

Feeding Mannan Oligosaccharides to Sows During Gestation and Lactation Alters Immune Cells in Milk

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Story in Brief

Thirty sows were used to determine the effects of feeding mannan oligosaccharides (Bio-Mos) during gestation and lactation on immune cell populations and cytokine concentrations in colostrums and milk. The treatments were a common gestation diet (control) or the common diet supplemented with Bio-Mos. Samples (40 to 50 mL) of colostrum (d 0) and mature milk (d 14) were obtained from each sow during lactation. Concentrations of immunoglobulins, cytokines, and the proportion of specific leukocytes were determined in the samples. Sows supplemented with Bio-Mos had fewer mummies at farrowing than control sows ($P < 0.04$); however, Bio-Mos supplemented sows had greater weight loss during lactation than control sows ($P < 0.01$). The concentration of immunoglobulins ($P < 0.001$) and all leukocytes ($P < 0.02$) in mature milk was lower than in colostrum. No effect of Bio-Mos supplementation was detected in milk IFN- γ , IL-2, IL-10, or TGF- β concentrations. The proportion of macrophages (CD14+ leukocytes) decreased in mature milk compared to colostrum of control sows (31.0 vs. 5.2%), while the proportion of this cell type remained elevated in Bio-Mos supplemented sows (25.5 vs. 20.6%) (treatment x stage of lactation interaction, $P < 0.04$). Supplementing the sow's diet with Bio-Mos during gestation and lactation increased the proportion of macrophages in mature milk. Further research will reveal if this change in mature milk is beneficial to the development of the nursing piglet's innate immunity.

Introduction

Intake of colostrum by the newborn piglet is important both for its immediate survival and its ability to thrive after weaning (Rooke and Bland, 2002). Furthermore, the immunological components of colostrum (i.e., leukocytes, cytokines, growth factors, etc.) contribute to the development of the piglet's immune system (Le Jan, 1996). However, the effects of the diet on the populations of immune cells and cytokines within the sow's colostrum and milk have not been extensively studied. The potential changes in the concentration of the immune components in colostrum and milk may impact pig performance during the lactation period and subsequent performance to market weight.

Bio-Mos (Alltech, Inc., Nicholasville, Ky.) has been reported to alter microbial populations in the gastrointestinal tract, by its ability to attach to type-1 fimbriae on the cell surface of specific bacteria, interfering with the ability of the bacteria to colonize the intestinal tract (Spring et al., 2000). By supplementing the sow's diet with Bio-Mos, to alter pathogen exposure, it may be possible to positively alter immune cell components of the sow's milk. Therefore, the aim of this study was to evaluate if there are any benefits on the immune variables of colostrum and milk from supplementing the sow's diet with Bio-Mos.

Experimental Procedures

Animals and diets. Thirty sows from one farrowing group were randomly assigned to one of two treatments. The treatments were a common gestation diet or the common diet supplemented with Bio-Mos. Treatments began 3 weeks prior to lactation, and continued through weaning. The control diet was supplemented with Bio-Mos (2 kg/ton of feed) at the expense of corn and administered

as the treatment diet. The gestation diets were limit-fed, in which the amount provided was based on sow condition. Sows continued on their respective treatment during the lactation phase (1 kg Bio-Mos/ton of feed), where sows were provided feed ad libitum until weaning.

Performance and immunological measurements. Initial body weight of sows was determined when sows began receiving dietary treatments during gestation (approximately d 86 of gestation). Sow body weight was determined again when the sows were moved into the farrowing facility and at weaning. Feed intake of individual sows was determined during gestation and lactation. The number of pigs born, pigs born alive, stillborns, and mummies was determined at farrowing, as well as live pig birth weight and weaning weight. The number of days for sows to return to estrus following weaning was also determined.

Samples (40 to 50 mL) of colostrum (d 0) and mature milk (d 14) were obtained from each sow. Sub-samples were centrifuged, the fat layer discarded, and the supernatant collected for the determination of IgM, IgG, and IgA immunoglobulin concentrations, and the concentration of specific cytokines (INF- γ , IL-2, IL-10, and TGF- β). Leukocytes were isolated from each milk sample, and the leukocyte population was defined by single stain flow cytometry. Monoclonal antibodies were used to determine the populations of T cell subpopulations (CD3, CD4, CD8), macrophages (CD14), IL-2 receptor on T cells (CD25), gamma/delta T cells (TCR $\gamma\delta$), and the presence of major histocompatibility complex-II (MHC-II) on leukocytes.

