RESEARCH REPORT
ANIMAL SCIENCE

Title: Impact of disease on amino acid requirements of growing pigs. NPB 13-082 revised

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Industry Summary:
A total of 40 gilts (initial BW 9.4 ± 0.9 kg) were used in three experiments (Exp) to evaluate the impact of disease induced either by bacterial lipopolysaccharide (LPS) or porcine reproductive and respiratory syndrome virus (PRRSV) on the measures of immune function, as well as protein and amino acid (AA) utilization. An isotope tracer technique was employed in all of the Exp to evaluate the impact of disease on kinetics/utilization of nine AA (Lys, Met, Thr, Trp, Ile, Leu, Val, Phe, Gln) simultaneously. In Exp 1, apparent ileal digestibility (AID) and protein retention were determined in saline-treated pigs. An isotope infusion rate and the best blood sampling frequency for studying AA kinetics in plasma were also determined in Exp 1. In Exp 2 and 3 effects of LPS and PRRSV on measures of immune function, as well as protein and AA utilization (i.e. protein digestibility and retention as well as AA utilization) were evaluated, respectively. Blood chemistry, hematology, and body temperature results indicated that both models of disease induced effective immune system stimulation. However, compared to LPS, PRRSV model elicits a more severe response with a more negative impact on the performance of pigs. Relative to feed-restricted control (550 g/d feed allowance), LPS did not reduce the daily feed intake. However, PRRSV-challenged pigs consumed only 55 % of their daily feed allowance. Compared to control, protein retention was decreased in PRRSV-challenged pigs (37 %), while LPS resulted in a numeric decrease in protein retention (6.4 %). AID of protein was decreased by LPS and PRRSV (16 and 26 %, respectively). Relative to control, LPS reduced the utilization of Lys and Phe by 17.4 and 37.6 %, respectively. The utilization rate of other AA was not affected by LPS. PRRSV significantly increased plasma Lys concentration, while it had no effect on Lys rate of utilization, suggesting a substantial decrease in Lys needs in PRRSV-challenged pigs. Relative to control, the PRRSV challenge increased the utilization rate of Met (111 %), and Thr (55 %). The increased Met and Thr utilization in PRRSV-challenged pigs could be associated with the enhanced use of Met and Thr for synthesis of immune system metabolites and increased catabolism of these AA. These factors may increase dietary Met and Thr requirements of PRRSV-challenged pigs, relative to requirements for other AA (e.g. Lys). Collectively, results of the current project suggest that LPS and PRRSV model of ISS effectively alter the measures of immune function and metabolism. However, it seems that the effect on AA utilization is either disease-specific, or is a function of disease severity.

Key findings:

- Disease alters metabolism of growing pigs and impacts their ability to utilize dietary protein and AA.
- Disease reduces (apparent) ileal digestibility of dietary protein and AA in growing pigs.
- Disease reduces lysine requirements in growing pigs.
- Infection with PRRSV increases requirements for methionine and threonine in growing pigs.

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Keywords: PRRSV, LPS, amino acid, utilization, requirement

Scientific Abstract:

