Journal of Animal Science, 2020, Vol. 98, No. 4, 1–10

doi:10.1093/jas/skaa082
Advance Access publication March 16, 2020
Received: 8 January 2020 and **Accepted:** 12 March 2020
Non Ruminant Nutrition

NON RUMINANT NUTRITION

Increasing the ratio of SID lysine to metabolizable energy improves pig performance during a viral challenge

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Abstract

Porcine reproductive and respiratory syndrome virus (**PRRSV**) compromises pig performance. However, increasing standardized ileal digestible Lys per Mcal metabolizable energy (**SID Lys:ME**) above requirement has been shown to mitigate reduced performance seen during a porcine reproductive and respiratory syndrome (**PRRS**) virus challenge. The objective of this study was to evaluate the effects of increasing the dietary SID Lys:ME from 100% National Research Council (**NRC**) requirement to 120% of the requirement in vaccinated (vac+; modified live vaccine Ingelvac PRRS) and non-vaccinated (vac–; no PRRS vaccine) grower pigs subjected to a PRRSV challenge. In addition, the dietary formulation approach to achieve the 120% ratio by increasing Lys relative to energy (**HL**) or diluting energy in relation to Lys (**LE**) was evaluated. This allowed us to test the hypothesis that pigs undergoing a health challenge would have the ability to eat to their energy needs. Within vaccine status, 195 mixed-sex pigs, vac+ (35.2 ± 0.60 kg body weight [BW]) and vac– (35.2 ± 0.65 kg BW) were randomly allotted to one of three dietary treatments (2.67, 3.23, or 3.22 g SID Lys:ME) for a 42-d PRRS virus challenge study representing 100%, 120%, and 120% of NRC requirement, respectively. Pigs were randomly allotted across two barns, each containing 24 pens with 7 to 10 pigs per pen (8 pens per diet per vaccine status). On day post-inoculation 0, both barns were inoculated with PRRSV and started on experimental diets. Within vaccine status, weekly and overall challenge period pig performance were assessed. In both vac+ ($P < 0.05$) and vac– ($P < 0.05$) pigs, the HL and LE diets increased end BW and overall average daily gain (ADG) ADG compared with pigs fed the control diet ($P < 0.05$). Overall, average daily feed intake (ADFI) during the challenge period was greater ($P < 0.05$) for pigs fed the LE diet compared with pigs fed control and HL treatments, regardless of vaccine status (20% and 17% higher ADFI than the control in vac+ and vac– pigs, respectively). The HL vac+ pigs had the greatest gain to feed (G:F) compared with the control and LE pigs (0.438 vs. 0.394 and 0.391 kg/kg, respectively; $P < 0.01$). Feed efficiency was not impacted ($P > 0.10$) by treatment in the vac– pigs. In summary, PRRSV-challenged grower pigs consumed feed to meet their energy needs as indicated by the increase in ADFI when energy was diluted in the (LE) diet, compared with control pigs. In both PRRS vac+ and vac– pigs subsequently challenged with PRRSV, regardless of formulation approach, fed 120% SID Lys:ME diets resulted in enhanced overall growth performance.

Keywords: lysine, metabolizable energy, porcine reproductive and respiratory syndrome virus, pig

Abbreviations

AA	amino acids
ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CP	crude protein
Ct	cycle threshold
DM	dry matter
dpi	day post-inoculation
GE	gross energy
G:F	gain to feed ratio
HL	high Lys
IgG	immunoglobulin G
ISUVDL	Iowa State University Veterinary Diagnostic Laboratory
LE	low energy
LPS	lipopolysaccharide
LS	least-squares
ME	metabolizable energy
MLV	modified live vaccines
NRC	National Research Council
PCV	porcine circovirus
PRRS	porcine reproductive and respiratory syndrome
PRRSV	porcine reproductive and respiratory syndrome virus
RT-PCR	real-time polymerase chain reaction
SID	standardized ileal digestibility
S:P	sample to positive ratio
vac–	vaccine negative
vac+	vaccine positive

Introduction

Porcine reproductive and respiratory syndrome (**PRRS**) is a disease caused by the PRRS virus (**PRRSV**) pathogen. This disease is arguably the most economically significant health challenge to the swine industry (Holtkamp et al., 2013; Nathues et al., 2017) as it antagonizes all stages of production causing increased morbidity, mortality, and decreased growth (Lunney et al., 2010). With moderate success, the swine industry has employed vaccine strategies to reduce the occurrence of PRRS in swine herds (Meng, 2000; Zuckermann et al., 2007; Renukaradhya et al., 2015). Commercially available vaccines, either modified live vaccines (**MLV**) or autogenous vaccines developed from indigenous field isolates, have been widely researched resulting in varying efficacy (Osorio et al., 1998; Mavromatis et al., 1999; Jeong et al., 2018). In today's swine industry, it is common practice for herds to be vaccinated against PRRSV in an effort to mitigate the negative growth performance anticipated by a PRRSV challenge. However, due to the variable efficacy of PRRSV vaccines, nutritional strategies may also be an effective way to improve performance during a PRRSV challenge.

Nutritional requirements for healthy pigs are well established by the National Research Council (NRC, 2012); however, nutrient requirements for pigs undergoing a health challenge are widely unknown, and this includes amino acids (**AA**). In a healthy pig, Lys is the first-limiting AA when feeding corn–soybean meal-based diets. However, AA utilization for swine with an activated immune system is not as well understood (NRC, 2012). In practical diet formulation, AA requirements are expressed in relation to energy as a ratio (i.e., standardized ileal digestible Lys per Mcal metabolizable energy [**SID Lys:ME**]). This ensures that a constant AA intake is achieved by the pig independent of the dietary energy level fed and related adjustment to feed intake, which is key to support optimal feed intake and growth. However, stimulation of the immune system due to a pathogen challenge can result in reduced voluntary feed intake and as a result lower energy and AA intake (Johnson, 2002; Doeschl-Wilson et al., 2009) that causes growth rate reductions (Greiner et al., 2001; Rochell et al., 2015; Schweer et al., 2018a). Furthermore, it has been suggested that under unrestricted feed conditions, healthy pigs will attempt to consume the amount of feed required to satisfy their requirement for energy and nutrients (Schiavon et al., 2018). However, it is unclear if pigs are able to adjust their feed intake to meet their energy needs under stress or disease.

