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Supplemental vitamin C and yeast cell wall β -glucan as growth enhancers in newborn pigs and as immunomodulators after an endotoxin challenge after weaning¹

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ABSTRACT: To test possible dietary immune modulators, 32 crossbred male pigs were given 1 of 4 dietary treatments (8 pigs/treatment): control, *Saccharomyces cerevisiae* with β -glucan (Energy Plus, Natural Chem Industries LTD, Houston, TX; 0.312 g/kg of BW, 2.5% of diet), vitamin C (Stay C 35, DSM Nutritional Products Inc., Prisippany, NJ; 75 ppm), or β -glucan plus vitamin C together (combination; 0.312 g/kg of BW and 75 ppm, respectively). Supplements were given in whole milk within 36 h of birth and then daily for 2 wk until weaning, when the supplement was given in feed for an additional 2 wk. Growth was recorded during the 4 wk of supplement delivery. An i.v. lipopolysaccharide challenge (LPS; 150 μ g/kg) was given 14 d postweaning at 0900. Behavior was observed, and blood samples were collected every 30 min for 4 h via a jugular catheter from -1 (0800) to 3 (1200) h relative to challenge (-60, -30, 0, 30, 60, 90, 120, 150, and 180 min), and tissues were collected after exsanguination. Beta-glucan (glucan and combination) increased ($P < 0.05$) BW and ADG compared with vitamin C and control. Cortisol concentrations showed an interaction ($P < 0.05$) of the β -glucan and vitamin C. Intestinal expression of tumor-necrosis factor (TNF)- α mRNA was greatest for vitamin C and

β -glucan compared with control and combination, and liver TNF- α mRNA expression showed a main effect ($P < 0.01$) of β -glucan. Lung expression of TNF- α mRNA exhibited a vitamin C effect ($P < 0.01$). In contrast, spleen had greater ($P < 0.01$) relative abundance of TNF- α mRNA in β -glucan pigs. Intestinal expression of IL-1Ra mRNA was greater ($P < 0.05$) for vitamin C and β -glucan treatments compared with the control and combination pigs. Liver expression of IL-1 receptor antagonist mRNA exhibited a vitamin C effect ($P < 0.01$). Lying and sleeping behaviors differed ($P < 0.05$) among treatments early in the observations (0700 to 0720), then sporadically until 50 min after the LPS injection. The vitamin C group slept less ($P < 0.05$) on those occasions. The time spent lying was least ($P < 0.05$) for the glucan and combination pigs immediately after the injection. These results show a complex interaction between vitamin C and this yeast product after LPS challenge, with differential expression in tissues by 2 h after LPS injections. The combination enhanced postweaning growth and reduced TNF- α expression of the intestinal and liver tissues, suggesting an important immunomodulatory role of the combination treatment.

Key words: growth, immune modulator, pig, vitamin C, yeast beta-glucan

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INTRODUCTION

Piglets are protected from many pathogens by passive transfer of immunoglobulins (Stokes and Bailey, 1998)

for the first few weeks of life, but these begin to wane just days after birth. Early stimulation of the immune system may enhance innate immune defenses during this period of immune development that is punctuated with many stressors. Additionally, enhanced innate immunity also may be beneficial through weaning.

Beta-glucan is an immunomodulator that can be extracted from the cell walls of yeasts, fungi, and some grains. It has enhanced the innate immune system providing protection against bacterial (Onderdonk et al., 1992), protozoan (Goldman and Jaffe, 1991), and viral diseases (Rouhier et al., 1995). Mechanisms of action

¹Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

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include activation of macrophages, neutrophils, and natural killer cells, along with B and T lymphocytes and increased phagocytosis and cytokine production in macrophages in vivo (Seljelid et al., 1987) and in vitro (Hoffman et al., 1993).

Other immunomodulators include antioxidant molecules, such as ascorbic acid (vitamin C). Vitamin C efficiently scavenges superoxide, hydrogen peroxide, hypochlorite, the hydroxyl and peroxy radicals, and singlet oxygen (Sies et al., 1992). As an antioxidant, vitamin C plays a key role in immune cell function. Protection against extracellular free radicals increases structural integrity of cells and tissues. Vitamin C protects cells from lipid peroxidation and increases the fluidity of cell membranes, thus enhancing the immune response (Bendich, 1993). In macrophages, vitamin C also increases phagocytosis, chemotaxis, and cell adherence, all of which are important in immune cell function (Del Rio et al., 1998).

The objective in the current study was to investigate the efficacy of β -glucan and vitamin C, alone and in combination, as growth or as immune enhancers in young pigs before an endotoxin challenge and as immune enhancers during an endotoxin challenge.

