Journal of Animal and Veterinary Advances 4 (9): 805-817, 2005
© Grace Publications, 2005

Effects of an *Actinobacillus pleuropneumoniae* Challenge on Growth, Nitrogen Balance, Insulin-like Growth Factor-I, Acute Phase Proteins and Final Body Composition of Swine

[1]J.A. Loughmiller, [2]S.S. Dritz, [1]J.L. Nelssen, [1]M.D. Tokach,
[1]R.D. Goodband,[3]B.W. Fenwick, [4]A. Weber and [4]T.L. Mcdonald
[1]Department of Animal Sciences and Industry, [2]Food Animal Health and Management Center
[3]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan 66506
[4]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha 68198

Abstract: Forty seven pigs (30±1 kg) were used to determine the effects of acute *Actinobacillus pleuropneumoniae* challenge on growth, N balance, acute phase proteins, plasma IGF-I and carcass characteristics. Pigs were challenged (APP; n=30; intranasally 5×10^7 cfu) on d 0, unchallenged and fed *ad libitum* (AL; n=7), or unchallenged and pair-fed the feed intake of a challenged pig (PF; n=10). Mortality was 23% for APP, but none occurred in unchallenged groups. A treatment×time interaction was observed for serum haptoglobin ($p<0.01$) which was higher for APP vs AL and PF on d 1 and 5. Plasma IGF-I (treatment and quadratic time effects, $p<0.03$) was lowest on d 1 for APP vs AL and PF ($p<0.01$). The IGF-I levels tended to remain lower for APP than for AL, but not PF, through d 9 ($p<0.10$). Treatment and linear time effects were observed ($p<0.05$) for ADG, which was lower ($p<0.01$) for APP vs AL from d 0 to 3. Lower ADG was observed from d 4 to 7 for PF vs APP ($p<0.06$) and PF vs AL ($p<0.04$). Day 0 to 17 ADG was higher for AL vs APP and PF ($p<0.03$). Treatment did not affect G/F ($p>0.10$). A treatment×time interaction was observed for N retained ($p<0.05$). Greater N retention was observed for AL vs APP ($p<0.01$) from d 0 to 7. Results indicate that growth and N balance reductions resulted from reduced ADFI and increased endogenous N losses. Reductions were primarily short-term, because growth performance and N retention recovered by d 14 after challenge.

Key words: Pigs, growth, N balance, IGF-I, acute phase proteins, respiratory disease

INTRODUCTION

Chronic disease challenges that restrict lean growth potential of swine have been minimized by wide-scale adoption of all-in/all-out, multisite production and segregated early weaning[1,2]. However, short duration, acute disease challenges still occur in these high-health production systems. They usually result from a pathogen infecting immune-naive groups of pigs. The pathogen spreads rapidly throughout the group and within a short period, immunity develops and performance partially recovers. Although lean growth rate has been improved dramatically in high-health production systems, acute disease challenges may be responsible for a large majority of the variation in lean growth rate between groups of pigs and among individuals within a group.

Previous studies have shown that protein metabolism is influenced negatively in immune stimulated pigs[3,4]. However, those experiments either utilized a noninfectious challenge or were designed to characterize chronic immune challenges typical in continuous-flow production systems. Because acute disease challenges affect current production systems, determining their effects on growth performance and N balance should help industry professionals further understand the effects of an immune response in swine. Previous study in our laboratory indicated that the effects of an acute *Salmonella typhimurium* challenge were overcome quickly and did not reduce long-term performance[5]. Because acute disease challenges are typically enteric or respiratory, we followed our enteric disease challenge experiment with the current experiment to determine the effects of an acute respiratory disease challenge on growing pig performance. Therefore, our objective was to characterize the effects of an acute *Actinobacilllus pleuropneumoniae* challenge in growing pigs by measuring changes in growth performance, protein metabolism using N balance techniques, carcass composition and organ weights. Furthermore, we wanted to document the changes in IGF-I and acute phase proteins before, during and after an acute disease challenge.

MATERIALS AND METHODS

Animal care and use: The Kansas State University Institutional Animal Care and Use Committee approved this experimental protocol.

Corresponding Author: R.D. Goodband, Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506

Animals and experimental design: Forty-seven pigs (initially 30±1 kg; PIC L326×C22) were obtained from the university swineherd and allotted to one of three experimental treatments. All pigs were verified *A. pleuropneumoniae* negative by serological testing prior to being selected for this study[6]. After serological verification, pigs were selected in blocks of 11 or 12 from the same 7 d farrowing group and were assigned randomly to either the *A. pleuropneumoniae* challenge or one of two control treatments. Within the two control treatments, pigs were assigned randomly to have *ad libitum* access to feed or to be pair-fed the net feed intake of an assigned *A. pleuropnuemoniae*-challenge pig. Each pair-fed control pig was matched to one *A. pleuropneumoniae* challenged pig throughout the entire experiment. Because we expected greater variation in performance and mortality with *A. pleuropneumoniae* challenge, 30 pigs were assigned to that group, 10 pigs to the pair-fed control group and 7 to the control *ad libitum*-feed group pigs.

Water was supplied at a 3.5:1 ratio with feed on a wt:wt basis[7]. All pigs were fed and watered twice daily at 0730 and 1930. Orts were collected, dried and weighed back daily. All pigs were fed a common corn-soybean meal diet formulated to contain 1.15% total lysine with no synthetic amino acids, added fat, or antibiotics (Table 1). Five 4-d collection periods immediately followed a 3-d adjustment period (d -4 to -1, d 0 to 3, d 4 to 7, d 8 to 11 and d 14 to 17) with the *A. pleuropneumoniae* challenge occurring on d 0. These times were selected to provide one prechallenge, one challenge, two recovery and one post-recovery periods. All pigs were euthanized on d 27 by intravenous sodium pentobarbital administration (390 mg mL^{-1} conc.; administered 1 mL/5 kg BW) and disposed of according to NIH-CDC biosafety level 1 procedures[8] at the Kansas State University Veterinary Diagnostic Laboratory.

Pair-feeding procedure: The pair-feeding was performed to control for responses that depend on feed intake. Each pair-fed control pig was matched to one *A. pleuropneumoniae*-challenged pig on experimental d -4 based upon initial BW and remained paired throughout the entire experiment, unless the challenged pig died. In that case, the control pig was reassigned to a previously unpaired challenge pig that most closely matched the body weight and previous feed intake pattern of the deceased pig. Throughout the experiment, five control pair-fed pigs were rematched because of mortality of the challenged pig. Pair feeding was accomplished by feeding the pair-fed control pig the previous 24 h net feed intake of its assigned *A. pleuropneumoniae* challenged pig.

Bacterial challenge: For this experiment, *A. pleuropneumoniae* serotype 1 reference strain 4074 was thawed and subcultured on chocolate agar (Remel, Lenexa, KS) for isolation and on blood agar (Remel, Lenexa, KS) to confirm the purity. After 24 h incubation at 37 C in 6% CO_2, an isolated colony was selected from the chocolate agar and inoculated into 25 mL of RPMI+ (GibcoBRL, Grand Island, NY) made according to package instructions and supplemented with 2.5% fetal bovine serum (Sigma, St. Louis, MO). This culture was incubated to stationary phase at 37° C with shaking. Ten millilitre of the stationary phase culture was inoculated into 100 mL of warm RPMI+ and incubated for 4 h at 37 C. Each animal received a 4 mL dose of the 4 h culture via intranasal inoculation (approximately 5×10^7 cfu). Negative control animals received 4 mL of sterile RPMI+ intranasally. Pigs assigned to the challenge treatment were inoculated intranasally with doses of 5×10^7 cfu *A. pleuropneumoniae* in 4 mL culture media. Control pigs were treated similarly with 4 mL sterile medium.