Nutritional strategies have previously been studied to promote earlier viral clearance and recovery that also enhance pig performance and well-being. One strategy has been to increase dietary soybean meal (Boyd and Zier-Rush, 2014; Rochell et al., 2015). Soybean meal is the primary dietary protein and AA source in traditional corn–soybean meal-based swine diets. It has been reported that increasing soybean meal from 17.5% to 29% reduced viremia load and improved growth in PRRSV-infected nursery pigs in an experimental setting (Rochell et al., 2015). However, it is unclear if the improved performance is due to increased concentration of crude protein (**CP**) and AA, or the increase in bioactive antioxidant compounds (i.e., isoflavones) found within soybean meal. The latter has yielded mixed results in PRRSV-infected pigs (Greiner et al., 2000; Smith et al., 2019).

Furthermore, based on previous work from our group, we determined that the potential benefits of feeding increased soybean meal during a PRRSV challenge are likely not related to the digestibility of nutrients or AA (Schweer et al., 2018b). Additionally, basal endogenous losses of AA were only nominally different in PRRSV-challenged pigs compared with healthy control pigs and this translated to minimal differences in standardized ileal digestibility (**SID**) of most AA (Schweer et al., 2018b). To further examine the impact of soybean meal, we have also studied how the relationship of Lys to energy impacts health-challenged pig performance. Using break point analysis, our group has reported that increasing SID Lys:ME to 110% to 120% above the NRC (2012) requirement resulted in improved growth performance and feed efficiency in grower pigs subjected to a PRRSV challenge, while unchallenged pigs did not benefit from a higher plane of AA (Schweer et al., 2018a). The increased Lys:ME ratio was achieved primarily by intact protein sources, while synthetic AA levels remained relatively constant. Reduction in feed intake during a disease challenge reduces the nutrient availability to tissues, thus being the primary cause of reduced lean tissue accretion observed during a viral challenge (Helm et al., 2019). Therefore, we hypothesized that decreasing dietary energy concentrations may be beneficial during immune stimulation to help mitigate anorexia (i.e., improve feed intake). Moreover, it is unclear if the improved growth performance during a PRRSV challenge is attributed to increases in dietary SID AA (increase in CP), or if reducing ME to achieve the same ratio, thereby promoting feed intake, would yield similar results.

Therefore, the objective of this study was to evaluate the effects of increasing SID Lys:ME in PRRSV-vaccinated and non-vaccinated pigs facing a subsequent PRRSV challenge on growth performance. Furthermore, we hypothesized that irrespective of how an increase in the SID Lys:ME (i.e., 120%) is achieved, by either an increase in g SID Lys or a reduction in ME would result in increased growth performance in PRRSV-infected pigs compared with that of pigs fed a 100% SID Lys:ME diet. Lastly, we hypothesized that health-challenged pigs would exhibit the ability to eat to their energy needs.

Materials and Methods

All procedures adhered to the ethical and humane use of animals for research and were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC# 18-158). This study was conducted from September 2018 to March 2019 in Ames, IA.

Animal housing and experimental design

Four hundred non-vaccinated, mixed-sex (purebred Duroc sires by commercial Yorkshire–Landrace F1 females; 5.4 ± 1.23 kg BW), 19- to 21-d old weaned PRRS-naïve pigs were randomly selected from a single source sow farm and transported to Ames, IA. Upon arrival, all weaned pigs were randomly split by litter across two barns with identical configuration (i.e., ventilation, temperature set points, pen configuration, feeders, and waterers). Each barn had 24 pens; however, only 12 pens in each barn were utilized for the nursery acclimation phase and each pen was double stocked to contain 15 to 17 pigs. All pens were identical in size (3.66 × 2.44 m), with fully slatted concrete flooring and two water cups. Each barn was climate controlled to thermoneutral conditions with propane heaters and wall ventilation fans which were adjusted accordingly as pig age increased. On day 1 post-placement, one barn was vaccinated intramuscularly with 1 mL of a modified live PRRS vaccine (Ingelvac PRRS MLV, Boehringer Ingelheim, St. Joseph, MO), while the other barn was not PRRSV vaccinated. Throughout the 42-d nursery acclimation period, all pigs were fed identical diets in three dietary phases and all diets met or exceeded the nutritional requirements of the pig (NRC, 2012).

On day 42 post-weaning (25.6 ± 4.31 kg BW), pig numbers were reduced in all nursery pens to carry out the experimental phase during the grower period. This was achieved by randomly selecting 7 to 10 pigs within pen and barn (vaccine status) and placing them into clean, unused pens within the same barn. The grower phase of the study was carried out using 48 identical pens (3.66 × 2.44 m wide, with fully slatted floors), containing a double-sided 36 cm feeder and two nipple waterers. Within vaccine status, there were 24 pens in which all pigs received a common corn–soybean meal-based grower diet that met or exceeded the nutritional requirement (NRC, 2012) for weight range of pigs up until 14 d prior to PRRSV inoculation.