MATERIALS AND METHODS

Animals and Treatments

All animal procedures were reviewed and approved by the University of Missouri Animal Care and Use Committee.

Crossbred male pigs (n = 32) from the University of Missouri Swine Farm were assigned to 1 of 4 dietary treatments (n = 8 pigs/treatment group): control (no supplementation), β -glucan (0.312 g/kg of BW equivalent to 2.5% of diet as-fed; Energy Plus, Natural Chem Industries, LTD, Houston, TX), vitamin C (75 ppm as-fed; Stay C 35, DSM Nutritional Products Inc., Parsippany, NJ), and β -glucan plus vitamin C (combination; 0.312 g/kg of BW and 75 ppm, respectively). Piglets were from 8 litters, with each treatment represented at least once in each litter, except for 1 litter with 3 piglets. This litter had control, β -glucan, and combination treatments. Therefore, 1 other litter had 2 pigs given the vitamin C treatment, for a total of 5 treatments within that litter.

Piglets were with the sows and nursing from d 1 to 14 of treatment. Additionally, controls were gavaged with 10 mL of whole bovine milk only (from a grocery store), and the other treatment groups had treatment supplements added to 10 mL of whole milk (DM analysis estimated at 29% ether extract, 39.2% protein, 5.9% ash, and 0.21% fiber; NRC, 1989). The gavage was administered within the first 36 h of birth, and then once daily for the first 2 wk. Treatments were not begun until 36 h after birth to allow for adequate colostrum intake and absorption. Body weights were taken every third day for the first 2 wk to adjust the oral dosages

Table 1. Dietary composition, as-fed basis

Item	%
Ground corn	45.82
Soybean meal	20.00
Edible dried whey	20.00
Menhaden select fish meal	5.00
Blood cells	1.75
Corn oil	3.00
Cornstarch	2.00
Limestone	0.50
Dicalcium phosphate	0.39
Salt	0.30
Vitamin premix ¹	0.025
Trace mineral premix ¹	0.15
L-Lysine·HCl	0.38
DL-Methionine	0.24
L-Threonine	0.22
Total	100
Calculated nutrient profile	
CP, %	20.72
ME, kcal/kg	3,481
Total lysine, %	1.60
Total methionine + cystine, %	0.96
Total threonine, %	1.04
Total tryptophan, %	0.81
Total valine, %	1.05
Total isoleucine, %	0.81
Total calcium, %	0.80
Available phosphorous, %	0.40

¹Mineral and vitamin mix provided per kilogram of diet: vitamin A, 11,000 IU; cholecalciferol, 1,100 IU; vitamin E, 22 IU; menadione, 4 mg; riboflavin, 8.25 g; niacin, 33 mg; D-pantothenic acid, 28 mg; Zn from ZnSO₄·7H₂O; 33 mg of Mn from MnSO₄·5H₂O; 165 mg of Fe from FeSO₄·5H₂O; 0.3 mg of I from KI; 0.3 mg of Se from Na₂SeO₃·5H₂O; and Santoquin, 50 mg.

of the supplements. Pigs were weaned at 2 wk of age and placed in individual pens. After weaning, weekly BW were recorded and treatment dosages adjusted accordingly. After weaning, treatments were added to the starter diet (Table 1), rather than to milk, and fed ad libitum for an additional 2 wk. Pigs were blocked by farrowing date into 2 groups separated by a day.

Sampling Procedures

On d 13 postweaning, a jugular vein of each pig was cannulated using the nonsurgical procedure described by Carroll et al. (1999). The next day, all pigs were challenged i.v. with 150 μ g of lipopolysaccharide/kg of BW (**LPS**; *Escherichia coli* serotype O111:B4; Sigma L-2630, Sigma Chemical, St. Louis, MO) dissolved in 0.9% (wt/vol) NaCl in water immediately after collection of the blood sample at challenge time 0. Blood samples were collected into 5-mL heparinized and 3-mL sodium citrate vacuum tubes every 0.5 h for 4 h (0800 to 1200) via the jugular catheter (-1, -0.5, 0, 0.5, 1, 1.5, 2, 2.5, and 3 h relative to LPS challenge).

The samples in sodium citrate tubes were collected for hematological analyses (IDEXX, Westbrook, MA). Hematocrits, peripheral blood mononuclear counts, granulocyte counts, and fibrinogen concentrations were

determined from these samples. Heparinized blood samples were centrifuged at $450 \times g$ for 30 min. Plasma was recovered and stored at -80°C . After the 4-h LPS challenge, animals were euthanized by captive bolt stunning followed by exsanguination, and tissue samples were collected from the intestine, spleen, liver, and lung.

