RESEARCH REPORT
ANIMAL SCIENCE

Title: Refining a model to investigate the interactive effects of health status and nutrition on nutrient and energy utilization in pigs **- NPB: #14-237**

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Industry Summary

Each year the National Pork Board identifies infectious disease prevention as a high-priority research area. The prevention and treatment of diseases common to swine operations represents a significant cost, both in terms of vaccinations, management intervention, and reduced performance. The negative economic impact of important pathogens is well documented. Potentially, the gastrointestinal tract and the host microbial populations represent a focal point defining the pigs' resistance or susceptibility to pathogens. Therefore, this project is designed to quantify important aspects (e.g., diet complexity, growth performance, digestibility, microbial ecology, and nutrient metabolism) of the interactions between nutrition and health. It is anticipated that this project will further refine disease challenge models and will be a powerful tool to help unveil possible alternative strategies that will help mitigate the economic burden caused by PCV2 as well as other infectious agents (e.g., PRRSV, etc.).

Key Words: digestibility, health, microbiota, pig, PCV2

Scientific Abstract

To refine a model to investigate the interactive effects of health status and nutrition on nutrient and energy utilization in pigs, three experiments were conducted. For Exp. 1, a total of 156 crossbred barrows (Large White x Landrace) were screened for PCV2 specific immunoglobulin G and M. Pigs (n = 100) with a sample-to-positive ratio (S/P) lower than 1.26 for passive IgG, and 1.0 for passive IgM were used for the study. At d 17 of age, 40 piglets were vaccinated for PCVAD with a single dose of Ingelvac CircoFLEX vaccine and the remaining 60 pigs were not vaccinated for PCVAD. At weaning, all pigs were fed a simple 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 =100; average BW = 7.1 kg; average age = 34.3 d) were sorted by initial BW and PCV status (vaccinated or inoculated) and randomly assigned to 24 pens (4-5 pigs/pen). At the conclusion of Exp. 1, pigs were selected from Exp. 1 to be used in the subsequent 2 experiments based on a statistical analysis to determine growth residuals between actual and predicted final BW using initial and final BW, day of infection, litter, pen, and maternal IgG from Exp. 1. For Exp. 2, vaccinated (n=16) and inoculated (n=16) pigs (average BW, 31.5±1.26 kg), with a low net residual BW were selected, and were housed 2 pigs/pen by treatment for a total of 36 pigs. For Exp. 3, additional vaccinated (n =16) and inoculated (n =16) pigs were selected from Exp. 1 pigs. The pigs selected for Exp. 3 included an equal number of pigs with high (positive) BW residuals (average BW, 33.6±3.22 kg) and with low (negative) residuals (average BW, 27.6±4.48), greater or lesser final BW compared with predicted BW, respectively. Therefore, Exp. 3 included 4 treatment groups: 1) vaccinated pigs with high BW residuals; 2) vaccinated with low BW residuals; 3) inoculated with high BW residuals; and 4) inoculated with low BW residuals. Pigs were individually housed in a different room within the UNL Animal Science Complex. Growth performance, apparent total tract digestibility and carcass traits (Exp. 2 and 3 only) were evaluated. For Exp. 1, no differences in BW, ADG, ADFI, or G:F were detected. Apparent total tract digestibility for GE and DM at 14 dpi was increased ($P < 0.045$ and $P < 0.042$, respectively for GE and DM) in inoculated pigs compared to vaccinated pigs. For Exp. 2, ADFI was similar for both treatment groups with the exception that ADFI was greater ($P < 0.01$) for inoculated pigs during the finisher 1 (wk 6 to 10) phase compared to vaccinated pigs. For feed efficiency, vaccinated pigs had greater ($P < 0.05$) efficiency compared to inoculated pigs during the last three grow-finish phases. No differences were observed in DM or GE digestibility with the exception that vaccinated pigs had greater ($P < 0.05$) DM and GE apparent total tract digestibility compared to inoculated pigs at the end of the Finisher 1 (wk 10) phase. No differences were observed for carcass traits. For Exp. 3, indicated that the pigs with initial low residual body weight consumed more feed, had greater ADG and feed efficiency, and had numerically increased final BW irrespective of PCV status (vaccinated vs inoculated). No differences in digestibility for GE and DM were observed. High residual inoculated pigs and low residual vaccinated pigs tended to be leaner compared to their counterparts.

Introduction

How are the nutrient requirements of a pig affected by a health challenge? In 2001, Reeds and Jahoor discussed aspects of the metabolic response to disease. Central to this idea is that animals faced with a health challenge experience discrepancies between 'nutrient need' (i.e., metabolic pathways of nutrient utilization) and the dietary nutrient requirement (i.e., the quantity of a nutrient that must be supplied in the diet to support the need). In the context of a health challenge there are changes in specific nutrient utilization pathways and a requirement for greater exogenous input (e.g., limiting amino acids). However, there is limited information with respect to specific changes in utilization pathways and specific changes in nutrient requirements for health-challenged animals. 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 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 dollars/pig which translates to a total loss of up to 2 billion dollars 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 during or subsequent to infection. 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.

This proposal describes a systematic approach to advancing the knowledge base about nutritional requirements of health-challenged pigs. It is apparent that the relationships among nutrient metabolism, host microbial ecology, and disease is important and a greater understanding of the physiological changes that take place before, during, and after a disease/stress event is warranted. Greater understanding of the impact of disease on metabolism will allow us to develop practical mitigation strategies in pigs. This research will help to define the short- and long-term impact disease challenge has on nutrient utilization in pigs, and it will help to refine the impact of disease challenge on digestibility and nitrogen balance. Presently, there is a paucity of data documenting nutrient/energy requirements for health-challenged pigs and no provisions are currently considered in the latest revision of swine nutrient requirements (NRC, 2012) for sick or stressed pigs.

PCV2 challenge model. Recently, experimental challenges were carried out for 4 weeks in 10 experimental batches of pigs infected with PCV2b strain at an average of 43 d of age (n = 974). Weekly measures of viremia, PCV2 specific antibodies (IgM and IgG) and average daily gain (ADG) were collected. Viremia was the best indicator of decreased ADG (the main performance indicator of disease susceptibility) following infection; a moderate negative relationship between viremia and ADG were observed starting with viremia at d 14 and ADG during the last two weeks of challenge (-0.31 to -0.39, $P < 0.001$). The correlation between viral load and overall ADG (0 – 28 d) was -0.36. In a subsequent experiment, using the same PCV2 challenge model, we tested the hypothesis that diet complexity affects growth performance of infected pigs (Mastromano et al., 2014). At least two important observations resulted from this work. First, we established the PCV2 model with respect to its deleterious effects on growth as experimental PCV2 infection resulted in reduced growth performance. Specifically, PCV2 infected pigs had reduced ($P < 0.04$) ADFI and reduced ($P< 0.03$) ADG compared to vaccinated pigs. Second, we have established that the diet may be an important factor in the overall outcomes of health, growth performance, nutrient digestibility, and microbial profile.