Housing and N balance: The experiment was conducted from December 1998 through April 1999. All pigs were housed in the same facility within two environmentally controlled rooms separated by a common hallway with constant lighting. Pigs were housed based upon health status and were kept in individually adjustable stainless steel metabolism cages (1.5 m×0.9 m) that allowed separate collection of feces and urine. The marker to marker method (0.5% ferric oxide in the first meal of each consecutive period and the eighth subsequent meal occurring on d 11 and d 17) was used to determine the beginning and end of feces collection for a period. Feces were collected twice daily and stored at -10°C. At the end of each period, feces were homogenized and subsampled. Urine was collected daily in polypropylene bottles containing 75 mL of 6 N HCl. Ten percent of the daily urine volume was

Table 1: Diet composition[a]

Ingredient	Percent
Corn[b]	64.04
Soybean meal, 46.5% CP[b]	32.89
Monocalcium phosphate	1.22
Limestone	1.10
Salt	0.35
Vitamin premix[c]	0.25
Trace mineral premix[d]	0.15

[a]Diet was formulated to contain 1.15% lysine, 0.75% Ca and 0.65% P

[b]Calculated values for lysine from NRC[39] and NCR-42[40] for corn and soybean meal, respectively, were used in diet formulation

[c]Provided the following per kilogram of complete diet: vitamin A, 11,023 IU; vitamin D_3, 1654 IU; vitamin E, 44 IU; menadione (menadione sodium bisulfate complex), 4.4 mg; riboflavin, 9.9 mg; pantothenic acid, 33 mg; niacin, 55 mg; vitamin B_{12}, 44 mcg

[d]Provided the following per kilogram of complete diet: Mn, 40 mg; Fe, 165 mg; Zn, 165 mg; Cu, 17 mg; I, 298 mcg; Se, 298 mcg

subsampled and stored at -10°C until laboratory analysis. Urine was thawed and then centrifuged at 2000×*g* to remove particulate matter before further subsampling prior to analysis. Feed samples were ground through a 1mm screen before analysis. Feed, feces and urine were analyzed for N on an as-is basis to minimize any loss of gaseous ammonia before analysis[7] and also for DM and GE according to AOAC[9] procedures.

Assays for insulin-like growth factor-I and acute phase proteins: Plasma and serum samples were harvested from blood samples drawn via jugular venipuncture using heparinized and nonheparinized vacuum tubes on d -3, 1, 5, 9 and 15 at least 4 h after the 0630 feeding. The samples were analyzed for concentrations of insulin-like growth factor (IGF-I), serum haptogolobin and AGP. Plasma IGF-I concentrations were determined using a commercially available two-site immunoradiometric assay (Diagnostic Systems Laboratories, Webster TX). This assay was validated for porcine IGF-I in our laboratory[10] and has a detection limit of 0.4 ng mL^{-1}. Serum haptoglobin concentrations were determined colorimetrically using procedures adapted from Smith *et al.*[11] that have a designed sensitivity from 0 to 150 mg Hgb dL^{-1}. Serum amyloid A concentrations were determined using a commercially available ELISA (Tridelta Development Ltd., Republic of Ireland) with a designed sensitivity from 5 to 500 mcg mL^{-1}. Samples above the detection limit were diluted at 1:500 instead of 1:250 to ensure that the concentration would fall within detectable limits[12]. Serum AGP concentrations were determined using a commercially available radial immunodiffusion assay (Cardio-Tech Services, Louisville KY) with detection limits from 50 to 1,500 mcg mL^{-1}.

Statistics: All data were analyzed as an unbalanced randomized complete block design using a PROC MIXED ANOVA procedure with repeated measures and a Satterthwaite error correction[13,14]. In addition, the mixed procedure utilized a maximal likelihood estimation of missing observations to account for a 23% mortality rate in the *A. pleuropneumoniae* challenged pigs during the experiment. The model included the treatment effect (animal within treatment variance) and the effects of time period and the treatment×time period interaction. Comparisons between treatments within sampling times were made only when a treatment×time interaction or a treatment effect was found. Individual pig was the experimental unit. Periodic samples by pig were used for the repeated measures. Pigs were blocked by time and initial weight and were assigned randomly to individual treatments within each block. Linear and quadratic polynomial contrasts were used to determine the effects of *A. pleuropneumoniae* challenge over time on all response criteria.

RESULTS

Acute phase proteins: A treatment×time interaction was observed for serum haptoglobin and amyloid A concentrations ($p<0.01$; Table 2). Serum haptoglobin levels were elevated for the *A. pleuropneumoniae*-challenged pigs versus the ad-libitum fed control pigs and the pair-fed control pigs on d 1, d 5 and d 9 ($p<0.01$) and were highest on d 5. Serum amyloid A levels rose more rapidly after the challenge than did serum haptoglobin levels. Serum amyloid A levels of the *A. pleuropneumoniae*-challenged pigs were elevated above those of control pair-fed pigs and *ad libitum* pigs on d 1 ($p<0.001$) and d 5 ($p<0.004$) and were highest on d 1.

A treatment effect was observed for serum AGP levels ($p<0.01$). This was a result of numerically higher AGP levels for the ad-libitum fed control pigs versus either the *A. pleuropneumoniae*-challenged pigs ($p<0.01$) or the pair-fed control pigs ($p<0.01$) throughout the duration of the experiment. In addition, serum AGP levels decreased over time similarly in all three treatments (linear, $p<0.01$).

Insulin-like growth factor I: A treatment effect and a quadratic time effect were observed for IGF-I concentrations ($p<0.03$). Levels of insulin-like growth factor-I were lowest for *A. pleuropneumoniae*-challenged pigs on d 1 compared to *ad libitum*-fed control pigs ($p<0.003$) and pair-fed control pigs ($p<0.001$). The levels of *A. pleuropneumoniae*-challenged pigs tended to remain lower than those of *ad libitum*-fed control pigs through d 9 ($p<0.10$).

Growth Performance: A treatment effect and a linear time effect ($p<0.05$) were observed for ADG (Table 3). The treatment effect was a result of decreased ADG from d 0 to 3 for the *A. pleuropneumoniae* challenged pigs ($p<0.003$) and the pair-fed control pigs ($p<0.04$) versus the *ad libitum* fed control pigs. Decreased ADG also occurred from d 4 to 7 for the pair-fed control pigs versus the *A. pleuropneumoniae*-challenged pigs ($p<0.06$) and the *ad libitum*-fed control pigs ($p<0.04$). The decreased ADG associated with the disease challenge and pair feeding carried through for the d 0 to 17 overall postchallenge period. Average daily gain was lower for the *A. pleuropneumoniae*-challenged pigs ($p<0.02$) and the pair-fed control pigs ($p<0.03$) than for the *ad libitum*-fed control pigs.

Table 2: Effects of *A. pleuropneumoniae* challenge and feeding regimen on selected acute phase proteins and insulin-like growth factor-I in 32 to 54 kg pigs[a]

		A. pleuropneumoniae	Nonchallenged				
	Days Post-challenge	*Ad libitum*$_1$	*Ad libitum*$_2$	Pair-fed$_3$	Probability (p<)		
Criteria	n	30	7	10	1 vs. 2	1 vs. 3	2 vs. 3
Haptoglobin	-3	48.8±05.1	49.9±09.0	48.9±07.7	0.910	0.990	0.93
(mg Hgb dL^{-1}[b])	1	85.3±05.1	49.4±09.0	50.3±07.7	0.001	0.001	0.93
	5	102.1±05.5	42.1±09.0	47.0±07.7	0.001	0.001	0.66
	9	63.8±05.8	37.9±09.0	33.3±07.7	0.010	0.001	0.68
	15	39.8±05.6	27.5±09.0	32.1±07.7	0.210	0.370	0.68
Amyloid A	-3	5.4±04.2	13.0±08.2	8.6±06.5	0.390	0.660	0.67
(μg mL^{-1}[b])	1	47.9±04.1	7.0±08.2	5.1±06.5	0.001	0.001	0.85
	5	34.0±04.4	8.1±08.2	8.8±06.5	0.004	0.001	0.94
	9	10.7±04.7	1.8±08.2	2.3±06.5	0.300	0.280	0.97
	15	4.7±04.5	1.5±08.2	6.0±06.5	0.710	0.860	0.64
AGP	-3	610.0±70.0	720.0±99.0	570.0±89.0	0.210	0.600	0.15
(μg mL^{-1}[c])	1	615.0±71.0	689.0±99.0	608.0±89.0	0.410	0.930	0.44
	5	563.0±73.0	676.0±99.0	605.0±89.0	0.210	0.600	0.49
	9	550.0±75.0	673.0±99.0	483.0±89.0	0.180	0.430	0.07
	15	488.0±73.0	586.0±99.0	392.0±89.0	0.280	0.230	0.06
IGF-I	-3	240.0±22.0	238.0±37.0	274.0±34.0	0.980	0.340	0.45
(ng mL^{-1}[d])	1	155.0±22.0	274.0±37.0	275.0±34.0	0.003	0.001	0.96
	5	173.0±23.0	239.0±37.0	221.0±34.0	0.100	0.170	0.70
	9	236.0±24.0	306.0±37.0	228.0±34.0	0.080	0.840	0.10
	15	263.0±24.0	288.0±37.0	270.0±34.0	0.540	0.850	0.70

