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β-1-3-Glucan Effect on Sow Antibody Production and Passive Immunization of the Piglet

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Abstract: Beta-1, 3-glucans (β-glucan) are complex, glucose homopolymers known to modulate immune capabilities when administered intravenously, intramuscularly or orally. This study investigated the β-glucan effect on sow antibody production and subsequent passive immunization of neonatal piglets. Crossbred sows (n = 24; parity3-9) were mated and 5 weeks prior to farrowing assigned to one of four treatments (n = 2 sows/treatment within each farrowing groups; n = 8 total sows/treatment). Treatments included: Corn-soy fed Control group, β-glucan (5 mg kg^{-1} body weight), App (Actinobacillus pleuropneumoniae; Pneu-Pac™; Schering-Plough) vaccination (5 and 2 weeks pre-farrowing) and β-glucan (5 mg kg^{-1} body weight) + App vaccination (5 and 2 weeks pre-farrowing). All sows were maintained on treatments from 5 weeks pre-farrowing to 2 weeks post-farrowing. Piglet performance was not affected ($p>0.05$) by treatment as indicated by similar birth and weaning weights between treatment groups. Independent of treatment, IgA, IgG and IgM in colostrum were elevated and declined rapidly as the age at weaning approached. The concentration and rate of decline in IgA, IgG and IgM in colostrum, milk and piglet serum were not affected ($p>0.05$) by treatment. Vaccination against App (treatments 3 and 4) resulted in an increase ($p<0.05$) in IgG and IgA specific for App serotypes 1, 5 and 7 in colostrum and milk and a corresponding increase ($p<0.05$) in serum in piglets at 4 d of age. However, β-glucan did not enhance specific App antibodies in piglet serum.

Key words: β-glucan, pig, colostrum, passive immunization

INTRODUCTION

In utero, the fetal pig does not experience antigenic challenge due to the epitheliochorial placenta type of the porcine species, which prevents transplacental passage of immunoglobulin molecules (Brambell, 1970; Watson, 1980; Straw *et al*., 1989; Chappuis, 1998; Roitt *et al*., 1998). As a result, the neonate is born immunologically naïve. During the first weeks of life the components of the neonates immune system are immature (Schwager and Schulze, 1997). While, antibodies are produced by the neonate, they do not reach effective concentrations until 3-4 weeks of age (Roitt *et al*., 1998). Therefore, the neonate dependents on immunoglobulin acquisition post-natally in the intestinal epithelium following the ingestion of colostrum (Schanbacher *et al*., 1997). The permeability of the intestine to protein molecules is high during the first 24-48 h after birth making this a critical time for long-term growth and survival of the neonate (Murata and Namioka, 1977). Additionally, trypsin inhibitors present in the colostrum and reduced intestinal proteolytic activity during this sensitive period of time contribute to the absorption of intact immunoglobulins (Chappius, 1998; Kiriyama, 1992; Murata and Namioka, 1977). Colostrum is the source of immunoglobulins IgA, IgG and IgM that confer passive immunity to the neonate; moreover, it contains viable leukocytes capable of expressing cell-mediated immunity (Hanson *et al*., 1984; Salmon, 1999; Goldman *et al*., 1998). Hence, piglets acquire immunity passively through maternal immunoglobulins and immunologic factors in colostrum that protect against pathogens until their own immune system matures.

Previous observations indicate that oral administration of a highly purified β-glucan can increase immunoglobulin production in response to vaccination in young pigs. Beta-1, 3-glucans are complex glucose homopolymers, extracted and purified from the cell wall of

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yeast (Williams *et al*., 1996), that are reported to have broad anti-infective properties without inducing leukocyte activation or stimulation of pro-inflammatory cytokines (Cisneros *et al*., 1996; Onderdonk *et al*., 1992; Antje *et al*., 1995). Moreover, immunoglobulin production is enhanced in response to vaccination in young pigs treated orally with highly purified β-glucan. Therefore, the study conducted herein was to determine the effect of β-glucan, as an oral adjuvant, on immunoglobulin production in late-gestating and early lactating sows as a means to increase colostral immunoglobulin concentration and subsequently enhancing the passive immunization in the neonate (Michalek *et al*., 1998).