After a 14-d acclimation period (day 56 post-weaning) to the grower pens, all pigs in both barns (vaccinated 35.2 ± 0.60 kg BW; non-vaccinated 35.2 ± 0.65 kg BW) were randomly allotted to one of three dietary treatments with eight pens per treatment per vaccine status. The three treatments per vaccine status were: 1) control, a diet formulated to contain 2.69 g SID Lys:ME [control diet representing 100% Lys:ME based on NRC (2012)]; 2) high Lys (**HL**), a diet containing 3.23 g SID Lys:ME achieved via increased inclusion of soybean meal and synthetic AA (120% ratio from control); and 3) low energy (**LE**), a diet containing 3.22 g SID Lys:ME achieved by reducing dietary ME via the inclusion of 18% fine grade, washed, and dried sand (120% ratio from control). The three diets (Table 1) were formulated to contain 2.69, 3.23, and 3.22 g SID Lys:ME, representing 100%, 120%, and 120% of requirements for 35 to 75 kg BW pigs. This SID Lys:ME requirement was based on breakpoint analysis from the Schweer et al., (2018a) projections for 35 to 75 kg BW pigs, adjusted for NRC (2012) and Maschhoffs' verified internal nutrient requirements. The three diets were meal form and formulated to meet or exceed NRC (2012) nutrient and energy requirements and contained similar total calcium, available phosphorus, and ratios of SID Thr, Trp, Met, Ile, and Val to SID Lys to avoid secondary AA deficiencies (Table 1).

Table 1. Experiential diet composition, as fed basis, 35 to 70 kg

	g SID Lys:Mcal ME		
Ingredients, %	2.69 (control)	3.23 (HL)	3.22 (LE)
Corn	75.91	68.89	56.22
Soybean meal, 48% CP	19.35	26.46	21.95
Limestone	0.94	0.93	0.84
Monocalcium phosphate, 21%	0.74	0.60	0.90
Salt	0.46	0.46	0.47
Sand	—	—	18.00
Fat, animal-vegetable blend	1.68	1.62	0.84
L-Lysine sulfate (54.6%)	0.52	0.55	0.41
L-Threonine	0.11	0.12	0.09
DL-Methionine	0.11	0.16	0.12
L-Valine	0.02	0.03	0.01
Vitamin premix[1]	0.03	0.03	0.03
Trace mineral premix[2]	0.08	0.08	0.08
Copper sulphate, 25.2%	0.06	0.06	0.06
Phytase 500 FTU/kg	0.01	0.02	0.00
Calculated composition			
DM, %	86.28	85.45	88.88
CP, %	14.77	17.60	14.48
ME, Mcal/kg	3.31	3.31	2.67
NE, Mcal/kg	2.58	2.54	2.04
Total calcium, %	0.58	0.58	0.58
Available phosphorus, %	0.24	0.24	0.24
Lys, Total %	0.99	1.18	0.96
SID AA			
Lys	0.89	1.07	0.86
Thr:Lys	0.61	0.61	0.61
Met+Cys:Lys	0.57	0.57	0.57
Trp:Lys	0.16	0.17	0.18
Ile:Lys	0.56	0.58	0.59
Val:Lys	0.65	0.65	0.65
SID Lys:ME, g/Mcal	2.69	3.23	3.22
Analyzed composition			
DM, %	87.03	87.06	87.05
CP, %	14.29	16.74	17.05
GE, Mcal/kg	3.87	3.86	3.08
Lys, Total %	0.77	1.22	1.08
Total AA:Lys			
Thr:Lys	0.86	0.56	0.53
Met+Cys:Lys	0.78	0.56	0.61
Ile:Lys	0.81	0.58	0.58
Val:Lys	0.88	0.65	0.64

[1]Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 5,291 IU as vitamin A acetate; vitamin D3, 827 IU as vitamin D-activated animal sterol; vitamin E, 26 IU as α-tocopherol acetate; menadione, 1.5 mg as menadione dimethylpyrimidinol bisulfite; vitamin B12, 0.02 mg; riboflavin, 6.0 mg; pantothenic acid, 22 mg as calcium pantothenate; niacin, 30 mg.
[2]Provided the following quantities of trace minerals per kilogram of complete diet: Fe, 124 mg as iron sulfate; Zn, 124 mg as zinc oxide; Mn, 29 mg as manganese sulfate; Cu, 12 mg as copper sulfate; I, 0.22 mg as calcium iodate; and Se, 0.22 mg as sodium selenite.

On day 56 post-weaning, corresponding with day post inoculation (**dpi**) 0, all pigs in both barns were inoculated intramuscularly with 1 mL of a live virulent PRRSV isolate (open reading frame 5, 1–18–4) containing 10^6 genomic PRRSV units per mL. For the next 42 dpi, pig BW, pen feed intake, and feed efficiency were collected and calculated weekly on dpi 0, 7, 14, 21, 28, 35, and 42. Pigs were allowed unrestricted access to feed and water throughout the 42-d PRRSV challenge. In

addition, deceased pigs from the LE dietary treatment were gross necropsied to determine if sand had caused any irritation to the digestive tract. There was no gross visible evidence of sand-induced irregularities of gastrointestinal tracts in these pigs.

Diet analysis

The three experimental diets used during the PRRSV challenge were analyzed for energy and nutrient composition. Analysis of dietary gross energy (**GE**) content was determined using bomb calorimetry (Oxygen Bomb Calorimeter 6200, Parr Instruments, Moline, IL). Diet samples were analyzed for dietary dry matter (**DM**) using method 934.01 according to AOAC (2007). Dietary AA and N analysis were conducted by University of Missouri Experimental Station Chemical Laboratories (Columbia, MO). AA and N analyses were performed using method 994.12, 999.13, and 990.03 according to AOAC (2007) methods, and CP was calculated (N × 6.25).

