Effect of Galacto-mannan-oligosaccharides or Chitosan Supplementation on Cytoimmunity and Humoral Immunity in Early-weaned Piglets

Y.-L. Yin¹, ², *, Z. R. Tang¹, Z. H. Sun¹, Z. Q. Liu¹, T. J. Li¹, R. L. Huang¹, Z. Ruan², Z. Y. Deng²
B. Gao³, L. X. Chen⁴, G. Y. Wu¹, ⁵ and S. W. Kim⁶

¹ Laboratory of Animal Nutrition and Human Health and Key Laboratory of Subtropical Agro-ecology, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, Hunan, 410125, China

ABSTRACT : Immunomodulatory feed additives might offer alternatives to antimicrobial growth promoters in pig production. This experiment was designed to determine the effects of dietary galacto-mannan-oligosaccharide (GMOS) and chitosan oligosaccharide (COS) supplementation on the immune response in early-weaned piglets. Forty 15-day-old piglets (Duroc×Landrace×Yorkshire) with an average live body weight of 5.6±0.51 kg were weaned and randomly assigned to 4 treatment groups that were fed maize-soybean meal diets containing either basal, 110 mg/kg of lincomycin, 250 mg/kg of COS or 0.2% GMOS, respectively, over a 2-week period. Another six piglets of the same age were sacrificed on the same day at the beginning of the study for sampling, in order to obtain baseline values. Interleukin (IL)-1β gene expression in peripheral blood monocytes, jejunal mucosa and lymph nodes, as well as serum levels of IL-1β, IL-2 and IL-6, IgA, IgG, and IgM, were evaluated for 5 pigs from each group at 15 and 28 days of age. The results indicate that weaning stress resulted in decreases in serum antibody and cytokine levels. Dietary supplementation with GMOS or COS enhanced (p<0.05) IL-1β gene expression in jejunal mucosa and lymph nodes, as well as serum levels of IL-1β, IL-2, IL-6, IgA, IgG and IgM compared to supplementation with lincomycin. These findings suggest that GMOS or COS may enhance the cell-mediated immune response in early-weaned piglets by modulating the production of cytokines and antibodies, which shows that GMOS or COS have different effects than the antibiotic on animal growth and health. (Key Words : Oligosaccharides, Interleukin-1β, -2 and -6, Gene Expression, Immune Function, Piglets)

INTRODUCTION

The immune system, especially acquired immunity, plays an important role in protecting piglets against pathogenic infection (Li et al., 2007a). However, acquired immunity is underdeveloped at the age of 3 to 4 weeks when piglets are usually weaned on commercial farms.

* Corresponding Author: Y.-L. Yin. Tel: +86-7314619703, Fax: +86-7314612685, E-mail: yyulong2003@yahoo.com.cn

1 The key Laboratory of Food Science of Ministry of Education of China, Department of Food Science & Engineering, Nanchang University, Nanchang, China.
2 Guangzhou Tanke Industry Co Ltd, Guangzhou, Guangdong, 510627, China.
3 Guang An Biological Technique Company, Changsha, Hunan Province, 410135, China.
4 Department of Animal Science, Texas A&M University, College Station, TX 7843-2471, USA.
5 Department of Animal Science, North Carolina State University, Raleigh, North Carolina, 27695 USA.

Received July 20, 2007; Accepted December 2, 2007
be developed. One such promising solution appears to be dietary supplementation with indigestible oligosaccharides. Galacto-manna-oligosaccharide (GMOS) is often obtained from galacto-manna-polysaccharide of the gum of sesbania after hydrolysis by the manna-polysaccharide enzyme. Chitosan (COS) is generated from chitin by deacetylation. Oligosaccharides are not digested by mammalian enzymes and are delivered to the large intestinal tract where they act as selective nutrients for certain bacterial populations (Tokunaga and Hosoya, 1989). Galacto-manna-oligosaccharide and COS might act as growth-promoters without the disadvantages associated with antibiotics. Recently, oligosaccharides have been used to enrich beneficial bacterial populations (i.e. lactobacilli and bifidobacteria) in domestic livestock and humans (Monsan, 1950; Orban et al., 1997). The most recent studies have suggested that lactobacilli bacteria can activate macrophages and stimulate their functions (Kitazawa et al., 2002; Morita et al., 2002). The implantation of bifidobacteria may prevent the development of tumors, stimulate the immune system and modulate intestinal colonization by clostridia (Sekine et al., 1985; Bezirtzoglou et al., 1989). Additionally, the results of several studies have indicated that dietary oligosaccharides can improve immune functions in mice and humans (Pierre et al., 1997; Van Loo et al., 1999; Guigoz et al., 2002).

