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

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**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. Therefore, 3- to 4-week-old early-weaned piglets have low antiviral ability (van Heugten et al., 1996; Kong et al., 2007a, b, c). Early weaning affects passive immunity, stresses piglets with an immature immune system and results in reduced feed intake and feed efficiency. These adverse effects can be alleviated through the use of growth-promoters (Touchette et al., 2002; Kong et al., 2006). The outcomes are enhanced feed intake and feed efficiency, and reduced incidence of diseases in early-weaned piglets.

Traditionally, antibiotics have been added to pig starter diets because of their ability to promote growth performance. However, drug residues in edible meat products and their potential contribution to the emergence of antibiotic-resistant bacteria threaten human health (National Research Council, 1980; Fuller, 1992), which is a serious practical issue that faces animal agriculture (Bach, 2001). Therefore, the use of antibiotics in animal feeds has been prohibited in Europe and limited in many other nations. Consequently, alternative solutions, which promote both the safety of consumers and the profitability of farmers, must

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

| Ingredients | Diet | | | |
|---|---|---|---|---|
| | Control | Antibiotics | COS[a] | GMOS[b] |
| Corn (crude protein, 8%) | 50.01 | 49.76 | 49.81 | 49.98 |
| Soybean meal (crude protein, 43%) | 14.20 | 14.20 | 14.20 | 14.20 |
| Fish meal (crude protein, 65%) | 6.00 | 6.00 | 6.00 | 6.00 |
| Soybean expanded (crude protein, 40%) | 10.11 | 10.00 | 10.00 | 10.00 |
| Dried whey | 9.00 | 9.00 | 9.00 | 9.00 |
| Dried cream | 8.00 | 8.00 | 8.00 | 8.00 |
| Limestone[c] | 0.50 | 0.50 | 0.50 | 0.50 |
| Monocalcium phosphate[d] | 1.00 | 1.00 | 1.00 | 1.00 |
| Propionic acid[e] | 0.10 | 0.10 | 0.10 | 0.10 |
| Antioxidant[f] | 0.02 | 0.02 | 0.02 | 0.02 |
| Vitamin premix[g] | 0.04 | 0.04 | 0.04 | 0.04 |
| Choline chloride (50%)[h] | 0.08 | 0.08 | 0.08 | 0.08 |
| Trace mineral premix[i] | 0.30 | 0.30 | 0.30 | 0.30 |
| NaCl[j] | 0.20 | 0.20 | 0.20 | 0.20 |
| Flavor[k] | 0.06 | 0.06 | 0.06 | 0.06 |
| L-lysine·HCl (lysine, 71%)[1] | 0.31 | 0.31 | 0.31 | 0.31 |
| L-methionine (methionine, 98%)[m] | 0.06 | 0.06 | 0.06 | 0.06 |
| L-threonine (threonine, 98%)[n] | 0.12 | 0.12 | 0.12 | 0.12 |
| Lincoycin mixture[o] | 0.00 | 0.25 | 0.00 | 0.00 |
| COS | 0.00 | 0.00 | 0.025 | 0.00 |
| GMOS | 0.00 | 0.00 | 0.00 | 0.20 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Nutritional contents (calculated, as-fed basis) | | | | |
| Dry matter (%) | 91.00 | 90.89 | 91.11 | 90.99 |
| Digestible energy (MJ/kg) | 15.33 | 15.33 | 15.33 | 15.33 |
| Crude protein (%) | 19.00 | 19.00 | 19.00 | 19.00 |
| Total calcium (%) | 0.71 | 0.71 | 0.71 | 0.71 |
| Available phosphate (%) | 0.48 | 0.48 | 0.48 | 0.48 |
| Lysine (%) | 1.35 | 1.35 | 1.35 | 1.35 |
| Methionine (%) | 0.42 | 0.42 | 0.42 | 0.42 |
| Threonine (%) | 0.90 | 0.90 | 0.90 | 0.90 |

[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-oligosaccharidse was provided by the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

[g] Provided the following per kilogram of complete feed: 11,000 IU vitamin A; 1,100 IU vitamin $D_3$; 22 IU vitamin E; 4 mg menadione as dimethylpirimidinol bisulfate; 0.03 mg vitamin B12; 28 mg d-pantothenic acid; and 33 mg niacin.

[i] 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), 297 μg CaI2, and 297 μg Se (Na2SeO3).

[o] Lincomycin mix supplied 44 g/kg pure lincomycin; pure lincomycin in medicated diet was 110 mg/kg.