Changes in plasma free AA kinetics reflect modification of AA metabolism in different metabolic states. Exposure to disease-causing agents can alter (increase or decrease) requirements for amino acids (AA), both qualitatively (metabolic demand; i.e. AA use) and quantitatively (dietary AA requirements). The knowledge about the effect of disease/health status on AA requirements of swine is limited and often ignores the relationship between the AA. Thus, the primary objective of the current study was to identify AA whose utilization is influenced by disease. In recent years, there has been debate on developing an industry-applicable model of disease for studying nutrient needs of pigs during immune system stimulation (ISS). Thus, the secondary objective of the study was to compare a pre-established non-pathogenic LPS model of ISS against PRRSV model for studying nutrient metabolism in growing pigs. A total of 40 PRRSV-negative gilts (initial BW 9.4 ± 0.9 kg) of commercially relevant genetics were used in three experiments (Exp). In all Exp pigs were fed a corn-SBM based diet (ME 14 MJ/kg, SID Lys 11.5 g/kg), and feed restricted (550 g/d). In Exp 1, a total of 10 saline-treated gilts were used to establish a base line for N retention and AID of nutrients. In addition, four pigs were surgically catheterized and infused with a bolus dose of [U-^{13}C, U-^{15}N] AA mixture (Lys, Met, Thr, Trp, Ile, Leu, Val, Phe, Gln) to determine the best isotope infusion rate and blood sampling frequency for studying AA kinetics in plasma. In Exp 2 and 3 ten and twenty gilts, respectively, were surgically catheterized in the left and right external jugular. ISS was induced by repeated i.m. injection of increasing amounts of LPS (initial dose of 30 µg/kg BW), and i.m. injection of live PRRSV in Exp 2 and 3, respectively. In Exp 2 and 3, blood samples were collected via the catheters every 48 h during pre- and post-challenge period and assayed for blood chemistry, hematology, and serum viral load. Body temperature was monitored on a daily basis. In both Exp, N-balances were determined during a 3 d pre-challenge period and a 3 d post-challenge period. At the end of each N-balance period a single dose of [U-^{13}C, U-^{15}N] AA mixture was infused i.v. to study plasma AA kinetics. A series of blood samples were taken at 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, and 60 minutes post-infusion to determine the plasma isotopic enrichments of ^{13}C- and ^{15}N-AA. A double-exponential model was fitted in data in order to estimate parameters from which the plasma AA rate of disappearance (i.e. AA flux) was calculated. At the end of each post-challenge N-balance period pigs were euthanized and ileal digesta was collected for measurement of AID of nutrients. All pigs in Exp 3 became PRRSV-positive within 2-4 d post challenge as assessed by qPCR titers. No differences were observed in body temperature, blood parameters, N-balance, and feed intake between Exp 1 and pre-challenge period of Exp 2 and 3. Blood chemistry, hematology, and body temperature results indicated that both LPS and PRRSV models induced effective ISS in pigs. However, compared to LPS, PRRSV model elicits a more severe response with a more negative impact on protein retention and AID of dietary protein in pigs. The LPS challenge numerically reduced N retention (from 9.5 to 8.9 g/d), and rate of disappearance (µmol/kg BW/h) of Lys (from 394 to 325) and Phe (from 126 to 79), without having an effect on the rate of disappearance of other AA from plasma. The PRRSV challenge reduced PD from 9.6 to 6.1 g/d, SE 0.73, but increased the rate of disappearance (µmol/kg BW/h) of Met (from 108 to 228) and Thr (from 83 to 129). PRRSV challenge also reduced Lys utilization but had no effect on the rate of disappearance of other AA. The increased Met and Thr utilization in PRRSV-challenged pigs could be associated with enhanced use of Met and Thr for the synthesis of immune system metabolites and increased catabolism of these AA. This may increase dietary Met and Thr requirements of PRRSV-challenged pigs, relative to the requirements for other AA (e.g. Lys). Collectively, results of the current project suggest that LPS and

PRRSV model of ISS effectively alter the measures of immune function and metabolism. However, it seems that the effect on AA utilization is either disease-specific, or is a function of disease severity.

Introduction:

In spite of extensive bio-security measures in the U.S. swine industry, we are still faced with disease challenges that impact sustainability and profitability of pork production. Hyper-activation of the immune system, as a result of exposure to pathogenic immunogens, can alter (increase or decrease) requirements for amino acids (AA), both qualitatively (metabolic demand; i.e. AA use) and quantitatively (dietary AA requirements). As is clearly stated in NRC (2012), the knowledge about the effect of disease/health status on nutrient, especially AA, requirements of swine is limited and often ignores the relationship between the AA. This lack of knowledge hampers the ability of feed manufacturers and commercial pig producers to formulate optimal diets that closely match nutrient requirements of pigs during disease and recovery. In recent years, there has been much debate on developing an industry-applicable model of disease to study nutrient metabolism in pigs during the hyper-activation of the immune system. Repeated injection with increasing amounts of bacterial lipopolysaccharide (LPS) provides a simple, non-pathogenic and inexpensive model for studying the impact of chronic disease on nutrient metabolism and evaluating the impact of potential (nutritional) interventions to alleviate the impact of disease on pigs' productivity. Although, it has been argued that the LPS model might not be a good indicator of immune system stimulation in commercial settings, where multiple pathogens are often involved, one important advantage of the LPS model is that it can be used on farm or in a controlled research setting without infecting other pigs. Furthermore, models in which animals are exposed to live pathogens are often associated with variable responses and require a large number of animals. Moreover, strict measures of biosecurity are needed when live pathogens are administered to animals. Thus, a reliable chronic disease model, which induces repeatable responses and is equivalent to chronic disease, needs to be developed for studying nutrient metabolism in pigs.