MATERIALS AND METHODS

Experimental design: To test the effect of β-glucan on maternal immunoglobulin production and subsequent passive immunization of neonatal pigs, 24 crossbred sows (Parity 3-9, 3 farrowing groups, n = 9, 8 and 7 sows/farrowing group) from the Texas A and M University-Kingsville Swine Center were utilized. Sows were fed a corn-soy (CS) based diet and maintained as per current industry practices. Five weeks prior to the farrowing dates, sows were assigned, by parity, to one of four treatment groups including: CS fed Control group (n = 6), β-glucan (5 mg kg^{-1} body weight; n = 5), App (Actinobacillus pleuropneumoniae; Pneu-Pac™; Schering-Plough) vaccination (5 and 2 weeks pre-farrowing; n = 6) and β-glucan (5 mg kg^{-1} body weight) + App vaccination (5 and 2 weeks pre-farrowing; n = 7). Sows in treatment groups 2 and 4 received β-glucan as a feed additive until 2 weeks post-farrowing. Vaccination against App was used as a sentinel for antibody transfer (Baarsch *et al*., 1995; Haesebrouch *et al*., 1997). Sows were moved to farrowing crates one week prior to their respective farrowing date. All sows farrowed at night or in the early morning, hence, the morning following farrowing was designated as D 0.5. Piglets within each litter were weighed and tattooed for permanent identification. Milk samples were obtained from the sow on D 0.5, 4, 8, 16 and on D of weaning. Samples were collected from the anterior region of the udder following the removal of the piglets for 45 min and administration of oxytocin (20 USP), a stimulator of mammary myoepithelial cell contraction. Samples were stored at -80°C until analysis for immunoglobulins IgG, IgA and IgM. Blood samples were collected via jugular venipuncture from each piglet on D 4, 8, 16 and on D of weaning. Serum was harvested and stored at -80°C until analysis for immunoglobulins IgG, IgA and IgM.

Serum and milk analysis: Serum and milk concentrations of immunoglobulins were determined using a double antibody sandwich enzyme link immunosorbent assay (ELISA) specific for porcine immunoglobulins as described by Nemec *et al*. (1994) and Leiner *et al*. (1999). Immunoplates (Nunc Maxisorp, 439454) were incubated overnight with affinity purified goat anti-pig IgG, IgM, or IgA (100 μL $well^{-1}$; Bethyl Laboratories, Montgomery, TX) in 0.1 M $NaCO_3$ (pH 8.2). Plates were washed 3 times with wash buffer (phosphate buffered saline; PBS + 0.05% Tween-20). Blocking buffer (PBS + 1% bovine serum albumin) was added (200 μL $well^{-1}$), to block non-specific binding and incubated for 30 min. Plates were washed three times with wash buffer and serum samples, diluted in PBS + Tween-20 (0.05%) + BSA (1%), were added to the plates and incubated for 2 h at room temperature. Following 3 washes with wash buffer, affinity purified goat anti-pig IgG, IgM, or IgA-Fc conjugated with horseradish peroxidase (1/2000) was added (100 μL $well^{-1}$) and incubated for 1 h at room temperature and then washed 4 times. Enzyme substrate buffer (ABTS [2,2-azino-bis3-ethylbenzthiazoline-6-sulfonic acid] + 0.0015% H_2O_2, pH 4.5) was added (100 μL $well^{-1}$) and incubated for 1 h at room temperature. Optical densities were determined at 405 nm (EL 311; Bio-Tek Instruments Inc., Winooski, VT). Sample IgG, IgM and IgA concentrations were determined by comparison to standard curves generated with purified swine immunoglobulins (Bethyl Laboratories, Montgomery, TX).