Cytokines (IL-1β, IL-2 and IL-6) play a central role in the cell-mediated immune response, and also participate in

### Table 1. Formulation (%) of the experimental diets for early-weaned piglets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>Antibiotics</th>
<th>COS</th>
<th>GMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (crude protein, 8%)</td>
<td>50.01</td>
<td>49.76</td>
<td>49.81</td>
<td>49.98</td>
</tr>
<tr>
<td>Soybean meal (crude protein, 43%)</td>
<td>14.20</td>
<td>14.20</td>
<td>14.20</td>
<td>14.20</td>
</tr>
<tr>
<td>Fish meal (crude protein, 65%)</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
</tr>
<tr>
<td>Soybean expanded (crude protein, 40%)</td>
<td>10.11</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Dried whey</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Dried cream</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Choline chloride (50%)</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Trace mineral premix</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Flavor</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>L-lysine-HCl (lysine, 71%)</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>L-methionine (methionine, 98%)</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>L-threonine (threonine, 98%)</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Lincomycin mixture</td>
<td>0.00</td>
<td>0.25</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>COS</td>
<td>0.00</td>
<td>0.00</td>
<td>0.025</td>
<td>0.00</td>
</tr>
<tr>
<td>GMOS</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

#### Nutritional contents (calculated, as-fed basis)

<table>
<thead>
<tr>
<th>Nutritional contents</th>
<th>Control</th>
<th>Antibiotics</th>
<th>COS</th>
<th>GMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>91.00</td>
<td>90.89</td>
<td>91.11</td>
<td>90.99</td>
</tr>
<tr>
<td>Digestible energy (MJ/kg)</td>
<td>15.33</td>
<td>15.33</td>
<td>15.33</td>
<td>15.33</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>19.00</td>
<td>19.00</td>
<td>19.00</td>
<td>19.00</td>
</tr>
<tr>
<td>Total calcium (%)</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
</tr>
<tr>
<td>Available phosphate (%)</td>
<td>0.48</td>
<td>0.48</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.35</td>
<td>1.35</td>
<td>1.35</td>
<td>1.35</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.42</td>
<td>0.42</td>
<td>0.42</td>
<td>0.42</td>
</tr>
<tr>
<td>Threonine (%)</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
</tr>
</tbody>
</table>

---

a COS: Chitosan obtained from chitin was provided by Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Liaoning province, Dalian City, China.
b GMOS: Galacto-mannan-oligosaccharide was provided by the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

c Provided the following per kilogram of complete feed: 11,000 IU vitamin A; 1,100 IU vitamin D₃; 22 IU vitamin E; 4 mg menadione as dimethylpirimidinol bisulfate; 0.03 mg vitamin B₁₂; 28 mg d-pantothenic acid; and 33 mg niacin.
d Provided the following per kilogram of complete feed: 600 mg Zn (ZnSO₄), 165 mg Fe (FeSO₄), 33 mg Mn (MnSO₄), 16.5 mg Cu (CuSO₄), 29.7 μg CaI₂, and 297 μg Se (Na₂SeO₃).
e Provided by Tanko Industry Company, GongZhou City, China.
f Provided the following per kilogram of complete feed: 165 mg Zn (ZnSO₄), 165 mg Fe (FeSO₄), 33 mg Mn (MnSO₄), 16.5 mg Cu (CuSO₄), 29.7 μg CaI₂, and 297 μg Se (Na₂SeO₃).
g Provided by Tanko Industry Company, GongZhou City, China.

---

the maintenance of tissue integrity (Li et al., 2007a). Changes in the intestinal cytokine network may occur in early-weaned piglets for several reasons. First, abrupt changes in dietary and environmental factors lead to both morphological and functional adaptations in the gut (Pié et al., 2004). Second, the numbers of T-cells, B-cells, and macrophages increase in the intestinal mucosa of early-weaned piglets (Wu, 1995; 1996).

The objective of this study was to investigate the effects of two oligosaccharides (chitosan and galacto-mannan-oligosaccharide) on IL-1β gene expression and serum concentrations of cytokines (IL-1β, IL-2 and IL-6) and antibodies (IgA, IgG and IgM) in early-weaned piglets. The findings may be useful to elucidate the cellular and molecular mechanisms responsible for enhanced immune functions in animals receiving dietary supplementation with indigestible oligosaccharides.