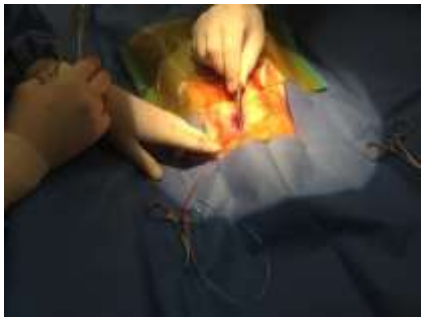
Objectives:

a. To evaluate the qualitative and quantitative impact of disease on amino acid requirements of growing pigs.

b. To compare a pre-established non-pathogenic (E.coli lipopolysaccharide; LPS) model of disease against the porcine reproductive and respiratory syndrome virus (PRRSV) model for studying nutrient metabolism in growing pigs.

Material & Methods:

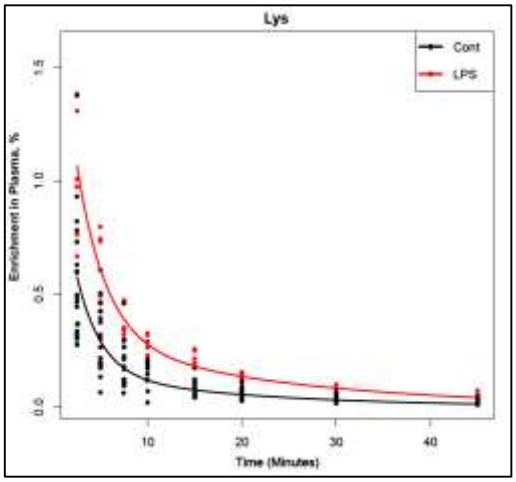
A total of 40 gilts (initial BW 9.4 ± 0.9 kg) were used in three experiments (Exp) to evaluate the impact of disease induced by bacterial lipopolysaccharide (LPS) or porcine reproductive and respiratory syndrome virus (PRRSV) on measures of immune function, and protein and amino acid (AA) utilization.



Exp 1: preliminary study. Ten gilts were used to establish a base line for apparent ileal digestibility (AID) of nutrients in healthy pigs, as well as an isotope infusion rate and the best blood sampling frequency for studying amino acid (AA) kinetics. Pigs were fed a corn-SBM based diet (ME 14 MJ/kg, SID Lys 11.5 g/kg), feed restricted (550 g/d), and housed individually in metabolism crates. The preliminary study was essential for establishing an isotopic enrichment curve. The enrichment curve was used to study amino acid kinetics/utilization during disease induced either by PRRSv or LPS model. To establish the enrichment curve four pigs were surgically catheterized in left and right external jugular. Pigs were allowed to recover from the surgery for 6 d. A 3 d N-balance was then conducted to determine the rate of N retention using all 10 pigs. Pigs were received injection (i.m.) with sterile saline to account for stress caused by injection. At the end of the study, the catheterized pigs were infused with the mixture of nine universally-labeled AA with ^{13}C- and ^{15}N-. Isotopic enrichment of labeled AA (tracer: tracee ratio) in plasma was achieved using gas chromatography mass spectrometry of extracted and derivatized AA. Enrichment curves and optimal infusion rate for Lysine (Lys), tryptophan (Trp), threonine (Thr), methionine (Met), phenylalanine (Phe), glutamine (Gln), leucine (Leu), Isoleucine (Ile), and Valine (Val) as well as blood sampling frequency were established using data collected in the preliminary study. At the end of the N-balance and infusion period pigs were euthanized by injection of lethal dose of sodium pentobarbital, and digesta was collected form last 1 meter of ileum. Apparent fecal digestibility and AID of dietary nutrients were determined using the indicator technique and TiO_2 (0.25 % of diet) as indigestible marker.

Exp 2: Effect of LPS challenge on amino acid utilization and whole-body nitrogen (N) economy of growing pigs. Ten gilts were surgically catheterized in the left and the right external jugular, individually housed in metabolism crates, and fed restricted (550 g/d) from a corn-SBM based diet (ME 14 MJ/kg, SID Lys 11.5 g/kg). ISS was induced by repeated intramuscular injection of increasing amounts of *E. coli* lipopolysaccharide (LPS; initial dose of 30 µg/kg BW) as previously described by Rakhshandeh et al. (2012). Pigs were allowed to recover from the surgery for 6 days. N-balances were determined during a 3 d pre inoculation period and a 3 d post inoculation period. Blood samples were collected via the catheters every 48 h during the pre- and post-challenge periods and assayed for blood chemistry, and hematology. At the end of each N-balance period a single dose of [U-^{13}C, U-^{15}N] AA mixture (Lys, Met, Thr, Trp, Ile, Leu, Val, Phe, Gln) was infused intravenously to study plasma AA kinetics. Serial blood samples were taken at time 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, and 60 minutes post infusion. The AA dilution technique was used to determine the rate of AA disappearance, a measure of AA utilization, from whole-body's general AA pool, i.e. plasma. Plasma [U-^{13}C, U-^{15}N] AA enrichments (i.e. tracer: tracee ratio) were measured by gas chromatography mass spectrometry (GC-MS). A double-exponential model was fitted in data by nonlinear least-

squares in order to estimate parameters from which the plasma AA rate of disappearance (i.e. AA flux; sum of disappearance of AA from the plasma pool towards protein synthesis and AA catabolism, µmol/kg BW/ h) was calculated.