Statistical analysis. The data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, N.C.). The experimental model used for the analysis of the immune variables consisted of dietary treatment (control and Bio-Mos), stage of lactation (colostrum and mature milk), and the interaction.

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Results and Discussion

Supplementing sows with Bio-Mos during gestation and lactation reduced ($P < 0.04$) the number of mummies and increased sow weight loss during lactation ($P < 0.01$; Table 1). The greater lactation weight loss of Bio-Mos supplemented sows did not translate into statistically heavier piglets at weaning, although weaning weight was 7 lb heavier per litter.

The concentration of the immunoglobulins, essential for passive immune protection throughout lactation, was not affected by dietary treatment (Table 2). There have been reports of Bio-Mos supplementation increasing the concentration of immunoglobulins in colostrum (Newman and Newman, 2001; Quinn et al., 2001). Consistent with previously published work (Klobasa et al., 1987), immunoglobulin concentrations decreased dramatically in mature milk compared to colostrum ($P < 0.001$). Bio-Mos supplementation during gestation and lactation did not impact IFN- γ , IL-2, IL-4, or TGF- β concentrations within the colostrum or milk (Table 2).

As expected, the proportion of all measured cell types decreased in the mature milk compared to colostrum ($P < 0.02$; Table 3). However, there was no treatment effect on CD8+ (cytotoxic T cells), CD3+ (T cells), CD4+ (T helper cells), CD25+ (activated lymphocytes), TCR $\gamma\delta$ (T cells with $\gamma\delta$ receptor), or MHCII+ (antigen presenting cells) proportions in the colostrum or milk (Table 3). Interestingly, the proportion of macrophages (CD14+ leukocytes) decreased in mature milk compared to colostrum of control sows (31.0 vs. 5.2%), while the proportion of this cell type remained elevated in Bio-Mos supplemented sows (25.5 vs. 20.6%) (treatment \times stage of lactation interaction, $P < 0.04$). At birth the piglet's helper T-cell (Th) population is predominantly differentiated into a Th2 subset. During mid- to late-lactation, as the animal is exposed to environmental pathogens, the cell population is directed towards a Th1 subset. Each of these cell types has specific immunological functions directed against certain types of immune

challenges. The principal function of Th2 cells is to stimulate cell-mediated (humoral) immune responses against allergens, helminths, and arthropods; while the principal function of Th1 cells is to stimulate phagocyte-mediated (cytotoxic T cells and macrophages) defense against bacterial and viral challenges (Abbas et al., 2000). In a swine production environment, bacterial and viral challenges cause significant economical losses from reduced animal performance. By altering the macrophage proportions in the mature milk with the supplementation of the sow's diet with Bio-Mos, we may have directed the development of a Th1 subset of cells, allowing the piglet to become more immunologically mature at an earlier age.

Implications

Supplementing the sow's diet with Bio-Mos during gestation and lactation increased the proportion of macrophages in mature milk. Further research in this area will reveal how this change may alter the development of the innate immune system in neonatal pigs.

Literature Cited

- Abbas, A.K., et al. 2000. Cellular and Molecular Immunology (4th Ed.).
 Klobasa, F. and J.E. Butler. 1987. Am. J. Vet. Res. 48:176-182.
 Le Jan, C. 1996. Vet. Res. 27:403-417.
 Newman, K.E. and M.C. Newman. 2001. J. Anim. Sci. 79(Suppl. 1):189 (Abstr.).
 Quinn, P.R., et al. 2001. J. Anim. Sci. 79(Suppl. 1):212 (Abstr.).
 Rooke, J.A. and I.M. Bland. 2002. Livestock Prod. Sci. 78:13-23.
 Spring, P., et al. 2000. Poult. Sci. 79:205-211.

Table 1. Sow and litter performance of sows fed a control or Bio-Mos supplemented diet during gestation and lactation.

