

Title: Development of a Nutrition × Health Interaction Model to Study Nursery Pig Performance - NPB #12-185

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revised

Industry Summary

The goal of this research project was to use Porcine Circovirus 2 (PCV2) challenge model as an approach to study the interaction of nutrition and disease (e.g., PRRS, mycoplasma pneumonia, salmonella, etc.). A total of 96 weaned barrows (age 27 to 40 d; BW 7.1 kg) were housed (4 pigs/pen) in an environmentally-controlled nursery with ad libitum access to feed and water over a 28-d study. Forty-eight pigs were vaccinated (VAC) for PCV2 prior to arrival, while the remaining 48 pigs (PCV) were inoculated with PCV2 on d 0. Pigs were randomly assigned to 1 of 3 dietary treatments: 1) complex diet (CO; lactose, spray-dried plasma, spray-dried whey); 2) simple diet (SI; corn and SBM); or 3) prebiotic diet (GS; SI + 2.5% Grobiotic-S). From the data obtained in this experiment, the main conclusions are as follows: 1) As evidenced by reduced BW, ADFI, and ADG in PCV2-inoculated pigs compared to PCV2-vaccinated pigs, the PCV2-challenge model that was implemented was efficacious with respect to the utility of this model for future efforts in exploring nutrition × health interactions; 2) As expected, changes in immune biomarkers were consistent with PCV2 disease progression; and 3) The observation that there are transient and conflicting effects of time, diet, and PCV-status on feed efficiency indicates that digestibility may be reduced in PCV-challenged pigs and that this model may be used in the future to manipulate the diet to help facilitate greater assimilation of nutrients in pigs faced with a disease challenge

Key Words: diet complexity, health, pig, PCV2

Scientific Abstract

To investigate the effects of complex and prebiotic diets on nursery pigs inoculated with or vaccinated against PCV2 on growth performance and immune parameters, 96 weaned barrows (age 27 to 40 d; BW 7.1 kg) were housed (4 pigs/pen) in an environmentally-controlled nursery with ad libitum access to feed and water over a 28-d study. Forty-eight pigs were vaccinated (VAC) for PCV2 prior to arrival, while remaining 48 pigs (PCV) were inoculated with PCV2 on d 0. Pigs were randomly assigned to 1 of 3 diets: complex (CO; lactose, spray-dried plasma, spray-dried whey), simple (SI; corn and SBM), or simple + 2.5% Grobiotic-S (GS). Blood samples were obtained twice per week (d -2 to 28) for serum

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cytokine and PCV2-specific immunoglobulin (Ig) G and M quantification. No significant time, diet, or PCV-status interactions were observed for growth performance. Body weight tended ($P < 0.06$; d 14 to 28) to be greater in VAC (11.1 to 18.4 kg) compared to PCV (10.3 to 16.7 kg) pigs, and overall ADG ($P < 0.04$) and ADFI ($P < 0.03$) were greater in VAC (0.40 kg and 0.68 kg, respectively) compared to PCV (0.35 kg and 0.61kg, respectively) pigs. Overall, pigs fed CO (0.71 kg) had greater ($P < 0.02$) ADFI compared to GS (0.61 kg) pigs and tended ($P < 0.08$) to be greater than SI (0.63 kg) pigs. Pigs fed CO (0.60) had greater ($P < 0.05$) G:F (d 0 to 7) compared to GS (0.46) and SI (0.51); however, G:F ($P < 0.05$; d 21 to 28) was decreased in CO (0.59) pigs compared to SI (0.67) pigs. For IgG, a diet x PCV-status interaction was observed ($P < 0.04$); PCV pigs fed SI and GS had increased PCV2-specific IgG ($P < 0.05$) compared to CO pigs. No effects of PCV-status or diet were observed with respect to IL-12p40, IL-6, IL-8, IL-10, or TNF- α ; however, main effects of PCV-status (IFN- α ; $P < 0.01$) and diet (IL-1 β ; $P < 0.03$) were observed. Specifically, greater IFN- α was observed for PCV compared to VAC pigs on d 11 ($P < 0.02$) and 14 ($P < 0.02$), and pigs fed CO had greater overall IL-1 β compared to GS ($P < 0.04$) and SI ($P < 0.02$). Results indicate a complex nursery diet may improve post-weaning growth performance and affect immune response to PCV2 infection.