[a]Forty seven pigs were used in an unbalanced randomized complete block design with repeated measures, [b]Treatment × time (p<0.01); quadratic time effect (p<0.01), [c]Treatment effect (p<0.01); linear time effect (p<0.01), [d]Treatment effect (p<0.01); quadratic time effect (p<0.03)

Table 3: Effects of *A. pleuropneumoniae* challenge and feeding regimen on growth performance of 32 to 54 kg pigs[a]

		A. pleuropneumoniae	Nonchallenged		Probability (p<)		
Criteria	Days Post-challenge	*Ad libitum*$_1$	*Ad libitum*$_2$	Pair-fed$_3$	1 vs. 2	1 vs. 3	2 vs. 3
ADG (kg[b])	-4 to -1	0.83±0.10	0.89±0.16	0.76±0.14	0.75	0.68	0.560
	0 to 3	0.54±0.10	1.08±0.16	0.64±0.14	0.003	0.50	0.040
	4 to 7	0.86±0.10	1.00±0.16	0.56±0.14	0.44	0.06	0.040
	8 to 11	1.21±0.10	1.33±0.16	1.01±0.14	0.54	0.49	0.280
	14 to 17	0.97±0.10	1.20±0.16	1.15±0.14	0.21	0.26	0.820
	0 to 17	0.88±0.08	1.16±0.11	0.87±0.10	0.02	0.89	0.030
ADFI (kg[c])	-4 to -1	1.60±0.09	1.54±0.16	1.48±0.13	0.71	0.40	0.760
	0 to 3	1.22±0.09	1.73±0.16	1.29±0.13	0.003	0.64	0.020
	4 to 7	1.37±0.10	1.92±0.16	1.13±0.13	0.001	0.11	0.001
	8 to 11	1.72±0.10	2.05±0.16	1.46±0.13	0.05	0.09	0.003
	14 to 17	2.25±0.10	2.29±0.16	1.91±0.13	0.83	0.02	0.050
	0 to 17	1.62±0.07	1.99±0.11	1.44±0.09	0.01	0.09	0.010
G/F	-4 to -1	0.51±0.15	0.59±0.29	0.48±0.29	0.80	0.93	0.780
	0 to 3	0.24±0.17	0.63±0.29	0.42±0.24	0.22	0.52	0.560
	4 to 7	0.38±0.17	0.54±0.29	-0.22±0.24	0.61	0.04	0.040
	8 to 11	0.69±0.17	0.66±0.29	0.75±0.24	0.93	0.83	0.800
	14 to 17	0.43±0.17	0.53±0.29	0.62±0.24	0.74	0.49	0.810
	0 to 17	0.43±0.13	0.60±0.18	0.40±0.16	0.36	0.86	0.340

[a] Forty seven pigs were used in an unbalanced randomized complete block design with repeated measures,
[b]Treatment effect, (p<0.02); linear time effect, (p<0.01), [c]Treatment × time (p<0.06)

Treatment and linear and quadratic time effects were observed for ADFI (p<0.05). This interaction resulted from decreases in ADFI from d 0 to 3, d 4 to 7 and d 8 to 11 for both the *A. pleuropneumoniae*-challenged pigs and the pair-fed control pigs versus the *ad libitum*-fed control pigs (p<0.05). In addition, ADFI was lower for the pair-fed control pigs versus the *A. pleuropneumoniae* challenged pigs (p<0.02) and the *ad libitum* control pigs (p<0.05) from d 14 to 17. These differences resulted in lower d 0 to 17 ADFI for *A. pleuropneumoniae* pigs and pair-fed control pigs than for *ad libitum*-fed control pigs (p<0.01). Feed efficiency (G/F) was not affected by treatment. In general, differences in growth performance were due primarily to decreased ADFI.

N and DM digestibility and energy balance: Treatment and linear time effects were observed for DM digestibility (p<0.01; Table 4). Greater apparent DM digestibility for *A. pleuropneumoniae*-challenged pigs compared to *ad libitum*-fed control pigs was observed from d 0 to 3 (p<0.06) and d 14 to 17 (p<0.02). Similarly, apparent DM digestibility was greater for the *A. pleuropneumoniae*-challenged pigs versus the pair-fed control pigs from d 0 to 3 (p<0.03) and d 4 to 7 (p<0.05) and tended to be greater

Table 4: Effects of *A. pleuropneumoniae* challenge and feeding regimen on apparent dry matter and apparent nitrogen digestibility and apparent energy balance in 32 to 54 kg pigs[a]

		A. pleuropneumoniae	Nonchallenged		Probability (p<)		
Criteria	Days Post-challenge	*Ad libitum*$_1$	*Ad libitum*$_2$	Pair-fed$_3$	1 vs. 2	1 vs. 3	2 vs. 3
App. DM dig.,	-4 to -1	86.80±0.90	87.40±1.20	88.10±1.10	0.610	0.170	0.56
(%[b])	0 to 3	87.90±0.90	85.90±1.20	85.80±1.10	0.060	0.030	0.92
	4 to 7	87.00±0.90	86.10±1.20	85.10±1.10	0.440	0.050	0.41
	8 to 11	86.90±0.90	85.60±1.20	85.70±1.10	0.240	0.220	0.93
	14 to 17	87.00±0.90	84.20±1.20	85.40±1.10	0.020	0.090	0.35
	0 to 17	87.00±0.70	85.50±0.80	85.60±0.70	0.004	0.002	0.93
App. N dig.,	-4 to -1	82.10±1.00	81.60±1.50	82.60±1.30	0.740	0.660	0.54
(%[c])	0 to 3	86.60±1.00	83.00±1.50	83.40±1.30	0.010	0.010	0.84
	4 to 7	85.70±1.10	83.70±1.50	83.10±1.30	0.150	0.040	0.72
	8 to 11	85.30±1.10	83.20±1.50	84.40±1.30	0.130	0.440	0.47
	14 to 17	85.70±1.10	81.80±1.50	83.80±1.30	0.010	0.120	0.21
	0 to 17	85.90±0.90	82.90±1.10	83.60±1.00	0.001	0.001	0.38
Energy Intake							
GE (MJ d[b])	0 to 3	16.70±1.40	23.70±2.50	17.70±2.10	0.010	0.680	0.06
	4 to 7	18.60±1.50	26.20±2.50	15.40±2.10	0.010	0.190	0.001
	8 to 11	23.40±1.50	28.00±2.50	20.00±2.10	0.100	0.170	0.01
DE, % of Ge[d]	0 to 3	87.50±1.00	85.90±1.30	85.90±1.20	0.140	0.100	0.99
	4 to 7	86.90±1.00	86.40±1.30	85.20±1.20	0.660	0.070	0.30
	8 to 11	87.00±1.00	85.90±1.30	86.10±1.20	0.320	0.390	0.83
ME, % of GE	0 to 3	85.90±1.50	84.30±2.30	84.50±2.00	0.500	0.500	0.94
	4 to 7	83.50±1.50	85.30±2.30	81.90±2.00	0.450	0.430	0.20
	8 to 11	86.00±1.50	85.30±2.30	82.40±2.00	0.780	0.090	0.27
ME efficiency							
(kg gain/MJ Me[e])	0 to 3	0.02±0.03	0.06±0.05	0.04±0.04	0.510	0.720	0.76
	4 to 7	0.03±0.03	0.05±0.05	-0.07±0.04	0.760	0.040	0.06
	8 to 11	0.06±0.03	0.06±0.05	0.07±0.04	0.990	0.790	0.83

[a]Forty seven pigs were used in an unbalanced randomized complete block design with repeated measures, [b]Treatment effect (p<0.01); linear time effect (p<0.01), [c]Treatment effect (p<0.01); quadratic time effect (p<0.02), [d]Treatment effect (p<0.03), [e]Quadratic time effect (p<0.09)

from d 14 to 17 (p<0.09). These periodic differences resulted in increased apparent DM digestibility from d 0 to 17 for the *A. pleuropneumoniae*-challenged pigs versus both control treatments (p<0.01).