Objectives

1) Determine the effects of diet complexity on nutrient and energy utilization in growing-finishing pigs infected with PCV2.

a. How does diet complexity affect nutrient digestibility and growth performance in health-challenged pigs throughout the growing-finishing period?

b. How does diet complexity affect gut microbial profiles in health challenged pigs and do changes in microbial profiles correlate with changes in nutrient digestibility throughout the growing-finishing period?

c. What is the economic impact of disease challenge throughout the growing-finishing period?

2) Determine the effects of disease challenge on nutrient digestibility and nitrogen balance in growing pigs.

a. What effect does disease challenge have on nutrient digestibility and nitrogen balance?

b. Is there a correlation between microbial ecology and changes in nutrient digestibility?

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.

Experiment 1. Initially, a total of 156 crossbred barrows (Large White x Landrace) were screened for PCV2 specific immunoglobulin (**Ig)** G and M by ELISA (Ingenasa, Madrid, Spain) from blood samples obtained at 14 d (± 4 d) of age at the UNL ARDC Swine Unit (Mead, NE). Pigs (n = 100) with a sample-to-positive ratio (S/P) lower than 1.26 for passive IgG, and 1.0 for passive IgM were used for the study. At d 17 of age, 40 piglets were vaccinated for PCVAD with a single dose of Ingelvac CircoFLEX vaccine (Boehringer Ingelheim). The remaining 60 pigs were not vaccinated for PCVAD.

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 =100; average BW = 7.1 kg; average age = 34.3 d) were sorted by initial BW and PCV status (vaccinated or inoculated) and randomly assigned to 24 pens (4-5 pigs/pen).

During the 42-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 m^2 of floor space per pig. All pigs were allowed ad libitum access to feed and water. Pigs were fed a corn-soybean meal diet (Table 1) that met or exceeded NRC (2012) requirements with no antibiotic inclusion.

Experiment 2 and 3. The pigs from experiment 1 were selected using a statistical analysis to determine growth residuals between actual and predicted final BW using initial and final BW, day of infection, litter, pen, and maternal IgG from experiment 1.

For experiment 2, vaccinated (n=16) and inoculated (n=16) pigs (average BW, 31.5±1.26 kg), with a low net residual BW were selected, and were housed 2 pigs/pen by treatment for a total of 32 pigs. For experiment 3, an additional set of vaccinated (n =16) and inoculated (n =16) pigs were selected from experiment 1 pigs. The pigs selected for experiment 3 included an equal number of pigs with high (positive) BW residuals (average BW, 33.6±3.22 kg) and with low (negative) residuals (average BW, 27.6±4.48), greater or lesser final BW compared with predicted BW, respectively. Therefore, experiment 3 included 4 treatment groups: 1) vaccinated pigs with high BW residuals; 2) vaccinated with low BW residuals; 3) inoculated with high BW residuals; and 4) inoculated with low BW residuals. These 32 pigs were individually housed in a different room within the UNL Animal Science Complex.

For experiment 2 and 3, animals had free access to water and ad libitum feed intake. Pigs were sequentially fed the grower 1 diet for 2 weeks, and grower 2, finisher 1 and finisher 2 diet for 4 weeks (Table 2). Diets were corn-soybean meal based and designed to meet or exceed NRC (2012) requirements. In the last week of each phase, diets with titanium dioxide were used for digestibility analysis.

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 34.3 d of age) all naïve pigs (n = 60) were infected intranasally (2 mL) and intramuscularly (1 mL) with a titer of 10^4 $TCID_{50}$/mL.

Growth Performance. Pig BW and feed disappearance were measured at the beginning of experiment 1 and weekly afterwards for the 6 wk trial. For experiments 2 and 3, pig BW and feed disappearance were measured every 2 weeks. Pig BW and feed disappearance were used to calculate ADG, ADFI, and G:F.

Blood Collection. Blood samples were obtained from each pig (5-9 mL) via jugular venipuncture in serum separator tubes at d 0, 7, 14, 21, 28, 35, and 42 of experiment 1 and at the end of each diet phase in experiments 2 and 3. 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.

Sample Collection for Microbiome Analysis. On d 0, 14, 28, and 42 of experiment 1 and at the end of each diet phase for experiments 2 and 3, fresh total tract samples were collected from individual pigs for analysis. Samples were frozen afterwards at -80˚C for later analysis.

Digestibility. On d 14, 28, 42, fecal samples were collected from each pen for experiment 1. For experiments 2 and 3, fecal samples were collected by pen and individually, respectively, at the end of each diet phase. All samples were frozen at -20˚C for later analysis. Samples were later dried in a 100˚C forced-air oven for 3 d and then ground afterwards. Samples were analyzed for DM, titanium dioxide, and energy.

Carcass Traits. At the end of the finisher 2 phase, animals from Exp. 2 and Exp. 3 were analyzed for loin-eye area (LEA) and backfat at the 10^{th} rib using ultrasound probing. Animals were then sent to a commercial slaughter facility where hot carcass weight (HCW) was provided. To calculate lean content, the procedure 6 equation from Burson (2006) in PIG 12-04-06g was used: Lb. lean = 5.7769 + (0.401 x HCW, lbs) – (18.838 x 10^{th} rib fat depth, in.) + (4.357 x 10^{th} rib LEA, sq. in.) + (1.006 x sex of pig) {barrow = 1, gilt = 2}. Lean percentage was calculated as lean, percent = lean, lbs / HCW, lbs * 100.

Statistics. Data was analyzed with the MIXED procedure of SAS using LSMEANS with the Tukey-Kramer adjustment. In Exp 1., pen was used as the experimental unit for growth performance and digestibility while BW at wk 0 served as a covariate. Pig was used as the experimental unit for IgG and IgM with no covariate. In Exp 2., pen was used as the experimental unit for all data. Both Exp 1 and Exp 2 were analyzed for the primary effect of vaccination. In Exp 3., pig was used as the experimental unit for all data and BW wk 0 as a covariate. The primary of effects of vaccination, residual, and the interaction of vaccination and residual were used for Exp 3.

Results

Experiment 1. To determine the infectious status of the pigs, serum was collected and analyzed for viremia. As expected, all inoculated pigs (Fig. 1) showed greater viremia than the vaccinated pigs after 7 dpi through 42 dpi ($P < 0.05$). Serum IgM (Fig 2A) and IgG (Fig 2B.) antibodies, specific to PCV2, were also measured. Titers of IgM were increased as expected in the inoculated group compared to the vaccinated group ($P < 0.05$); however, no significant differences were found within the inoculated group. Titers of IgG increased relative to time of vaccination or inoculation. Taken together, this data demonstrates that experimental PCV2 inoculation alters PCV2 viremia and PCV2-specific IgG titers.