Introduction

Diet formulation and feeding a proper diet is critical to the health and well-being of young pigs. There are obvious benefits associated with feeding a balanced ration including improved feed efficiency, increased rate of growth and increased profitability. One consideration in feeding young pigs is diet complexity. Simple diets, formulated to meet the pigs nutritional requirements, are primarily comprised of corn and soybean meal. Complex diets containing milk products (e.g., dried whey) and specialty protein sources (e.g., fish meal, spray-dried animal plasma) have been used to stimulate feed intake and promote growth (Dritz et al., 1996; Mahan et al., 2004)

Porcine Circovirus type 2 (PCV2) is one of the top diseases causing economic loss to the pork industry. The PCV2 infection often facilitates other co-infections (Gillespie et al., 2009; Takada-Iwao et al., 2011; Opriessnig and Halbur, 2012) which is a necessary factor to form PCV2 associated diseases (PCVAD; e.g., post-weaning multi-systemic wasting syndrome). The prevention and treatment of PCVAD result in significant financial loss in terms of the vaccination, management intervention, and reduced performance (Alarcon et al., 2013). It has been estimated that the cost associated with PCVAD can range from 3 to 20 dollars/pig giving the total loss of up to 2 billion dollars in the U.S swine production (Gillespie et al., 2009). A major opportunity to reduce this economic burden is to define how nutrient requirements change during and subsequent to a PCVAD-related health challenge.

Sub-clinically infected pigs generally have reduced performance due to a combination of factors. First, the increased pro-inflammatory cytokine expression and secretion during infection induce a negative signal to alleviate hormone and growth factor action (Fernandez-Celemin et al., 2002; O'Connor et al., 2008) leading to the down-regulation of protein synthesis and up-regulation of protein degradation. Second, the over activation of immune system in responses to infection may lead to the reduced efficiency of energy and nutrient metabolism (Delmastro-Greenwood and Piganelli, 2013). Last, sub-clinically infected pigs have reduced feed consumption (Alarcon et al., 2013). Whereas the infected pigs apparently require different nutrient inputs compared to healthy pigs, no official feeding guidelines for infected pigs are established. The current proposal will help to elucidate how experimental PCV2 infection affects energy and nutrient digestibility and amino acid (Lysine) requirement in pigs. The results of this project will also expand the understanding of the interaction between diet quality and infection to construct an appropriate feeding program for infected pigs during and subsequent to a health challenge.

Producers typically manage post-weaning lag several ways including: 1) introducing newly-weaned pigs into clean (low pathogen load) facilities, and 2) providing nutrient-dense complex diets containing lactose (milk sugar), highly digestible proteins (e.g., fishmeal, spray- dried animal plasma), and antibiotics. Although most feed budgets allocate relatively small amounts feed during the nursery period (day 21 to day 45 to 55), these diets can be expensive. Also,

there has been increased pressure to decrease and even discontinue the use of nontherapeutic additions of antibiotics in swine diets. Therefore, management and nutritional strategies will be needed to replace current dietary antibiotic approaches.

Objectives

The proposed research project developed and refined a model to investigate nutrition x health interactions in the nursery pig. Specific objectives were: 1) to determine the effect of a complex nursery diet (compared to simple corn-soybean meal based one) on the development of gut microbial populations, pig immune parameters, and growth performance in nursery pigs inoculated for, or vaccinated against Porcine Circovirus 2 (PCV2); 2) use current genotyping technology to establish relationships between resistance or susceptibility to PCV2 (as indicated by specific titers) and how diet complexity may interact to affect post-weaning performance.

Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska, Lincoln.

Animals and Experimental Design. Initially, a total of one hundred and sixty weaned crossbred barrows (Large White x Landrace) were screened for PCV-2 specific Immunoglobulin (Ig) G and M by ELISA (Ingenasa, Madrid, Spain) from blood samples obtained at 14 d of age at the UNL ARDC Swine Unit (Mead, NE). Individuals (n = 96) with a sample-to-positive ratio (S/P) lower than 0.3 for passive IgG (all pigs were IgM negative) were included in the current experiment. At 16-17 days of age, one half (n = 48) of the pigs were vaccinated for PCVAD with a single dose of Ingelvac CircoFLEX vaccine (Boehringer Ingelheim). At weaning, all pigs were fed a standard corn-soybean meal diet (without antibiotics) until approximately 30 d of age at which time all pigs were transferred to the UNL Animal Science Complex (Lincoln, NE) where the experimental infection was conducted. Upon arrival at the UNL Animal Science complex, pigs (n = 96; ave 33.5 d of age; ave BW = 7.1 kg) were sorted by initial BW and PCV status (vaccinates or inoculates) and randomly assigned to 24 pens (4 pigs/pen; 4 pens/treatment) 2 d prior to the beginning of the experiment. Pens were randomly allotted to one of three dietary treatments: complex, simple, or simple + prebiotic (GroBioticS; International Ingredient Corp., St. Louis, MO). During the 28-d experiment, pigs were housed in a common room in 24 identical pens with a combination of slatted and solid surface flooring. The pens provided approximately 0.65 m² of floor space per pig. All pigs were allowed ad libitum access to diets and water.