At the end of the infusion, pigs were euthanized and samples of ileal digesta were collected from distal ileum, and the weight of organs was determined. Apparent fecal digestibility and AID of dietary nutrients were determined using the indicator technique and TiO_2 (0.25 % of diet) as indigestible marker. To ensure effectiveness of immune system stimulation, eye temperature of animals was measured hourly using the infrared imaging technique.

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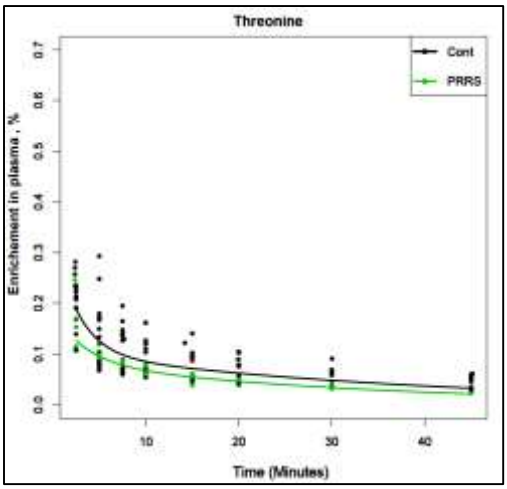
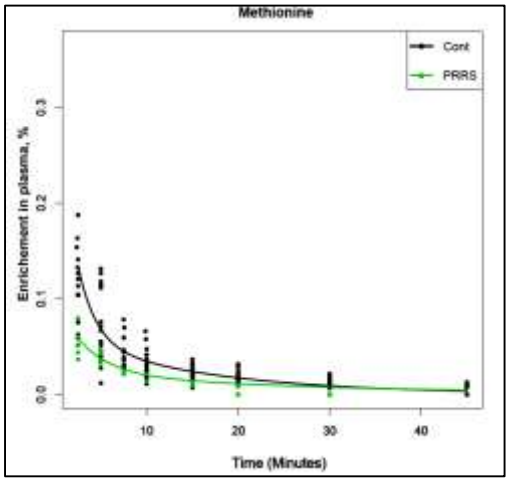
Exp 3: Effect of PRRSV challenge on amino acid utilization and whole-body nitrogen (N) economy of growing pigs. Twenty PRRSV-negative gilts were blocked by time, surgically catheterized, housed in metabolism crates, fed corn-SBM based diet (ME 14 MJ/kg, SID Lys 11.5 g/kg), feed restricted (550 g/d), and then inoculated intramuscularly with a live field PRRSV as described previously by Rakhshandeh et al. 2013. Blood samples were collected via the catheters at 0, 2, 4, 6, 8, and 9 days post inoculation, and assayed for blood chemistry, hematology, and serum viral load. N-balances were determined during a 3 d pre inoculation period and a 3 d post inoculation period. Post-challenge N-balance was started 6 d after inoculation with PRRV. In previous studies we observed a 6-7 d incubation period for PRRSV (Rakhshandeh et al. 2013. At the end of each N-balance period a single dose of [U-^{13}C, U-^{15}N] AA mixture (Lys, Met, Thr, Trp, Ile, Leu, Val, Phe, Gln) was infused intravenously to study plasma AA kinetics. Serial blood samples were taken at time 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, and 60 minutes post infusion. The AA dilution technique was used to determine the rate of AA disappearance, a measure of AA utilization, from whole-body's general AA pool, i.e. plasma. Plasma [U-13C, U-15N] AA enrichments (i.e. tracer: tracee ratio) were measured by gas chromatography mass spectrometry (GC-MS). A double-exponential model was fitted in data by nonlinear least-squares in order to estimate parameters from which the plasma AA rate of disappearance (i.e. AA flux; sum of disappearance of AA from the plasma pool towards protein synthesis and AA catabolism, µmol/kg BW/ h) was calculated.