Weekly growth performance and feed intake are shown in Figure 3 and Table 3. No difference in BW, ADG, ADFI, or G:F was detected throughout the six-week trial for PCV status ($P > 0.10$). Apparent total tract digestibility was determined for DM and GE at 14, 28, and 42 dpi. Digestibility for GE and DM at 14 dpi (Fig 4) was increased ($P < 0.045$ and $P < 0.042$, respectively for GE and DM) in inoculated pigs compared to vaccinated pigs. No differences were observed in apparent

total tract digestibility on d 28 or 42 post-infection. This data indicates that initial digestibility may be altered by PCV status, but no significant impact occurred relative to growth performance.

Experiment 2. To determine if PCV status had long-term effects on growth and finish potential, pigs with the low net residual BW were selected from both the vaccinated and inoculated pigs in experiment 1. Growth performance and BW for experiment 2 can be found in Table 4. Initial BW was not different between groups ($P > 0.10$) and remained that way throughout the experimental period. Average daily feed intake was similar for both treatment groups with the exception that ADFI was greater ($P < 0.01$) for inoculated pigs during the finisher 1 (wk 6 to 10) phase compared to vaccinated pigs. No differences were found between groups in any phase for ADG. For feed efficiency, vaccinated pigs had greater ($P < 0.05$) efficiency compared to inoculated pigs during the last three phases (Grower 2, Finisher 1 and 2).

Apparent total tract digestibility was assessed at the end of each diet phase (Table 5). No differences were observed in DM or GE digestibility with the exception that vaccinated pigs had greater ($P < 0.05$) DM and GE apparent total tract digestibility compared to inoculated pigs at the end of the Finisher 1 (wk 10) phase. Backfat, LEA, lean, and percent lean were determined at the end of the trial with inoculated pigs have numerically lower backfat and higher percent lean compared with vaccinated pigs; however, none of the traits were significant.

Experiment 3. To evaluate the effects of PCV-status on long term growth, digestibility and carcass traits in individually fed pigs, pigs with high and low residual BW were selected from experiment 1. Growth performance data for experiment 2 can be found in Table 6. Due to residual selection, initial BW was different between high and low residual groups ($P < 0.05$). Thus, initial BW was used as a covariate for performance data. Using this analysis, BW was not different through the first three phases of the trial. In the final finisher phase, low residual pigs had numerically higher BW than their high residual counterparts ($P > 0.10$). During the grower 1 phase (wk 1 to 2) an increase in ADFI for vaccinated pigs over inoculated pigs ($P < 0.05$) was observed. In the finisher phases, low residual pigs consumed more feed than the high residual pigs ($P < 0.05$). For ADG, only low residual pigs had greater ($P < 0.004$) gain compared to high residual pigs during finisher 1 (wk 6 to 10). During the grower 2 and finisher 1 phases, the low residual pigs had greater G:F than high residual pigs ($P < 0.05$).

Digestibility and carcass data are found in Table 7 and Figure 5. No differences in digestibility for GE and DM were found across all groups for all phases for main effects. No differences were found between treatment groups for LEA ($P > 0.10$); however, a tendency for a Trt x residual interaction was observed for percent lean where the high residual inoculated pigs and the low residual vaccinated pigs were leaner compared to their counterparts.

Discussion

The overall goal of this work is to help define the short- and long-term impact disease challenge has on nutrient utilization in pigs. Previous work by our group and funded by the Nebraska Pork Producers/National Pork Board (#12-185: Development of a Nutrition x Health Interaction Model to Study Nursery Pig Performance) was directed at establishing the PCV2 challenge model as an appropriate model to investigate nutrition and health interactions. This initial experiment lead to the following main conclusions: 1) The PCV2 challenge model is efficacious as evidenced by reduced BW, ADFI, and ADG in PCV2 challenged pigs; 2) As a result of transient and conflicting effects of time, diet, and PCV-status on feed efficiency, this model presents an opportunity to use this model to test the hypothesis that nutrient digestibility may be affected by health status; and 3) The microbiome of pigs may be altered in pigs when vaccinated against or inoculated with PCV2.

The third observation was not included in our final report for the #12-185 project but has since been finalized and published as part of the Digestive Physiology in Pigs proceedings in the Journal of Animal Science (van Sambeek et al.,

2016). Briefly, as you can see (Table 10) there are significant phenotypic associations with microbial operational taxonomic units relative to health status (PCV2 vaccinated vs PCV2 inoculated). From this, we hypothesized the nutrient digestibility may be impacted by health status as a function of changes in microbial community. However, in the initial experiment nutrient digestibility was not evaluated; thus, the phenotypic digestibility data could was not available to be used in the multivariate analysis.

In the current experiment, our first objective was to determine the effects of diet complexity on nutrient and energy utilization in growing-finishing pigs infected with PCV2. To accomplish this objective two groups of weaned pigs (vaccinated against or inoculated with PCV2) were fed a simple diet. A simple diet was used because, according to our previous experiment (#12-185), the simple diet afforded PCV2 challenged pigs with the greatest benefit with respect to overall BW, ADG, and feed efficiency during the nursery phase. Results from the current experiment, contrary to what we expected, indicate that there were no overall differences between vaccinated and inoculated pigs with respect to overall growth performance even though changes in viremia and immune parameters were clearly achieved by the PCV2 challenge. However, significant effects of GE and DM digestibility were observed after the initial challenge. Specifically, GE and DE digestibility was increased ($P < 0.045$ and $P < 0.042$, respectively for GE and DM) in inoculated pigs compared to vaccinated pigs on d 14.

One of the aspects of disease challenge experiments and models is that results tend to be highly variable. Many inter-related factors (genetics, diet, environment, etc.) combine to impact the final outcome of disease challenge. In addition, with respect to porcine circovirus specifically, pigs within a barn or even within a pen may be differentially affected by the presence of the virus. In light of the fact that in the current experiment, significant effects of PCV2 status did not give us the anticipated impact on overall growth performance, the original protocol was modified such that two additional experiments were designed in an attempt to parse out these variable effects. At the end of experiment 1 (the nursery phase), pigs that were either vaccinated against or inoculated with PCV2 were selected to evaluate the long-term effects on growth, nutrient digestibility, and carcass traits (through the grow-finish phase) in two separate experiments. For experiment 2, pigs with low net residual BW during the nursery phase were selected from vaccinated and inoculated nursery groups. For experiment 3, a study was designed to compare subsequent growth, digestibility and carcass traits in pigs that had low residual BW to pigs with high residual BW.

Results from experiment 2, where pigs were selected with low residual BW coming out of the nursery phase, indicate that vaccinated pigs may have greater feed efficiency in the final 3 phases of the grow-finish period. There were no differences in DM or GE apparent total tract digestibility with the exception that vaccinated pigs had greater DM and GE apparent total tract digestibility compared to inoculated pigs at the end of the Finisher 1 (wk 10) phase. In addition, no significant effects of vaccination or inoculation in pigs with initial low residual BW were observed with respect to carcass traits. Interestingly, an economic analysis (Table 8) of experiment 2 shows that whether pigs are harvested at similar weights or age, infection with PCV2 results in decrease of about $11 / head compared with vaccinating animals. This is mostly impacted by the increase in ADFI by inoculated animals where as carcass value contributes little to the difference.