Experimental Diets. Diets were formulated to meet or exceed NRC (2012) requirements with no antibiotic inclusion. Eight pens (n = 32 pigs) from each of the aforementioned 2 groups (vaccinates or inoculates) received either a complex nursery diet (fishmeal, spray dried whey, spray dried animal plasma, lactose, reduced corn and soybean meal), a simple nursery diet (no fishmeal, spray dried whey, spray dried animal plasma, lactose, or prebiotic), or the simple diet plus prebiotic (2.5% GroBiotic-S). Experimental diets were fed for a total of 30 d (2 d prior to inoculation and 4 wk subsequent to experimental infection). The ingredient composition and calculated analysis of experimental diets are presented in Table 1.

Experimental PCV2 Inoculation. The PCV2b isolate used in the experimental infection was recovered from a pig that had symptoms characteristic of PCV2 infection and is the same isolate used in previous experiments (McKnite et al., 2014). On d 0 (ave 33.5 d of age) all naïve pigs (n = 48) were infected intranasally (2 mL) and intramuscularly (1 mL) with a titer of 104.0 TCID₅₀/mL. Pigs were observed daily for clinical signs of infection and facial thermometers were used to monitor daily body temperature for 7 d post-inoculation.

Growth Performance. Pig BW and feed disappearance measurements were obtained at the beginning of the experiment and weekly thereafter (d 0, 7, 14, 21, and 28). Pig BW and feed disappearance data were used to calculate ADG, ADFI, and G:F.

Blood Collection. Blood samples were collected from each pig (3 to 5 mL) via jugular venipuncture on d -2 (two days prior to inoculation), 4, 7, 10, 14, 18, 21, and 28 before weighing using glass blood tubes containing no anticoagulant. Tubes containing blood samples were immediately placed on ice and allowed to clot overnight before harvesting serum by centrifugation (1,500 × g for 20 min at 4°C). Serum samples were aliquoted and stored at -80°C for subsequent analyses (described below).

Serum Immune Measures. For quantification of PCV2-specific IgG and IgM, serum samples obtained on d -2, 7, 14, 21, and 28 were utilized for quantification by ELISA (described above). Serum samples obtained on d 7, 11, 14, and 21 were analyzed by a Luminex Multiplex Immunoassay (ProcartaPlex Multiplex Immunoassays; Affymetrix eBioscience) according to the manufacturers instructions. The multiplex assay included the following cytokines: IL-12p40, IL-6, IL-10, TNF-alpha, IL-4, IFN-gamma, IL-8, IL-1beta, and IFN-alpha.

Tissue Collection. Tissue samples were collected from control pigs (n =12) 1 d prior to the start of the experiment and from experimental pigs (n = 1 pig/pen) at the conclusion of the experiment (d 28). Pigs were euthanized by exposure to CO₂. The sampling procedure was conducted as follows. Two-cm segments of duodenum (gut segment below pyloric opening) and ileum (gut segment before ileocecal valve) were collected for the determination of villous height (VH), villous circumference, crypt depth (CD), and villous height: crypt depth (VH:CD) ratio. Tissues were quickly fixed by addition of 5 to 10 mL 4% paraformaldehyde solution and stored at 4°C for 24 h before being washed by 70% ethanol. Samples were stored at 4°C until later analyses.

Gut Histological Measurements. Paraformaldehyde-fixed tissues were dehydrated by 3 washes of 100% ethanol and 3 washes of Citrisolv (Cat. No. 22-143-975; Fisherbrand) for 1 h per wash. Dehydrated tissues were infiltrated with warm paraffin overnight before being embedded in fresh paraffin (56°C) to generate paraffin blocks. The intestinal blocks were sectioned (7-cm thick) using the Reichert-Jung 2030 Biocut microtome (Rankin Biomedical Corporation, Holly, MI). The prepared slides were then de-paraffined and stained with haematoxylin and eosin. Images were recorded using an Olympus DP71 camera (Olympus, Center Valley, PA). The VH, CD, VH:CD ratio, and villous area were measured using Cell Sense standard software (Olympus, Center Valley, PA). For each intestinal sample, a minimum of 10 to 15 full villi and crypts were measured and averaged for statistical analysis. All measurements were performed without treatment awareness.

Statistical Analyses. All data were analyzed as a completely randomized design using the MIXED procedure (SAS Inst. Inc., Cary, NC). All means are presented as least-squares means (\pm SEM). Pen was considered an experimental unit and a random effect for growth performance and serum variables; whereas, pig is considered an experimental unit for gut histological analyses. The model included treatment as a fixed effect for growth performance, serum, and gut histological variables. For the immune variable data, the model included treatment and treatment × time (day) interaction. For the cell viability and proliferation data, each well was considered an experimental unit and a random effect. The statistical model included treatment as a fixed effect. The statistical model for all data consisted of the main effects of diet, LPS, time, and their interactions. Pen was considered a random effect for dietary factor.