Tissue Sample Collection

Intestinal tissue samples, collected from the mesenteric side of the ileum approximately 1 m cranial to the ileocecal junction, were washed and placed immediately into RNALater (Qiagen, Valencia, CA). A sample of the spleen, collected to represent a cross-section of the spleen, was placed immediately into RNALater. Liver tissue from 2 lobes were collected and placed into RNALater. Lung tissue from approximately 15 cm from the ventral surface of the most ventral lobe and 30 mm deep was removed. Approximately 3×3 cm of total area was collected from each tissue; care was taken to aseptically remove the tissue and to place it immediately into cold RNALater, which was stored on ice for transport to the laboratory.

Stress Hormone Response

Serum cortisol and ACTH were analyzed within single assays (Daniel et al., 1999) using commercially available RIA kits (Coat-a-count, Diagnostic Products Corp., Los Angeles, CA). Tumor-necrosis factor (TNF)- α was determined using a porcine-specific ELISA (Pierce-Endogen Inc., Woburn, MA.). The intraassay CV for cortisol and ACTH were 5.6 and 6.7%, respectively. The intraassay CV for TNF- α was 10.4%. The minimum detectable concentration was 8 pg/mL for ACTH, 2 ng/mL for cortisol, and 31.3 pg/mL for TNF- α . Assay sensitivities were 9 pg/mL, 0.11 $\mu\text{g/dL}$, and 5 pg/mL for ACTH, cortisol, and TNF- α , respectively.

RNA and PCR Procedures

Extraction of total RNA was performed on tissues that had been suspended in RNALater at the time of necropsy, and then washed according to the manufacturer's instructions. Extraction was performed using RNEasy Mini kits (Qiagen, Valencia, CA), and Quishredders (Qiagen) were used to homogenize the samples. Total RNA was used in a real-time reverse-transcription-PCR procedure. Primer and probe sequences (Table 2) were those determined using Primer Express software using the sequences for IL-1 β from Huether et al. (1993; M86725), IL-1 receptor antagonist protein from Yin and Murtaugh (unpublished, 1996; L38849), and TNF- α from Choi et al. (1991; X57321). Dyes and Quenchers were FAM (6-carboxyfluorescein) and VIC for genes of interest and reference genes, respectively, with MGB (minor groove binder, Applied Biosystems, Foster City, CA). The reference gene was hypoxanthine guanine phosphoribosyl transferase (HPRT).

Real-Time Reverse Transcription-PCR Conditions. The methods of Applied Biosystems User Bulletin #2 were used. Primers and probes were optimized for each gene of interest and the reference genes. After reverse transcription, a reaction mix of 25 μL of Universal Master Mix (Applied Biosystems), 5 μL each of optimum primer concentrations, 5 μL of optimum probe concentration, 5 μL of cDNA sample, and 5 μL of water were added to a 96-well PCR plate, for a total volume of 50 μL /well in duplicate. Reactions were run for 40 cycles using an ABI PRISM 7000 (Applied Biosystems). Standard curves (generated from a sample with a great abundance of the gene of interest) were constructed to obtain the relative abundance of the genes of interest, which was expressed as a ratio of the gene of interest to HPRT for each sample.

Behavior

Behavior was monitored 2 h before and 3 h after LPS challenge, for a total of 5 h of observations. Observations were taken using an instantaneous scan sample once every 10 min, beginning at 0700 and lasting until 1200, to measure activity and clinical responses to LPS, such as lying, sleeping, vomiting, and scours (Hurnik et al., 1995).

Statistical Analysis

Serum, hematology, and reverse transcription-PCR data were analyzed as a 2×2 factorial arrangement of treatments (β -glucan and vitamin C) using the repeated statement of the MIXED procedure of SAS (Samuels and Witmer, 1999), and using GLM (SAS Inst. Inc., Cary, NC) when time was not part of the model. When β -glucan and vitamin C interactions were detected, mean separations by LSD were used to determine treatment differences. Treatment, time, and group effects were included in the model. Gene expression data were analyzed using a randomized complete block design, with treatment and group effects in the model statement. Behaviors were analyzed using PROC FREQUENCY of SAS, with a 3-way χ^2 test.

RESULTS

Beta-glucan and vitamin C are used when referring to a main effects and interactions or to generic substances. Beta-glucan, vitamin C, combination, and control are used to refer to specific treatments.

