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Marcio Antonio Dornelles Goncalves

Steven S. Dritz

Joel M. DeRouchey

*See next page for additional authors*

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# Effects of algae-derived $\beta$ -glucans with zinc on nursery pig growth performance and immune response under commercial conditions

## Authors

Marcio Antonio Dornelles Goncalves, Steven S. Dritz, Joel M. DeRouchey, Michael D. Tokach, Robert D. Goodband, and Jason C. Woodworth

# Effects of Algae-Derived $\beta$ -Glucans with Zinc on Nursery Pig Growth Performance and Immune Response Under Commercial Conditions<sup>1,2</sup>

*M.A.D. Goncalves<sup>3</sup>, S.S. Dritz<sup>3</sup>, J.M. DeRouchey, M.D. Tokach, R.D. Goodband, and J.C. Woodworth*

## Summary

An experiment was conducted to determine the impact of increasing levels of Algamune ZPC (Algal Scientific Corporation, Plymouth, MI) on growth performance and porcine circovirus type 2 (PCV2)-specific immune response of nursery pigs housed under commercial conditions. Algamune ZPC is a polysaccharide-zinc complex feed additive composed of 35%  $\beta$ -1,3-glucan extracted from algae and 10% zinc. A total of 2,484 pigs (PIC 337 × 1050, initially 15.7 lb) were used in a 40-d trial. After feeding a common pelleted diet for 7 d after weaning, pigs were allotted to 1 of 6 dietary treatments in a randomized complete block design with 14 or 16 replicate pens and 27 pigs per pen. All pigs were vaccinated with PCV2 and *M. hyopneumoniae* vaccines (1 mL Foster PCV and 1 mL RespiSure-One; Zoetis, Florham Park, NJ) at d 3 after birth and at weaning. Blood samples of 72 pigs (12 pens per treatment) were collected on d 2, 18, and 38. The 6 experimental diets were fed in two phases (d 0 to 12 and 12 to 40). Dietary treatments included: a negative control diet fed in both phases (1,910 and 110 ppm of zinc oxide in Phase 1 and 2, respectively); the negative control diet with 104, 208, 423, and 625 ppm added Algamune ZPC for both Phase 1 and Phase 2; and a negative control diet with 423 ppm added Algamune ZPC fed during phase Phase 1 followed by the negative control in Phase 2.

From d 0 to 40, increasing Algamune ZPC tended to decrease then increase (quadratic,  $P = 0.09$ ) ADG and increase (linear,  $P = 0.10$ ) ADFI. No differences were observed in F/G. There were no differences ( $P > 0.54$ ) in ADG, ADFI or F/G in pigs fed 423 ppm Algamune ZPC in both phases compared with pigs fed 423 ppm Algamune ZPC only in Phase 1 and the negative control diet fed in Phase 2. The lowest removal rates were observed among pigs assigned to 423 ppm Algamune ZPC only in Phase 1 or in both phases (0 and 0.27%, respectively). No evidence of differences was detected in PCV2-neutralizing antibody titers on d 16, but the titers decreased on d 38 (linear,  $P = 0.04$ ) with increasing Algamune ZPC.

In conclusion, including up to 625 ppm of Algamune ZPC in nursery pig diets from 16 to 56 lb had minimal impact on growth performance. Also, modulation of the specific immune response to PCV2 on d 38 after weaning was negatively related to increasing Algamune ZPC under commercial conditions.

**Key words:**  $\beta$ -glucans, immune response, nursery

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<sup>2</sup> Appreciation is expressed to New Horizon Farms for use of pigs and facilities and to Lanny Bosma, Shannon Paulson, and Marty Heintz for technical assistance.