Blood collection and analysis

Two pigs in each pen were randomly selected and these same two pigs were snare-restrained and serial bled on dpi –7, 0, 7, 14, 21, 28, 35, and 42. Blood samples (8 to 10 mL) were collected from the jugular vein into serum tubes (BD Vacutainer, Franklin Lakes, NJ) for routine diagnostic testing. Blood samples from pigs at 0 dpi were collected immediately before inoculation. All blood samples were allowed to clot, then serum separated by centrifugation (2,000 × *g*, 15 min at 4 °C) pooled within the dietary treatment and vaccine status, and stored at –80 °C until analysis. Serum aliquots were submitted to the Iowa State University Veterinary Diagnostic Laboratory (**ISUVDL**), Ames, IA, for testing. Real-time polymerase chain reaction (RT-PCR) and serum antibody testing for PRRSV were performed using commercial reagents (VetMAX NA and EU PRRSV RT-PCR, Thermo Fisher Scientific, Waltham, MA) and a commercial ELISA kit (HerdCheck PRRS X3, IDEXX Laboratories, Inc., Westbrook, ME), respectively. A serum viremia cycle threshold (**Ct**) ≥ 37 was considered negative and serology antibody was considered negative when sample to positive ratio (S:P) ≤ 0.40.

Statistical analysis

Within vaccine status and with pen considered the experimental unit, all data were analyzed using a complete randomized design with the PROC MIXED procedure of Statistical Analysis System (SAS) 9.4 (SAS Inst. Inc., Cary, NC). All performance data were analyzed for the fixed effects of dietary treatment consisting of control, HL, and LE Lys:ME, representing 2.69, 3.23, and 3.22 g SID Lys:ME, respectively. Least-squares (**LS**) means were determined for each treatment using the LS means statement and differences in LS means were produced using the PDIFF option. Tukey's multiple comparison adjustment was used on each LS mean pairwise comparison. Data were reported as LS means and standard error of the mean. Differences were considered significant when $P < 0.05$ and a tendency when $0.05 < P < 0.10$.

Results

Diet analysis

During the PRRSV challenge period, the experimental diets were formulated to contain 2.69, 3.23, and 3.22 g SID Lys per Mcal ME (Table 1). Proximate and AA analyses of the diets were conducted to verify that the diets were formulated similar to the predicted values (Table 1). Analyzed GE of the diets were 3.87, 3.86, and 3.01 Mcal/kg, representing the control, HL, and LE dietary treatments, respectively. These results confirmed the formulated 20% reduction in dietary energy LE in comparison to the control and HL diets.

Population vaccine status, health, and response to PRRSV

Serum samples were pooled within dietary treatment and vaccine status to confirm weekly PRRSV viremia and antibody titers (dpi 0 to 42). The serology responses to the PRRS vaccine and the PRRSV challenge are reported in Table 2. Prior to PRRSV inoculation, PRRSV viremia was not detected in pigs irrespective of vaccine status based on serum Ct values ≥ 37. As expected, the PRRSV-vaccinated pigs had detectable PRRSV antibodies 56

Table 2. Overall effects of increasing the ratio of SID lysine and reduced ME on PRRSV viremia and antibody titers in PRRSV-infected pigs

	g SID Lys:Mcal ME					
	Vaccinated			Non-vaccinated		
Parameter[1]	2.69 (control)	3.23 (HL)	3.22 (LE)	2.69 (control)	3.23 (HL)	3.22 (LE)
PRRSV Ct value[2]						
Dpi 0	≥37.0	≥37.0	≥37.0	≥37.0	≥37.0	≥37.0
Dpi 7	25.8	25.3	24.1	17.6	16.5	19.6
Dpi 14	32.0	26.8	32.1	25.4	25.3	26.2
Dpi 21	35.4	35.6	≥37.0	27.3	20.1	26.8
Dpi 28	≥37.0	≥37.0	≥37.0	31.0	30.1	29.8
Dpi 42	≥37.0	≥37.0	≥37.0	≥37.0	36.7	≥37.0
PRRSV S/P ratio[3]						
Dpi 0	2.025	1.890	1.881	–0.006	–0.008	–0.005
Dpi 7	2.005	1.773	1.949	0.304	0.154	0.220
Dpi 14	2.011	1.943	1.995	1.266	1.158	1.307
Dpi 21	1.919	2.016	1.941	1.380	1.217	1.181
Dpi 28	2.185	2.049	1.859	1.273	1.242	1.279
Dpi 42	1.978	1.894	1.940	1.685	1.285	1.571

[1]Pooled serology within treatment and vaccine status over time, day post-inoculated (dpi).
[2]Ct ≥ 37.0 denotes PRRS negative.
[3]PRRSX3 antibody sample to positive (S/P) ratio, ≤0.40 denotes PRRS negative.

d post-vaccination, while the non-vaccinated pigs were deemed negative for PRRSV antibodies with S:P ≤ 0.40. The success of the PRRSV challenge was confirmed via PCR over the 42-d challenge period. By 7 dpi, irrespective of diet and vaccination status, PRRS viremia Ct values were reported in the range of 16 to 26 (considered positive if <37; Table 2). As expected, PRRSV Ct values increased (i.e., viremia decreased) as pigs seroconverted. Vaccinated pigs had detectable PRRSV antibodies (S:P ratio) prior to PRRSV inoculation, and PRRSV antibody levels increased throughout the challenge period and plateaued at 28 dpi, at which time all vaccinated pigs were considered non-viremic (Ct > 37; Table 2). As expected, non-vaccinated pigs experienced a longer duration and magnitude of PRRSV viremia based on diagnostics. Following PRRSV inoculation, antibody titers for non-vaccinated pigs increased throughout the challenge period (Table 2).