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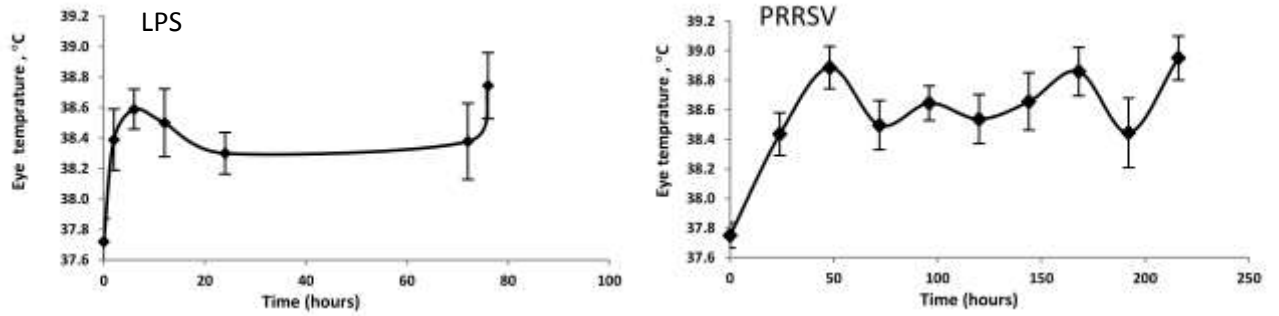
Results & Discussion

No differences were observed in eye temperature, blood parameters, N-balance, and feed intake (FI), between control and pre-ISS period of LPS and PRRSv-treated pigs (*P > 0.05*).

Impact of immune system stimulation (ISS) on measures of immune function

Repeated injection with increasing amounts of LPS and inoculation with PRRSV increased the eye temperature within 2 and 24 h period post immune system stimulation (ISS), respectively. Body temperature remained elevated during the post-ISS period (*P < 0.02*). Increased eye temperature, an indicator of internal body temperature, is modulated by action of pro-inflammatory cytokines, in particular IL-1β. Therefore, increased body and eye temperature reflects ISS mediated by pro-inflammatory cytokines. Relative to the pre-challenge period (ISS-



Effects of LPS and PRRS challenge on eye temperature. Infrared imaging technique was used to measure eye temperature. Data are least square mean ±SE.

), BUN levels and creatinine ratio were higher in LPS and PRRSV treated pigs, respectively, suggesting an increase in muscle proteolysis in ISS pigs (Table 1 and 2). Systemic inflammation in pigs is usually characterized by increased muscle proteolysis. Blood glucose concentration was lower only in LPS treated pigs. LPS challenge had no effect on hematology (Table 1). However, PRRSV challenge decreased the levels of hemoglobin in plasma (Table 2). Decreased hemoglobin levels can be attributed to reduced erythrocytes life span mediated by pro-inflammatory cytokines. In addition, reduced hemoglobin levels in ISS pigs can in part be attributed to reduced spleen weight (Table 4). Both models of ISS increased the anion gap, and tended to decrease the total CO_2 levels, suggesting a shift of aerobic to anaerobic metabolism in ISS pigs (Table 1 and 2). All pigs became PRRSV-positive within 2 days post inoculation as assessed by qPCR

*Table 1. Effect of immune system stimulation (**ISS**) induced by E. coli lipopolysaccharide (**LPS**) on blood parameters*[*]

	ISS-	ISS+	SE	*P*
Blood chemistry				
BUN[†], mg/dl	8.7	11.5	1.08	*0.032*
Glucose, mg/dl	89.4	61.7	4.44	*0.001*
Hematology				
Hct, % PCV	27.3	30.1	5.49	*0.644*
Hb, g/dl	12.1	11.2	1.33	*0.619*
Electrolytes				
Cl^-, mmol/l	102.9	102.0	1.44	*0.562*
Na^+, mmol/l	142.5	140.8	0.93	*0.209*
K^+, mmol/l	4.2	4.6	0.32	*0.214*
Acid/Base				
PCO_2, mmHg	43.6	36.1	2.66	*0.058*
HCO_3, mmol/l	26.2	25.3	2.56	*0.784*
TCO_2, mmol/l	29.5	27.2	1.04	*0.054*
Anion Gap, mmo/l	12.5	16.1	1.01	*0.016*

[*]*Data are least square mean ± largest standard error (SE).*
[†]*BUN, blood urea nitrogen; Hct, hematocrit; Hb, hemoglobin; Cl^-, chloride ion; Na^+, sodium ion; K^+, Potassium ion;PCO_2, partial pressure of carbon dioxide; HCO_3, bicarbonate; TCO_2, total carbon dioxide.*

titers. Serum viral load was highest at 4 days post inoculation and remained elevated until day 9 post inoculation. Collectively, these results indicated that both models of ISS induced effective immune system stimulation. However, compared to LPS, PRRSv model elicits a more severe response with a more negative impact on performance of pigs.