**MATERIAL AND METHODS**

**Animals, diets and experimental design**

Forty 15-day-old piglets (Duroc×Landrace×Yorkshire) with an average live body weight of 5.6±0.51 kg) were obtained from a local commercial swine herd and randomly divided into four groups. Experimental diets were formulated based on NRC requirements (National Research Council, 1998; Ruan et al., 2007). The study consisted of a group of pigs fed for 14 days (referred to as the 14CR), the antibiotic treatment (referred to as the ANT group), the COS-supplementation (0.025%) group, and the GMOS-supplementation (0.2%) group (Table 1). Each treatment had 10 replicates. Another group of similar aged piglets was sacrificed on the same day as the beginning of the study for obtaining baseline values. The animals were individually weighed, whereas feed intake and feed efficiency were determined for each pen on a weekly basis to monitor the growth of animals fed different diets for obtaining data in weeks 1 and 2. At the end of the 14-day period of feeding with the experimental diets, six piglets per treatment were sacrificed for sampling. The animal protocol was approved by the Animal Care Committee of the Institute of Subtropical Agriculture, The Chinese Academy of Sciences.

**Sampling and sample processing procedures**

Blood samples (5 ml) were collected via orbito-sinal puncture on days 0 and 14 after weaning from 5 pigs per treatment and stored in uncoated normal and EDTA-coated tubes. Serum was obtained by centrifugation at 3,000 rpm for 20 min and stored at -20°C until required for interleukin (IL) and Ig analysis. The blood samples (15 ml) stored in EDTA-coated tubes were prepared for the separation of peripheral blood monocytes. After blood samples were collected, piglets were sacrificed by the injection of 4% sodium pentobarbital solution (40 mg/kg BW) for the collection of tissue samples. One gram of jejunal mucosa and mesenteric lymph nodes were collected. The samples were immediately frozen in liquid nitrogen and stored at -70°C as described by Tang et al. (2005) until the extraction of total RNA.

A blood sample (15 ml) was mixed with 15 ml Hank’s reagent (Central Lab, XiangYa Medical College, Central South University, China) for the separation of peripheral blood monocytes. The mixture was added to the surface of 5 ml of mononuclear cell separation medium (Institute of Biomedical Engineering, The Chinese Academy of Medical Sciences) in a 50-ml centrifuge tube, and centrifuged at 2,000 rpm for 20 min. Monocytes in the middle layer of the tube were transferred into a 10-ml centrifuge tube, and the tube was centrifuged at 2,000 rpm for an additional 10 min. The cells were washed three times with 5 ml Hank’s reagent through centrifugation (2,000 rpm, 15 min). The monocytes were suspended in RPMI-1640 medium, which included 10% calf serum and stored at -80°C until the extraction of total RNA.

**Determination of serum IgG, IgA, IgM, IL-6, IL-2 and IL-1β**

Serum IgG, IgA and IgM were determined using radial immuno-diffusion kits (Triple J Farms, Bellingham, WA, 2005; Li et al., 2007b). Feed and water were provided to the pigs ad libitum. The piglets were checked daily for signs of disease and mortality. The animals were individually weighed, whereas feed intake and feed efficiency were determined for each pen on a weekly basis to monitor the growth of animals fed different diets for obtaining data in weeks 1 and 2. At the end of the 14-day period of feeding with the experimental diets, six piglets per treatment were sacrificed for sampling. The animal protocol was approved by the Animal Care Committee of the Institute of Subtropical Agriculture, The Chinese Academy of Sciences.
USA). Serum IL-6, IL-2, and IL-1β were analyzed using porcine IL-6, IL-2 and IL-1β RIA kits (Shanghai Institute of Biological Products, China), respectively, according to the manufacturer’s instructions.

**Quantification of porcine IL-1β mRNA**

The levels of porcine IL-1β mRNA in peripheral blood monocytes, jejunal mucosa and mesenteric lymph nodes were determined by quantitative reverse-transcribed PCR, where porcine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene.