Statistical analyses: Milk and serum samples were analyzed using the General Linear Model (GLM) procedure of SAS (1985). The partitioned sources of variation included treatment, farrowing group, parity and their respective interactions. Specific treatment comparisons were made using non-orthogonal contrasts. Comparisons included: Control (1) vs. App vaccine (3) = V, Control (1) vs. β-glucan (2) = B and App vaccine (3) vs. β-glucan + App vaccine (4) = BV.

RESULTS AND DISCUSSION

In contrast to Dritz *et al*. (1995), piglet performance was not altered ($p<0.05$) by β-glucan as indicated by similar birth weights (1.3±0.04 kg) and weaning weights (5.5±0.12 kg) among the four treatment groups (data not shown). However, this is likely attributed to the administration of β-glucan directly to the sow rather than the piglet, which diminished potentially unfavorable effects on piglet performance. Milk content of IgA, IgG and IgM were initially high but declined rapidly throughout lactation (Table 1). Immunoglobulin G was

Table 1: Effect of oral β-glucan fed to gestating sows on milk IgA, IgG and IgM production during D 0.5 to D of weaning

	Treatment[a]					
Time (day)	1	2	3	4	SEM[b]	Comparison[c]
IgA (mg mL^{-1})						
0.5	6.99	7.79	7.77	7.04	±0.72	
4	5.10	4.80	4.81	5.01	±0.44	
8	3.64	3.66	5.14	3.37	±0.23	V, BV
16	3.21	3.60	3.45	3.22	±0.25	
Weaning	3.04	3.46	4.95	3.93	±0.30	
IgG (mg mL^{-1})						
0.5	51.91	46.71	37.14	39.28	±4.26	
4	1.62	1.19	1.14	1.43	±0.15	
8	0.49	0.39	0.48	0.70	±0.06	
16	0.29	0.30	0.32	0.41	±0.03	
Weaning	0.26	0.43	0.34	0.42	±0.04	
IgM (mg mL^{-1})						
0.5	3.48	3.54	2.14	2.91	±0.18	V
4	2.54	2.26	1.82	2.25	±0.12	V
8	1.50	1.18	0.90	1.54	±0.08	V, BV
16	1.08	0.91	0.72	1.09	±0.05	V, BV
Weaning	1.21	1.16	0.84	1.44	±0.09	BV

[a]Sows were assigned to 1 of 4 treatments: 1) no App vaccine, 0 mg kg^{-1} β-glucan 2) no App vaccine, 5 mg kg^{-1} β-glucan 3) App vaccine at 5 and 2 weeks prior to farrowing, 0 mg kg^{-1} β-glucan 4) App vaccine at 5 and 2 weeks prior to farrowing, 5 mg kg^{-1} β-glucan. [b]Standard error of the least square mean. [c]Significant ($p<0.05$) treatment effect comparisons within day are marked: treatment 1 versus treatment 3 (+/- vaccine; V), treatment 1 versus treatment 2 (+/- β-glucan; B) and treatment 3 versus treatment 4 (+ vaccine +/- β-glucan; BV)

Table 2: Effect of oral β-glucan fed to gestating sows on piglet's serum IgA, IgG and IgM from D 0.5 to D of weaning

	Treatment[a]					
Time (day)	1	2	3	4	SEM[b]	Comparison[c]
IgA (mg mL^{-1})						
4	2.85	2.87	4.88	3.13	±0.25	V, BV
8	0.59	1.15	1.59	0.91	±0.11	V, BV
16	0.13	0.15	0.19	0.15	±0.01	
Weaning	0.15	0.15	0.16	0.14	±0.01	
IgG (mg mL^{-1})						
4	31.58	32.04	30.55	30.23	±1.28	
8	22.49	28.44	25.92	25.60	±1.02	
16	13.21	13.28	17.36	14.50	±0.92	
Weaning	8.82	8.13	10.20	7.95	±0.50	
IgM (mg mL^{-1})						
4	1.59	1.99	1.60	1.74	±0.14	
8	0.70	0.94	0.78	0.98	±0.08	
16	0.58	0.60	0.59	0.69	±0.05	
Weaning	0.79	0.64	0.70	0.78	±0.08	