Results from experiment 3, where pigs were either selected with low or high residual BW coming out of the nursery phase, indicate that the pigs with initial low residual body weight consumed more feed, had greater ADG and feed efficiency, and had numerically increased final BW irrespective of PCV status (vaccinated vs inoculate). However, no significant differences in digestibility were observed during experiment 3. With the exception that a tendency for a Trt x residual interaction was observed for percent lean where the high residual inoculated pigs and the low residual vaccinated pigs were leaner compared to their counterparts, no other effects on carcass traits were observed between treatment groups. In terms of feed cost and carcass value (Table 9), vaccinated pigs marginally cost more than

inoculated pigs, but the larger difference came from high residual pigs that had a greater feed efficiency, based on unadjusted data. As a result low residual pigs cost an extra $17.43 / head due to increased feed intake and days on feed day when pigs are sold at similar market weight. However, when sold on age basis, low residual pigs reduce feed cost but take a hit on carcass value resulting in $15.44 disparity between high and low residual pigs.

In summary, the effects of PCV2 challenge observed in the initial phase (nursery) were not as profound as what we have experienced with this model in previous experiments even though pigs clearly exhibited viremia similar to previous experiments. Because of this, the decision was made to modify the protocol to attempt to compare pigs with low and high BW residuals. Very few experiments have been conducted previously to investigate the interactive effects of health and nutrition let alone those effects relative to the entire life span of the growing pig. However, these can be useful for assessing differential production costs between treatments. Experiment two and three indicate that there may be some compensatory effects on growth irrespective of total tract nutrient digestibility. However, due to limitations created by the lack of an initial disease challenge effect on growth performance and due to limitations in using body weight residuals to select animals, more work is needed to investigate and verify the long-term interactive effects of health status and nutrition on nutrient and energy utilization in pigs.

With respect to the microbial analysis, DNA has been isolated from samples obtained in all three experiments. However, delays in completing the sequencing have been created due to a backlog in the sequencing queue. We anticipate that sequencing will be completed and a multivariate analysis conducted by the end of February, 2017.

Table 1. Diet formulation and chemical composition for experiment 1 (%, as-fed basis).[1]	
Ingredients, %	Diet
Corn	58.99
SBM, 47.7% CP	33.40
Dicalcium phosphate, 18.5%	1.73
Limestone	0.35
Salt	0.30
Vitamin premix[2]	0.25
Trace mineral premix[3]	0.15
Corn oil	3.85
Lysine-HCl	0.30
DL-Methionine	0.11
L-Threonine	0.07
Titanium dioxide	0.50
Calculated composition, %	
SID Lys	1.22
SID Thr	0.72
SID Trp	0.23
SID Met	0.39
CP	21.16
ME, kcal/kg	3.56
Ca	0.86
Available P	0.43

[1]Formulated using NRC (2012)

[2]Vitamin premix supplied per kg of diet: vitamin A (as retinyl acetate), 5500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as 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 B_{12} (as cyanocobalamin), 33.0 mg

[3]Trace mineral premix containing: copper (as $CuSO_4H_2O$), 10 mg/kg; iodine (as $Ca(IO_3) \cdot H_2O$), 0.25 mg/kg; iron (as $FeSO_4 \cdot 2H_2O$), 125 mg/kg; manganese (MnO), 15 mg/kg; selenium (Na_2SeO_3), 0.3 mg/kg; zinc ($ZnSO_4 \cdot H_2O$), 125 mg/kg

Table 2. Diet formulation and predicted composition for experiments 2 and 3[1].

	Grower 1	Grower 2	Finisher 1	Finisher 2
Ingredients, %				
Corn[2]	59.92	65.14	68.36	74.55
Soybean meal, 46.5% CP	33.00	28.00	25.00	19.00
Dicalcium phosphate, 18.5% P	1.00	0.90	0.70	0.55
Limestone	0.85	0.75	0.73	0.70
Salt	0.30	0.30	0.30	0.30
Vitamin premix[3]	0.25	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15	0.15
Corn Oil	4.00	4.00	4.00	4.00
Lysine·HCl	0.30	0.30	0.30	0.30
DL-Methionine	0.08	0.06	0.05	0.04
L-Threonine	0.15	0.15	0.15	0.14
L-Trp	0.00	0.00	0.01	0.02
Calculated composition, %				
CP	17.63	15.89	14.86	12.78
ME, kcal/kg	3474	3485	3496	3508
Ca	0.67	0.60	0.53	0.47
STTD P	0.32	0.29	0.25	0.22
SID Lys	1.22	1.09	1.02	0.87
SID Met	0.36	0.32	0.30	0.26
SID Thr	0.80	0.73	0.69	0.60
SID Trp	0.23	0.20	0.19	0.17

[1]Formulated using NRC (2012)

[2]Titanium dioxide added at 0.5% in diet, in place of corn

[3]Vitamin premix supplied per kg of diet: vitamin A (as retinyl acetate), 5500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as 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 B_{12} (as cyanocobalamin), 33.0 mg

[4]Trace mineral premix containing: copper (as $CuSO_4H_2O$), 10 mg/kg; iodine (as $Ca(IO_3) \cdot H_2O$), 0.25 mg/kg; iron (as $FeSO_4 \cdot 2H_2O$), 125 mg/kg; manganese (MnO), 15 mg/kg; selenium (Na_2SeO_3), 0.3 mg/kg; zinc ($ZnSO_4 \cdot H_2O$), 125 mg/kg

Figure 1. Weekly serum viremia of pigs vaccinated for or inoculated with PCV2 during experiment 1.