**Primer design**

The primers were designed using DNAMAN 4.15 software (Lynnon Biosoft, Canada) according to gene sequences in GenBank (http://www.ncbi.nlm.nih.gov; IL-1β, M86725; GAPDH, AF017079): primer for porcine IL-1β (Forward 5’-GGCTA ACTAC GGTGA CAACA ATAAT G-3’; Reverse 5’-CAGAT TCTTT CCCTT GATCC CTAA-3’; 485 bp: 277-761 bp), and porcine GAPDH (Forward 5’-GAAGG TCGGA GTGAA CGGAT T -3’ Reverse 5’-GCCTT CTCCA TGGTC GTGA -3’; 312 bp: 347-658 bp) synthesized by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. (China). The specificity of PCR primers for IL-1β and GAPDH was verified by examining PCR amplicons using DNA sequence analysis (Shanghai Sangon Biological Engineering Technology and Service Co. Ltd., China).

**RT-PCR**

Total RNA was reverse-transcribed into cDNA by an AMV First Strand cDNA Synthesis Kit (Bio Basic Inc. Canada, lot: BS252). The synthesized cDNA was amplified using the PCR reagent (Taq polymerase: MBI Fermentas, lot: EP0402; d NTP Mix: MBI Fermentas, USA: R0191). Each 25-μl PCR reaction contained the following: 12.3 μl of sterile de-ionized H2O; 2.5 μl of 10×PCR Buffer; 2.5 μl of dNTP mix (2 mmol/L); 1.25 μl each of forward and reverse IL-1βprimers (10 μmol/L); 0.8 μl each of forward and reverse GAPDH primers (10 μmol/L); 0.1 μl of TaqDNA polymerase (5 U/μl); 1.5 μl of MgCl2 (25 mmol/L); 2 μl of 125 pg-12.5 μg cDNA. The following procedure was used for amplification: 1 cycle at 95°C for 2 min; 30 cycles at 95°C for 45 sec, 60.6°C for 1 min and 30 sec, 72°C for 45 sec; and a final elongation step at 72°C for 10 min.

**Semi-quantification of PCR products**

Ten microliters of PCR products and 2 μl loading dye (25% bromophenol blue, 25% glycerol) were mixed. PCR products were electrophoresed on 1.5% agarose gel containing ethidium bromide (0.5 μg/ml) for 1 h at 100 V. A low DNA mass ladder (MBI Fermentas) was used as a molecular weight marker. DNA bands were visualized and densitometric analysis was performed on a UV transilluminator (UVP Bioimaging Systems, USA).

**Statistical analysis**

The data were analyzed statistically according to the General Linear Model Procedure of SAS (SAS Institute, Inc., Cary, NC, USA). The percentage data were subjected to log10 transformation prior to the analysis of variance. Differences in means among treatment groups were separated using the Duncan’s multiple range test (SAS). The following model was used:

\[ Y_{ij} = \mu + D_i + \varepsilon_{ij} \]

Where Y is the response parameter, D is the effect of treatment and \( \varepsilon_{ij} \) is the experimental error. Pen was considered as an experimental unit in calculating daily gain, feed intake and feed:gain. Differences among least-square treatment means were assessed using the Least Significant Differences (LSD) test (p<0.05) according to SAS.

**RESULTS AND DISCUSSION**

Average daily gain (ADG), feed intake and feed efficiency were computed and analyzed for week-1 and week-2 (Table 2). There was no difference in performance in week-1 (p>0.05). Piglets fed GMOS, COS, and
Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on mRNA levels for interleukin-1β (IL-1β) in peripheral blood monocytes in early-weaned piglets. Abbreviations: 0CR = Control group at day zero of the study; 14CR = Control group at day 14 of the study; ANT = Antibiotic supplementation group; COS, chitosan supplementation diet; and GMOS, galacto-mannan oligosaccharide supplementation diet. In Panel (A), agarose gel (1.5%) electrophoresis shows the results of RT-PCR analysis of IL-1β mRNA and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA from peripheral blood monocytes of the 5 dietary groups. In the left panel, lanes 1-9 represent DNA marker (lane 1), negative control (lane 2), 0CR (lanes 3-6, n = 4), and 14CR (lanes 7-9, n = 3). In the right panel, lanes 1-13 represent DNA marker (lane 1), negative control (lane 2), ANT (lanes 3-5, n = 3), GMOS (lanes 6-9, n = 4), and COS (lanes 10-13, n = 4). RT-PCR conditions are described in Section 2. Panel (B) shows a quantitative analysis of the relative abundance of peripheral blood monocyte IL-1β mRNA from the 5 dietary groups normalized by mRNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Values are the mean ± pooled STD (n = 4) expressed in arbitrary densitometric units. Means with a different superscript letter differ significantly (p<0.05).