Treatment and quadratic time effects were observed for apparent N digestibility (p<0.01, p<0.02, respectively). It was higher for the *A. pleuropneumoniae*-challenged pigs compared to the ad libitum-fed control pigs from d 0 to 3 (p<0.06) and d 14 to 17 (p<0.02). In addition, apparent N digestibility was higher for the *A. pleuropneumoniae*-challenged pigs versus the pair-fed control pigs from d 0 to 3 (p<0.03) and d 4 to 7 (p<0.05). These differences led to increased apparent N digestibility for the *A. pleuropneumoniae* challenged pigs versus both control treatments from d 0 to 17 (p<0.001).

Energy intake: Gross energy intake was affected by treatment (p<0.01) and increased over time within treatment (linear, p<0.01). It was higher for the *ad libitum*-fed control pigs than for the *A. pleuropneumoniae*-challenged pigs from d 0 to 3 (p<0.01) and d 4 to 7 (p<0.01) and tended to be higher from d 8 to 11 (p<0.10). Gross energy intake was also higher for the *ad libitum*-fed control pigs compared to the pair-fed control pigs from d 0 to 3 (p<0.06), d 4 to 7 (p<0.001) and d 8 to 11 (p<0.01).

Digestible energy, as a percent of GE intake, was affected by treatment (p<0.03). The *A. pleuropneumoniae*-challenged pigs tended to have greater DE intake than the pair-fed control pigs from d 0 to 3 (p<0.10) and 4 to 7 (p<0.07), but their intake was not higher than that of the *ad libitum*-fed control pigs (p<0.14).

Metabolizable energy intake, as a percent of GE intake, was not affected by treatment (p<0.18) or time (p>0.59). Metabolizable energy utilization efficiency also was unaffected by treatment (p<0.50) but tended to increase over time (quadratic, p<0.09).

Nitrogen balance: A tendency for a treatment×time interaction was observed for N intake (p<0.09; Table 5). The interaction was due to decreased N intake for the *A. pleuropneumoniae*-challenged pigs versus the *ad libitum*-fed control pigs from d 0 to 3 (p<0.008), 4 to 7 (p<0.001) and d 8 to 11 (p<0.05). In addition, the pair fed control pigs had lower N intake than the *ad libitum*-fed control pigs from d 0 to 3 (p<0.02), 4 to 7 (p<0.001), d 8 to 11 (p<0.003) and d 14 to 17 (p<0.05). These differences during the challenge and recovery periods resulted in higher N intake for the *ad libitum*-fed control pigs versus either the *A. pleuropneumoniae*-challenged pigs (p<0.004) or the pair-fed control pigs (p<0.001) from d 0 to 17.

Table 5: Effects of *A. pleuropneumoniae* challenge and feeding regimen on nitrogen balance and N retention efficiency in 32 to 54 kg pigs[a]

		A. pleuropneumoniae	Nonchallenged		Probability (p<)		
Criteria	Days Post-challenge	*Ad libitum* Trt 1	*Ad libitum* Trt 2	Pair-fed Trt 3	1 vs. 2	1 vs. 3	2 vs. 3
N intake (g d^{-1}[b])	-4 to –1	55.7±3.1	53.4±5.4	51.4±4.6	0.70	0.75	0.76
	0 to 3	44.8±3.2	60.2±5.4	44.8±4.6	0.008	0.60	0.02
	4 to 7	47.6±3.3	66.6±5.4	39.1±4.6	0.001	0.56	0.001
	8 to 11	59.8±3.3	71.1±5.4	50.8±4.6	0.05	0.30	0.003
	14 to 17	78.4±3.3	79.6±5.4	66.3±4.6	0.84	0.23	0.05
	0 to 17	57.3±2.3	69.2±3.7	50.1±3.2	0.004	0.05	0.001
Fecal N (g d^{-1}[c])	-4 to –1	10.0±0.58	9.7±1.1	8.9±0.95	0.77	0.79	0.61
	0 to 3	6.1±0.61	10.0±1.1	7.3±0.95	0.003	0.69	0.07
	4 to 7	6.8±0.62	10.7±1.1	8.2±0.95	0.003	0.98	0.002
	8 to 11	9.1±0.63	11.7±1.1	6.2±0.95	0.04	0.62	0.02
	14 to 17	11.4±0.63	14.3±1.1	10.9±0.95	0.02	0.98	0.02
	0 to 17	8.3±0.40	11.7±0.7	8.1±0.6	0.001	0.82	0.001
Urinary N (g d^{-1}[d])	-4 to –1	14.7±1.60	12.9±2.4	12.9±2.1	0.41	0.44	0.99
	0 to 3	17.2±1.70	16.2±2.4	12.8±2.1	0.67	0.04	0.19
	4 to 7	17.4±1.70	17.7±2.4	12.5±2.1	0.89	0.07	0.04
	8 to 11	20.7±1.70	22.5±2.4	15.5±2.1	0.43	0.01	0.006
	14 to 17	26.9±1.70	29.6±2.4	23.6±2.1	0.23	0.09	0.02
	0 to 17	20.4±1.40	21.5±1.8	16.1±1.7	0.48	0.002	0.002
Retained N (g d^{-1}[e])	-4 to –1	31.0±2.00	30.8±3.4	29.5±2.9	0.97	0.99	0.74
	0 to 3	21.6±2.10	33.9±3.4	24.7±2.9	0.001	0.81	0.03
	4 to 7	23.4±2.10	38.1±3.4	20.4±2.9	0.001	0.89	0.001
	8 to 11	30.0±2.20	36.8±3.4	27.1±2.9	0.06	0.98	0.02
	14 to 17	40.2±2.20	35.6±3.4	31.7±2.9	0.21	0.60	0.35
	0 to 17	28.6±1.70	36.1±2.4	26.0±2.1	0.002	0.21	0.001
N retention efficency							
% of N intake	-4 to –1	55.6± 6.2	57.7±11.8	57.7±9.9	0.87	0.85	0.99
	0 to 3	43.8± 6.5	56.4±11.8	53.3±9.9	0.34	0.41	0.84
	4 to 7	38.1± 6.6	57.3±11.8	18.8±9.9	0.15	0.10	0.01
	8 to 11	50.1± 6.8	52.2±11.8	50.7±9.9	0.87	0.96	0.92
	14 to 17	51.8±6.8	45.0±11.8	48.1±9.9	0.61	0.75	0.84
	0 to 17	45.8±4.5	52.8±7.1	42.8±6.1	0.35	0.66	0.24
% of absorbed N[f]	-4 to –1	67.9±8.0	70.6±15.5	69.7±13.0	0.87	0.90	0.96
	0 to 3	50.9±8.5	67.8±15.5	63.8±13.0	0.33	0.40	0.84
	4 to 7	43.3±8.6	68.5±15.5	16.0±13.0	0.15	0.08	0.01
	8 to 11	59.0±8.8	62.6±15.5	60.1±13.0	0.84	0.94	0.90
	14 to 17	60.6±8.8	55.0±15.5	57.2±13.0	0.75	0.82	0.91
	0 to 17	53.2±5.7	63.6±9.2	49.4±7.9	0.30	0.67	0.21