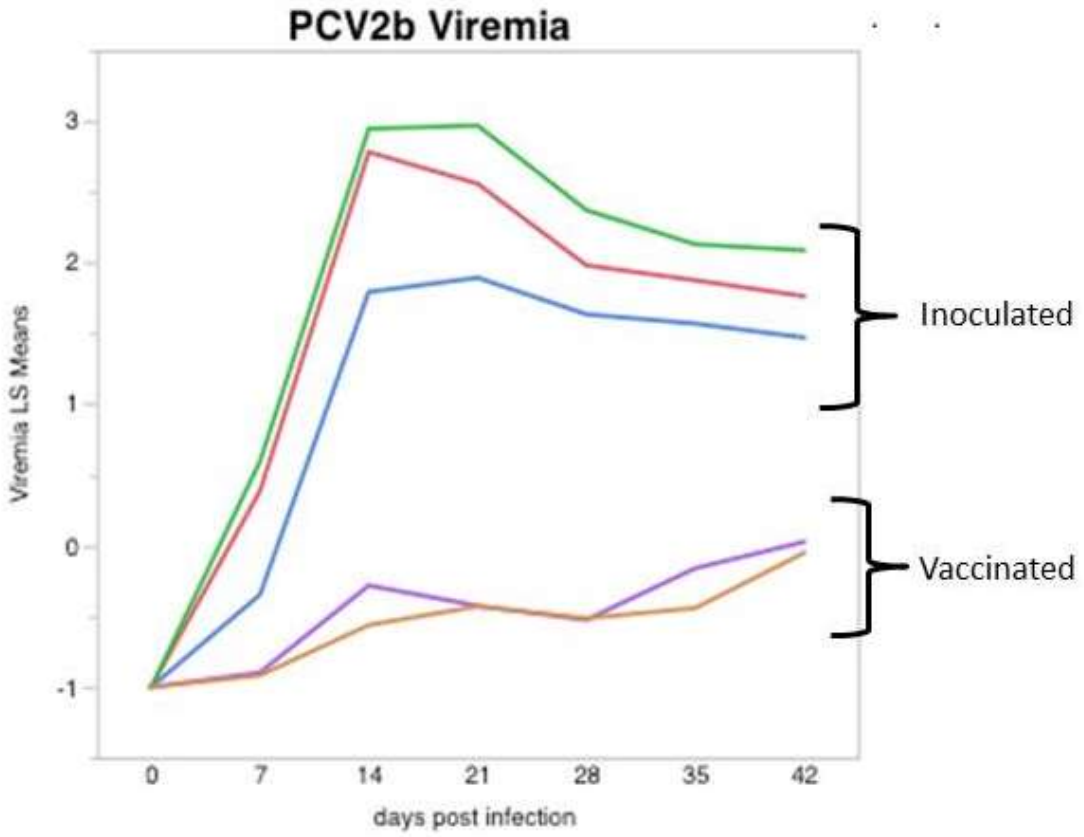
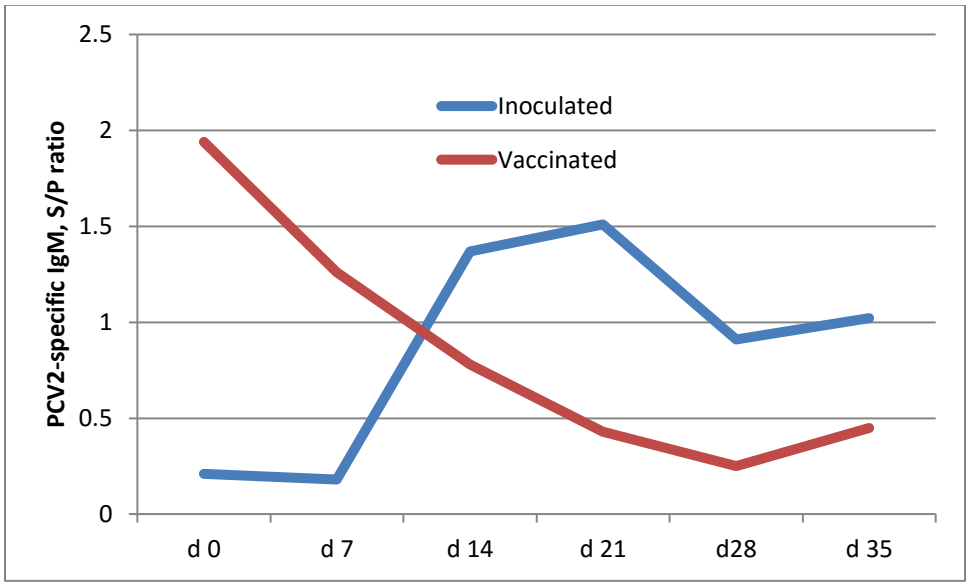


Figure 2. Serum titers of PCV2-specific IgM (A) and IgG (B) in pigs vaccinated for or inoculated with PCV2 during experiment 1.

A)



B)

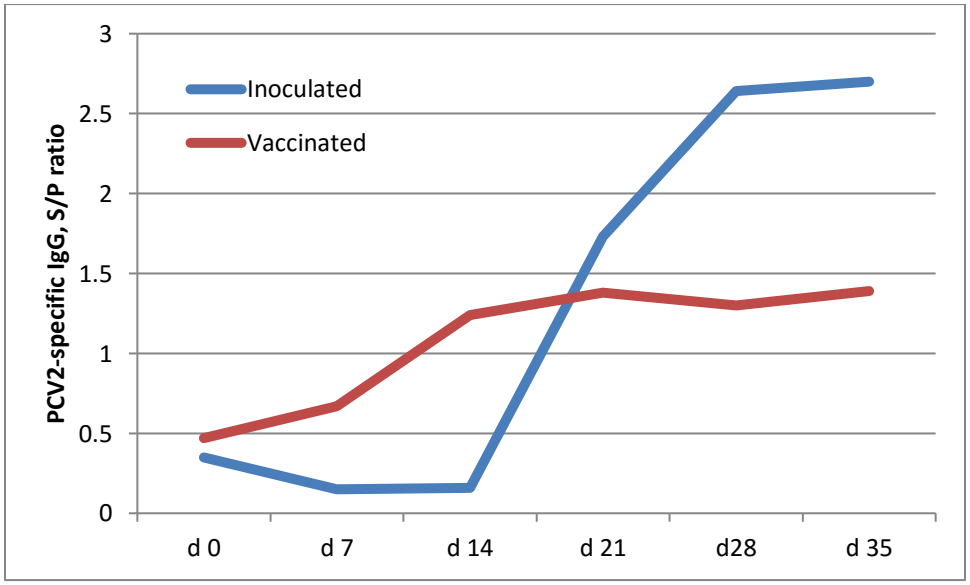


Table 3. Growth performance of pigs inoculated with or vaccinated for PCV2 in the nursery phase (Experiment 1).

Item	Inoculated	Vaccinated	SEM	*P*-value
d 0-7				
ADFI, kg/d	0.52	0.54	0.038	0.700
ADG, kg/d	0.23	0.23	0.017	0.977
G:F	0.45	0.45	0.048	0.998
d 7-14				
ADFI, kg/d	0.72	0.73	0.027	0.709
ADG, kg/d	0.44	0.43	0.020	0.763
G:F	0.61	0.59	0.024	0.532
d 14-21				
ADFI, kg/d	0.86	0.90	0.041	0.483
ADG, kg/d	0.53	0.56	0.027	0.452
G:F	0.62	0.63	0.042	0.837
d 21-28				
ADFI, kg/d	1.10	1.09	0.053	0.906
ADG, kg/d	0.56	0.61	0.030	0.164
G:F	0.51	0.58	0.047	0.311
d 28-35				
ADFI, kg/d	1.35	1.39	0.086	0.701
ADG, kg/d	0.75	0.80	0.036	0.259
G:F	0.59	0.59	0.056	0.922
d 35-42				
ADFI, kg/d	1.59	1.62	0.055	0.727
ADG, kg/d	0.86	0.88	0.033	0.662
G:F	0.55	0.55	0.034	0.978

Figure 3. Change in pig body weight over a six-week period when vaccinated for or inoculated with PCV2 during experiment 1. Data presented as least square means, $P > 0.10$.