<sup>3</sup> Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

## Introduction

Feed additives that could modulate the immune response of nursery pigs may serve as an alternative to growth-promoting antimicrobials.  $\beta$ -glucans are polysaccharides containing only glucose and are found as cellulose in plants; cell walls of yeast, fungi, or bacteria; and bran of cereal grains. Research has shown that dietary inclusion of 0.025% of yeast-derived  $\beta$ -glucans in nursery pig diets increased ADG, ADFI, and pig BW on d 28 after weaning (Dritz et al., 1995<sup>4</sup>). In addition, pigs fed 0.025%  $\beta$ -glucans had an increased mortality rate compared with pigs fed the negative control or 0.05%  $\beta$ -glucans, but Li et al. (2006<sup>5</sup>) observed an improvement in the immune system of pigs fed yeast-derived  $\beta$ -glucans. Most research has been performed with  $\beta$ -glucans extracted from specific yeast cell wall components. A new product, Algamune ZPC (Algal Scientific Corporation, Plymouth, MI), contains  $\beta$ -glucans extracted from algae and is a polysaccharide-zinc complex (35%  $\beta$ -1,3-glucan and 10% zinc).

Therefore, the objective of this experiment was to determine the impact of Algamune ZPC on growth performance, removal rate, and PCV2-specific immune response of nursery pigs housed under commercial conditions.

## Procedures

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee.

The study was conducted at a commercial research nursery in southwestern Minnesota. The facility was totally enclosed, environmentally controlled, and mechanically ventilated. Pens were distributed across 2 rooms and had completely slatted flooring and deep pits for manure storage. Each pen was equipped with a 5-hole stainless steel dry self-feeder and a pan waterer for ad libitum access to feed and water. Daily feed additions to each pen were accomplished through a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of providing and measuring feed amounts for individual pens. A total of 2,484 pigs (PIC 337  $\times$  1050, initially 15.7 lb BW) were used in a 40-d trial. Pigs were weaned at 19 d of age and were initially fed a common pelleted diet for 7 d before the start of the experiment. On d 7 after weaning, pigs were weighed and pens of pigs were allotted to 1 of 6 dietary treatments in a randomized complete block design. Each treatment had 14 or 16 replicate pens and 27 pigs per pen, with each pen containing a mix of barrows and gilts.

All pigs were vaccinated with porcine circovirus type 2 (PCV2) and *M. hyopneumoniae* vaccines (1 mL Foster PCV and 1 mL RespiSure-One; Zoetis, Florham Park, NJ) on d 3 after birth and at weaning. Blood samples of 72 pigs (12 pens per treatment, 1 pig per pen) were collected on d 2, 18, and 38 of the trial and were submitted to the Kansas State Veterinary Diagnostic Laboratory to measure PCV2 antibody titers using indirect immunofluorescence (IFA) assay.

<sup>4</sup> Dritz, S.S., J. Shi, T.L. Kielian, R.D. Goodband, J.L. Nelssen, M.D. Tokach, M.M. Chengappa, J.E. Smith, and F. Blecha. 1995. Influence of dietary beta-glucan on growth performance, nonspecific immunity, and resistance to *Streptococcus suis* infection in weanling pigs. J. Anim. Sci. 73:3341–3350.

<sup>5</sup> Li, J., D.F. Li, J.J. Xing, Z.B. Cheng, and C.H. Lai. 2006. Effects of  $\beta$ -glucans extracted from *Saccharomyces cerevisiae* on growth performance, and immunological and somatotrophic responses of pigs challenged with *Escherichia coli* lipopolysaccharide. J. Anim. Sci. 84:2374–2381.

All dietary treatments were corn-soybean meal–based with 5% select menhaden fish meal, 10% spray-dried whey, and 10% dried distillers grains with solubles (DDGS) in Phase 1 and 20% DDGS in Phase 2 (Table 1). Phase 1 diets were fed for 12 days, and Phase 2 diets were fed for 28 days. Algamune ZPC was added at the expense of corn. The 6 experimental diets included a negative control diet fed in both phases (1,910 and 110 ppm of zinc oxide in Phases 1 and 2, respectively); or the negative control diets with 104, 208, 423, or 625 ppm added Algamune ZPC for both Phase 1 and Phase 2; and the negative control diet with 423 ppm added Algamune ZPC fed during phase Phase 1, followed by the negative control diet without any added Algamune ZPC fed in Phase 2. To provide the diets with intermediate levels of  $\beta$ -glucans, the negative control diet and the 625 ppm Algamune ZPC were blended using the robotic feeding system. Diets were fed in meal form and were manufactured at the New Horizon Farms Feed Mill (Pipestone, MN). Pig weight and feed disappearance were measured on d 0, 7, 12, 20, 26, 33, and 40 of the trial to determine ADG, ADFI, and F/G. Pig inventory was continually monitored to determine removal rate and mortality.