Results

Growth performance data are summarized in Table 2. No significant diet × PCV-status (inoculated or vaccinated) interactions were observed for BW, ADFI, or G:F. With respect to BW, no main effect of diet was observed. However, vaccinated pigs tended ($P = 0.06$) to be heavier than inoculated pigs throughout the experimental period. With respect to ADFI, overall main effects of both diet ($P < 0.04$) and PCV-status ($P < 0.03$) were observed. Specifically, during Phase I

(d 7 to 14) pigs fed the complex diet had greater ADFI compared to all other treatments irrespective of PCV-status. Overall (d 0 to 28), PCV-inoculated pigs had decreased ADFI compared to PCV-vaccinated pigs. With respect to ADG, a tendency for a time × diet × PCV-status was observed ($P < 0.085$). A main effect of PCV-status ($P < 0.04$) was observed whereby PCV-vaccinated pigs had greater ADG compared to PCV-inoculated pigs during Phase 2 (d 21 to 28; $P < 0.01$) and for the overall experimental period (d 0 to 28; $P < 0.03$). With respect to G:F, an overall main effect of diet ($P < 0.02$) was observed whereby pigs fed the complex diet had greater G:F compared to all other treatments in Phase 1 (d 0 to 7; $P < 0.01$). Interestingly, this situation was reversed in Phase 2 (d 14 to 28) whereby pigs fed the simple diet had greater ($P < 0.01$) G:F compared to pigs fed the complex diet.

Serum immune biomarker data is summarized in Figures 1-3. Effects of diet complexity and PCV-status were evaluated with respect to both circulating PCV2-specific immunoglobulins (IgG and IgM) and non-specific serum cytokines (IL-12p40, IL-6, IL-10, TNF-alpha, IL-4, IFN-gamma, IL-8, IL-1beta, and IFN-alpha). Overall, with respect to PCV2-specific IgG, a significant diet × PCV-status interaction ($P < 0.04$) was observed. Specifically, PCV-inoculated pigs fed the simple diet had greater concentrations of IgG compared to PCV-inoculated pigs fed the complex diet on d 28 ($P < 0.05$). For IgM, there were no interactive effects of time, diet, or PCV-status. However, a main effect of PCV-status ($P < 0.001$) was observed whereby PCV-inoculated pigs had greater overall concentrations of IgM compared to PCV-vaccinated pigs. With respect to serum cytokines, there were no significant effects for IL-12p40, IL-6, IL-10, TNF-alpha, IL-4, IFN-gamma, or IL-8 (data not shown). However, a significant main effect of diet was observed for IL-1beta as pigs fed the complex diet had greater concentrations of this cytokine compared to all other treatments ($P < 0.03$). For IFN-alpha, a main effect ($P < 0.01$) of PCV-status was observed whereby PCV-inoculated pigs had greater overall IFN-alpha concentrations compared to PCV-vaccinated pigs. In addition, a tendency ($P < 0.08$) for a diet × PCV-status was observed for IFN-alpha whereby PCV2-inoculated pigs had greater concentrations of IFN-alpha compared to all other treatments on d 14.

Gut microbial populations. Samples have been sequenced and data is currently being analyzed.

Discussion

How are nutrient requirements of a pig affected by a health challenge? The economic losses associated with swine diseases are a significant issue in the U.S. pork industry. For example, the economic losses associated with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) are estimated to be \$640 million annually (USDA, 2008). In addition, Porcine Circovirus type 2 (PCV2) is also one of the top 3 diseases causing economic loss in the pork industry. It has been estimated that the cost associated with Porcine Circovirus Associated Diseases (PCVAD) can range from \$3 to \$20/pig which translates to a total loss of up to \$2 billion in U.S pork production systems (Gillespie et al., 2009). The bottom line is that health challenges are expensive and little progress has been made with respect to defining nutrient requirements of sick animals. The appropriate model to be used for this type of research is debatable. However, research conducted at the University of Nebraska has demonstrated the validity of using Porcine Circovirus 2 (PCV2) inoculation as a means to investigate the variation in the immune response to the virus and the etiology of the expression of PCVAD. Recently, this model has also been used by our research group to investigate the interactive effects of nutrition and health on the expression of PCVAD. The overall goal of this proposal was to use this model as an initial approach to develop subsequent efforts to study the interaction of nutrition and disease. To achieve this goal, we tested the hypothesis that diet complexity alters development of gut microbial populations, pig immune parameters, and growth performance in nursery pigs inoculated for, or vaccinated against Porcine Circovirus 2 (PCV2).