Diagnostic testing also indicated that all pigs, irrespective of PRRS vaccination status, became naturally infected with porcine circovirus 2 (**PCV2**) between dpi 7 and 14, as confirmed by PCR; all pigs had not received PCV2 vaccinations prior to this experiment. As a result of this PRRSV and PCV2 coinfection, the PRRSV vaccinated and non-vaccinated barns experienced 11 and 22 mortalities, respectively, equating to 5.6% and 11.3% mortality over the test period. However, mortality was not different across dietary treatment (data not shown). A common cause of mortality, as reported by necropsy and diagnostics via the ISUVDL, was attributed to systemic effects of PRRSV and PCV2, with *Streptococcus suis* sepsis resulting in rapid death. Due to the severity of disease from unintended PCV2 infection, intentional PRRSV challenge, and secondary bacterial infections, all pigs were placed on water amoxicillin (Vet Rx Pharmacy, St. Peter, MN) from 14 to 21 dpi to decrease the impact of opportunistic secondary bacterial pathogens. From 22 to 30 dpi, all pigs received sodium salicylate (Aurora Pharmaceutical LLC., Northfield, MN) through the water with a daily target dose of 50 mg/kg body weight to help mitigate any febrile response associated with the multifactorial infection.

Performance: PRRSV-vaccinated pigs

Prior to the disease challenge period (dpi 0), all pigs were fed a common nursery diet and no differences in pig performance

Table 3. Effects of increasing the ratio of SID lysine to ME on growth performance in PRRSV-infected, vaccinated growing pigs

	g SID Lys:Mcal ME				
Parameter	2.69 (control)	3.23 (HL)	3.22 (LE)	SEM	*P*-value
Nursery[1]					
Start BW, kg	5.5	5.4	5.3	0.115	0.318
ADG, kg	0.482	0.490	0.478	0.017	0.883
ADFI, kg	0.755	0.798	0.760	0.018	0.277
G:F	0.720	0.708	0.709	0.022	0.911
End BW, kg	25.7	25.9	25.1	0.647	0.651
PRRSV Challenge[2]					
Dpi 0 to 7					
ADG, kg	0.416	0.633	0.511	0.062	0.071
ADFI, kg	1.120^{b}	1.411^{a}	1.324^{ab}	0.063	0.014
G:F	0.375	0.452	0.396	0.050	0.534
End BW, kg	37.4^{b}	40.6^{a}	38.3^{b}	0.532	0.002
Dpi 7 to 14					
ADG, kg	0.407	0.506	0.520	0.087	0.615
ADFI, kg	1.221^{b}	1.462^{ab}	1.494^{a}	0.073	0.033
G:F	0.327	0.336	0.344	0.061	0.980
End BW, kg	40.6^{b}	44.1^{a}	42.0^{ab}	0.809	0.021
Dpi 14 to 21					
ADG, kg	0.790	0.966	0.949	0.092	0.348
ADFI, kg	1.729	1.745	2.027	0.090	0.052
G:F	0.458	0.536	0.467	0.041	0.355
End BW, kg	45.8^{b}	50.8^{a}	48.6^{b}	0.857	0.002
Dpi 21 to 28					
ADG, kg	0.968	1.016	1.090	0.092	0.647
ADFI, kg	2.102^{b}	2.221^{ab}	2.525^{a}	0.092	0.013
G:F	0.474	0.459	0.445	0.036	0.846
End BW, kg	52. 7^{c}	58.6^{a}	56.6^{b}	0.937	0.001
Dpi 28 to 35					
ADG, kg	0.912	1.045	0.967	0.072	0.434
ADFI, kg	2.398^{c}	2.438^{b}	2.792^{a}	0.078	0.004
G:F	0.376^{b}	0.430^{a}	0.346^{c}	0.021	0.035
End BW, kg	59.5^{b}	66.0^{a}	63.3^{a}	1.037	0.001
Dpi 35 to 42					
ADG, kg	0.873	1.073	1.070	0.083	0.178
ADFI, kg	2.456^{c}	2.590^{b}	3.053^{a}	0.068	<.0001
G:F	0.354	0.415	0.350	0.026	0.181
End BW, kg	66.6^{b}	73.5^{a}	70.8^{a}	1.194	0.003

[1]Nursery period (−56 to −14 dpi), all pigs fed common diet; *n* = 4 pens per treatment and 15 to 17 pigs per pen.
[2]Challenge period (0 to 42 dpi), all pigs fed experimental diets; *n* = 8 pens per treatment and 7 to 10 pigs per pen.
[a, b, c]Means with differing superscripts indicate a significant ($P < 0.05$) difference.

parameters within the vaccinated pens were detected ($P > 0.10$; Table 3). From 0 to 7 dpi, there was a tendency ($P = 0.071$) for ADG to be increased by 150% in the HL pigs compared with the control treatment, while LE was not different from either treatment ($P > 0.05$). Growth rates were similar between treatments for all other weekly weigh periods ($P > 0.10$; 7 to 42 dpi). An increase ($P < 0.05$) in ADFI was observed weekly throughout the challenge period, with the exception of dpi 14 to 21 in which a tendency for ADFI was observed ($P < 0.10$) as a result of the LE treatment compared with the control and HL dietary treatments. From 28 to 35 dpi, G:F was greatest for pigs fed the HL dietary treatment, lowest for pigs fed the LE treatment, and intermediate for those fed control diet; however, G:F differences were not detected in any other weekly growth periods ($P > 0.05$).

For the overall challenge period (Table 4), increasing SID Lys:ME to 120% of NRC (2012) requirement during the 42-d PRRSV challenge period increased ADG ($P < 0.01$), regardless of how the 120% ratio was achieved by either increasing g SID Lys (HL) or decreasing ME (LE). Overall ADFI increased by 19.8% as a result of LE dietary treatment compared with control ($P < 0.01$), whereas the HL treatment was similar to the control. When expressing overall ADFI on a ME intake per day, the HL pigs had significantly higher ME intakes compared with the LE ($P < 0.05$), with the control pigs being intermediate (Table 4). An increase in overall G:F was observed in pigs fed the HL treatment compared with pigs fed the control and LE treatments ($P < 0.01$), which were not different from each other. End BW of pigs fed HL and LE treatments were improved 6.9 kg and 4.2 kg, respectively, in comparison to the control ($P < 0.05$).