Diet samples were taken from 6 feeders per dietary treatment on each weigh day and combined to form a composite sample within each phase. Samples of the diets were submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CP, Ca, P, ash, and crude fat.

Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. For statistical analysis of growth performance, the fixed effect was the dietary treatment, and room and weight block within room were included in the model as a random effects. The effects of increasing dietary Algamune ZPC on performance criteria were determined by linear and quadratic polynomial contrasts with coefficients adjusted for unequally spaced inclusions. A single degree of freedom contrast was used to compare performance of pigs fed 423 ppm in both phases with pigs fed 423 ppm in Phase 1 and the negative control diet in Phase 2. Results for treatment criteria were considered significant at  $P \leq 0.05$  and tendencies from  $P > 0.05$  to  $P \leq 0.10$ .

For the immune response analysis, PCV2 antibody titers were transformed by obtaining the log base 2 of the reciprocal of the highest dilution antibody detected in the sample. PCV2 antibody titer results were then analyzed using repeated measures to determine the effects of Algamune ZPC level on response criteria over time and the treatment  $\times$  time interactions. The fixed effects used were dietary treatment, serum sampling period, and treatment  $\times$  period interaction. Log-transformed PCV2 neutralizing antibody titers at day 2 were included as a covariate. Weight block within serum sampling period and pen within dietary treatment were included in the model as random effects.

## Results and Discussion

Chemical analyses of the complete diets (Table 2) showed that, as expected, DM, CP, Ca, P, ash, and crude fat levels were similar across dietary treatments.

From d 0 to 40, increasing Algamune ZPC tended to decrease then increase (quadratic,  $P = 0.09$ ) ADG and increased (linear,  $P = 0.10$ ) ADFI (Table 3). No differences were observed in F/G. There were no differences in ADG, ADFI, or F/G in pigs fed 423 ppm

Algamune ZPC in both phases compared with pigs fed 423 ppm Algamune ZPC only in Phase 1 and the negative control diet in Phase 2. Hiss and Sauerwein (2003<sup>6</sup>) also observed a tendency for increased ADFI without changing feed efficiency when feeding  $\beta$ -glucans.

Although removal rates were low across all treatments compared with industry standards, the numerically lowest removal rates were in pigs assigned to the dietary treatment of 423 ppm Algamune ZPC only in Phase 1 or in both phases (0.0 and 0.27%, respectively).

No statistical differences were detected in PCV2-neutralizing antibody titers on d 16, but titers decreased on d 38 (linear,  $P = 0.04$ ) with increasing Algamune ZPC. The mean of all bleeding periods showed a tendency (linear,  $P = 0.07$ ) toward decreased PCV2-neutralizing antibody titers as Algamune ZPC concentration increased. This result was unexpected, because increasing  $\beta$ -glucans was expected to increase specific immune response, but these results agree with the study conducted by Hiss and Sauerwein (2003<sup>6</sup>), in which no modulation of specific immune response was observed. Cheng et al. (2004<sup>7</sup>) found evidence of higher activity of cell-mediated immune response in poultry when feeding  $\beta$ -glucans compared with control pigs. In a study conducted in weaned pigs by Li et al. (2005<sup>8</sup>), a modulation of both humoral and cellular immunity was observed when feeding  $\beta$ -glucans. This result indicates that  $\beta$ -glucans can improve immunity of pigs. There was no difference in PCV2-neutralizing antibody titers between pigs fed 423 ppm in both phases and pigs fed 423 ppm in Phase 1 and the negative control diet in Phase 2.