A primary objective of this work was to demonstrate the utility of this approach (i.e., the PCV2-challenge model used) for investigating diet × health interactions and to be able to revisit this approach to develop potential mitigation strategies. From the data collected, one conclusion that can be made with respect to the utility of this model is that the model was efficacious as evidenced by reduced BW, ADFI, and ADG in PCV2-inoculated pigs compared to PCV2-vaccinated pigs. It is also important to note that, as expected, changes in immune biomarkers (IgG, IgM, IL-1beta, and

IFN-gamma) are consistent with PCV2 disease progression. Interestingly, and contrary to our hypothesis, the incidence of diet × PCV-status interactions was not as common as expected. However, the observation that there are transient and conflicting effects of time, diet, and PCV-status on feed efficiency presents an opportunity to use this model to test an interesting hypothesis. That is, we hypothesize that digestibility may be reduced in PCV-inoculated pigs and that there is an opportunity to manipulate the diet to help facilitate greater assimilation of nutrients in pigs faced with a disease challenge. Accordingly, nutrient requirements (e.g., standardized ileal digestible lysine) may be different between infected and healthy pigs.

Overall, results from this work indicate that a complex nursery diet may improve post-weaning growth performance and affect immune response to PCV2 infection. Further work is needed to determine the effects of diet complexity and disease-status on nutrient and energy utilization, nutrient digestibility, and nitrogen balance in nursery pigs as well as grow-finish pigs.

Table 1. Ingredient and chemical composition of the experimental diets (% , as-fed basis).

	Complex	Simple	Simple + Prebiotic
Ingredients, %			
Corn	53.27	56.49	56.49
Soybean meal, 46.5% CP	21.50	33.40	33.40
Spray-dried porcine plasma	3.00	0.00	0.00
Select menhaden fish meal	8.00	0.00	0.00
Spray dried whey	7.00	0.00	0.00
DairyLac 80 ¹	6.00	0.00	0.00
GroBiotic-S ²	0.00	0.00	2.50
Corn starch	0.00	3.00	0.50
Dicalcium phosphate, 18.5% P	0.00	1.73	1.73
Limestone	0.53	0.35	0.35
Salt	0.30	0.30	0.30
Vitamin premix ³	0.25	0.25	0.25
Trace mineral premix ⁴	0.15	0.15	0.15
Corn Oil	0.00	3.85	3.85
Lysine·HCl	0.00	0.30	0.30
DL-Methionine	0.00	0.11	0.11
L-Threonine	0.00	0.07	0.07
Calculated composition, %			
SID Lys	1.22	1.22	1.22
SID Thr	0.78	0.72	0.72
SID Trp	0.25	0.23	0.23
SID Met	0.36	0.39	0.39
CP	19.46	17.59	17.59
ME, kcal/kg	3,370	3,370	3,370
Ca	0.69	0.69	0.69
Available P	0.43	0.43	0.43

¹ DairyLac 80 is a sweet and dried whey soluble product (International Ingredient Corporation, St. Louis, MO) containing 3.2% CP, and 0.06% Lys (analyzed composition) and 80% lactose.

² GroBiotic®-S (International Ingredient Corporation, St. Louis, MO) is a natural prebiotic for nursery pigs comprised of yeast fractions, whey and fermentation solubles.

³Supplied per kg of diet: vitamin A (as retinyl acetate), 5,500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as α -tocopheryl acetate), 30 IU; vitamin K (as menadione dimethylpyrimidinol bisulfate), 4.4 mg; riboflavin, 11.0 mg; d-pantothenic acid, 22.05 mg; niacin, 33.0 mg; vitamin B₁₂ (as cyanocobalamin), 33.0 mg.

⁴Supplied per kg of diet: copper (as CuSO₄·5H₂O), 10 mg; iodine (as Ca (IO₃)·H₂O), 0.25 mg; Iron (FeSO₄·2H₂O), 125 mg; manganese (MnO), 15 mg; Selenium (Na₂SeO₃), 0.3 mg; Zinc (ZnSO₄·H₂O), 125 mg.

Table 2. Effect of diet complexity on growth performance of barrows vaccinated against or inoculated with PCV2¹.