[c, d, e, f, g, h, i, j, k, l, m, n, o] Provided by Tanko Industry Company, GongDong Province, GongZhou City, China.

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

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 sampling (0 CR group), in order to obtain baseline values for the piglets of this trial.

Chitosan was provided by Dalian Chemical and Physical Institute (Chinese Academy of Sciences, China) and is a 6-sugar unit of N-acetyl glucosamine with β-(1-4)-linkages. This COS has a molecular mass of $10^3$-$10^4$ Daltons. Galactomannan (Institute of Microbiology, Chinese Academy of Sciences, Beijing, China) is a type of oligosaccharide obtained from galacto-manna-poly-saccharide of sesbania gum after degradation by the manna-polysaccharide enzyme. Total sugar content and availability in the product exceed 80% and 70%, respectively. The linkage of mannose was split using the specificity of the manna-polysaccharide enzyme in the hydrolysis of galacto-mannan-polysaccharide to give galacto-mannan-oligo-saccharide. The product is a mixture of GMOS, poly-saccharide monosaccharide (galactose and mannose) and other soluble substances. The molecular weight of GMOS is about 200-2,000 (Tang et al., 2005; Huang et al., 2007).

The piglets had free access to creep feed during suckling. The four groups of piglets were individually allocated randomly into pens with one pig per pen in a temperature-controlled room, as described by Tang et al. (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.

### 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,

**Table 2.** Performance of the piglets fed the basal diet, basal diet supplemented with galacto-mannan-oligosaccharides (GMOS), basal diet supplemented with chitosan (COS), and basal diet supplemented with lincomycin

| Items | Lincomycin | Basal | GMOS | COS | SEM* | p |
|---|---|---|---|---|---|---|
| Phase 1 (week 1) | | | | | | |
| ADG (g) | 102 | 98 | 101 | 103 | 9.34 | 0.753 |
| ADFI (g) | 220 | 219 | 223 | 219 | 20.34 | 0.234 |
| F:G | 2.15 | 2.23 | 2.20 | 2.12 | 0.09 | 0.457 |
| Phase 2 (week 2) | | | | | | |
| ADG (g) | 225[a] | 168[b] | 220[a] | 230[a] | 8.66 | 0.045 |
| ADFI (g) | 500 | 456 | 468 | 509 | 18.56 | 0.862 |
| F:G | 2.20[a] | 2.71[b] | 2.12[a] | 2.21[a] | 0.06 | 0.047 |

* Pooled standard error of the mean. [a, b] Values sharing different superscript letters within a row are different ($p<0.05$).

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 $H_2O$; 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 $MgCl_2$ (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 $\log_{10}$ 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:

$$Y_{ij} = \mu + D_j + \varepsilon_{ij}$$

Where Y is the response parameter, $D_j$ 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

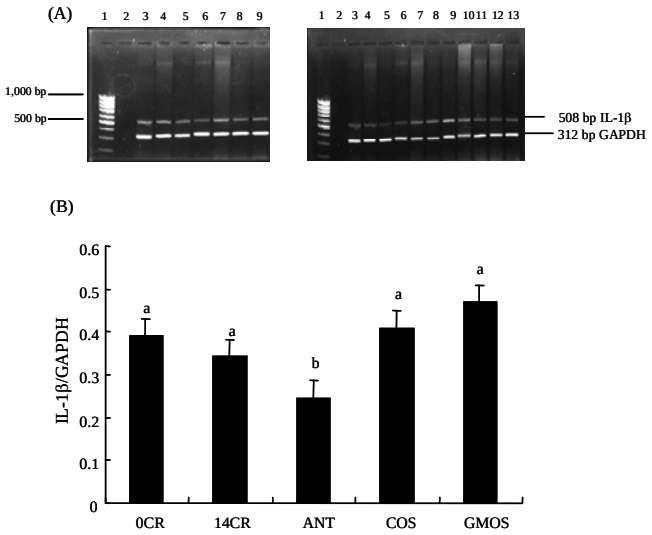


**Figure 1.** Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on mRNA levels for interleukin-1β (IL-1β) in peripheral blood monocytes in early-weaned piglets. Abbreviations : 0CR = Control group at day zero of the study; 14CR, = Control group at day 14 of the study; 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 the results of 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 ($p<0.05$).