Average Daily Gain and Body Weight

Body weights were recorded every 3 d for the first 2 wk of age while the piglets remained with the sows and then weekly after weaning. Average daily gain was calculated for each of the 3-d periods and then on a weekly basis. The ADG was influenced by treatment, with a main effect of β -glucan to increase ($P < 0.01$)

Table 2. Sequence of PCR primers and TaqMan probes specific for porcine cytokines

Cytokine	Sequence (5'-3')	Probe sequence (5'-3')	GenBank accession number
IL-1 β , f ¹	TCTGCCCTGTACCCCAACTG	TACATCAGCACCTCTC (VIC/MGB) ²	M86725
IL-1 β , r ³	CCCAGGAAGACGGGCTTT		
IL-1Ra, ⁴ f	CCTTTTCCTGTCCACTCAGAGA	TGCCTGCCACCCC (VIC/MGB)	L38849
IL-1Ra, r	GAAGGCTTGCATCCTGCAA		
TNF- α , ⁵ f	AACCCTCTGGCCCAAGGA	TCAGATCATCGTCTCAAAC (VIC/MGB)	X57321
TNF- α , r	GGCGACGGGCTTATCTGA		
HPRT, ⁶ f	CAATGCAAACCTTGCTTTCCTT	TCAAGCAGCATAATC (FAM/MGB)	U32316
HPRT, r	GCTTGCAACCTTGACCATCTTT		

¹f = Forward primer.

²For each probe, the label and type of probe are given in parentheses.

³r = Reverse primer.

⁴Ra = Receptor antagonist.

⁵TNF- α = Tumor necrosis factor-alpha.

⁶HPRT = Hypoxanthine guanine phosphoribosyl transferase.

ADG relative to the control and vitamin C-supplemented pigs. Average daily gain was greater ($P < 0.05$) in pigs that received the combination supplement compared with the control and vitamin C groups. There was an overall time effect ($P < 0.01$) on ADG, as well as a trend ($P = 0.06$) for a time \times treatment interaction. The greatest effect of the combination was observed from d 15 to 18 after weaning, with greater ADG for piglets on the combination treatment (0.13 kg/d) compared with 0.02, 0.03, and 0.04 kg/d for vitamin C, control, and β -glucan treatments, respectively.

Hematology Data

Hematology data were measured as a change from baseline after LPS administration because there were significant differences in baseline values. Hematocrit percents and fibrinogen were not influenced by the supplements. There was a trend for β -glucan to have a decrease ($P = 0.07$) in granulocyte cell numbers (data not shown) relative to the vitamin C-supplemented pigs. Lymphocyte (data not shown) counts tended to decrease ($P = 0.07$) more with β -glucan supplementation than with the vitamin C supplementation.

Stress Hormone Response

Given that there was no time effect during the prechallenge period, the first 3 baseline samples (-1, -0.5, and 0 h) were averaged and then subtracted from each subsequent measurement taken during the challenge to control for baseline differences. There was no difference among treatments for plasma ACTH concentration (data not shown). There was an overall time effect such that plasma concentrations of ACTH increased ($P < 0.01$) in all groups after the LPS challenge. However, there was no treatment \times time interaction.

There was no treatment difference in basal (prechallenge) cortisol concentrations. After the challenge,

plasma cortisol profiles (Figure 1) showed a trend ($P = 0.06$) for an effect of vitamin C and a trend for a β -glucan and vitamin C interaction ($P = 0.07$) in response to the challenge. The cortisol response for the vitamin C-supplemented pigs was less ($P < 0.01$) than the control pigs using mean separations. There was an overall time effect ($P < 0.01$) for cortisol such that plasma concentrations of cortisol increased in all groups after the LPS challenge. There was no time \times treatment interaction.

Immune Measures

There was a tendency ($P = 0.08$) for a vitamin C and β -glucan interaction in plasma TNF- α concentrations (Figure 2) after the LPS administration. The vitamin C-supplemented pigs had lower TNF- α concentrations relative to the controls, using mean separation. There was a trend ($P = 0.09$) for a time \times treatment interaction, such that treatments were not different at time 0 nor at 3 h postchallenge, but differed among treatments at 0.5, 1, 1.5, 2, and 2.5 h postchallenge. However, TNF- α mRNA expression was greatest for vitamin C-supplemented and β -glucan supplemented pigs compared with control and combination groups in intestine samples (Table 3), and similarly, the liver TNF- α (Table 3) expression was greatest ($P < 0.01$) for the vitamin C group compared with control and combination treatments, with a main effect of β -glucan ($P < 0.01$). Lung tissue mRNA (Table 3) had greater ($P < 0.01$) TNF- α expression for vitamin C and combination groups (main effect of vitamin C). In contrast, spleen tissue (Table 3) of β -glucan-supplemented pigs had a greater ($P < 0.01$) relative abundance of TNF- α .