[a]Sows were assigned to 1 of 4 treatments: 1) no App vaccine, 0 mg kg^{-1} β-glucan 2) no App vaccine, 5 mg kg^{-1} β-glucan 3) App vaccine at 5 and 2 weeks prior to farrowing, 0 mg kg^{-1} β-glucan 4) App vaccine at 5 and 2 weeks prior to farrowing, 5 mg kg^{-1} β-glucan. [b]Standard error of the least square mean. [c]Significant ($p<0.05$) treatment effect comparisons within day are marked: treatment 1 versus treatment 3 (+/- vaccine; V), treatment 1 versus treatment 2 (+/- β-glucan; B) and treatment 3 versus treatment 4 (+ vaccine +/- β-glucan; BV)

higher than IgA and IgM in colostrum on D 0.5 having a mean value of 43.76±4 mg mL^{-1}; however, by D 4 of lactation, it decreased to 3.1% of its initial concentration. Immunoglobulin G continued to decrease ($p<0.05$) reaching nadir concentrations on D 8 of lactation. This is similar to a study by Frenyo *et al.* (1981) that reported a decrease in milk IgG to 3.2% of the initial concentration by D 5 of lactation. The total concentration of IgG and rate of decline were not affected by treatment. The physiological process of providing large amounts of IgG in colostrum might be an adaptation that compensates the piglets inability to obtain antibodies prior to birth and to provide the piglet with protection against systemic infectious pathogens in the first days of life. Likewise, the quick decline in IgG content coincides with the subsequent shut-off of immunoglobulin absorption in the gut of the neonate (Butler, 1979).

In colostrum, the initial IgA mean value was 7.4±0.72 mg mL^{-1} and appeared to begin decreasing on D 4; however, by D 8 concentrations remained steady throughout the lactation period. Treatment did not affect total concentrations of IgA in milk or serum. By D 8 of lactation, a suppression of IgA occurred in the App vaccinated sows receiving β-glucan (treatment 4; Table 2). However, this was the only time that this phenomenon appeared to have occurred; therefore, it does not likely represent an effect of either App vaccination or β-glucan. Immunoglobulin IgA was the predominant antibody in the milk throughout lactation, which is supported by numerous studies (Porter, 1973; Watson, 1980; Salmon, 1999; Butler, 1979). Moreover, IgA was higher ($p<0.05$) in colostrum than in the serum of piglets, which is similar to a previous report by Bourne and Curtis (1973). A consistent level of IgA in milk throughout lactation is an important component in mucosal immunity that ensures the piglet with enteric and mucosal protection. Immunoglobulin IgA is expressed in the mucosa as a secretory IgA and instead of being absorbed through the intestinal tract it lines the mucosa and prevents mucosal infectious agents from adhering to the epithelium (Porter, 1972).

Immunoglobulin IgM concentrations were the lowest in colostrum with an initial mean value of 3.02±0.18 mg mL^{-1} (Table 1). App vaccination resulted in a slight ($p = 0.1$) suppression in colostral concentrations of IgM (treatment 3), which was maintained throughout lactation. In contrast, β-glucan appeared to overcome ($p<0.05$) the suppression on IgM content (treatment 4) on D 8, D 16 and at weaning (Table 1). It is not clear how β-glucan reversed the suppression on IgM following App vaccination. Similar to a report by Gomez *et al.* (1998), serum IgG was higher ($p<0.05$) than IgA and IgM in all piglets until weaning; reaching peak concentrations on D 4 and declining until weaning (Table 2).