Figure 1. Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on mRNA levels for interleukin-1β (IL-1β) in the jejunal mucosa of early-weaned piglets. Abbreviations are the same as in Figure 1. In Panel (A), agarose gel (1.5%) electrophoresis shows the results of RT-PCR analysis of IL-1β mRNA and mRNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the jejunal mucosa of the 5 dietary groups. In the left panel, lanes 1-9 represent DNA marker (lane 1), negative control (lane 2), 0CR (lanes 3-6, n = 4), and 14CR (lanes 7-9, n = 3). In the right panel, lanes 1-13 represent DNA marker (lane 1), negative control (lane 2), ANT (lanes 3-5, n = 3), GMOS (lanes 6-9, n = 4), and COS (lanes 10-13, n = 4). RT-PCR conditions are described in Section 2. Panel (B) shows a quantitative analysis of the relative abundance of the jejunal mucosal IL-1β mRNA from the 5 dietary groups normalized by the mRNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Values are the mean ± pooled STD (n = 4) expressed in arbitrary densitometric units. Means with a different superscript letter differ significantly (p<0.05).

Figure 2. Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on mRNA levels for interleukin-1β (IL-1β) in jejunal mucosa of early-weaned piglets. Abbreviations are the same as in Figure 1. In Panel (A), agarose gel (1.5%) electrophoresis shows the results of RT-PCR analysis of IL-1β mRNA and mRNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the jejunal mucosa of the 5 dietary groups. In the left panel, lanes 1-9 represent DNA marker (lane 1), negative control (lane 2), 0CR (lanes 3-6, n = 4), and 14CR (lanes 7-9, n = 3). In the right panel, lanes 1-13 represent DNA marker (lane 1), negative control (lane 2), ANT (lanes 3-5, n = 3), GMOS (lanes 6-9, n = 4), and COS (lanes 10-13, n = 4). RT-PCR conditions are described in Section 2. Panel (B) shows a quantitative analysis of the relative abundance of the jejunal mucosal IL-1β mRNA from the 5 dietary groups normalized by the mRNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Values are the mean ± pooled STD (n = 4) expressed in arbitrary densitometric units. Means with a different superscript letter differ significantly (p<0.05).

Dietary supplementation with oligosaccharides or antibiotic had no effect on IL-1β mRNA in peripheral blood mononuclear cells (PBMC) (p>0.05; Figure 1). However, as shown in Figure 2 and 3, supplementation with either GMOS or COS increased IL-1β mRNA levels in PBMC or IL-1β mRNA levels in jejunal mucosa and mesenteric lymph nodes (p<0.05). An increase in the mRNA level for IL-1β is expected to increase the translation of the gene and, therefore, the production of the IL-1β protein. Consistent with this view, we found that serum levels of IL-1β, IL-2, and IL-6 in piglets fed the GMOS or COS diet were higher than those in piglets fed the negative and positive control diets on day 14 (p<0.05; Figures 4, 5 and 6). Our values for serum IL-1β, IL-2 and IL-6 in control piglets were similar to those previously reported (0.19±0.06 ng/ml, 5.0±1.5 ng/ml, and 108.85±41.48 pg/ml, respectively). The magnitude of the increase in mRNA levels in the intestinal mucosa and lymph nodes did not precisely match that in serum levels of IL-1β and other cytokines. This result may be explained by the fact that the half-lives of mRNA and proteins for cytokines differ markedly and cytokines can be produced by various cell types (Li et al., 2007a). Furthermore, rates of synthesis of cytokines in response to dietary supplementation with GMOS or COS may vary with the anatomical site of cells. It also should be noted that if inflammatory cytokines increased with the indigestible oligosaccharides, it is possible that the effect might result from the activation of some intestinal bacteria which then stimulated an immune defense response (Yang et al., 2007). Due to our limited resources, we were not able to analyze gene expression in all cell types in the piglets. Nonetheless, our findings demonstrate for the first time that dietary...
supplementation with indigestible oligosaccharides enhanced the expression of the gene for IL-1β in the intestinal mucosa and mesenteric lymph nodes of early-weaned piglets. This may contribute to increased levels of IL-1β and other cytokines in serum.