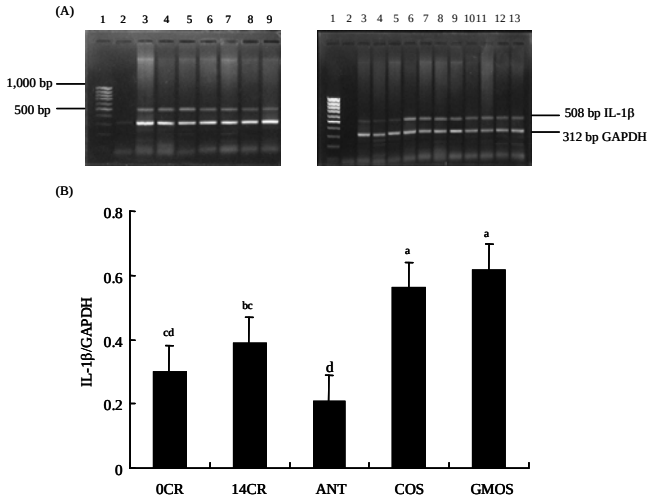


**Figure 2.** Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on mRNA levels for interleukin-1β (IL-1β) in the jejunal mucosa of early-weaned piglets. Abbreviations are the same as in Figure 1. In Panel (A), agarose gel (1.55%) electrophoresis shows the results of 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 jejunum mucous 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 ($p<0.05$).

antibiotics had greater ADG and better F:G ($p<0.05$) than those fed the basal diet during week-2.

Dietary supplementation with oligosaccharides or antibiotic had no effect on IL-1β mRNA in peripheral blood mononuclear cells (PBM) ($p>0.05$; Figure 1). However, as shown in Figure 2 and 3, supplementation with either GMOS or COS increased IL-1β mRNA levels in PBM 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

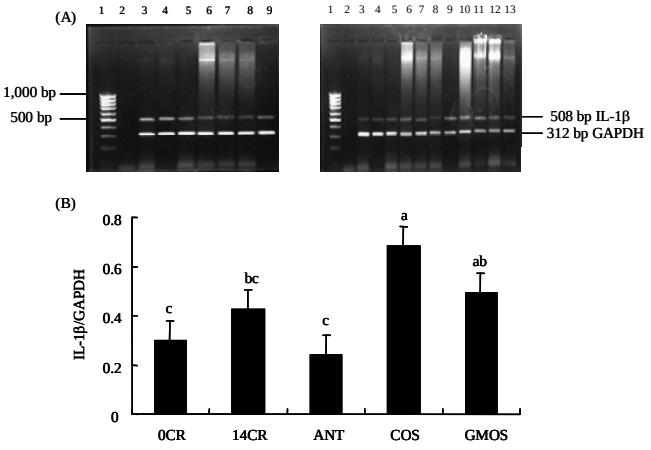


**Figure 3.** Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on mRNA levels for interleukin-1β (IL-1β) in mesenteric lymph nodes of early-weaned piglets. Abbreviations are the same as in Figure 1. In Panel (A), agarose gel (1.5%) electrophoresis shows the results of RT-PCR analysis of IL-1β mRNA and mRNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the mesenteric lymph nodes 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 (10-13, n = 4). RT-PCR conditions are described in Section 2. Panel (B) shows a quantitative analysis of the relative abundance of the mesenteric lymph node 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 ($p<0.05$).

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 $T_h$ 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 (Blok et al., 2002). In addition, IL-2 is a key cytokine with broad-spectrum and crucial immunomodulatory activities. One such activity is to enhance the function of T cells, T suppressor cells, macrophages and natural killer cells, thereby inducing T cells to generate interferons and activating $T_h$ cells. Interleukin-6 has many other physiological functions: (1) It is essential for the differentiation of B cells, and (2)

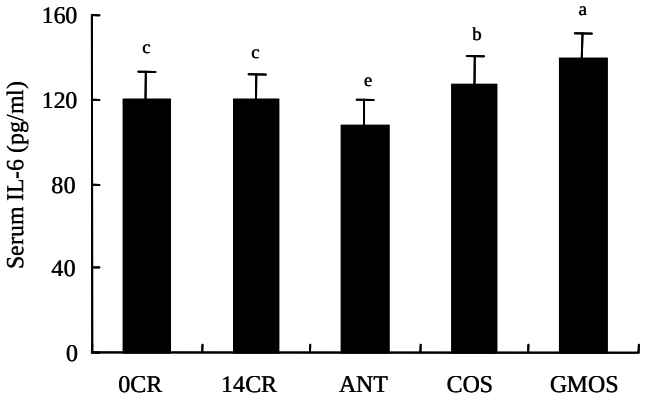


**Figure 4.** Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on serum IL-6 concentrations in early-weaned piglets. Abbreviations are the same as in Figure 1. Values are the mean±pooled STD (n = 4) expressed in arbitrary densitometric units. Means with a different superscript letter differ significantly ($p<0.05$).