Performance: PRRSV-non-vaccinated pigs

In the non-vaccinated pigs, prior to the disease challenge period (dpi 0), there were no differences in pig performance parameters ($P > 0.10$; Table 5). Throughout the challenge period, pigs remained PRRSV seropositive until 42 dpi (Table 2), confirming PRRSV inoculation was successful. Weekly growth performance results are shown in Table 5. From 0 to 7, 21 to 28, and 28 to 35 dpi, ADG increased in pigs fed the HL and LE dietary treatments relative to control ($P < 0.05$), with no differences between treatments during the other weekly weigh periods. There were no differences ($P > 0.05$) in ADFI between treatments during the first four weekly weigh periods. An increase in ADFI was observed from 28 to 35 and 35 to 42 dpi as an effect of LE dietary treatment ($P < 0.01$). From 0 to 7, 21 to 28, and 28 to 35 dpi, G:F was increased in pigs fed the HL and LE diets compared with control ($P < 0.05$); with no other G:F differences observed between treatments throughout other weekly growth periods.

Overall growth performance results are shown in Table 4. Overall, increasing SID Lys:ME to 120% of NRC (2012) requirement during the 42-d PRRSV challenge period increased ADG ($P < 0.05$), regardless of how the 120% ratio was achieved by either increasing g SID Lys or decreasing ME. Overall ADFI increased 16.6% as a result of LE dietary treatment with respect to control ($P < 0.01$); with no difference seen between HL and control ($P > 0.05$). Further, during the overall challenge period, daily ME intake (Mcal/d) tended ($P = 0.077$) to differ, with the LE pigs having the lowest ME intake per day compared with the control and HL pigs (Table 4). Dietary treatment had no effect on overall G:F ($P > 0.10$). End BW of pigs fed HL and LE treatments were improved 5.4 and 5.2 kg, respectively, in comparison to control ($P < 0.05$).

Discussion

It is well established that Lys is the first-limiting AA in healthy pigs, and to ensure that the targeted amount of Lys is being consumed by the pig, diets are formulated on a ratio of Lys to energy (i.e., g SID Lys:ME). Previous breakpoint analysis from our group (Schweer et al., 2018a) has reported that during both an experimental and natural PRRSV challenge, increasing SID Lys:ME 10% to 20% above NRC (2012) requirements resulted in improved growth performance and feed efficiency. This increase in Lys:ME is presumably accounting for the reduced feed and Lys intake (Schweer et al., 2017), thus preserving lean tissue. When formulating to 100% of NRC requirement in PRRSV-challenged pigs, Lys intake would be reduced, which is thought to contribute to a depleted AA pool which likely results in a reduction of lean tissue accretion (Helm et al., 2019). Therefore, our objective herein was to confirm the performance benefit of increasing the dietary SID Lys:ME in PRRSV vaccinated and non-vaccinated grower pigs experiencing a PRRSV challenge.

Table 4. Overall effects of increasing the ratio of SID lysine to ME on growth performance in PRRSV-infected pigs

	g SID Lys:Mcal ME[1]				
Parameter	2.69 (control)	3.23 (HL)	3.22 (LE)	SEM	*P*-value
Vaccinated[2]					
Start BW, kg	34.7	36.1	34.7	0.600	0.178
End BW, kg	66.6[b]	73.5[a]	70.8[a]	1.194	0.003
ADG, kg	0.728[b]	0.873[a]	0.851[a]	0.033	0.013
ADFI, kg	1.838[b]	1.978[b]	2.202[a]	0.054	0.001
ME intake/d, Mcal	6.19[ab]	6.54[a]	5.88[b]	0.172	0.029
G:F	0.394[b]	0.438[a]	0.391[b]	0.010	0.005
Non-vaccinated[2]					
Start BW, kg	35.4	36.1	34.0	0.647	0.104
End BW, kg	60.4[b]	65.8[a]	65.6[b]	1.245	0.021
ADG, kg	0.572[b]	0.680[a]	0.687[a]	0.030	0.024
ADFI, kg	1.563[b]	1.621[b]	1.823[a]	0.047	0.003
ME intake/d, Mcal	5.17	5.37	4.87	0.139	0.077
G:F	0.334	0.384	0.368	0.014	0.135

[1]n = 8 pens/treatment and 7 to 10 pigs per pen.
[2]Overall challenge period (0 to 42 dpi), pigs fed experimental diets.
[a, b]Means with differing superscripts indicate a significant ($P < 0.05$) difference.

Table 5. Effects of increasing the ratio of SID lysine to ME on growth performance in PRRSV-infected, non-vaccinated growing pigs