Interleukin-1 β and its receptor antagonist mRNA expressions were varied among the tissues. Interleukin-1 β mRNA expression in the intestine (Table 3) tended to be greater ($P = 0.09$) for vitamin C and β -glucan groups compared with the control and combination supplemented groups, and IL-1Ra expression was greater

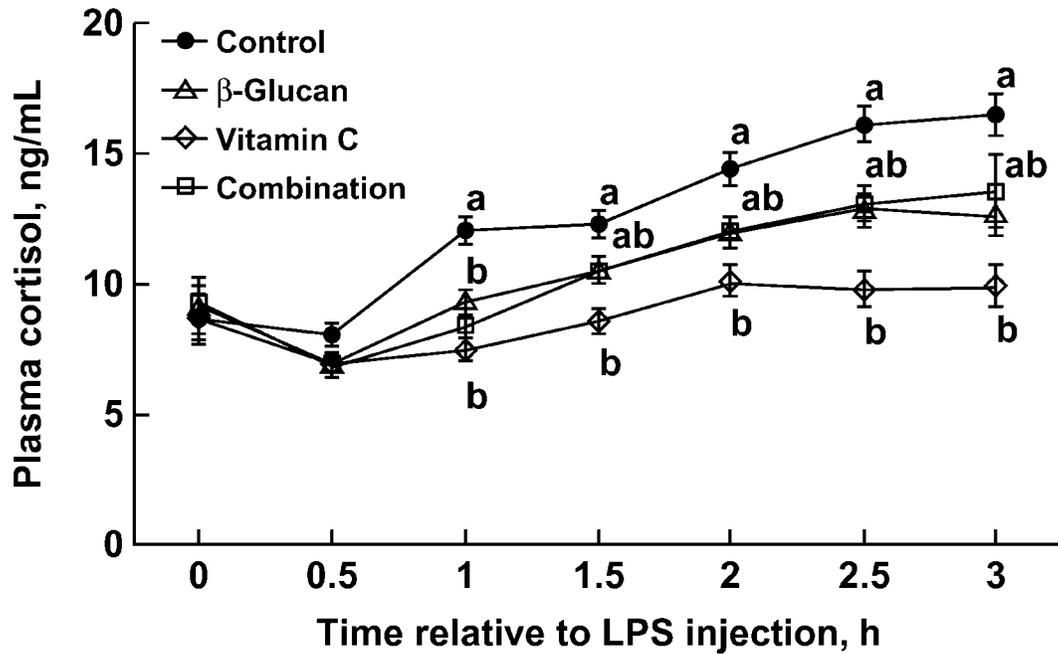


Figure 1. Cortisol response profiles after a lipopolysaccharide (LPS) injection of pigs fed the control, β -glucan (2.5%), vitamin C (vitamin C, 75 ppm), or both β -glucan and vitamin C (combination; β -glucan, 2.5% and vitamin C, 75 ppm) supplements for 4 wk beginning 36 h after birth. Main effect of vitamin C ($P = 0.06$); β -glucan \times vitamin C interaction ($P = 0.07$); time effect ($P < 0.01$). ^{a,b}Means within a time with differing superscripts are different ($P < 0.05$). $n = 8$ pigs per treatment.

