO from d 109 of gestation failed to influence the immunoglobulin composition of mammary secretions. In contrast, Mitre et al. (2005) demonstrated that shark-liver oil supplementation from d 80 of gestation increased colostral IgG concentrations and serum IgG concentrations in suckled piglets. The supplementation duration in the current study may have been insufficient to induce a beneficial shift in colostral IgG concentrations, as previous studies demonstrating positive effects have started supplementation earlier in gestation (Mitre et al., 2005).

In agreement with previous studies (Fritsche et al., 1993b; Rooke et al., 2000), dietary provision of long-chain n-3 PUFAs increased EPA and DHA proportions of sow milk in a predictable manner and was reflected in piglet serum at weaning (Lauridsen and Jensen, 2007). It has previously been reported that FO supplementation may alleviate the negative impact induced on piglet performance experienced during an immunological challenge via an altered release of pro-inflammatory cytokines (Calder, 2002; Liu et al., 2003).

Thies et al. (1999) reported that FO inclusion decreased leukocyte phagocytosis and lymphocyte proliferation in pigs. Rather, in the current study, maternal FO supplementation enhanced percentage positive leukocytes and lymphocytes phagocytizing E. coli at weaning. Phagocytosis is an essential component of host immunity responsible for detection and elimination of invading pathogens (Paape et al., 2003). The increased lymphocyte phagocytosis in piglets from sows fed diets supplemented with FO may contribute toward an enhanced cell-mediated immune response against infectious organisms.

Maternal SWE supplementation decreased piglet eosinophil numbers, suppressed lymphocyte phagocytosis, and increased leukocyte phagocytosis at weaning. Beta-glucans have been demonstrated to enhance the functional activity of macrophages in vitro (Chang et al., 2010); however, animal studies pertaining to enhance phagocytosis and cell-mediated immunity after dietary β-glucan supplementation are limited. Thus, the current study provides novel information on the effect of SWE and FO supplementation on phagocytosis activity in suckled piglets at weaning. Porcine colostrum and milk not only contain highly digestible nutrients but also include numerous growth factors (Xu et al., 2000), cytokines, and chemokines (Nguyen et al., 2007) that are potentially susceptible to dietary modulation. Therefore, it is plausible that SWE supplementation influenced phagocytosis by altering the synthesis of key cytokines/chemokines that regulate phagocytic events into mammary secretions; however, only immunoglobulin quantification was examined in sow milk in the current study.

In summary, our results demonstrate that SWE dietary supplementation to sows from d 109 of gestation until weaning (d 26) increased colostrum IgG concentrations. Furthermore, piglets suckling SWE-supplemented sows had greater serum IgG on d 5 and 12, indicating an enhanced humoral immune function, which can improve host defense against invading pathogens. These results provide new insight on the effect of maternal SWE and FO supplementation on phagocytic activity of piglet white blood cells at weaning. Dietary SWE supplementation increased piglet lymphocyte phagocytosis, whereas FO supplementation increased leukocyte and lymphocyte phagocytosis at weaning. This increased phagocytic activity against E. coli is an important observation as piglets are increasingly susceptible to infectious disease immediately postweaning. However, improvements observed on piglet immune competence in the present study accompanied no improvement on suckling piglet performance. On the basis of these results, it remains unclear whether any beneficial effects on suckling piglets may be extended to the postweaning period. Further studies are required to
explore the potential application of these diets to alleviate the negative impact of a disease challenge pre- and postweaning.

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