Trait	Sow treatment		P-value
	Control	Bio-Mos	
Sow performance			
Initial gestation weight, lb	541.6 \pm 13.9	555.0 \pm 12.1	0.47
Final gestation weight, lb	566.7 \pm 13.8	584.2 \pm 12.1	0.35
Gestation weight gain, lb	25.2 \pm 2.6	29.2 \pm 2.3	0.25
Sow weaning weight, lb	493.4 \pm 14.7	475.8 \pm 12.9	0.38
Lactation weight loss, lb	-73.4 \pm 9.4	-109.3 \pm 8.2	0.01
Sow ADFI, lb/d	9.95 \pm 0.73	8.93 \pm 0.64	0.30
Return to Estrus, d	5.34 \pm 0.16	5.08 \pm 0.14	0.23
Sow litter performance			
Number born alive	12.19 \pm 0.77	12.38 \pm 0.67	0.86
Number stillborn	1.81 \pm 0.52	1.31 \pm 0.46	0.48
Number of mummies	0.71 \pm 0.20	0.13 \pm 0.18	0.04
Litter weight, lb	38.66 \pm 2.33	40.03 \pm 2.04	0.66
Piglet weight, lb	3.21 \pm 0.17	3.30 \pm 0.15	0.71
Number weaned	9.23 \pm 0.47	9.56 \pm 0.41	0.59
Age of pig at weaning, d	21.05 \pm 0.51	21.13 \pm 0.45	0.91
Litter weaning weight*, lb	126.0 \pm 9.0	133.0 \pm 7.9	0.57
Pig weaning weight*, lb	13.60 \pm 0.57	13.86 \pm 0.50	0.60

*Analyses have both age at weaning and pigs born alive included as covariates.

Table 2. Immunoglobulin and cytokine concentrations from the colostrum and mature milk of sows fed a control or Bio-Mos supplemented diet during gestation and lactation.

Trait	Sow treatment		P-value	Stage of lactation		P-value
	Control	Bio-Mos		Colostrum	Mature milk	
Immunoglobulins, ng/mL						
IgG	3252.4 ± 375	3208.1 ± 325	0.93	6430.3 ± 308	30.2 ± 389	0.0001
IgM	309.2 ± 34	343.8 ± 30	0.45	480.8 ± 28	172.2 ± 36	0.0001
IgA	986.4 ± 66	967.8 ± 58	0.83	1610.5 ± 55	343.7 ± 69	0.0001
Cytokines, pg/mL						
INF- γ	1057 ± 311	901 ± 270	0.71	1081 ± 256	866 ± 323	0.59
IL-2*	25.5 ± 1.6	35.5 ± 1.5	0.76	18.2 ± 1.5	57.5 ± 1.6	0.07
IL-10 ^a	17.2 ± 2.6	5.6 ± 2.3	0.37	7.5 ± 2.2	12.8 ± 2.7	0.86
TGF- β	109 ± 28	135 ± 24	0.49	115 ± 23	130 ± 29	0.67

^a Data were transformed [$\log(x + 1)$] for statistical analysis due to heterogeneity of variance.

Table 3. Phenotypic expression of cell surface markers (as indicated by single-stain flow cytometric analysis) on leukocytes from the colostrum and mature milk of sows fed a control or Bio-Mos supplemented diet during gestation and lactation.

Trait, % ^a	Sow treatment		P-value	Stage of lactation		P-value
	Control	Bio-Mos		Colostrum	Mature milk	
CD3	4.3 ± 1.17	5.0 ± 1.19	0.70	6.6 ± 1.10	2.7 ± 1.26	0.02
CD4	7.7 ± 1.70	8.4 ± 1.69	0.79	11.9 ± 1.56	4.1 ± 1.82	0.01
CD8	14.1 ± 2.37	11.5 ± 2.34	0.45	20.4 ± 2.18	5.2 ± 2.53	0.01
CD14 ^b	18.1 ± 3.57	23.0 ± 3.51	0.33	28.2 ± 3.27	12.9 ± 3.80	0.01
CD25	12.8 ± 2.18	10.5 ± 2.15	0.47	20.3 ± 2.00	3.0 ± 2.32	0.01
TCR $\gamma\delta$	11.3 ± 2.32	10.8 ± 2.29	0.88	19.4 ± 2.13	2.8 ± 2.47	0.01
MHC II	14.2 ± 2.33	13.2 ± 2.30	0.77	24.1 ± 2.13	3.3 ± 2.48	0.01

^a Populations of T cell subpopulations (CD3, CD4, CD8), macrophages (CD14), IL-2 receptor on T cells (CD25), gamma/delta T cells (TCR1), and the presence of major histocompatibility complex-II (MHC-II) on leukocytes.

^b Treatment x stage of lactation interaction (P = 0.04) discussed in text.