	g SID Lys:Mcal ME				
Parameter	2.69 (control)	3.23 (HL)	3.22 (LE)	SEM	P-value
Nursery[1]					
Start BW, kg	5.3	5.3	5.5	0.245	0.777
ADG, kg	0.478	0.472	0.488	0.009	0.506
ADFI, kg	0.749	0.743	0.777	0.013	0.201
G:F	0.774	0.730	0.731	0.025	0.431
End BW, kg	25.4	25.1	26.1	0.487	0.350
PRRSV Challenge[2]					
Dpi 0 to 7					
ADG, kg	–0.022[b]	0.119[ab]	0.275[a]	0.064	0.014
ADFI, kg	0.839	0.879	1.001	0.052	0.083
G:F	–0.011[b]	0.121[ab]	0.270[a]	0.070	0.034
End BW, kg	35.2	36.9	36.0	0.663	0.228
Dpi 7 to 14					
ADG, kg	0.265	0.319	0.340	0.061	0.669
ADFI, kg	0.826	0.804	0.938	0.052	0.183
G:F	0.342	0.385	0.369	0.066	0.898
End BW, kg	37.0	39.1	38.3	0.821	0.232
Dpi 14 to 21					
ADG, kg	0.759	0.667	0.617	0.094	0.569
ADFI, kg	1.412	1.463	1.587	0.069	0.209
G:F	0.528	0.451	0.390	0.050	0.180
End BW, kg	42.9	43.8	42.6	1.156	0.766
Dpi 21 to 28					
ADG, kg	0.587[b]	0.782[ab]	0.894[a]	0.069	0.017
ADFI, kg	1.848	1.872	2.130	0.093	0.087
G:F	0.317[b]	0.414[ab]	0.425[a]	0.028	0.023
End BW, kg	47.2	50.1	49.3	1.306	0.302
Dpi 28 to 35					
ADG, kg	0.842[b]	1.086[a]	0.937[ab]	0.058	0.025
ADFI, kg	2.153[b]	2.283[b]	2.551[a]	0.045	<0.001
G:F	0.392[b]	0.477[a]	0.366[b]	0.026	0.018
End BW, kg	53.1[b]	57.8[a]	55.7[b]	1.212	0.041
Dpi 35 to 42					
ADG, kg	1.003	1.109	1.056	0.074	0.607
ADFI, kg	2.297[b]	2.423[ab]	2.724[a]	0.087	0.009
G:F	0.439	0.454	0.388	0.023	0.139
End BW, kg	60.4[b]	65.8[a]	63.6[b]	1.245	0.021

[1]Nursery period (–56 to –14 dpi), all pigs fed common diet; *n* = 4 pens per treatment and 15 to 17 pigs per pen.
[2]Challenge period (0 to 42 dpi), all pigs fed experimental diets; *n* = 8 pens/treatment and 7 to 10 pigs per pen.
[a, b]Means with differing superscripts indicate a significant ($P < 0.05$) difference.

Furthermore, we hypothesized that irrespective of how the 120% SID Lys to ME ratio was achieved via diet formulation, either by increasing Lys or reducing ME, it would result in increased growth performance in PRRSV-infected pigs compared with the NRC (2012) recommended Lys:ME requirement.

It is inevitable throughout the swine industry that growing pigs will experience a performance-impacting disease challenge. A PRRSV challenge is shown to attenuate growth rates 30% to 59% compared with healthy controls (Che et al., 2011; Rochell et al., 2015; Schweer et al., 2016). The differences in severity of this negative impact on growth performance is thought to be a result of pig age, viral strain, and PRRS viral clearance rates (Murtaugh et al., 2002). In recent years, Rochell et al. (2015) and Schweer et al., (2018a) have reported that dietary treatment can aid in improving growth performance and feed efficiency of pigs experiencing PRRSV challenge. In particular, Schweer et al. (2018a) reported that increasing the dietary SID Lys:ME by 10% to 20% above NRC (2012) requirement in 25 to 50 kg pigs increased growth performance and feed efficiency. However, it is unclear if the improved growth performance during this PRRSV challenge was attributed to increase in SID AA, CP, or other functional factors associated with soybean meal.

In this research, due to the intentional formulation of the diets, CP levels remained similar in both the control and LE diets, along with relatively similar soybean meal inclusion levels of 19.35% and 21.95%, respectively. However, the HL diet was formulated to have an increased CP level with the increased inclusion of soybean meal (26.5%) in comparison to control and LE diets. Soybean meal contains naturally occurring bioactive components, that is, isoflavones, that have antiviral activity in PRRSV-challenged pigs (Greiner et al., 2001); however, no differences in viremia (i.e., PCR Ct values) or antibody titers were observed due to dietary treatment in this study. When feeding diets divergent in soybean meal inclusion levels to newly weaned pigs, feeding diets with a high and low soybean meal inclusion level to newly weaned pigs, pigs fed high soybean meal diets had a reduction in immune stress and increased ADG during a PRRSV challenge

(Rochell et al., 2015). When utilizing soybean meal to increased Lys:ME ratio, various other essential and nonessential AA are likely also increasing in the diet which may be beneficial. It has been shown that during a lipopolysaccharide (LPS) challenge pigs fed increased levels of Met and Met + Cys resulted in increased protein deposition, indicating that the optimal Met:Met + Cys is greater during immune system stimulation (Litvak et al., 2013). Additionally, Thr and Trp are two AA that play an important role in the immunity of animals (Li et al., 2007). Threonine is a major component of plasma immunoglobulin G (**IgG**) and has shown to enhance antibody production and serum IgG levels in young pigs challenged with *Escherichia coli* (Wang et al., 2006). Additionally, Trp is a precursor of serotonin (5-hydroxytrptamine) and feed intake regulation. Limited research has been conducted to evaluate the effects of altering dietary Thr and Trp during immune challenge. However, when evaluating the effects of Thr and Trp supplementation on the attenuation of immunological challenge-induced growth reduction in PRRS-vaccinated pigs, Xu et al. (2014) reported increased feed intake and improved ADG in Thr and Trp-supplemented pigs compared with control after PRRS vaccination. Altogether, increasing soybean meal inclusion in the diet likely increases the intake of multiple AA, not just Lys, thus reducing the need for lean tissue catabolism and preserving lean tissue during a disease challenge.

