

# The Effect of Phosphorylated Mannans on Growth and Immune Responses of Weanling Pigs

M.E. Davis<sup>1</sup>, C.V. Maxwell<sup>1</sup>, D.C. Brown<sup>1</sup>, G.F. Erf<sup>2</sup>, and T.J. Wistuba<sup>1</sup>

## Story in Brief

Phosphorylated mannans (MAN) derived from the yeast cell wall of *Saccharomyces cerevisiae* may have the potential to beneficially modulate immune function in the weanling pig, possibly providing an alternative to the use of dietary growth-promoting antibiotics. In this study, 32 pigs (19 d of age and 12.6 lb initial BW) were randomly assigned to 16 pens in an environmentally-controlled nursery to determine the effects of dietary supplementation with mannans on growth and immune function. Average daily gain and F/G were improved ( $P < 0.05$ ) when pigs were fed diets supplemented with mannans from day 0 to 14 and in the overall experiment after weaning. Percentage of neutrophils was lower ( $P = 0.08$ ) and percentage of lymphocytes was higher ( $P < 0.05$ ) in blood from pigs fed mannans compared to those fed the basal diet. Lamina propria macrophages isolated from pigs fed diets containing mannans phagocytosed a greater ( $P = 0.05$ ) number of sheep red blood cells than those isolated from pigs fed the basal diet. On d 19 after weaning, pigs fed diets supplemented with MAN had a greater ( $P < 0.10$ ) proportion of CD14<sup>+</sup> lamina propria leukocytes than control pigs. On d 21 after weaning, the proportion of CD14<sup>+</sup>MHCII<sup>+</sup> lamina propria leukocytes was lower ( $P < 0.10$ ) when pigs were fed MAN compared to control pigs. Supplementation of mannans in the diets of weanling pigs improves gain and efficiency, and modulates the immune capabilities of the weanling pig, both systemically and enterically.

## Introduction

The addition of antibiotic growth promoters to swine diets is a common practice, particularly to the diets of newly-weaned pigs. However, there has been increasing pressure on the industry to decrease or discontinue these additions because of the potential development of antibiotic resistance within bacterial populations. The need for alternative methods to improve growth and efficiency of swine production and to modulate the pig's natural ability to fight disease has prompted the scientific investigation of several feed additives and their ability to positively alter immune function. In a similar manner to the action of antibiotics added to the diet, phosphorylated mannans (MAN) have the ability to alter the microbial population in the intestinal tract, and have also been reported to alter immune function in swine. However, the mechanism by which MAN functions to modulate health is not well defined. Before the benefits of MAN can be successfully utilized in swine production systems, a better understanding of its mode of action is needed. Thus, the specific objectives of this study were to: 1) evaluate the effects of MAN on gain, feed intake, and feed efficiency of pigs weaned to a conventional, on-site nursery facility, and 2) measure the immunomodulatory effects of MAN.

## Experimental Procedures

A total of 32 pigs averaging 19 d of age and 12.6 lb of initial BW were weaned and randomly distributed within 16 pens, so that two pigs were contained in each pen. Dietary treatments consisted of a typical starter pig diet (10% SBM, 17.5% whey, 8.5% fish meal, 3.7% spray-dried animal plasma, 2% spray-dried blood cells, and antibiotics) with or without the addition of 0.3% MAN. Diets were assigned to pens in a completely randomized design. Dietary treatments were administered throughout the entire experimentation period.

Pig BW and feed intake were determined on d 14 and 21 of the experiment to calculate ADG, ADFI, and F/G. On d 14, pigs were bled via vena cava puncture to obtain a whole blood sample in tubes containing EDTA for the isolation of peripheral blood mononuclear cells to determine lymphocyte proliferation response and the phagocytic ability of blood monocytes/macrophages.

Pigs were euthanized by lethal injection of sodium pentobarbital on d 19, 21, 24, and 26 after weaning, so that four randomly selected pens (two pens representing each dietary treatment) were sampled on each day. Prior to euthanization, pigs were bled via vena cava puncture to determine differential blood leukocyte concentrations and flow cytometric analysis. After euthanization, 40 cm of jejunal tissue was obtained from each pig, and lamina propria leukocytes were isolated to determine macrophage phagocytic ability and for flow cytometric analysis.

Data were analyzed as a completely randomized design with pen as the experimental unit. The model included the effects of dietary treatment when analyzing ADG, ADFI, F/G, and monocyte/macrophage phagocytosis. Initial BW was used as a covariate when analyzing ADG, ADFI, and F/G. The model included dietary treatment, sampling day, and the treatment x day interaction when analyzing differential blood leukocytes and flow cytometric data. Data were analyzed using the General Linear Model procedure of SAS (SAS Inst., Inc., Cary, NC).