Item	Treatment ²						SEM	P-value		
	Vaccinates			Inoculates				Main effect ³		
	Complex	Simple	Prebiotic	Complex	Simple	Prebiotic		Diet	PCV	D × P
BW, kg										
d 0	7.2	7.3	7.2	7.0	7.0	7.0	0.10	0.95	0.19	0.62
d 7	9.2	8.8	8.7	8.7	8.5	8.1	0.20	0.01	0.11	0.57
d 14	11.4	11.0	10.8	10.9	10.2	9.8	0.30	0.02	0.07	0.68
d 21	14.5	14.6	13.2	13.5	12.9	12.7	0.50	0.16	0.10	0.52
d 28	18.7	19.5	16.9	17.2	16.6	16.4	0.60	0.08	0.05	0.18
Phase 1										
d 0 to 7										
ADG, g	265.3	207.3	217.0	251.8	223.3	169.7	18.3	0.01	0.33	0.25
ADFI, g	444.8	437.3	431.3	426.8	416.3	425.0	27.8	0.94	0.51	0.96
G:F	0.60	0.47	0.51	0.59	0.54	0.41	0.04	0.01	0.67	0.20
d 7 to 14										
ADG, g	320.5	316.0	295.5	314.5	243.0	237.5	33.0	0.30	0.11	0.58
ADFI, g	653.8	509.3	585.7	585.5	476.8	434.0	35.8	0.01	0.01	0.29
G:F	0.49	0.61	0.51	0.53	0.51	0.55	0.05	0.59	0.87	0.23
d 0 to 14										
ADG, g	293.0	261.5	256.5	283.2	233.0	203.5	18.4	0.02	0.06	0.51
ADFI, g	549.3	473.2	493.8	506.1	446.5	429.5	23.3	0.01	0.03	0.73
G:F	0.55	0.54	0.49	0.56	0.52	0.48	0.03	0.08	0.83	0.84
Phase 2										
d 14 to 21										
ADG, g	442.8	523.0	351.8	377.8	391.0	426.8	54.6	0.46	0.37	0.18
ADFI, g	878.8	769.5	598.5	726.8	614.3	644.8	72.8	0.07	0.16	0.31
G:F	0.51	0.68	0.53	0.52	0.63	0.67	0.06	0.06	0.50	0.23
d 21 to 28										
ADG, g	603.8	694.7	516.3	529.5	525.0	528.5	33.5	0.06	0.01	0.05
ADFI, g	1032.1	985.7	864.3	894.6	828.6	892.6	58.1	0.36	0.09	0.25
G:F	0.59	0.70	0.61	0.59	0.64	0.60	0.04	0.14	0.46	0.71
d 14 to 28										
ADG, g	523.5	608.7	433.8	453.3	458.2	477.5	36.2	0.13	0.06	0.05
ADFI, g	955.3	872.3	731.4	810.7	721.4	768.8	60.3	0.11	0.10	0.24
G:F	0.55	0.68	0.57	0.55	0.63	0.63	0.03	0.01	0.80	0.14
Overall (d 0 to 28)										
ADG, g	408.0	435.2	345.2	368.3	345.2	340.7	23.3	0.10	0.03	0.21
ADFI, g	752.3	656.4	612.9	658.4	584.0	599.2	33.6	0.02	0.04	0.48
G:F	0.55	0.61	0.53	0.57	0.58	0.56	0.02	0.06	0.96	0.38

^{a,b}within a row, means without a common superscript differ ($P < 0.05$) within the porcine circovirus (PCV) challenge group.

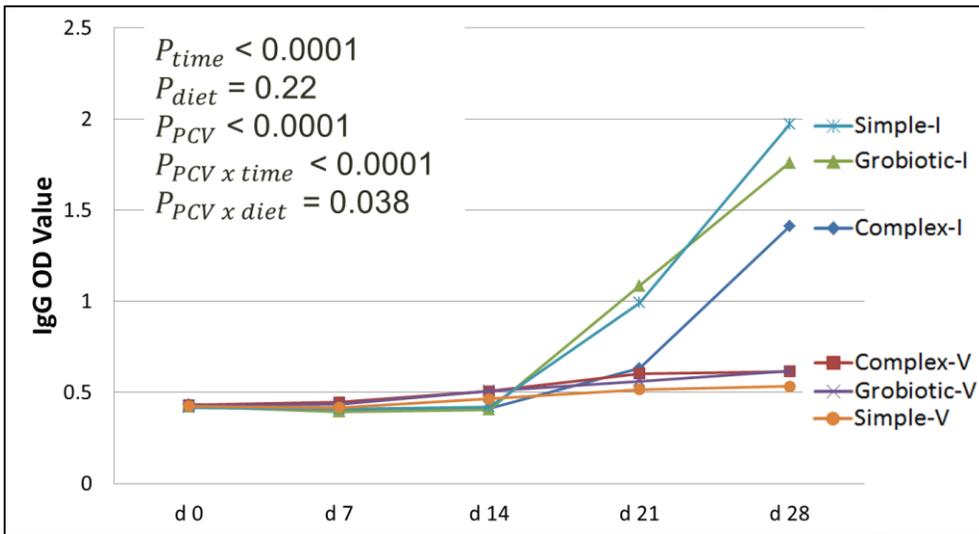
¹ 16 pigs/treatment.