[a]Forty seven pigs were used in a unbalanced randomized complete block design with repeated measures, [b]Treatment × time interaction (p<0.09), [c]Treatment effect (p<0.01); quadratic time effect, (p<0.01), [d]Treatment effect (p<0.01); quadratic time effect, (p<0.01), [e]Treatment × time interaction (p<0.01) [f]Quadratic time effect (p<0.08)

Table 6: Effects of *A. pleuropneumoniae* challenge and feeding regimen on carcass characteristics and organ weights of 32 to 54 kg pigs[a]

	A. pleuropneumoniae	Nonchallenged		Probability (p<)		
Criteria	Ad libitum Trt 1	Ad libitum Trt 2	Pair-fed Trt 3	1 vs. 2	1 vs. 3	2 vs. 3
d 19 10th rib fat depth, mm	9.57±0.85	11.43±1.34	9.68±1.15	0.20	0.93	0.30
d 19 LMA, cm^2	30.09±0.7	32.00±1.4	32.50±1.1	0.48	0.24	0.78
d 19 Organ wt (g)						
Heart	277.00±0.21	332.00±0.32	280.00±0.28	0.10	0.90	0.19
Liver	1516.00±0.78	1605.00±0.98	1440.00±0.90	0.29	0.30	0.09
Spleen	169.00±0.22	204.00±0.35	190.00±0.30	0.36	0.53	0.75
Kidneys	322.00±0.12	315.00±0.22	313.00±0.18	0.77	0.66	0.94
Lungs	768.00±0.47	685.00±0.87	596.00±0.72	0.41	0.05	0.45
Stomach[b]	372.00±0.12	335.00±0.16	386.00±0.16	0.02	0.36	0.01
Small Intestine	1387.00±0.73	1284.00±0.95	1370.00±0.86	0.22	0.81	0.38
Total Organ Wt.	4727.00±0.100	4891.00±0.190	4441.00±0.177	0.45	0.17	0.09
Organs as % BW	9.79±0.54	9.35±0.63	9.46±0.62	0.36	0.49	0.84

[a]Forty seven pigs were used in a unbalanced randomized complete block design, [b]Treatment effect (p<0.03)

Furthermore, N intake was lower for the pair-fed control pigs compared to the *A. pleuropneumoniae*-challenged pigs from d 0 to 17 (p<0.05).

Treatment and quadratic time effects were observed for fecal N (p<0.01). It was lower for the *A. pleuropneumoniae*-challenged pigs and the pair-fed control pigs than for the *ad libitum*-fed control pigs during all postchallenge time periods, resulting in lower fecal N levels from d 0 to 17 (p<0.001).

Treatment and quadratic time effects were observed for urinary N (p<0.01). The differences were results of pair-fed pigs lower urinary N levels compared

to the *ad libitum*-fed control pigs during the three time periods from d 4 to 17 ($p<0.04$) or the *A. pleuropneumoniae*-challenged pigs during d 0 to 3 ($p<0.04$), d 8 to 11 ($p<0.01$) and d 0 to 17 ($p<0.01$). Tendencies for lower urinary N levels for the pair-fed controls than for the *A. pleuropneumoniae*-challenged pigs were observed both from d 4 to 7 ($p<0.07$) and d 14 to 17 ($p<0.09$). Urinary N was not different for the *A. pleuropneumoniae*-challenged pigs versus the *ad libitum*-fed control pigs from d 0 to 17 after challenge ($p<0.48$).

A treatment×time interaction was observed for retained N ($p<0.05$). Greater N retention was observed for the *ad libitum*-fed control pigs than for the *A. pleuropneumoniae*- challenged pigs from d 0 to 3, 4 to 7 ($p<0.001$) and d 8 to 11 ($p<0.06$). In addition, *ad libitum*-fed control pigs had greater N retention than the pair-fed control pigs from d 0 to 3 ($p<0.03$), 4 to 7 ($p>0.001$) and 8 to 11 ($p<0.02$). The decreased N retention after challenge resulted in lower N retention for both *A. pleuropneumoniae*-challenged pigs ($p<0.002$) and the pair-fed control pigs ($p<0.001$) compared to the *ad libitum* control pigs from d 0 to 17.

Nitrogen retention efficiency, as a percentage of N intake, was not affected by treatment ($p<0.48$) or time ($p<0.17$). The retention efficiency of absorbed N also was unaffected by treatment ($p<0.42$); however, retention efficiency of absorbed N tended to change over time (quadratic, $p<0.08$).

Carcass composition and organ weights: Carcass characteristics and total organ weights were not affected by treatment, except for stomach weight ($p<0.03$; Table 4). Stomach weight was greater for pair fed control pigs than for *ad libitum*-fed control pigs ($p<0.01$) and *A. pleuropneumoniae*-challenged pigs ($p<0.02$).

DISCUSSION

Acute phase proteins: The increases in serum haptoglobin concentrations on d 1, d 5 and d 9 associated with the *A. pleuropneumoniae* challenge were similar to results of earlier research. Heegard *et al.*[15] and Hall *et al.*[16] also reported elevated serum haptoglobin levels immediately following an acute *A. pleuropneumoniae* challenge in pigs. Increased serum haptoglobin concentrations are associated with increased immune function during an acute phase immune response because interleukin-6 stimulates increased hepatic synthesis of acute phase proteins[17-19]. Using procedures similar to those used in this study, Loughmiller *et al.*[5] observed elevated serum haptoglobin levels following an acute *S. typhimurium* challenge in pigs, compared to both pair-fed and *ad libitum*-fed controls. Dritz *et al.*[20] also found a two- to threefold increase in serum haptoglobin levels in pigs challenged with a noninfectious endotoxin compared to nonchallenged counterparts.

The rise in serum amyloid A levels in the *A. pleuropnuemoniae* challenged pigs, but not in the unchallenged control pigs, after challenge is also consistent with an acute phase immune response. Serum amyloid A is typically one of the fastest reacting acute phase, with levels increasing up to 1000-fold within 4 to 5 h after immune stimulation[21]. Heegaard *et al.*[15] used a gel electrophoresis procedure to evaluated the effects of an acute *A. pleuropneumoniae* challenge on acute phase proteins in pigs. They also reported that serum amyloid A levels were elevated on d 1 and 2 after challenge. In addition, the lack of a serum haptoglobin or amyloid A response in the nonchallenged pigs indicates that our biosecurity procedures were adequate to prevent their unintentional infection.

The lack of a serum AGP response to *A. pleuropneumoniae* supports previous research indicating that AGP is more specific to immune stimulation than other acute phase proteins. Eckersall *et al.*[22] and Lampreave *et al.*[23] both reported that AGP levels in pigs did not rise following inflammation associated with a turpentine injection. In contrast, they found increased levels of other acute phase proteins including C-reactive protein and haptoglobin. Further, Webel *et al.*[11] did not observe increased serum AGP concentrations following an endotoxin challenge in growing pigs. Itoh *et al.*[24] reported increased AGP levels in conventionally reared pigs as compared to specific-pathogen-free reared pigs. However, in pigs with gross lung lesions associated with chronic pleuropneumonia, serum AGP levels were within normal ranges. In another study, AGP levels were elevated in response to an acute *S. typhimurium* challenge[5]. Similarly, Williams *et al.*[3] observed increased serum AGP concentrations in pigs continuously reintroduced to immune stimulation compared to segregated early-weaned counterparts.