## Results and Discussion

Pigs fed diets supplemented with MAN had greater ( $P < 0.05$ ) ADG and lower ( $P < 0.05$ ) F/G, and tended to have greater ( $P = 0.11$ ) ADFI than pigs fed the control diet from d 0 to 14 after weaning (Table 1). Although neither ADG, ADFI, nor F/G were altered ( $P > 0.40$ ) as a result of dietary treatment from d 14 to 21 after weaning, the improvement in ADG and F/G was maintained in the overall experiment ( $P < 0.05$ ). This was reflected by the greater ( $P < 0.05$ )

<sup>1</sup> Department of Animal Science, Fayetteville

<sup>2</sup> Center for Excellence in Poultry Science, Fayetteville

BW of pigs fed MAN on d 14 and d 21 of the experiment compared to pigs fed the control diet. This observation is consistent with previous research, in which the addition of MAN to weanling pig diets was reported to increase gain and efficiency (Davis et al., 2002; Kim et al., 2000).

The percentage of lymphocytes increased ( $P < 0.05$ ) and the percentage of neutrophils decreased ( $P = 0.08$ ) in blood obtained from pigs fed MAN compared to control pigs (Table 2). Although not significantly different, the increase in the concentration of lymphocytes and the decrease in the concentration of neutrophils resulted in a numerically lower ( $P = 0.14$ ) neutrophil-to-lymphocyte ratio when pigs were fed diets containing MAN compared to control pigs. An increase in the neutrophil:lymphocyte ratio is associated with stress in poultry (Gross and Siegel, 1983), and pigs exposed to stress have been reported to have an elevated proportion of neutrophils in the blood (Morrow-Tesch et al., 1994). The alterations in blood leukocyte proportions observed in this study indicate that MAN supplementation may alleviate some of the effects of weaning stress in young pigs.

Dietary treatments provided to pigs following weaning did not alter ( $P > 0.50$ ) the percentage of phagocytic monocytes/macrophages, or the number of sheep red blood cells (SRBC) consumed by phagocytic monocytes/macrophages isolated from peripheral blood (Table 3). Although the percentage of phagocytic macrophages isolated from the jejunal lamina propria was not altered by dietary treatment, phagocytic macrophages isolated from pigs fed MAN consumed a greater ( $P = 0.05$ ) number of SRBC per phagocytic macrophage than pigs fed diets without MAN (Table 3). On d 19 after weaning, pigs fed diets supplemented with MAN had a greater ( $P < 0.10$ ) proportion of CD14<sup>+</sup> lamina propria leukocytes than control pigs (Table 4). On d 21 after weaning, the proportion of CD14<sup>+</sup> leukocytes isolated from the peripheral blood ( $P < 0.05$ ) and CD14<sup>+</sup>MHCII<sup>+</sup> lamina propria leukocytes ( $P < 0.10$ ) was lower when pigs were fed MAN compared to control pigs. Gelderman et al. (1998) reported that macrophage function can be enhanced by the binding of mannose to receptors on the macrophage cell surface. The

increase in the ability of jejunal lamina propria macrophages isolated from pigs fed MAN to phagocytose SRBC in this experiment may be a result of the exposure of macrophages to MAN in the enteric environment. Basta et al. (1999) reported that CD14 expression on monocytes was down-regulated as they differentiated into macrophages, and their phagocytic and bacteriocidal activities increased. Therefore, low CD14 and MHC-II expression on the cell surface of monocytes/macrophages may be indicative of their predominantly phagocytic activities. The decrease in the proportions CD14<sup>+</sup>MHCII<sup>+</sup> lamina propria leukocytes observed in this study when pigs were fed MAN compared to control pigs suggests an association with increased macrophage activation in the form of phagocytosis. Moreover, this association is corroborated by the greater number of SRBC phagocytosed by phagocytic macrophages isolated from the jejunum of pigs fed MAN.

## Implications

Phosphorylated mannans have the potential to serve as a growth-enhancing additive in the diets of newly-weaned pigs. Although the addition of MAN to weaned pig diets altered the immune characteristics measured in this study, further investigation is warranted to discern the mechanism by which mannans alter systemic and enteric immune function.