*Table 2. Effect of immune system stimulation (**ISS**) induced by porcine reproductive and respiratory syndrome virus (**PRRSV**) on blood parameters*[*]

	ISS-	ISS+	SE	*P*
Blood chemistry				
BUN[†], mg/dl	11.8	11.0	0.73	*0.490*
Creatinine, mg/dl	0.68	0.86	0.05	*0.007*
BUN:Creatinine	17.8	12.9	1.23	*0.003*
Glucose, mg/dl	78.2	84.8	5.68	*0.382*
iCa, mmol/l				
Hematology				
Hct, % PCV	24.8	22.9	2.65	*0.592*
Hb, g/dl	12.1	8.1	0.95	*0.001*
Electrolytes				
Cl^-, mmol/l	102.9	102.0	1.44	*0.562*
Na^+, mmol/l	142.2	142.2	1.04	*0.965*
K^+, mmol/l	4.0	4.2	0.14	*0.321*
Acid/Base				
TCO_2, mmol/l	29.5	28.2	0.58	*0.084*
Anion Gap, mmo/l	13.0	16.6	1.11	*0.009*

[*]*Data are least square mean ± largest standard error (SE).*
[†]*BUN, blood urea nitrogen; Hct, hematocrit; Hb, hemoglobin; Cl^-, chloride ion; Na^+, sodium ion; K^+, Potassium ion; TCO_2, total carbon dioxide; iCa, ionized calcium.*

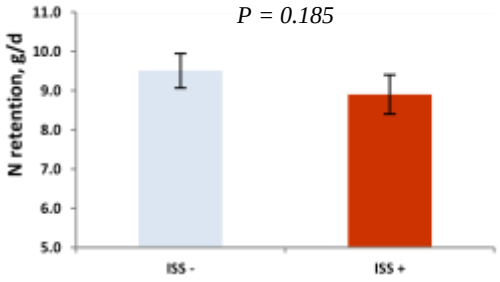
Impact of ISS on dietary N utilization

Relative to pre-challenge, LPS did not reduce daily feed intake (*P = 0.410*). However, PRRSV-challenged pigs consumed only 55 % of their daily feed allowance (*P < 0.012*). Ileal digestibility of dietary protein was decreased by LPS and PRRSV; 16 and 26 %, respectively, relative to 0.76 for control (i.e. pigs in preliminary study; n=10; Table 3 and 4). Reduced AID of dietary protein in post challenge period can be attributed to reduced feed intake, and ISS-induced increased gut endogenous protein and AA losses. However, the latter requires further investigation. N-retention was decreased in LPS and PRRSv-treated pigs (20 and 64 %, respectively, relative to 11 g/d for pre-challenge period; Table 3 and 4). Reduced N-retention during the post-ISS period can in part be attributed to the reduced feed intake. However, when N-retention values were corrected for daily N intake, the N-retention was still lower in post-ISS compared to pre-ISS period (6.4 and 37 % in LPS and PRRSV-treated pigs, respectively; Table 3 and 4). The latter can mainly be associated with increased protein degradation and reduced protein synthesis in skeletal muscle. Systemic inflammation in pigs is usually characterized by reduced N retention and efficiency of AA utilization for whole-body N retention.

*Table 3. Effect of immune system stimulation (**ISS**) induced by E. coli lipopolysaccharide (**LPS**) on nitrogen (**N**) retention, ileal digestibility, and organ weight**

	ISS-	ISS+	SE	*P*
N utilization, g/d				
N intake	18.3	15.2	1.82	*0.107*
N excretion	8.4	7.2	0.52	*0.064*
N balance	11.0	8.7	0.95	*0.035*
Organ weight, g				
Liver	508	560	33.0	*0.407*
Spleen	127	77.2	7.75	*0.001*
Ileal N digestibility, %	76	64	3.73	*0.001*

Data are least square mean ± largest standard error (SE).