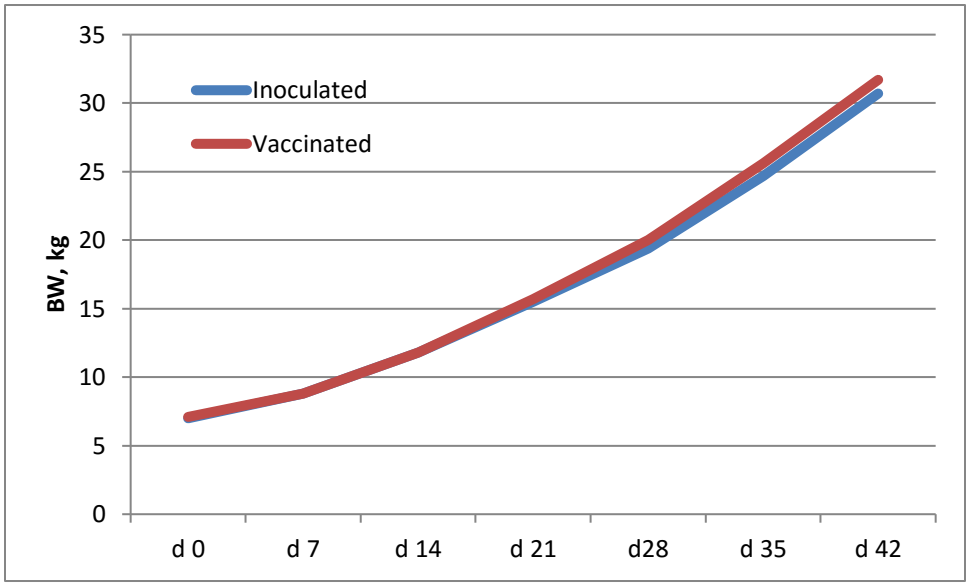


Figure 4. Apparent total tract digestibility coefficients for GE and DM during six-week period during experiment 1. Data presented as least square means and SEM error bars.

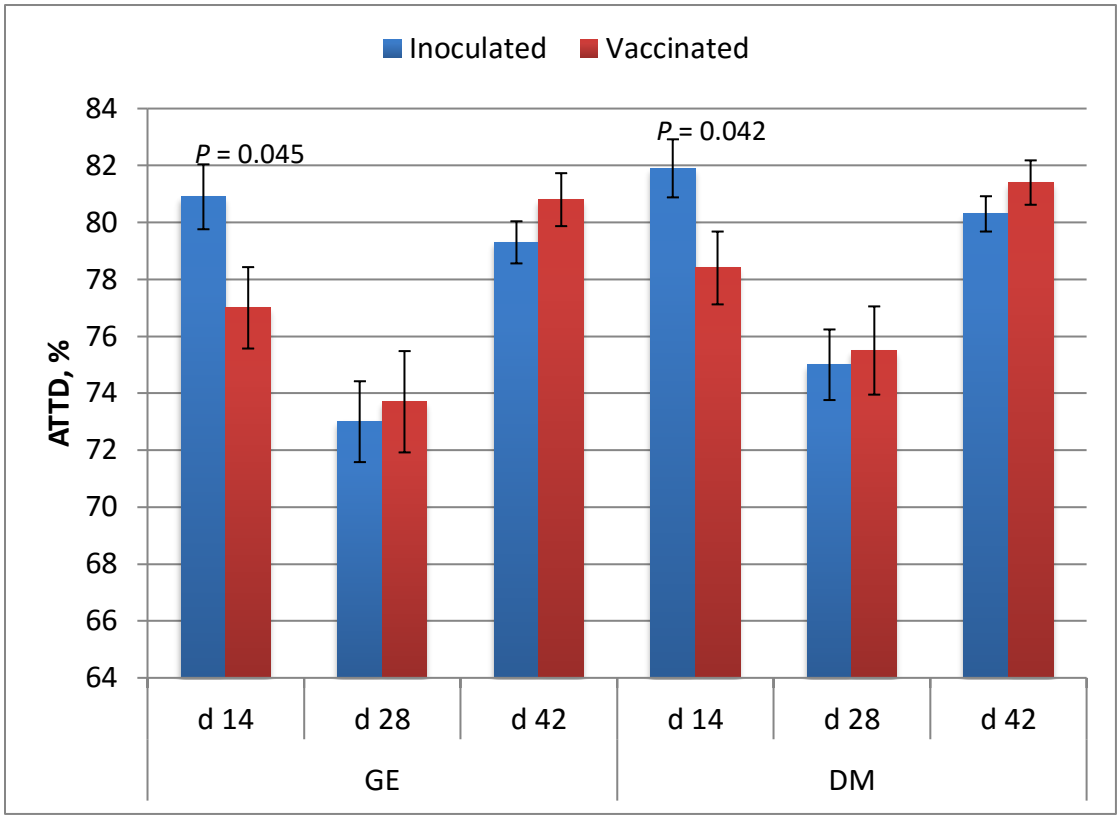


Table 4. Growth performance of pigs during the grow-finish phase that were previously vaccinated for or inoculated with PCV2 and selected for low net residual BW during the nursery phase (Experiment 2).

Treatment	Inoculated	Vaccinated	SEM	*P*-value
BW, kg				
wk 0	38.5	37.7	1.44	0.688
wk 1 to 2	52.9	51.1	1.69	0.670
wk 2 to 6	76.3	75.4	1.45	0.660
wk 6 to 10	106.2	106.6	1.84	0.891
wk 10 to 14	132.2	134.0	2.67	0.636
ADFI, kg/d				
wk 1 to 2	2.63	2.54	0.087	0.504
wk 2 to 6	3.21	3.08	0.094	0.326
wk 6 to 10	3.89	3.36	0.128	0.010
wk 10 to 14	4.06	3.66	0.196	0.177
ADG, kg/d				
wk 1 to 2	0.97	0.96	0.028	0.736
wk 2 to 6	1.15	1.18	0.028	0.382
wk 6 to 10	1.03	1.06	0.053	0.671
wk 10 to 14	0.93	0.96	0.045	0.741
G:F				
wk 1 to 2	0.41	0.39	0.027	0.637
wk 2 to 6	0.36	0.39	0.009	0.056
wk 6 to 10	0.27	0.32	0.013	0.008
wk 10 to 14	0.23	0.26	0.010	0.032

Table 5. Carcass traits and apparent total tract digestibility (ATTD) of DM and GE during the grow-finish phase that were previously vaccinated for or inoculated with PCV2 and selected for low net residual BW during the nursery phase (Experiment 2).