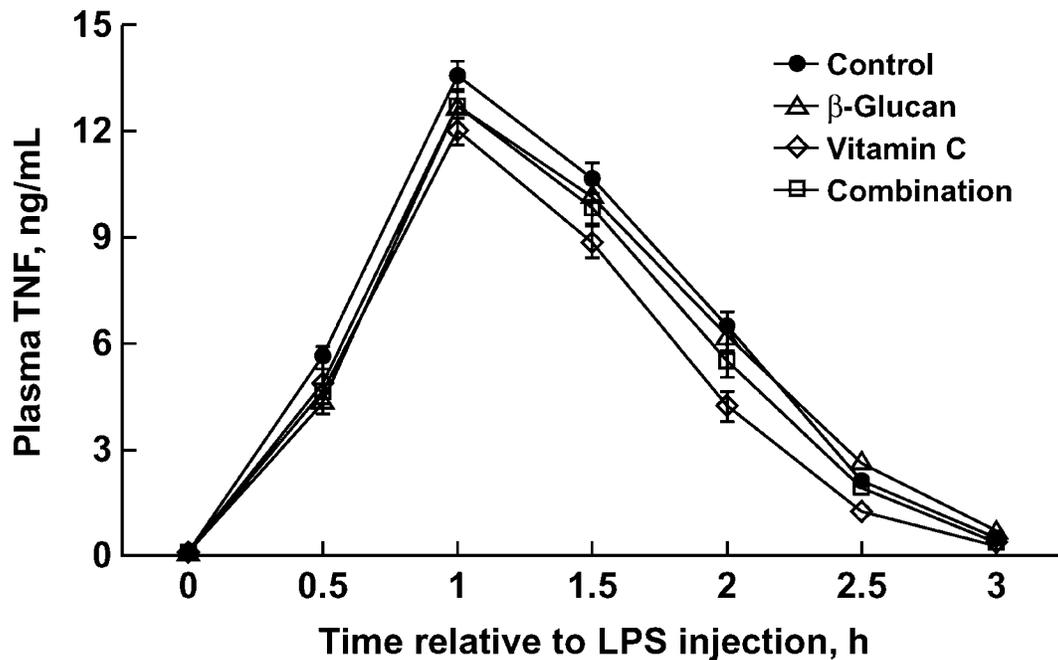


Figure 2. Plasma tumor necrosis factor (TNF)-alpha concentrations after a lipopolysaccharide (LPS) injection of pigs fed the control, β -glucan (2.5%), vitamin C (75 ppm), or both β -glucan and vitamin C (combination; β -glucan, 2.5%, and vitamin C, 75 ppm) supplements for 4 wk beginning 36 h after birth. Beta-glucan \times vitamin C interaction ($P = 0.08$); time effect ($P < 0.01$); time \times treatment interaction ($P = 0.09$). $n = 8$ pigs per treatment.

Table 3. Relative abundance (mean \pm pooled SE) of cytokine mRNA expression from intestine, liver, lung, and spleen samples using hypoxanthine guanine phosphoribosyl transferase as an internal standard

Tissue	Item	Treatment ¹				SE	Significance ²		
		Control	β -glucan	Vitamin C	Combination		β -Glucan	Vitamin C	Interaction
Intestine	TNF- α ³	0.59 ^b	1.46 ^{ab}	3.28 ^a	0.51 ^b	0.34	*	*	**
	IL-1 β	4.75	16.28	14.68	5.37	5.86			†
	IL-1Ra ⁴	11.5 ^b	28.7 ^{ab}	29.8 ^a	10.2 ^b	7.93			*
	IL-1 β :Ra	0.43	0.44	0.38	0.47	0.07			
Liver	TNF- α	2.97 ^a	3.22 ^a	7.39 ^b	1.43 ^a	1.02	**		**
	IL-1 β	13.1	4.5	10.7	8.5	3.6			
	IL-1Ra	434	723	2,115	1,231	301		**	†
	IL-1 β :Ra	0.02	0.01	0.01	0.01	0.004		**	†
Lung	TNF- α	0.52	1.01	3.5	2.79	0.76		**	†
	IL-1 β	1.25	0.74	0.80	0.83	0.21			
	IL-1Ra	8.79	7.75	10.52	9.41	1.29			
	IL-1 β :Ra	0.16	0.09	0.08	0.11	1.03			
Spleen	TNF- α	9.9	29.6	18.9	27.1	3.8	**		
	IL-1 β	1.75	2.99	2.46	2.78	0.64			
	IL-1Ra	8.5	10.52	10.22	7.41	1.86			
	IL-1 β :Ra	0.20	0.44	0.26	0.60	0.17			†

^{a,b}Means within the same amplicon and tissue with differing superscripts differ ($P < 0.01$).

¹Piglets were given no oral supplement (control), or β -glucan (glucan, 2.5%), vitamin C (75 ppm), or both (combination, β -glucan, 2.5% and vitamin C, 75 ppm) in milk replacer for 2 wk then in feed for 2 wk. $n = 8$ pigs per treatment.

²Means within an amplicon differ, † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$. Means separations were used for treatments when interactions of β -glucan and vitamin C were detected.

³TNF- α = Tumor necrosis factor-alpha.

⁴Ra = Receptor antagonist.

($P < 0.05$) for vitamin C and β -glucan compared with the control and combination supplemented groups. Liver IL-1 β mRNA expression (Table 3) was not different among treatments, but the IL-1Ra mRNA expression was greatest ($P < 0.01$) for vitamin C and combination (vitamin C main effect). The lung (Table 3) and spleen (Table 3) tissues did not have different IL-1 β and IL-1Ra mRNA expression due to treatments.

Behavior

Pigs spent most of their time sleeping before the challenge (Figure 3). However, the percentage of pigs awake at several observations before LPS injection differed among treatments. More pigs ($P < 0.05$) on the β -glucan supplement were sleeping at 0710. At 0850, 50 and 37% of the control and vitamin C pigs slept, respectively, whereas 100% of the combination and β -glucan pigs slept. The LPS i.v. challenge was given at 0900. The injection awoke many of the pigs; more slept ($P < 0.05$) at 0900 than at 0910. By 0940, the preinjection differences in sleeping patterns were evident; vitamin C pigs slept less ($P < 0.05$) than the combination or β -glucan pigs, and at 0950 the vitamin C pigs slept less ($P < 0.05$) than the β -glucan pigs (50 and 100%, respectively).