Cytokines such as IL-1β are known to mediate the inflammatory response. They act through the following complex mechanisms: (1) by promoting the proliferation and differentiation of thymocytes and mature T-cells; (2) by inducing T-cells to generate IL-2 and activating T<sub>H</sub> cells to release factors required for the function of B-cells; (3) by enhancing B-cell differentiation and, therefore, promoting the production of antibodies; and (4) by inhibiting the growth of tumor cells and killing them (Bloks et al., 1998; Bassaganya-Riera et al., 2001). This reflects a physiological response of the host to immunological stressors (Arthington et al., 1996; Wu, 1996; Hicks et al., 1998). In apparent contrast to the present findings, Davis et al. (2002) reported that lymphocyte proliferation did not respond to dietary supplementation with mannan-oligosaccharides (MOS) in piglets. Moreover, Davis et al. measured the proliferation of lymphocytes more than 2 weeks after weaning. Available evidence shows that an increase in lymphocyte proliferation occurred in piglets when they were challenged by disease, weaning or other stressors (Arthington et al., 1996; Wu, 1996; Hicks et al., 1998; Bassaganya-Riera et al., 2001). This reflects a physiological response of the host to immunological stressors.
challenges. Consistent with the present results, Yasunori and Yoshiyuki (2004) found that low-molecular-weight chitosans or oligochitosan might be particularly useful for preventing tumor growth in mice through the activation of intestinal immune functions. Additionally, Xin et al. (2000) demonstrated that dietary COS supplementation could promote the generation of IL-1 and phagocyte-activated factor. Further, Kelly et al. (2002) reported that dietary supplementation with MOS could have beneficial effects on intestinal microbial populations and systemic immune functions.

Our values for serum levels of IgG, IgM, and IgA in control piglets are similar to those previously reported by other investigators, which ranged from 6.0 to 16.0, 0.6 to 2.0 and 2.0 to 5.0 g/L, respectively (Gomez et al., 1998). A significant finding in the present study is that serum levels of IgG, IgA and IgM were markedly decreased in piglets (p<0.01) in response to early weaning (Figure 7). Importantly, dietary supplementation with GMOS or COS increased serum concentrations of these antibodies (p<0.05; Figure 7). As indicated above, the major underlying mechanisms for the effects of indigestible oligosaccharides are likely to be related to changes in the intestinal and systemic immune network. Plasma IgG, IgA and IgM are the major serum immunoglobulins that protect the extravascular compartment against pathogenic viruses and microorganisms (Li et al., 2007a). Gomez et al. (1998) reported that plasma IgG, by preventing the intestinal gut surface from bacterial damage, helps maintain optimal intestinal function and gastrointestinal growth, which in turn is beneficial for piglet health and growth performance.

Notably, dietary supplementation with MOS enhanced leucocyte activity in fish (Yoshida et al., 1995), bile IgA levels in broiler chickens (Savage et al., 1997), lymphocyte PHA transportation and phagotrophy of leucocytes in broiler chickens (Spring et al., 2000), as well as serum IgG levels in pigs (White et al., 2002).

In summary, the present data on serum concentrations of IL-1, IL-6 and Ig, as well as expression of the IL-1β gene in jejunal mucosa and leukocytes indicate that dietary supplementation with GMOS and COS has beneficial effects on animal growth and health that differ from those of antibiotics. This suggests that the underlying mechanisms of actions in swine nutrition differ between oligosaccharides and antibiotics. Further studies are needed to clarify these complex issues.

ACKNOWLEDGMENT

The authors of Y. L. Yin and Q. Liu gratefully acknowledges the support of K. C. Wong Education Foundation, Hong Kong. This research was jointly supported by grants from The Chinese Academy of Sciences and Knowledge Innovation Project (contract No. KSCX2-YW-N-051, KZCX3-SW-441, YW-N-022, and KSCX2-SW323), National Basic Research Program of China (contract No. 2004CB117502), the National Natural Science Foundation of China (contract No. 30528006, 30671517, 30700581, 30771558 and 30371038), Fund of Agricultural Science and Technology outcome application (contract No. 2006GB24910468), National Scientific and Technological Supporting Project (2006BAD12B07 and 2006BAD12B02-5-2), Guang Dong Province and Guang Zhou City Project (contract No. 2006B200330005), Program for Gangjiang Scholars and Innovative University
Research Team (contract No. 65292 and IRT0540), Hunan Projects 2007FJ1003, 06FJ3046, Institute of Subtropical Agriculture, CAS projects (ISACX-LYQY-QN-0703 and ISACX-LYQY-QN-0701).

REFERENCES


Orban, J. I., J. A. Patterson, O. Adeola, A. L. Sutton and G. N.