## Literature Cited

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**Table 1. Growth response of weanling pigs fed phosphorylated mannans or a control diet.<sup>a</sup>**

Item	CONTROL	MAN	SEM	P=
d 0 to 14				
ADG, lb	0.34	0.56	0.07	0.049
ADFI, lb	0.58	0.69	0.04	0.106
Feed/gain	1.76	1.21	0.11	0.005
D 0 to final				
ADG, lb	0.52	0.69	0.05	0.033
ADFI, lb	0.79	0.86	0.04	0.243
Feed/gain	1.56	1.26	0.06	0.008
Pig weight, lb				
Initial	11.90	13.11	0.50	0.108
d 14	17.26	20.32	0.95	0.050
d 21	22.43	26.11	1.05	0.034

<sup>a</sup> Values are means of eight pens representing each dietary treatment. Initial BW was used as a covariate when analyzing ADG, ADFI, feed/gain, and pig BW.

<sup>3</sup> CD14<sup>+</sup> indicates leukocytes positive for the cluster of differentiation-14 cell surface marker, indicative of monocytes/macrophages.

<sup>4</sup> MHCII<sup>+</sup> indicates leukocytes positive for major histocompatibility complex-II, an antigen-presenting molecule on the surface of many types of leukocytes.

**Table 2. Differential leukocyte proportions of weanling pigs fed phosphorylated mannans or a control diet.<sup>a</sup>**

Item	Control	Mannan	SEM	P=
Leukocytes (%) <sup>b</sup>				
Neutrophils	52.8	45.3	2.7	0.080
Lymphocytes	42.8	50.7	2.0	0.026
Monocytes	0.68	0.4	0.23	0.401
Eosinophils	3.7	3.6	0.8	0.956
Neutrophil-to-lymphocyte ratio	1.39	1.05	0.15	0.142

<sup>a</sup> Values are means of eight pens representing each dietary treatment.

<sup>b</sup> Leukocyte proportions are presented as a percentage of total white blood cell counts within the peripheral blood.

**Table 3. Macrophage phagocytosis response of weanling pigs fed phosphorylated mannans or a control diet.<sup>a</sup>**

Item	CONTROL	MAN	SEM	P=
Macrophage phagocytosis <sup>b</sup>				
Blood				
% Phagocytic	15.7	16.5	1.73	0.769
Avg. SRBC <sup>c</sup>	1.62	1.70	0.07	0.432
Lamina propria				
% Phagocytic	24.2	26.7	1.94	0.366
Avg. SRBC <sup>c</sup>	2.31	2.63	0.11	0.051

<sup>a</sup> Values are means of eight pens representing each dietary treatment.

<sup>b</sup> Percentage of phagocytic macrophages and average number of sheep red blood cells phagocytosed from macrophages isolated from the blood at d 14 after weaning.

<sup>c</sup> Values represent the average number of sheep red blood cells (SRBC) consumed by phagocytic macrophages.

**Table 4. The effect of phosphorylated mannan (MAN) supplementation on the proportions of blood and jejunal lamina propria CD14<sup>+</sup> and CD14<sup>+</sup>MHCII<sup>+</sup> leukocytes in weanling pigs at 19, 21, 24, and 26 d after weaning.<sup>a</sup>**

MAN (%)	Day	Blood		Lamina propria	
		CD14 <sup>+</sup>	CD14 <sup>+</sup> MHCII <sup>+</sup>	CD14 <sup>+</sup>	CD14 <sup>+</sup> MHCII <sup>+</sup>
0	19	2.6 ± 0.6	7.9 ± 1.7	6.9 ± 1.6 <sup>z</sup>	2.9 ± 1.5
0.3		4.3 ± 0.6	6.4 ± 1.7	13.5 ± 1.6 <sup>y</sup>	7.4 ± 1.5
0	21	27.1 ± 1.0 <sup>w</sup>	23.0 ± 1.0	32.3 ± 4.2	18.6 ± 3.2 <sup>y</sup>
0.3		20.3 ± 1.0 <sup>x</sup>	21.1 ± 1.0	17.9 ± 4.2	3.3 ± 3.2 <sup>z</sup>
0	24	9.8 ± 3.8	4.3 ± 1.2	9.4 ± 1.4	5.9 ± 1.7
0.3		14.3 ± 3.8	6.0 ± 1.2	5.6 ± 1.4	2.2 ± 1.7
0	26	28.2 ± 2.4	15.6 ± 2.6	6.4 ± 3.1	3.3 ± 2.3
0.3		28.5 ± 2.4	24.2 ± 2.6	8.5 ± 3.1	4.6 ± 2.3

<sup>a</sup> Blood and jejunal tissue were obtained on d 19, 21, 24 and 26 after weaning from eight pigs (four from each dietary treatment). Leukocytes from both tissues were immunofluorescently stained with mouse anti-pig monoclonal antibodies specific for CD14 and MHCII. Values represent the mean proportions ± SEM.

<sup>w,x</sup> For each day, means within a column without a common superscript differ ( $P < 0.05$ ).

<sup>y,z</sup> For each day, means within a column without a common superscript differ ( $P < 0.10$ ).