The percentage of pigs lying at 0700 was less ($P < 0.05$) for the control compared with the β -glucan pigs (data not shown). Again at 0800, fewer ($P < 0.05$) control

pigs were lying than the β -glucan or combination pigs. At the time of the injection, most of the pigs were lying (100 and 88% of control and β -glucan and combination, respectively), but fewer ($P < 0.05$) of the β -glucan and combination pigs (13 and 25%, respectively) were lying at 0910. However, at 0920, fewer ($P < 0.05$) of the vitamin C pigs were lying compared with the combination pigs (50 and 100%, respectively). There was no difference among treatments in the frequency of pigs vomiting, scouring, walking, nosing, or playing at any time point over the 5-h observation period.

DISCUSSION

Pigs had greater ADG when given β -glucan with vitamin C. Most notable was the reduced lag after weaning for the pigs given the combination supplement. The greater ADG is the same effect seen in a previous study with preruminant dairy calves given the same products (McKee et al., 2000) and is in contrast to weanling pigs that did not have a gain or feed efficiency response to β -glucan feeding (Mao et al., 2005). Taken together, it seems that feeding the β -glucan before weaning is beneficial for gain or feed efficiency and that supplemental ascorbic acid is needed to obtain the greatest response to β -glucan feeding. How β -glucan plus ascorbic acid affects the use of liquid diets is not known. The energy component of the yeast cell wall product added approximately 0.015 kg/d of gain. In the current

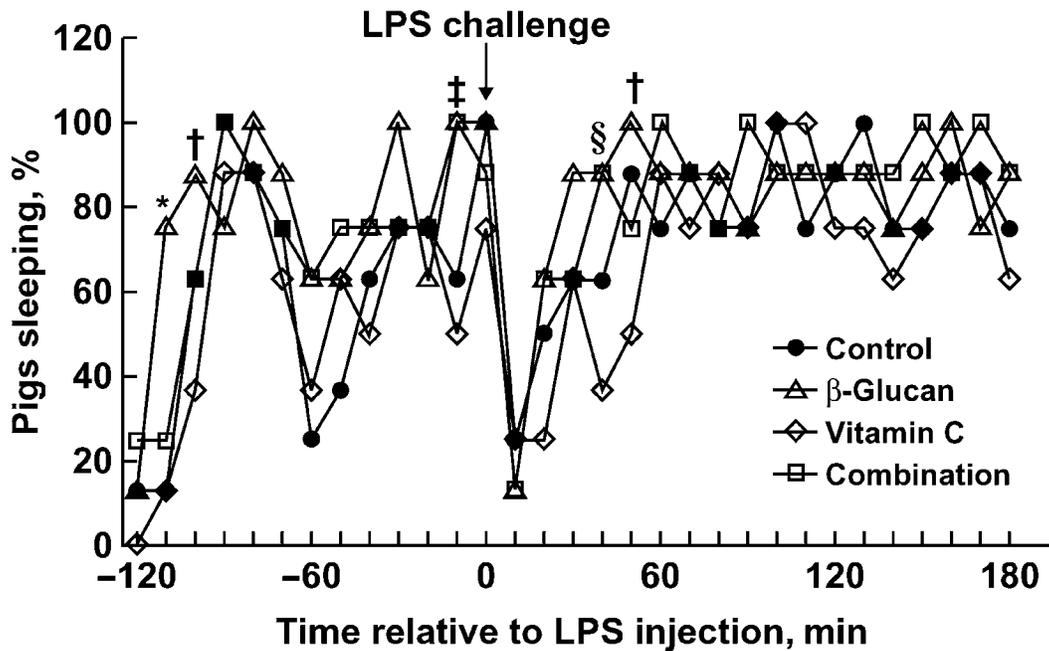


Figure 3. Percentage of pigs sleeping after a lipopolysaccharide (LPS) injection of pigs fed the control, β -glucan (glucan, 2.5%), vitamin C (vitamin C, 75 ppm), or both β -glucan and vitamin C (combination; β -glucan, 2.5% and vitamin C, 75 ppm) supplements for 4 wk beginning 36 h after birth. χ^2 analysis at $P < 0.05$ for: * β -glucan vs. 3 other treatments; †vitamin C vs. glucan; ‡vitamin C and control vs. glucan and combination; and §, vitamin C vs. both β -glucan treatments. $n = 8$ pigs per treatment.

study, pigs given vitamin C treatment had BW gain comparable with the controls. This is in contrast to the finding of Mahan et al. (1994) that swine given supplemental vitamin C had improved BW gain and gain to feed response for the first 2 wk postweaning. Yen and Pond (1981, 1984, 1987) found inconsistent results with vitamin C supplementation in weanling pigs. In 1981, they found increased ADG and ADFI; however, in 1984, ADG and ADFI were reduced when supplemented with vitamin C. Differences among studies such as these can often be attributed to fluctuating environmental stressors including variations in the endemic pathogen loads within different environments. However, these data must be interpreted with caution because the pigs in the current study were housed individually after weaning. Therefore, the normal mixing stress and competition for feed that occurs at weaning was not experienced by the pigs of the current study.