In contrast to concentrations of IgG in sows' milk, piglet serum IgG did not drastically decrease. Neither App vaccination nor β-glucan affected total serum IgG concentrations. Unlike IgG, serum IgA decreased ($p<0.05$)

Table 3: Influence of oral β-glucan fed to gestating sows on Actinobacillus pleuropneumoniae (App) IgG, serotypes 1, 5 and 7 on piglet serum at 4 D of age

	Treatment[a]					
Serotypes	1	2	3	4	SEM[b]	Comparison[c]
1	0.124	0.080	0.423	0.404	0.028	V
5	0.363	0.261	0.584	0.608	0.034	V
7	0.359	0.249	0.543	0.579	0.038	V

[a]Sows were assigned to 1 of 4 treatments: 1) no App vaccine, 0 mg kg^{-1} β-glucan 2) no App vaccine, 5 mg kg^{-1} β-glucan 3) App vaccine at 5 and 2 weeks prior to farrowing, 0 mg kg^{-1} β-glucan 4) App vaccine at 5 and 2 weeks prior to farrowing, 5 mg kg^{-1} β-glucan. [b]Standard error of the least square mean. [c]Significant ($p<0.05$) treatment effect comparisons within day are marked: treatment 1 versus treatment 3 (+/- vaccine; V), treatment 1 versus treatment 2 (+/- β-glucan; B) and treatment 3 versus treatment 4 (+ vaccine +/- β-glucan; BV)

Table 4: Influence of oral β-glucan fed to gestating sows on Actinobacillus pleuropneumoniae (App) IgG, serotypes 1, 5 and 7 on piglet serum at 4 D of age

	Treatment[a]					
Serotypes	1	2	3	4	SEM[b]	Comparison[c]
1	0.016	0.006	0.194	0.145	0.005	V, BV
5	0.077	0.066	0.439	0.337	0.010	V, BV
7	0.123	0.136	0.462	0.376	0.009	V, BV

[a]Sows were assigned to 1 of 4 treatments: 1) no App vaccine, 0 mg kg^{-1} β-glucan 2) no App vaccine, 5 mg kg^{-1} β-glucan 3) App vaccine at 5 and 2 weeks prior to farrowing, 0 mg kg^{-1} β-glucan 4) App vaccine at 5 and 2 weeks prior to farrowing, 5 mg kg^{-1} β-glucan. [b]Standard error of the least square mean. [c]Significant ($p<0.05$) treatment effect comparisons within day are marked: treatment 1 versus treatment 3 (+/- vaccine; V), treatment 1 versus treatment 2 (+/- β-glucan; B) and treatment 3 versus treatment 4 (+ vaccine +/- β-glucan; BV)

(Table 2) compared to a moderate decline that occurred in sows' milk. Serum concentrations of IgA are typically low as they reside primarily in and around mucosal epithelia. In contrast to IgA concentrations in milk, serum IgA was higher ($p<0.05$) on D 4 and D 8 in the App vaccinated treatment groups, independent of β-glucan treatment. At present, the difference between milk IgA and piglet serum IgA in the App vaccinated groups is not clear. Treatments did not affect total serum concentrations or the rate of decline of IgA in milk or piglet serum.

Serum concentrations of IgM were highest on D 4 and significantly ($p<0.05$) declined until weaning (Table 2). Contrary to the concentration of IgM in milk in the App vaccinated sows, serum IgM was not suppressed, which is a difference that is not presently clear. Treatments did not affect serum concentrations or the rate of decline of IgM in milk or piglet serum.

Vaccination against App (treatments 3 and 4) resulted in an increase ($p<0.05$) in IgG and IgA specific for App serotyes 1, 5 and 7 in colostrum and milk (Table 3). This corresponded to an increase ($p<0.05$) in piglet serum on D 4 (Table 4). In summary, β-glucan can be efficacious as an oral adjuvant to enhance immunoglobulin production in response to vaccination. However, at the dosage used in the current study, β-glucan was not effective at enhancing passive immunization in piglets.

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