To further test the benefit of increasing the Lys:ME of PRRSV-challenged pigs, 3.22 g SID Lys:ME was also achieved via a dilution of energy (LE dietary treatment), as discussed previously. This LE diet resulted in increased ADG compared with the control diet and resulted in similar ADG to the HL treatment. Although increased CP and AA may be beneficial, these data indicated that the Lys:ME is critical to driving the improved performance responses in a PRRSV-challenged pig. By default, the 20% reduced ME diet (LE) also indicates that viral-challenged pigs were able to adjust their voluntary feed intake to eat to their energy needs. The theory of pigs eating to their energy needs implies that a dilution of dietary energy would result in an increase in feed intake. Reduction in ADFI in newly weaned pigs has been reported as a result of increased energy concentration in the diet in both healthy and immune-challenged pigs when compared with diets with lower energy concentration (van Heugten et al., 1996; Oresanya et al., 2007). In *E. coli* LPS-challenged nursery pigs, feed intake was reduced in pigs fed high energy diets; however, energy intake was equal between high and low energy diets, indicating immune-stimulated pigs have the ability to adjust their voluntary feed intake to meet their energy needs regardless of dietary energy concentration (van Heugten et al., 1996). In the current study, we report that a 20% reduction in dietary ME increased ADFI 20% and 17% in PRRS-vaccinated and non-vaccinated pigs, respectively, in the face of a PRRSV challenge. These results are in agreement with a previous dilution study conducted by Baker et al. (1968) in which 53 kg pigs fed a diet with 20% inclusion of sand resulted in a 20% increase in ADFI, in non-disease-challenged pigs. Collectively, these results indicate the pig's ability to adjust their voluntary feed intake to achieve a level of energy needs in both healthy and disease-challenged situations. Thus, increasing dietary energy concentrations would likely result in a reduction in feed intake to maintain a constant daily energy intake.

Although the highest ADG in vaccinated (2.60 g SID Lys:ME) and non-vaccinated (3.22 g SID Lys:ME) pigs did not result from the same dietary treatment, growth was increased the greatest at a similar total Lys intake of 24.1 g/d in both PRRSV-vaccinated and non-vaccinated pigs. The NRC (2012) recommends a Lys intake of 16.9 g/d in 35 to 75 kg pigs; however, two diets in the current study were formulated to 120% of NRC requirement for the disease challenge period which equated to ~20.3 g Lys/d. Although growth rate and PRRSV status of the pigs differ, these results are similar to Schweer et al. (2018a) in which growth was optimized at similar total daily Lys intake in control and PRRSV-infected pigs. Interestingly, the results from the current study and Schweer et al. (2018a) differ from previous work reporting that Lys requirement (g/d basis) is reduced in immune-stimulated pigs compared with nonimmune-stimulated pigs (Williams et al., 1997; Zimmerman et al., 1997). This is thought to be attributed to increased lean tissue deposition in pigs with low immune system activity compared with those with high immune stimulation. Nonetheless, the results from this study support the theory that in the event of a stressor such as a disease challenge, AA requirements may change due to increased metabolic activity and the repartitioning of nutrients away from lean tissue accretion (i.e., protein catabolism), thus indicating the importance and impact of feed intake during a disease challenge. Overall, by decreasing ME in the diet to achieve 120% of NRC (2012) SID Lys:ME requirement, we were able to increase ADFI attenuating a portion of the growth depression commonly observed during a PRRSV challenge.

In today's swine industry, PRRS vaccination strategies are commonly implemented to serve as a line of protection in the event of a PRRSV challenge; however, available vaccines have varying efficacy (Osorio et al., 1998; Mavromatis et al., 1999; Meng, 2000). The efficacy of PRRSV vaccines is commonly assessed by evaluating the vaccines' ability to reduce viremia after the challenge, which is crucial for mitigating the negative effects associated with PRRSV. In young, naive pigs, it is often a concern that early vaccination is ineffective due to the immature immune system's inability to effectively respond and build immunity. However, a study conducted by Jeong et al. (2018) concluded that PRRS MLV vaccination of pigs as early as day 1, and as late as day 182 of age, resulted in improved growth performance in the face of a natural PRRSV challenge. Although not the object of paper, the PRRSV-vaccinated group had reduced mortality and improved growth performance compared with non-vaccinated pigs throughout the 42-d challenge period; however, PCV2 likely had a major impact on mortality in this study. These findings are in agreement with previous findings (Park et al., 2014; Jeong et al., 2018; Oh et al., 2019).

In summary, this work validates that during a controlled PRRSV challenge (also naturally co-challenged with PCV2), increasing SID Lys:ME to 120% in grower pigs aids in the mitigation of negative growth performance associated with mixed infections including PRRSV challenge (Schweer et al., 2018a). Irrespective of vaccination status, a 20% dilution of energy in the diet resulted in increased feed intake, translating to an increase in ADG and end BW in comparison to a control throughout a PRRSV challenge. The results from this study support the theory that in the event of a disease challenge, AA requirements may change due to increased metabolic activity and the repartitioning of nutrients away from lean tissue accretion, indicating the importance and impact that feed intake has during a disease challenge. Feed efficiency was most improved as a result of the HL dietary treatment, suggesting that from a feed efficiency standpoint, increasing SID Lys was the most beneficial mitigation strategy rather than diluting ME. However, in non-vaccinated pigs, both the HL and LE treatment resulted in similar increases in ADG and end BW, suggesting that during a severe health challenge reducing dietary energy is also

an effective strategy to achieve a 120% SID Lys:ME. The utilization of sand to dilute dietary energy is not a practical approach. However, the utilization of dietary fiber to dilute energy could be a more practical industry approach. Overall, increasing SID Lys:ME 20% above the recommended NRC (2012) requirement in PRRSV-infected pigs resulted in increased growth performance in comparison to control. This performance was observed irrespective of vaccination status or the dietary strategy used to achieve the 120% SID Lys:ME.

Acknowledgment

The funding for this research project was provided by the National Pork Board Grant (18.119) and The Maschhoffs LLC.

Conflict of interest statement

All authors declare no conflicts of interest regarding the diet ingredients and health challenge presented herein. However, O.F.M and C.M.S are employed by The Maschhoffs LLC.

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