Vitamin C-supplemented pigs in this study had greater granulocyte numbers and lymphocyte numbers before the LPS challenge compared with the other treatment groups. Three hours after the LPS was given, granulocyte and lymphocyte numbers decreased for all treatments, although the vitamin C-supplemented pigs were able to preserve more cells than the other treatment groups. This is likely due to the antioxidant properties of vitamin C, thereby increasing cell structural integrity and preventing cell death, allowing more cells to contribute to this immune response.

We found no difference in ACTH among treatments despite the fact that treatment differences were observed for cortisol profiles after the LPS challenge. This may be due to the time of sampling or to the dynamics associated with ACTH secretion. Cortisol concentrations increased in all treatment groups 1 h after receiving LPS. However, pigs treated with vitamin C alone had lower concentrations of cortisol than controls. Weanling pigs fed β -glucans had reduced cortisol responses after an i.m. LPS challenge (Mao et al., 2005). Cytokines that affect the hypothalamic-pituitary-adrenocortical axis were attenuated in the β -glucan fed pigs of that study. This was in contrast to the cytokine response of the current study.

This study showed that vitamin C-supplemented pigs tended to have less TNF- α in the plasma compared with the other treatment groups. This may indicate that the greater cortisol concentrations in the β -glucan, combination, and control treatment groups could be attributed to more cytokine release peripherally, initiating a greater response at the hypothalamic-pituitary-adrenocortical axis. Vitamin C also had an impact on plasma TNF- α protein.

The influence of the supplements on cytokine expression varied among tissues. An interaction of the supplements persisted in intestine and liver samples. Beta-glucan enhanced TNF- α expression and the ratio of IL-1 β to its receptor antagonist in the spleen, but vitamin C had the predominant effect in lung tissues. Vitamin

C has been shown to be important in the reduction of lung damage caused by smoking (Block et al., 2004; de Sousa et al., 2005). In contrast, plasma TNF- α was reduced by vitamin C treatments. These data point to the importance of the microenvironment effect on the measurements. For example, the immune measurements (cytokine expression) from lung tissues do not always correspond to immune cell measures in the intestine or spleen tissues.

Pigs experiencing an immune challenge typically display a febrile response as well, which is induced by pyrogenic cytokines such as IL-1 and TNF- α (Hart 1988; Johnson et al., 1993). Clinical signs that animals exhibit during an immune response such as inactivity, sleepiness, and decreased eating and drinking behavior are believed to be conducive to survival in the wild (Hart, 1988). In this study, the vitamin C-supplemented pigs tended to spend only one-half of the time lying compared with the other treatment groups 20 min after receiving LPS. They also slept less at 40 and 50 min after the LPS injection, which was around the time of peak concentrations of cortisol and TNF- α . This response could be attributed to the fact that the vitamin C-supplemented animals had less cytokine production available to reach the blood brain barrier, thereby reducing the sleeping behavior that is demonstrated with increased amounts of TNF- α and IL-1 β .

In the current study, feed was removed during the blood sampling period; therefore, we could not measure eating behavior. Although water was available, no drinking behavior was observed in the 5-h period with the 10-min scan samples. Calapai et al. (1991) showed that TNF- α and LPS reduce water and feed intake in rats. However, this is in contrast to the finding of Madsen and Kristensen (2005) who observed increased water consumption as an indicator of a diseased or stressed pig. In the present experiment, the sickness behavior was induced. The findings may have been different if we were monitoring a naturally occurring pathogenic state of the animal, with a lengthened time of onset. For instance, White et al. (2002) found immunoglobulin G concentrations were enhanced by including a yeast product in diets of weanling pigs, but microbial and growth measures were not changed from their controls. Those authors believe that the mechanism of immunoglobulin enhancement was by enhanced immune response to pathogens rather than to the yeast product itself.

In conclusion, β -glucan and vitamin C supplementation had immunomodulating effects in pigs with some evidence of a synergistic effect of the combination. This possible synergism merits further investigation to determine if the action of these 2 supplements fed together is effective in promoting pig growth and health under commercial handling systems.

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