Treatment	Inoculated	Vaccinated	SEM	*P*-value
Backfat, cm	2.33	2.54	0.112	0.191
LEA, cm2	54.8	54.0	1.25	0.659
Lean, kg	54.7	54.2	0.85	0.700
Lean, %	51.5	50.4	0.55	0.168
DM ATTD, %				
wk 2	79.5	79.6	0.84	0.902
wk 6	82.1	82.9	0.43	0.178
wk 10	82.2	84.1	0.52	0.027
wk 14	82.9	84.3	0.60	0.128
GE ATTD, %				
wk 2	79.3	79.3	0.90	0.957
wk 6	82.3	83.1	0.49	0.286
wk 10	82.1	84.1	0.60	0.040
wk 14	82.5	84.0	0.64	0.116

Table 6. Growth performance of pigs during the grow-finish phase that were previously vaccinated for or inoculated with PCV2 and selected for high or low residual BW (Experiment 3).

Treatment Residual	Inoculated		Vaccinated		SEM	*P*-value		
	H	L	H	L		Trt	Residual	Trt*Residual
BW, kg								
wk 0	43.1	27.2	40.8	33.2	1.84	0.334	0.001	0.034
wk 1 to 2	52.1	51.7	52.0	52.1	1.20	0.827	0.902	0.775
wk 2 to 6	80.4	72.5	77.5	75.5	3.38	0.964	0.223	0.280
wk 6 to 10	108.3	107.8	105.9	107.7	3.53	0.628	0.881	0.678
wk 10 to 14	133.6	138.7	130.2	134.8	4.19	0.235	0.332	0.937
Wk 10 to 14[1]	140.9	129.6	135.0	131.8	3.49	0.604	0.048	0.262
ADFI, kg/d								
wk 1-2	2.27	1.95	2.35	2.31	0.102	0.007	0.141	0.097
wk 2 to 6	3.02	2.98	2.88	3.04	0.082	0.594	0.618	0.245
wk 6 to 10	3.17	3.75	3.25	3.60	0.170	0.812	0.028	0.400
wk 10 to 14	3.39	3.84	3.25	3.68	0.166	0.243	0.033	0.932
ADG, kg/d								
wk 1-2	1.16	1.10	1.15	1.15	0.087	0.781	0.750	0.684
wk 2 to 6	1.14	1.24	1.08	1.19	0.049	0.123	0.102	0.930
wk 6 to 10	0.91	1.29	0.96	1.14	0.056	0.383	0.004	0.082
wk 10 to 14	0.92	1.04	0.84	0.92	0.077	0.091	0.290	0.700
G:F								
wk 1-2	0.51	0.57	0.49	0.50	0.037	0.102	0.419	0.366
wk 2 to 6	0.38	0.42	0.38	0.39	0.012	0.084	0.047	0.143
wk 6 to 10	0.28	0.35	0.30	0.32	0.013	0.406	0.008	0.037
wk 10 to 14	0.27	0.27	0.26	0.25	0.017	0.139	0.772	0.988

[1]BW values not adjusted with wk 0 BW covariate

Table 7. Carcass traits and apparent total tract digestibility (ATTD) of DM and GE of pigs during the grow-finish phase that were previously vaccinated for or inoculated with PCV2 and selected for high or low residual BW (Experiment 3).

Treatment	Inoculated		Vaccinated		SEM	*P*-value		
Residual	H	L	H	L		Trt	Residual	Trt*Residual
Backfat, cm	2.53	2.72	2.35	2.79	0.188	0.661	0.160	0.403
LEA, cm2	51.1	56.1	51.1	50.3	2.51	0.124	0.484	0.155
Lean, kg	52.5	52.7	53.1	51.4	1.49	0.713	0.678	0.430
Lean, %	49.8	50.5	50.5	48.5	1.04	0.375	0.623	0.098
DM ATTD, %								
wk 2	84.1	84.7	84.9	84.3	1.16	0.798	0.994	0.503
wk 6	83.3	83.7	83.9	84.3	0.89	0.359	0.662	0.998
wk 10	84.2	84.9	83.8	84.6	0.88	0.550	0.490	0.910
wk 14	85.3	85.9	84.0	85.3	0.88	0.157	0.358	0.618
GE ATTD, %								
wk 2	83.5	84.7	84.7	84	1.34	0.820	0.855	0.380
wk 6	83.6	84.1	84.4	84.7	0.92	0.301	0.743	0.889
wk 10	84.3	84.9	83.9	84.5	0.70	0.552	0.598	0.950
wk 14	85.2	85.8	83.7	85.2	0.93	0.140	0.311	0.536

Figure 5. Pig carcass traits for Experiment 3. Data presented as least square means and SEM error bars for group PCV-status (inoculated = NOV and vaccinated = VAC), and residual (high and low), respectively.

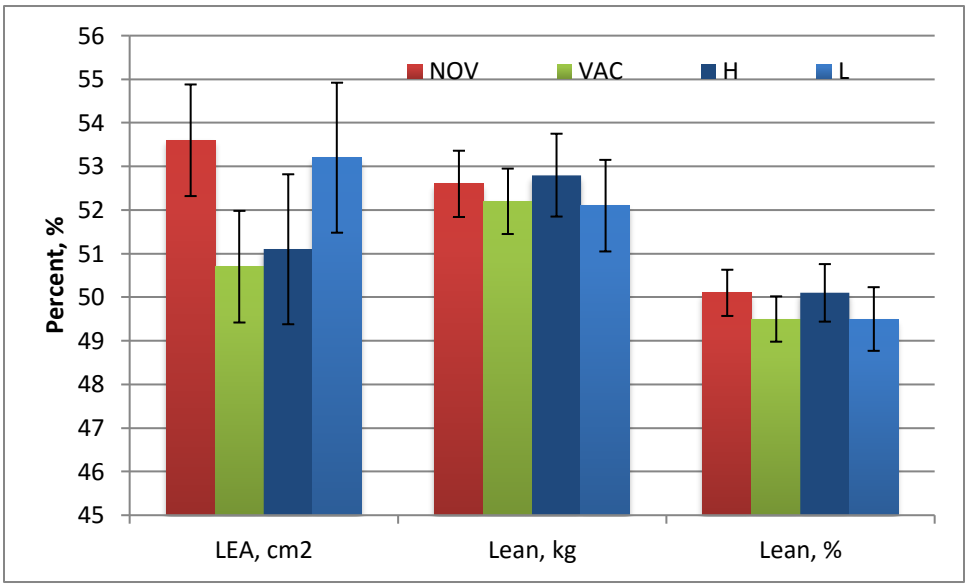


Table 8. Differences in feed cost and carcass value for Experiment 2

Harvest at same live weight	Vaccinated	Inoculated	Difference
ADFI, kg/d	3.25	3.56	0.32
Feed Days	93	94	1
Total Feed, kg	302	335	32.93
Feed Cost @ $0.33/kg	$99.70	$110.56	$10.87
Barn Cost @ $0.10/pig/d housing	$9.30	$9.40	$0.10
Carcass Value @54.94/cwt[1]	$117.42	$117.14	$(0.27)
Estimated cost of PCV2 infection / head			$11.24

Harvest at same age	Vaccinated	Inoculated	Difference
Feed Cost $0.33/kg @ 93 days	99.70	109.39	$9.69
Body Weight, lb	282.7	280.1	-2.64
Carcass Weight, lb	213.7	211.7	-2.00
Carcass Value, @ $54.94/cwt[1]	117.42	116.32	$(1.10)
Estimated cost of PCV2 infection / head			$10.79

[1]National Base Average price reported 12 Dec 2015
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Table 9. Differences in feed cost and carcass value for Experiment 3.