In conclusion, adding up to 625 ppm of Algamune ZPC in nursery pig diets from d 7 to 40 after weaning (16 to 56 lb BW) had minimal impact on growth performance, and modulation of the specific immune response to PCV2 on d 38 after weaning was negatively related to increasing Algamune ZPC in diets under commercial conditions. Further research is needed to evaluate the effects of Algamune ZPC on nursery pig performance and immune response in herds with a higher degree of pathogen challenge than used in this study.

<sup>6</sup> Hiss, S., and H. Sauerwein. 2003. Influence of dietary  $\beta$ -glucan on growth performance, lymphocyte proliferation, specific immune response and haptoglobin plasma concentrations in pigs. *J. Anim. Phys. Anim. Nutr.* 87:2–11.

<sup>7</sup> Cheng, Y.H., D.N. Lee, C.M. Wen, and C.F. Weng. 2004. Effects of  $\beta$ -glucan supplementation on lymphocyte proliferation, macrophage chemotaxis and specific immune responses in broilers. *Asian-Aust. J. Anim. Sci.*, 17:1145–1149.

<sup>8</sup> Li, J., J. Xing, D. Li, X. Wang, L. Zhao, S. Lv, and D. Huang. 2005. Effects of  $\beta$ -glucan extracted from *Saccharomyces cerevisiae* on humoral and cellular immunity in weaned piglets. *Arch. Anim. Nutr.*, 59:303–312.

**Table 1. Diet composition (as fed basis)**

Item	Phase 1 <sup>1</sup>		Phase 2 <sup>2</sup>	
	Negative control	Algamune ZPC 625 ppm	Negative control	Algamune ZPC 625 ppm
Corn	44.73	44.66	45.73	45.67
Soybean meal (46.5% CP)	28.05	28.05	31.41	31.41
Dried distillers grains with solubles	10.00	10.00	20.00	20.00
Select menhaden fish meal	5.00	5.00	--	--
Spray-dried whey	10.00	10.00	--	--
Dicalcium phosphate (18.5 % P)	0.25	0.25	0.55	0.55
Limestone	0.78	0.78	1.15	1.15
Salt	0.30	0.30	0.35	0.35
L-lysine	0.28	0.28	0.45	0.45
DL-methionine	0.065	0.065	0.053	0.053
L-threonine	0.04	0.04	0.06	0.06
Zinc oxide	0.25	0.25	--	--
Trace mineral premix	0.100	0.100	0.100	0.100
Vitamin premix	0.125	0.125	0.125	0.125
Algamune ZPC <sup>3</sup>	--	0.0625	--	0.0625
Phytase <sup>4</sup>	0.038	0.038	0.025	0.025
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible (SID)				
amino acids, %				
Lysine	1.30	1.30	1.25	1.25
Isoleucine:lysine	68	68	69	69
Leucine:lysine	141	141	155	155
Methionine:lysine	33	33	31	31
Met & cys:lysine	57	57	57	57
Threonine:lysine	63	63	63	63
Tryptophan:lysine	19.1	19.1	19.2	19.2
Valine:lysine	75	75	77	77
Total lysine, %	1.49	1.49	1.45	1.45
ME, kcal/lb	1,504	1,503	1,492	1,491
SID Lysine:ME, g/Mcal	3.92	3.92	3.80	3.80
CP, %	24.4	24.4	24.6	24.6
Ca, %	0.73	0.73	0.67	0.67
P, %	0.64	0.64	0.56	0.56
Available P, %	0.50	0.50	0.40	0.40

<sup>1</sup>A common diet was fed for the first 7 d after weaning and followed by the Phase 1 diet fed from d 0 to 12 of the study.

<sup>2</sup>Phase 2 was fed from d 12 to 40 of the study.

<sup>3</sup> Algamune ZPC (Algal Scientific Corporation, Plymouth, MI) is a zinc metal polysaccharide complex that contains  $\beta$ -1,3-glucan from algae.

<sup>4</sup> OptiPhos 2000 (Huvepharma, Sheridan, IN) provided phytase at 853 and 569 phytase units (FTU)/lb with a release of 0.14% and 0.13% of available P for Phase 1 and Phase 2 diets, respectively.