²Pigs were either vaccinated against PCV2 (n = 48; Ingelvac CircoFLEX, Boehringer Ingelheim) prior to the beginning of the experiment or inoculated with a field isolate of PCV2 (n = 48; PCV2b isolate used in the experimental infection was recovered from a pig that had symptoms characteristic of PCV2 infection) on d 0 of the experiment and fed either a complex nursery diet (fishmeal, spray dried whey, spray dried animal plasma, lactose, reduced corn and soybean meal), a simple nursery diet (no fishmeal, spray dried whey, spray dried animal plasma, lactose, or prebiotic), or the simple diet plus prebiotic (2.5% GroBiotic-S).

³PCV = PCV challenge effect; DIET = diet effect; P × D = interaction between PCV and diet effects.

Figure 1. Effect of diet complexity on immunoglobulin G (Panel A; SEM = 0.05) and M (Panel B; SEM = 0.03) profiles in barrows vaccinated against or inoculated with PCV2.

A)



B)

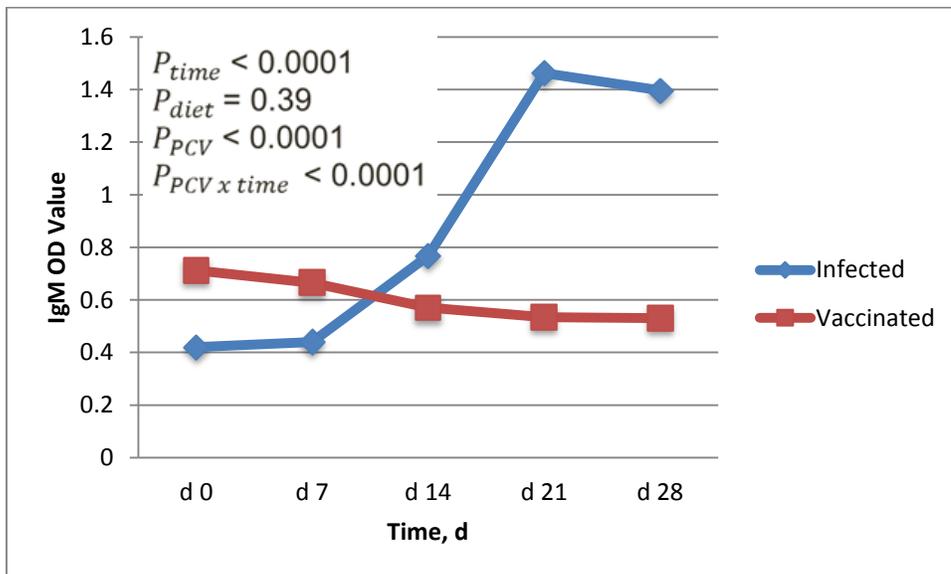
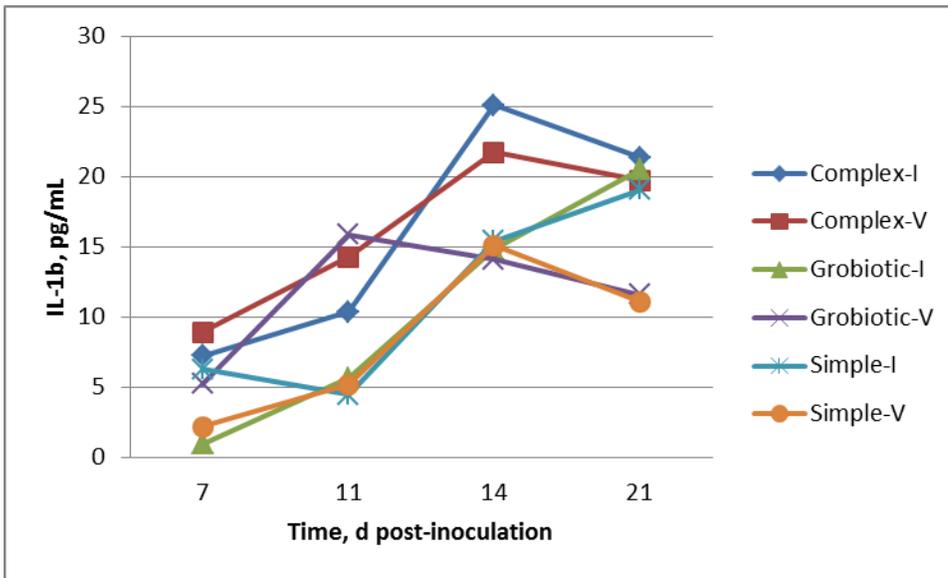


Figure 2. The interactive effects of diet complexity on circulating concentrations of interleukin-1 beta in barrows vaccinated against or inoculated with PCV2 (Panel A; SEM = 5.9 pg/mL). Panel B represents the main effects of dietary treatment (P < 0.03; SEM = 1.7 pg/mL) on interleukin-1beta in pigs vaccinated against or inoculated with PCV2.