Insulin-like growth factor-I: The decrease in the IGF-I concentration of *A. pleuropneumoniae*-challenged pigs on d 1 versus the control *ad libitum*-fed pigs and the pair -fed control pigs is consistent with research reviewed by Spurlock[24]. The review indicated that during an acute immune response, levels of proinflammatory cytokines increased and appear to affect the growth hormone-IGF-I axis, leading to decreased levels of IGF-I but not necessarily growth hormone. Feed intake, insulin and thyroid hormones T_3 and T_4 are all additional factors in

the regulation of plasma IGF-I concentrations and the combination of the apparent proinflammatory cytokine response, with low feed intake decreases circulating IGF-I levels[25-28]. The numerical decreases in IGF-I levels of the pair-fed control pigs suggest that although feed intake moderates the IGF-I response, the proinflammatory cytokines associated with the immune response appear to exert a greater effect on circulating IGF-I levels. Decreased IGF-I levels in response to an acute disease challenge also were observed by Balaji *et al.*[10]. They reported that plasma IGF-I concentrations in 15 kg pigs temporarily declined after an acute *S. typhimurium* challenge. In contrast, Loughmiller *et al.*[5] did not find a similar decrease in 30 kg pigs subjected to an acute *S. typhimurium* challenge with a similar dose and administration, the larger size of the pigs. In addition, the greater duration of feed intake reduction in present study versus that of Loughmiller *et al.*[5] probably contributed to the difference in the IGF-I responses to an acute *A. pleuropneumoniae* challenge versus an acute *S. typhimurium* challenge. Although Loughmiller *et al.*[5] observed a decrease in feed intake associated with an acute *S. typhimuirum* challenge, it occurred for only the initial d 0 to 3 postchallenge period, whereas the results reported herein show feed intake reductions from d 0 to 11 postchallenge. Thus, the combined effects of the acute *S. typhimurium* challenge and the brief decrease in feed intake were not sufficient to cause a decrease in plasma IGF-I. However, the combination of an acute *A. pleuropneumoniae* challenge and decreased feed intake from d 0 to 11 appears to be associated with decreased plasma IGF-I levels on d 1 and numerically lower levels on d 5 and 9 postchallenge.

Growth Performance: Our results indicate that the effects of an acute respiratory challenge were of sufficient duration and intensity that differences in ADG and ADFI occurred for the overall postchallenge period. However, ADG had recovered by d 8 after challenge, so these on growth performance appear to be primarily short-term. Although recovery from an acute *A. pleuropneumoniae* challenge takes longer, the recovery in ADG after the effects of the pathogen are overcome is similar to that observed by Loughmiller *et al.*[5] using an acute *S. typhimurium* challenge.

Although differences were apparent in d 14 to 17 ADFI for the *A. pleuropneumoniae*-challenged pigs and the pair-fed control pigs, no difference occurred in ADFI between *A. pleuropneumoniae* pigs that were paired and their unchallenged pair-fed counterparts (2.16 and 1.91 kg, respectively; $p>0.24$). Additionally, although slight tendencies existed for differences from d 8 to 11 and d 0 to 17, the ADFI were similar for the *A. pleuropneumoniae*-challenged pigs and the pair-fed controls ($p<0.27$). This similarity indicates that the pair feeding successfully controlled for effects dependent on changes in feed intake throughout the experiment.

Most of the reductions in ADG associated with an acute *A. pleuropneumoniae* challenge apparently are due to reduced ADFI. These reductions presumably are caused by increased concentrations of proinflammatory cytokines that reduce feed intake[17]. This result is similar to observations of Dritz *et al.*[20] and van Heugten *et al.*[29,30], who each used a noninfectious endotoxin challenge to stimulate an immune response. Although van Heugten *et al.*[29,30] found short-term decreases in growth performance, they did not differentiate effects of feed intake reductions from effects specific to the immune response. Similar to our results, Dritz *et al.*[20] reported that the majority of the decreased performance during the acute phase immune response could be associated with reductions in feed intake.

The lack of variation in G/F further indicates that differences in growth performance associated with an acute *A. pleuropneumoniae* challenge are primarily due to changes in feed intake after the challenge. However, the large numerical differences between treatments from d 4 to 7 after challenge were due primarily to the negative G/F reported for the pair-fed controls during this period. This estimated value is a result of negative gain for some pigs during that period. Thus, when the mathematical mean was calculated, a negative value resulted, which was not consistent with the least square means for ADG and ADFI during the same period. Because removing these data also would have produced an estimate with limited value, we chose to leave it as is, while recognizing that true feed efficiency is likely quite higher.

The lack of effect on G/F is similar to previous results from our laboratory using an acute *S. typhimurium* challenge[5]. They did not observe treatment variations in G/F and determined that differences in growth performance postchallenge were primarily due to changes in feed intake. In contrast, Dritz *et al.*[20] and Williams *et al.*[3] reported decreased G/F in immune-stimulated pigs compared to nonchallenged counterparts. Dritz *et al.*[20] further reported decreased G/F in endotoxin-challenged pigs in excess of that observed in their pair-fed nonchallenged counterparts. The differences observed in both studies likely were results of multiple endotoxin injections over several days or the chronic pathogenic challenge associated with their respective immune stimulation models. Because the pigs were immunostimulated repeatedly, their immune systems were likely at high activity rates throughout those studies.

Although present results and those of Loughmiller *et al.*[5] indicate short-term numerical differences in G/F after an acute disease challenge, the immune activity associated with an acute disease challenge, without reexposure, apparently does not elicit a long-term effect on G/F.

The gradual recovery in growth performance of the *A. pleuropneumoniae*-challenged pigs in comparison to both nonchallenged control treatments suggests that although the immune activation associated with the disease challenge subsided rapidly, lung lesions likely took longer to resorb[31]. These dense, fluid-filled lesions likely hampered the pigs' respiratory exchange capacity. This probable reduction lowered the ability of the body to aerobically process nutrients, thus lowering the cellular requirement for new fuel substrate and feed intake stimulation. As the lungs recovered, the pigs were able to more fully express their growth potential, leading to the recoveries in ADG and ADFI following the d 0 to 3 challenge period. The similar performance after recovery indicates that the immune system in an inactive state, even after stimulation, does not impose a large burden upon the pig's metabolic processes. These results also indicate that when an environmental stress is removed, performance will improve up to the limits imposed by the current environment[32,33] and physiological changes associated with the immune response.

Digestibility: Similar to growth performance, apparent DM and N digestibility were not affected by treatment prior to the disease challenge. The increased apparent DM digestibility of the *A. pleuorpneumoniae*-challenged pigs versus both control treatments appeared to result from more than reduced feed intake. If reduced feed intake was the primary cause, then the apparent DM digestibility for the *A. pleuropmeumoniae*-challenged pigs and the pair-fed control pigs would have been similar after challenge. The increased demand for nutrients to support the immune response many have either lowered the endogenous losses in the gut lumen or stimulated increased nutrient uptake. Although we do not know the cause with certainty, the increased apparent DM digestibility is consistent with the growth performance results. The *A. pleuropneumoniae* challenged pigs had lower ADFI during several periods after challenge; however, ADG was different only from d 0 to 3 after challenge. Thus, the increased apparent DM digestibility appears to have partially ameliorated the effects of lower ADFI on ADG. Similarly, the lower ADG of the pair-fed controls versus the *ad libitum*-fed controls from d 0 to 7 postchallenge is consistent with the lack of differences in apparent DM digestibility between these two groups. In contrast, Loughmiller *et al.*[5] reported that apparent DM digestibility in the pair fed control pigs was higher than that in either the *S. typhimurium*-challenged pigs or *ad libitum*-fed control pigs immediately postchallenge. However, this increased apparent DM digestibility was compounded by the effects of an acute enteric disease challenge upon intestinal morphology. After the gastrointestinal tract recovered from that challenge, they also observed numerically increased apparent DM digestibility in the challenged pigs versus the *ad libitum*-fed and pair-fed control treatments.

Similar to the response in apparent DM digestibility, apparent N digestibility was increased for the *A. pleuropneumoniae*-challenged pigs after challenge, but not for the pair-fed control pigs. This similarity of response in apparent nutrient digestibility again suggests that either endogenous secretions or actual nutrient digestibility are affected by the immune response to an acute disease challenge. Because previous studies evaluating apparent N digestibility utilized enteric disease challenges, it is difficult to compare their apparent N digestibility results with ours. Both Loughmiller *et al.*[5] and Williams *et al.*[3] observed decreased apparent N digestibility immediately postchallenge, likely due to the intestinal inflammation, crypt cell and enterocyte necrotic lesions and sloughing of microvilli that occur during an enteric disease challenge[34].