Harvest at same live weight	Vaccinated	Inoculated	Difference	High	Low	Difference
ADFI, kg/d	3.15	3.18	0.03	3.04	3.29	0.25
Feed Days	93	92	-1	88	96	8
Total Feed, kg	293	292	-0.52	267	316	48.17
Feed Cost @ $0.33/kg	96.67	96.50	$(0.17)	88.24	104.14	$15.90
Barn Cost @ $0.10/pig/d housing	9.30	9.20	$(0.10)	8.80	9.60	$0.80
Carcass Value @54.94/cwt[1]	117.78	117.78		117.78	117.05	$(0.73)
Difference between groups			$(0.27)			$17.43
Harvest at same age	Vaccinated	Inoculated	Difference	High	Low	Difference
Feed Cost $0.33/kg @ 93 and 88 days	96.67	97.55	$0.88	88.24	95.46	$7.22
Body Weight, lb	281.4	283.6	2.2	283.6	263.8	-19.8
Carcass Weight, lb	212.7	214.4	1.7	214.4	199.4	-15.0
Carcass Value, @ $54.94/cwt[1]	116.87	117.78	$0.91	117.78	109.56	$(8.22)
Difference between groups			$(0.04)			$15.44

[11]National Base Average price reported 12 Dec 2015 www.dailylivestockreport.com

Table 10. Associations of operational taxonomic units (OUT) with phenotypic data using multivariate analysis by linear associations (MaAsLin) in a previous PCV2 challenge experiment (NPB #12-185).

Variable	Feature	Value[2]	Phylum	Family	Genus	Species	Coefficient	*P*-value	*q*-value
Day × PCV	OTU102	`day*PCV`d14_I	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	*salivae*	–0.063	0.001	0.025
Day × PCV	OTU102	`day*PCV`d28_V	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	*salivae*	–0.084	0.001	0.030
IgM	OTU11	IgM	*Firmicutes*	*Oscillospiraceae*	*Oscillibacter*	*valericigenes*	–0.094	0.005	0.034
Day × PCV	OTU154	`day*PCV`d14_V	*Firmicutes*	*Clostridiaceae*	*Clostridium*	*saccharobutylicum*	–0.071	0.001	0.023
Day × PCV	OTU196	`day*PCV`d28_V	*Firmicutes*	*Ruminococcaceae*	*Ruminococcus*	*bromii*	0.067	0.000	0.000
Day × PCV	OTU231	`day*PCV`d28_V	*Firmicutes*	*Ruminococcaceae*	*Ruminiclostridium*	*thermosuccinogenes*	0.105	0.000	0.015
Day × PCV	OTU364	`day*PCV`d0_V	*Firmicutes*	*Lachnospiraceae*	*Anaerostipes*	*caccae*	0.062	0.000	0.001
Day × PCV	OTU364	`day*PCV`d28_I	*Firmicutes*	*Lachnospiraceae*	*Anaerostipes*	*caccae*	0.059	0.000	0.002
Day × PCV	OTU364	`day*PCV`d28_V	*Firmicutes*	*Lachnospiraceae*	*Anaerostipes*	*caccae*	0.051	0.000	0.009
Day × PCV	OTU37	`day*PCV`d14_I	*Firmicutes*	*Clostridiaceae*	*Clostridium*	*autoethanogenum*	0.051	0.000	0.011
Day × PCV	OTU37	`day*PCV`d28_V	*Firmicutes*	*Clostridiaceae*	*Clostridium*	*autoethanogenum*	0.071	0.001	0.019
IgM	OTU39	IgM	*Firmicutes*	–	*Intestinimonas*	*butyriciproducens*	–0.073	0.002	0.013
Day × PCV	OTU426	`day*PCV`d0_V	*Firmicutes*	*Oscillospiraceae*	*Oscillibacter*	*ruminantium*	0.079	0.000	0.001
Day × PCV	OTU426	`day*PCV`d14_I	*Firmicutes*	*Oscillospiraceae*	*Oscillibacter*	*ruminantium*	0.068	0.000	0.012
Day × PCV	OTU426	`day*PCV`d28_V	*Firmicutes*	*Oscillospiraceae*	*Oscillibacter*	*ruminantium*	0.063	0.000	0.013
Day × PCV	OTU45	`day*PCV`d14_V	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	*stercorea*	–0.089	0.000	0.009
Day × PCV	OTU45	`day*PCV`d28_V	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	*stercorea*	–0.114	0.001	0.023
IgG	OTU45	IgG	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	*stercorea*	0.214	0.007	0.041
Day × PCV	OTU50	`day*PCV`d14_V	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	*denticola*	0.066	0.000	0.002
Day × PCV	OTU52	`day*PCV`d28_I	*Firmicutes*	*Clostridiaceae*	*Saccharofermentans*	*acetigenes*	–0.056	0.001	0.028
Day × PCV	OTU713	`day*PCV`d14_V	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	*scopos*	0.063	0.000	0.006
Day × PCV	OTU713	`day*PCV`d28_I	*Bacteroidetes*	*Prevotellaceae*	*Prevotella*	*scopos*	0.058	0.000	0.013
Day × PCV	OTU801	`day*PCV`d14_I	*Firmicutes*	*Veillonellaceae*	*Megasphaera*	*elsdenii*	0.063	0.000	0.010
Day × PCV	OTU801	`day*PCV`d14_V	*Firmicutes*	*Veillonellaceae*	*Megasphaera*	*elsdenii*	0.060	0.000	0.017
Day × PCV	OTU801	`day*PCV`d28_I	*Firmicutes*	*Veillonellaceae*	*Megasphaera*	*elsdenii*	0.062	0.000	0.011
Day × PCV	OTU814	`day*PCV`d0_V	*Bacteroidetes*	*Cytophagaceae*	*Cytophaga*	*xylanolytica*	0.060	0.001	0.044
IgG	OTU9	IgG	*Bacteroidetes*	*Sphingobacteriaceae*	*Solitalea*	*koreensis*	0.407	0.002	0.014

[1]All OTU are >1% of OTU relative abundance.

[2]V = vaccinated; I = inoculated with porcine circovirus 2.