A)



B)

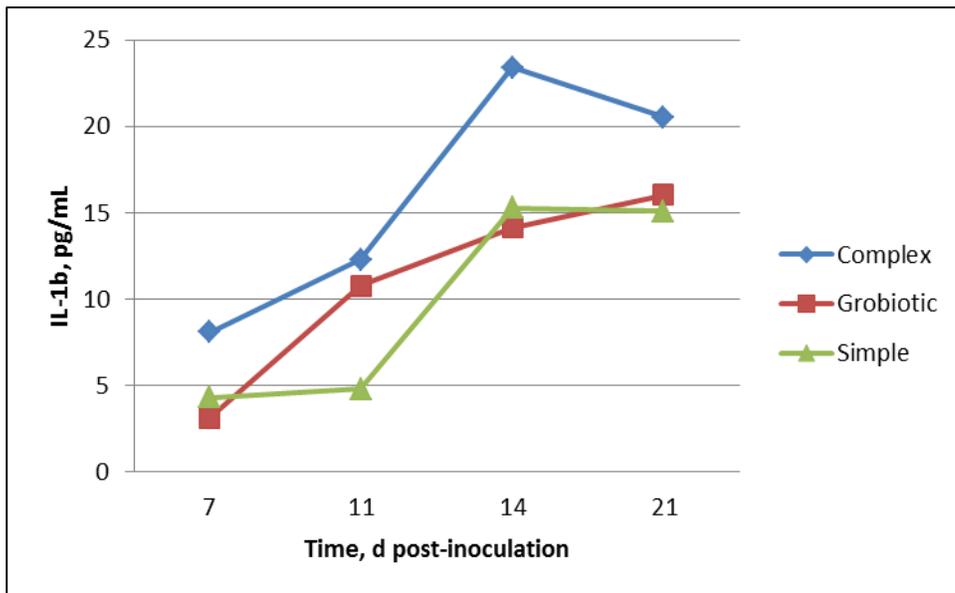
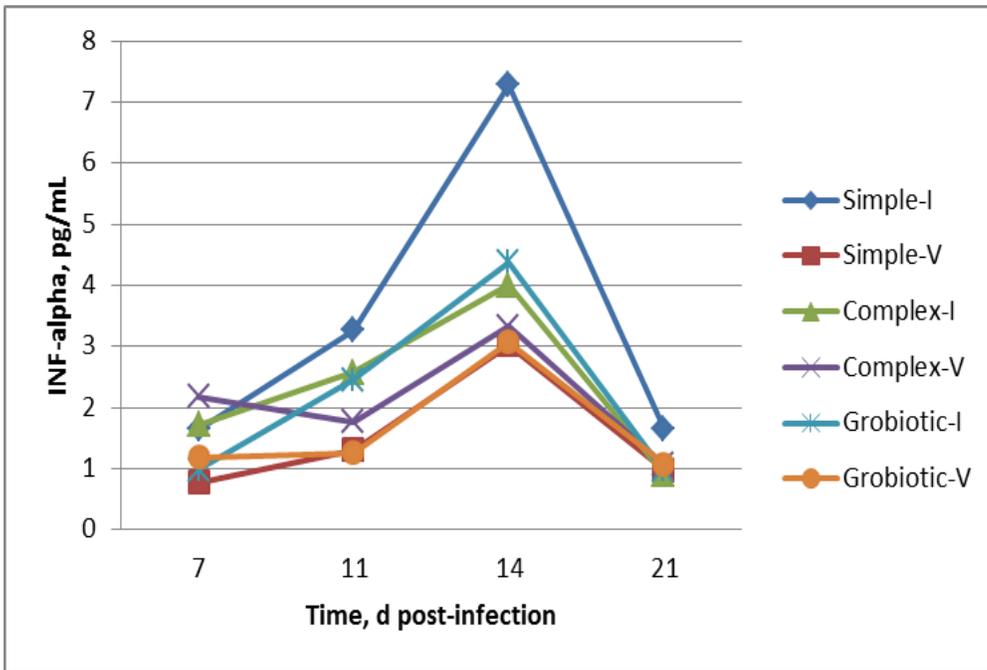


Figure 3. The interactive effects of diet complexity on circulating concentrations of interferon-alpha in barrows vaccinated against or inoculated with PCV2 (Panel A; SEM = 0.9). Panel B represents the main effects of PCV2 ($P < 0.01$; SEM = 0.2) on interferon-alpha in pigs vaccinated against or inoculated with PCV2.

A)



B)