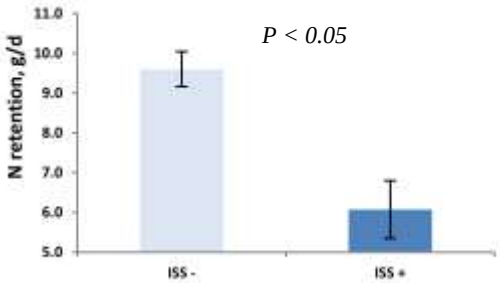


Nitrogen retention corrected for N intake

*Table 4. Effect of immune system stimulation (**ISS**) induced by porcine reproductive and respiratory syndrome virus (**PRRSV**) on nitrogen (**N**) retention, ileal digestibility, and organ weight**

	ISS-	ISS+	SE	*P*
N utilization, g/d				
N intake	18.3	10.0	1.78	*0.001*
N excretion	8.3	6.6	0.53	*0.005*
N balance	10.9	3.9	1.52	*0.001*
Organ weight, g				
Liver	508	557	33.1	*0.402*
Spleen	127	78.3	7.75	*0.001*
Ileal N digestibility, %	76	56	3.44	*0.001*

Data are least square mean ± largest standard error (SE).



Nitrogen retention corrected for N intake

Impact of ISS on amino acid utilization

The ultimate goal of the present study was to quantify the effect of ISS on AA utilization in growing pigs. Changes in plasma free AA kinetics (i.e. AA flux or rate of disappearance) reflect modification of AA utilization in different metabolic states. In the current study, isotope tracer technique was utilized to determine the rate of AA utilization in ISS pigs.

The rate of disappearance of an AA reflects the amount of free AA that out fluxes per unit of time from the blood plasma pool. The rate of disappearance includes the use of AA for protein synthesis and catabolism, and does not distinguish between the two fluxes. The rate of disappearance of AA in plasma in combination with the concentration of AA is, however, more useful for quantifying changes in AA metabolism than merely plasma AA concentrations. Changes in AA metabolism, e.g. an increased protein synthesis rate, can occur without concomitant changes in plasma AA concentrations, as AA concentrations can be maintained when fluxes from protein intake, breakdown and synthesis of body protein, and catabolism of AA are changing. Yet, changes in plasma AA concentrations have been used previously as a measure to evaluate the effects of ISS on AA metabolism. In the present study, the lower rate of disappearance for Lys in LPS challenged pigs was not associated with a change plasma concentration of Lys (Figure 1 and 2). Therefore, the use of AA plasma concentrations as a single measure to quantify the effects of ISS on AA metabolism can be misleading.

The double exponential model accurately describes the decrease in the ^{13}C- and ^{15}N- enrichment of individual plasma AA after the injection of the bolus with [U-^{13}C, U-^{15}N]-labelled AA. The average root mean square prediction error of the studied AA ranged between 4.8 and 6.9%, with 0.95% of the prediction error attributable to random variation.

Effect of LPS model of ISS on AA utilization: LPS-induced ISS reduced the rate of disappearance of Lys and Phe, without affecting their plasma concentrations, suggesting a decrease in Lys and Phe utilization. Rates of disappearance of Met were numerically higher in ISS+ (LPS challenged) than ISS- pigs but it did not reach statistical significance (Figure 1). Although lower numerically, rates of disappearance of other AA were not affected by ISS (Figure 1). Reduced plasma AA flux most probably can be associated with reduced utilization of AA for protein synthesis in skeletal muscles.

Effect of PRRSV model of ISS on AA utilization: The PRRSV challenge increased the rate of disappearance (µmol/kg BW/h) for Met (from 108 to 228, SE 26.7), and Thr (from 83 to 129, SE 11.5; Figure 4). PRRSV significantly increased the plasma Lys concentrations, while it had no effect on Lys rate of disappearance, suggesting a substantial decrease in Lys utilization in ISS pigs (Figure 3 and 4). The plasma flux of other AA was not affected by PRRSV (Figure 3). These results suggest that PRRSV infection reduces N-retention and alters metabolism of Met, and Thr growing pigs. The increased Met, Thr, and Phe flux in PRRSV challenged pigs could be associated with enhanced utilization of Met, and Thr for synthesis of immune system metabolites and/or increased catabolism of these AA. This may increase dietary Met, Thr, and Phe requirements of health-challenged pigs, relative to requirements for other AA (e.g. Lys). Further study is required to quantify the impact of PRRSV on dietary requirements for Met, Thr, Phe, and Lys. This will be addressed in the second phase of the current project.

Taken together, results of the current project suggest that LPS and PRRSV model of ISS effectively alter the measures of immune function and metabolism. However, it seems that the effect on AA utilization is either disease specific, or is a function of disease severity.