**Table 2. Chemical analysis of diets containing Algamune ZPC (as fed-basis)<sup>1,2</sup>**

Item, %	Phase 1						Phase 2					
	Negative control	Algamune ZPC, ppm					Negative control	Algamune ZPC, ppm				
		104	208	423	625	423/0 <sup>3</sup>		104	208	423	625	423/0 <sup>3</sup>
DM	89.8	89.6	90.0	89.4	89.7	89.3	90.0	89.6	89.3	89.3	89.6	89.6
CP	24.5	24.7	24.5	23.9	23.5	24.0	24.0	25.0	25.4	25.5	24.5	24.5
Ca	0.82	0.85	0.83	0.83	0.79	0.86	0.79	0.80	0.78	0.83	0.83	0.83
P	0.63	0.60	0.60	0.64	0.62	0.62	0.54	0.52	0.54	0.55	0.55	0.55
Fat	3.8	3.7	3.5	3.5	3.9	3.7	3.9	3.8	3.8	3.7	4.0	4.0
Ash	5.8	6.1	6.0	6.0	5.9	6.0	5.3	5.3	5.2	5.4	5.3	5.3

<sup>1</sup> A composite sample consisting of 6 subsamples was performed a proximate analysis by Ward Laboratories, Inc. (Kearney, NE).

<sup>2</sup> Phase 1 and 2 diets were fed from d 0 to 12 and from d 12 to 40 of the study, respectively.

<sup>3</sup> 423 ppm of Algamune ZPC (Algal Scientific Corporation, Plymouth, MI) was fed in Phase 1 followed by the negative control diet in Phase 2.

**Table 3. Effects of Algamune ZPC on nursery pig growth performance and immune response under commercial conditions<sup>1,2</sup>**

	Negative control	Algamune ZPC, ppm					SEM	Probability, <i>P</i> <		
		104	208	423	625	423/0 <sup>5</sup>		Algamune ZPC <sup>3</sup>		423 ppm entire × only in Phase 1
								Linear	Quadratic	
d 0 to 40										
ADG, lb	1.01	0.99	0.99	1.00	1.02	1.01	0.03	0.21	0.09	0.65
ADFI, lb	1.52	1.49	1.51	1.51	1.55	1.53	0.06	0.10	0.13	0.54
F/G	1.51	1.51	1.52	1.52	1.53	1.52	0.01	0.32	0.80	0.67
BW, lb										
d 0	15.8	15.8	15.7	15.7	15.6	15.7	0.27	0.18	0.74	0.97
d 40	56.7	55.8	56.0	56.2	56.9	56.5	2.63	0.39	0.09	0.67
Removal rate, %										
d 40	0.50%	0.70%	0.47%	0.27%	0.47%	0.00%	--	--	--	--
Log-transformed PCV2-neutralizing antibody titers <sup>4</sup> , log <sub>2</sub>										
d 16	6.7	7.2	6.9	6.8	6.2	6.6	0.65	0.38	0.49	0.79
d 38	7.0	8.0	7.5	6.8	5.9	7.1	0.66	0.04	0.18	0.75
Overall	6.9	7.6	7.2	6.8	6.0	6.8	0.52	0.07	0.21	0.98

<sup>1</sup> A total of 2,484 nursery pigs (PIC 337 x 1050, initially 15.7 lb BW) with 27 pigs per pen and 14 or 16 pens per treatment.

<sup>2</sup> Algamune ZPC (Algal Scientific Corporation, Plymouth, MI) is a zinc metal polysaccharide complex that contains β-1,3-glucan from algae.

<sup>3</sup> Contrasts were determined using negative control and the different levels of Algamune ZPC inclusion.

<sup>4</sup> A total of 72 pigs (12 pens per treatment, 1 pig per pen) were sampled. An increase in the log-transformed PCV2-neutralizing antibody titer indicates an increased concentration of neutralizing antibody.

<sup>5</sup> 423 ppm of Algamune ZPC was fed in Phase 1 followed by the negative control diet in Phase 2.