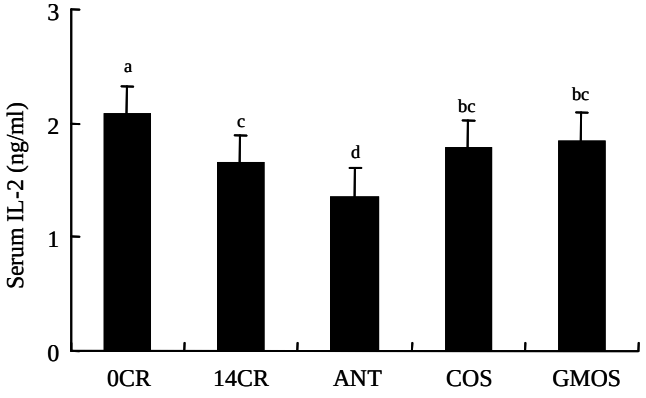


**Figure 5.** Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on serum IL-2 concentrations in early-weaned piglets. Abbreviations are the same as in Figure 1. Values are the mean±pooled STD (n = 4) expressed in arbitrary densitometric units. Means with a different superscript letter differ significantly ($p<0.05$).

recombinant IL-6 can activate T cell receptors for gene expression and induce T cells to generate IL-2 (Davis et al., 2002). The complex network of cytokines regulates the immune response in the host to prevent susceptibility to disease and enhance resistance to infections.

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

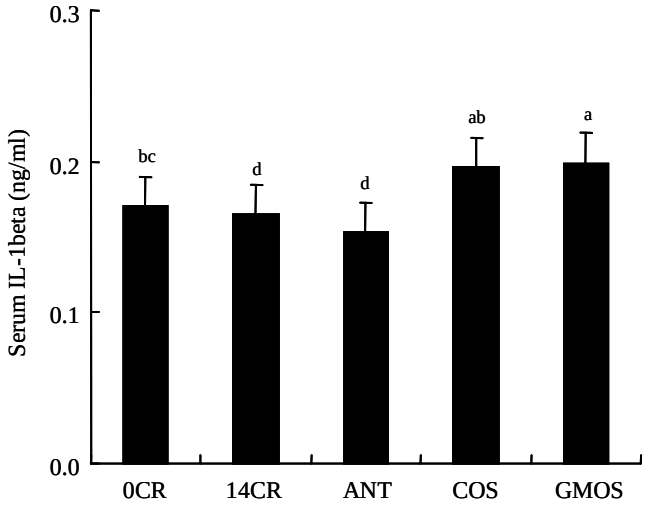


**Figure 6.** Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on serum IL-1β concentrations in early-weaned piglets. Abbreviations are the same as in Figure 1. Values are the mean±pooled TD (n = 6 for the 0CR group, 5 for the other groups). (a-f) Means with a different superscript letter differ significantly ($p<0.05$).

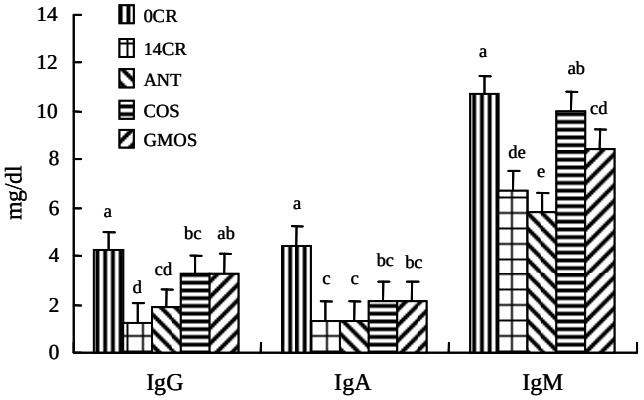


**Figure 7.** Effects of dietary supplementation with chitosan and galacto-mannan oligosaccharides on serum IgA, IgM, and IgG concentrations in early-weaned piglets. Abbreviations are the same as in Figure 1. Values are the mean±pooled STD (n = 4) expressed in arbitrary densitometric units. Means with a different superscript letter differ significantly ($p<0.05$).

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 immunoglobins 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.

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