Energy balance: The average DE and ME intakes of pigs in all three treatments from d 0 to 3 and d 8 to 11 postchallenge are in general agreement with estimated DE and ME intakes of pigs ranging from 10 to 50 kg[35]. This agreement suggests that energy intake was not limiting the performance of the *ad libitum*-fed control pigs. The tendency for increased DE intake, as a percent of GE intake, of the *A. pleuropneumoniae* challenged pigs versus the pair-fed control pigs from d 0 to 3 and d 4 to 7 is consistent with the increased apparent DM and N digestibility observed during the same periods. Although no differences in ME intake, as a percent of GE intake, occurred, the numerical tendencies in ME intake suggest that the higher DE intake in the *A. pleuropneumoniae*-challenged pigs was utilized to support the immune response and subsequent recovery.

Similar to the results of van Huegten *et al.*[29], Williams *et al.*[36] and Loughmiller *et al.* [5], we did not observe any differences in the efficiency of ME utilization for gain. This lack of differences indicates that decreased energy intake lowers growth rates during an immune challenge, not alterations in ME utilization for gain. Therefore, consistent with previous research, our data indicate that the inflammatory response to an acute

disease challenge affected protein metabolism, but not the efficiency of energy utilization for gain.

Nitrogen Balance: Similar to growth performance and apparent digestibility, the lack of differences in N balance between treatments prior to the disease challenge indicates that all pigs were in a similar metabolic state from d -4 to -1. Observed differences in N intakes were the results of decreased ADFI for the *A. pleuropneumoniae*-challenged pigs and their pair-fed counterparts from d 0 to 11 after challenge. Variations in fecal N concentrations were associated with differences in apparent N digestibility. Although they also can be explained lower N intake, differences in urinary N content between the pair-fed control pigs and the *A. pleuropnuemoniae*-challenged pigs indicate that changes in protein metabolism specific to the immune response occurred. This is further corroborated by the lack of differences in urinary N between the *A. pleuropneumoniae*-challenged pigs and the *ad libitum*-fed control pigs from d 0 to 17 postchallenge, even though large differences in N intake were observed after the disease challenge. The increased urinary N losses presumably were due to increased hepatic globular protein synthesis to support the immune response, which is reported to be driven by increased levels of proinflammatory cytokines during the acute phase[17,19,25]. The increased urinary N losses, combined with the acute phase protein responses, are consistent with reviewed research indicating that during an acute immune response, proinflammatory cytokines shift protein metabolism from growth and skeletal muscle accretion to immunological globular protein synthesis. The proinflammaotry cytokines likely stimulated an increased level of skeletal muscle catabolism to support the globular protein synthesis, causing most of the variation in urinary N losses between the pair-fed control pigs and the *A. pleuropneumoniae* challenged pigs. In contrast, study by Loughmiller *et al.*[5] found similar urinary N levels in pigs that were challenged with *S. typhimurium* pair-fed control pigs and *ad libitum*-fed control pigs. However, pigs receiving the *S. typhimurium* challenge had a quicker recovery rate so that most differences in N balance and growth occurred from d 0 to 3 postchallenge. In the present study, we observed differences over a longer duration, typically lasting until at least d 7 after challenge. Thus, the longer term reductions in N intake and increased urinary N losses reported herein indicate that the *A. pleuropneumoniae* challenge resulted in greater short-term endogenous N losses than an acute *S. typhimurium* challenge.

The decreased N retention from d 0 to 11 postchallenge was associated primarily with decreased feed intake and increased urinary N losses, presumably caused by the challenged pigs' feed refusal and increased muscle catabolism to support the immune response[17,19,25]. This is consistent with previous research in our laboratory, indicating that differences in N retention from an acute *S. typhimurium* challenge are due primarily to reductions in N intake[5]. In addition, Williams *et al.*[3] indicated that long-term N retention is lower in pigs with chronic immune stimulation. Similar to the recovery in N retention observed by Loughmiller *et al.*[5], present results indicate that once the immune challenge was overcome, immune system activity quickly subsided and N retention recovered before the end of the study. However, the overall decrease in N retention from d 0 to 17 after challenge indicates that the effects of an acute *A. pleuropneumoniae* challenge are longer lasting than the effects from an acute *S. typhimurium* challenge observed by Loughmiller *et al.*[5]. In addition, Williams *et al.*[3] reported that pigs receiving a high continual disease challenge from multiple pathogens had lower overall N balance and lysine utilization efficiency. The shorter duration of response in our study indicates that once the pig has overcome the disease challenge, immunological stimulation subsides and nutrients are partitioned again to growth processes.

Nitrogen retention efficiency: The lack of effect on in N retention efficiency, as a percent of either N intake or absorbed N, before and after the *A. pleuropneumoniae* challenge indicate that differences in N retention were associated primarily with variable N intakes. Similar to the response to *A. pleuropneumoniae*, an acute *S. typhimurium* challenge did not affect N retention efficiency in pigs[5]. These results indicate that the metabolic alterations associated with acute respiratory or enteric disease challenges redirect N utilization towards the immune response but do not significantly affect the efficiency of use. In contrast, van Heugten *et al.*[29] observed decreased protein utilization efficiency in growing pigs twice challenged with noninfectious endotoxin compared to nonchallenged controls injected with a sterile saline solution. Williams *et al.*[5] also reported decreased N retention efficiency from increased chronic immune activation. Thus, although protein utilization is not affected by a single acute disease challenge, protein utilization efficiency apparently is decreased from the repeated inflammatory responses when pigs are subjected to repeated immune stimulation.

Carcass characteristics and organ weights: The lack of long-term differences in carcass characteristics suggests that the short-term reduction in feed intake did not affect

postchallenge body composition. This observation is consistent with the lack of effects on feed efficiency, energy utilization efficiency and N retention efficiency. Thus an acute *A. pleuropneumoniae* challenge apparently does not alter the long-term performance or body composition of the challenged pigs. Conversely, Williams *et al.*[36] indicated that carcass characteristics were poorer and selected organ weights were heavier for chronically immune-stimulated pigs, indicating that long-term immune stimulation causes changes in body composition and, thus, affects the efficiency of lysine utilization[3]. This conclusion is consistent with previous observations of additively increased reductions in performance with multiple, nonimmulogical stresses[32]. Thus, the differences between our results and those of Williams *et al.*[37] illustrate that one-time immunological stresses do not impact long-term performance; however, continual, multiple, immunological challenges will reduce long-term performance and alter body composition.

These data provide further biological evidence that multisite swine production systems will improve production efficiency, even though acute disease outbreaks still occur. Although many variations such systems exist[38], they all are based upon the principle that similarly aged cohort pigs are reared on isolated sites to prevent vertical and horizontal introductions of pathogens. It is recognized that these systems only rarely completely eliminate pathogens from cohort groups. However, our data suggest that lean growth rates will recover after these outbreaks, if reintroduction of additional pathogens is avoided. Therefore, if the pig's immune system maintains the ability to overcome the pathogen, few long-term effects are expected.

CONCLUSIONS

The results of this study indicate that reductions in performance associated with an acute respiratory disease challenge are due to both feed intake reductions and the acute phase immune response. No compensatory effects were observed, so the long-term effects associated with an acute *A. pleuropneumoniae* challenge are primarily the economic effects of increased mortality and short-term performance losses. Because field conditions typically have multiple stresses, losses from reduced performance and mortality associated with an acute *A. pleuropneumoniae* challenge may be greater. However, in a near optimal environment, metabolic alterations associated with an acute *A. pleuropneumoniae* respiratory disease challenge in growing pigs appear to be primarily short-term.

REFERENCES

1. Dritz, S.S., J.L. Nelssen, R.D. Goodband, M.D. Tokach and M.M. Chengappa, 1994. Application of segregated early weaning technology in the commercial swine industry. Comp. Cont. Educ. Pract. Vet., 16: 677-685.
2. NAHMS., 1997. National animal health monitoring system, Part III: Changes in the US pork industry. USDA Animal and Plant Health Inspection Service Centers for Epidemiology and Animal Health, Fort Collins, CO.
3. Williams, N.H., T.S. Stahly and D.R. Zimmerman, 1997a. Effect of chronic immune system activation on body nitrogen retention, partial efficiency of lysine utilization and lysine needs of pigs. J. Anim. Sci., 75: 2472-2480.
4. Webel, D.M., B.N. Finck, D.H. Baker and R.W. Johnson, 1997. Time course of increased plasma cytokines, cortisol and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. J. Anim. Sci., 75: 1514-1520.
5. Loughmiller, J.A., S.S. Dritz, M.D. Tokach, R.D. Goodband, J.L. Nelssen, M. De La Llata and S.A. Moser, 1999. Enteric disease challenge effects on pig growth, N balance and immune indicators. J. Anim. Sci., 77: 71.
6. Montaraz, J.A., B. Fenwick, H. Hill and M. Rider, 1996. Evaluating antibody isotype-specific ELISA, complement fixation and Apx I hemolysin neutralization tests to detect serum antibodies in pigs infected with *Actinobacillus pleuropneumoniae*. Swine Health Prod., 4: 79-83.
7. Mohn, S. and C.F.M. de Lange, 1998. The effect of body weight on the upper limit to protein deposition in a defined population of growing gilts. J. Anim. Sci., 76: 124-133.
8. CDC, 1997. Biosafety in Microbiological and Biomedical Laboratories. 3rd Edn., Office of health and safety, Centers for Disease Control and prevention. Accessed Oct. 20, 1997. http://www.cdc.gov/od/ohs/biosfty/biosfty.htm
9. AOAC, 1990. Official Methods of Analysis (15th Edn.). Association of Official Analytical Chemists, Arlington, VA. pp:
10. Balaji, R., K.J. Wright, C.M. Hill, S.S. Dritz, E.L. Knoppel and J.E. Minton, 2000. Acute phase responses of pigs challenged orally with *Salmonella typhimurium*. J. Anim. Sci.,
11. Smith, J.E., P.S. Chavey and G.A. Andrews, 1998. Semiautomatic and robotic methods for determining serum haptoglobin levels. Vet. Clin. Pathol., 27: 11-14.

12. McDonald, T.L., A. Weber and J.W. Smith, 1991. A monoclonal antibody sandwich immunoassay for Serum Amyloid A (SAA) protein. J. Immunol. Met., 144: 149-155.
13. Littell, R.C., G.A. Milliken, W.W. Stroup and R.D. Wolfinger, 1996. SAS® system for mixed models. SAS Institute Inc., Cary, NC.
14. Mead, R., 1994. The Design of Experiments. Cambridge University Press, New York.
15. Heegaard, P.H.M., J. Klausen, J.P. Nielsen, N. Gonzalez-Ramon, M. Pineiro, F. Lampreave and M.A. Alava, 1998. The porcine acute phase response to infection with *Actinobacillus pleuropneumoniae*. Haptoglobin, C-reactive protein, major acute phase protein and serum amyloid. A protein are sensitive indicators of infection. Comp. Biochem. Physiol., 119: 365-373.
16. Hall, W.F., T.E. Eurell, R.D. Hansen and L.G. Herr, 1992. Serum haptoglobin concentration in swine naturally or experimentally infected with *Actinobacillus pleuropneumoniae*. JAVMA., 201: 1730-1733.
17. Klasing, K.C., 1988. Nutritional aspects of leukocytic cytokines. J. Nutr., 118: 1436-1446.
18. Cason, J., 1989. Immune reactions and host nutritional status: The role of interleukin-1. Nutr. Res., 9: 237-250.
19. Johnson, R.W., 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated view. J. Anim. Sci., 75: 1244-1255.
20. Dritz, S.S., K.Q. Owen, R.D. Goodband, J.L. Nelssen, M.D. Tokach, M.M. Chengappa and F. Blecha, 1996. Influence of lipopolysaccharide-induced immune challenge and diet complexity on growth performance and acute-phase protein production in segregated early-weaned pigs. J. Anim. Sci., 74: 1620-1628.
21. Gruys, E., M.J. Obwolo and M.J.M. Toussaint, 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: A review. Vet. Bull., 64: 1009-1018.
22. Eckersall, P.D., P.K. Saini and C. McComb, 1996. The acute phase response of acid soluble glycoprotein, α-1 acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, In: The pig. Vet. Immunol. Immunopathol., 51: 377-385.
23. Lampreave, F., N. Gonzalez-Ramon, S. Martinez-Ayensa, M.A. Hernandez, H.K. Lorenzo, A. Garcia-Gil and A. Pineiro, 1994. Characterization of the acute phase serum protein response in pigs. Electrophoresis, 15: 672-676.
24. Spurlock, M.E., 1997. Regulation of metabolism and growth during immune challenge: An overview of cytokine function. J. Anim. Sci., 75: 1773-1783.
25. Spurlock, M.E., 1997. Regulation of metabolism and growth during immune challenge: An overview of cytokine function. J. Anim. Sci., 75: 1773-1783.
26. Morovat, A. and M.J. Dauncey, 1998. Effects of thyroid status on insulin-like growth factor-I, growth hormone and insulin are modified by food intake. Eur. J. Endocrinol., 138: 95-103.
27. D'Ercole, A.J., 1996. Insulin-like growth factors and their receptors in growth. In: (Edn) R. Rosenfeild. Growth and Growth Disorders. Endocrinology and Metabolism Clinics of North America. W.B. Saunders Company, Philadelphia, PA. pp: 573-589.
28. Buonomo, F.C. and C. A. Baile, 1991. Influence of nutritional deprivation on insulin-like growth factor I, somatotropin and metabolic hormones in swine. J. Anim. Sci., 69: 755-760.
29. van Heugten, E., J.W. Spears and M.T. Coffey, 1994. The effect of dietary protein on performance and immune response in weanling pigs subjected to an inflammatory challenge. J. Anim. Sci., 72: 2661-2669.
30. van Heugten, E., M.T. Coffey and J.W. Spears, 1996. Effects of immune challenge, dietary energy density and source of energy on performance and immunity in weanling pigs. J. Anim. Sci., 74: 2431-2440.
31. Nicolet J., 1992. *Actinobacillus pleuropneumoniae*. In: A.D. Leman, B.E. Straw, W.L. Mengeling, S. D'Allaire and D.J. Taylor (Edn). Diseases of Swine, (7th Edn). Iowa State University Press, Ames IA., pp: 401-408.
32. Hyun, Y., M. Ellis, G. Riskowski and R.W. Johnson, 1998. Growth performance of pigs subjected to multiple concurrent environmental stressors. J. Anim. Sci., 76: 721-727.
33. Klasing, K.C., D.E. Laurin, R.K. Peng and D.M. Fry, 1987. Immunologically mediated growth depression in chicks: Influence of feed intake, corticosterone and interleukin-1. J. Nutr., 117: 1629-1637.
34. Schwartz, K.J., 1999. Salmonellosis. In: B.E. Straw, S. D'Allaire, W.L. Mengeleng and D.J. Taylor(Edn) Diseases of Swine (8th Edn). Iowa State University Press, Ames IA., pp: 535-562.
35. NRC, 1998. Nutrient requirements of swine (10th Edn.). National Academy Press, Washington, DC.
36. Williams, N.H., T.S. Stahly and D.R. Zimmerman, 1997b. Effect of chronic immune system activation on the rate, efficiency and composition of growth and lysine needs of pigs fed from 6 to 27 kg. J. Anim. Sci., 75: 2463-2471.

37. Williams, N.H., T.S. Stahly and D.R. Zimmerman, 1997c. Effect of level of chronic immune system activation on the growth and dietary lysine needs of pigs fed from 6 to 112 kg. J. Anim. Sci., 75: 2481-2496.
38. Harris, D.L., 2000. Multi-site Pig Production. Iowa State University Press, Ames, IA.
39. NRC, 1988. Nutrient requirements of swine (9th Edn.). National Academy Press, Washington, DC.
40. NCR-42. Committee on Swine Nutrition. 1992. Variability among sources and laboratories in chemical analysis of corn and soybean meal. J. Anim. Sci., 70: 70.