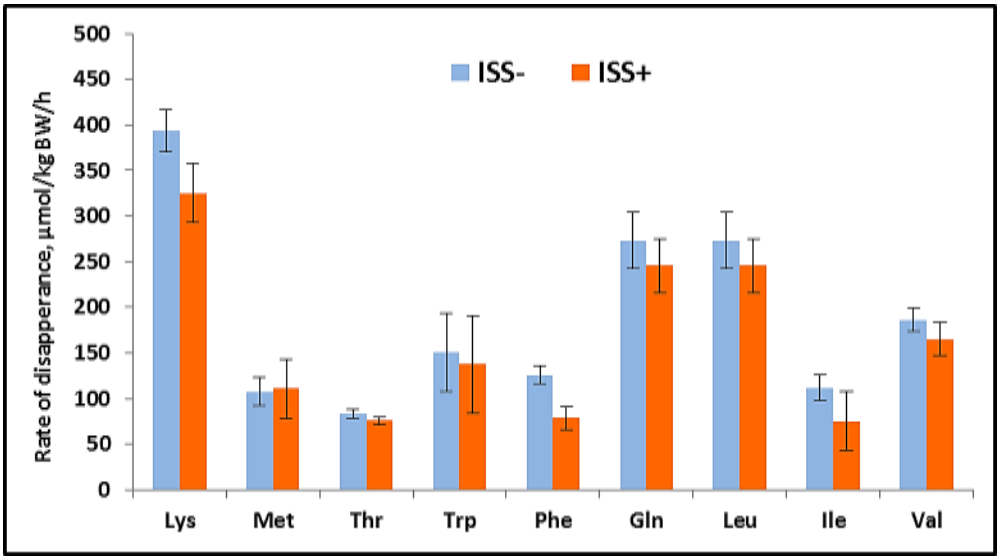


Figure1. Impact of immune system stimulation (ISS) induced by E. coli lipopolysaccharide (LPS) on rate of amino acid (AA) disappearance from plasma (i.e. AA flux). Data are least square mean ± standard error (SE) and corrected for AA intake. ISS was induced by repeated injection of increasing amounts of LPS. Plasma AA flux: the sum of disappearance of AA from the plasma pool towards polypeptide (e.g. protein) synthesis and catabolism.

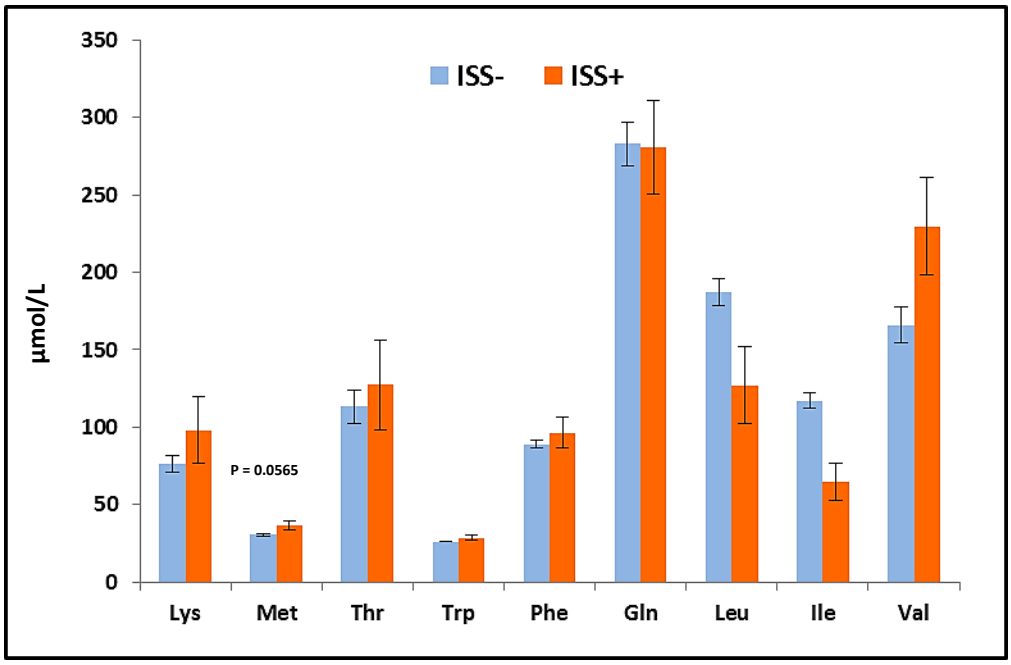


Figure 2. Impact of immune system stimulation (ISS) induced by E. coli lipopolysaccharide (LPS) on plasma amino acid (AA) concentrations. Data are least square mean ± standard error (SE) and corrected for AA intake. ISS was induced by repeated injection of increasing amounts of LPS.

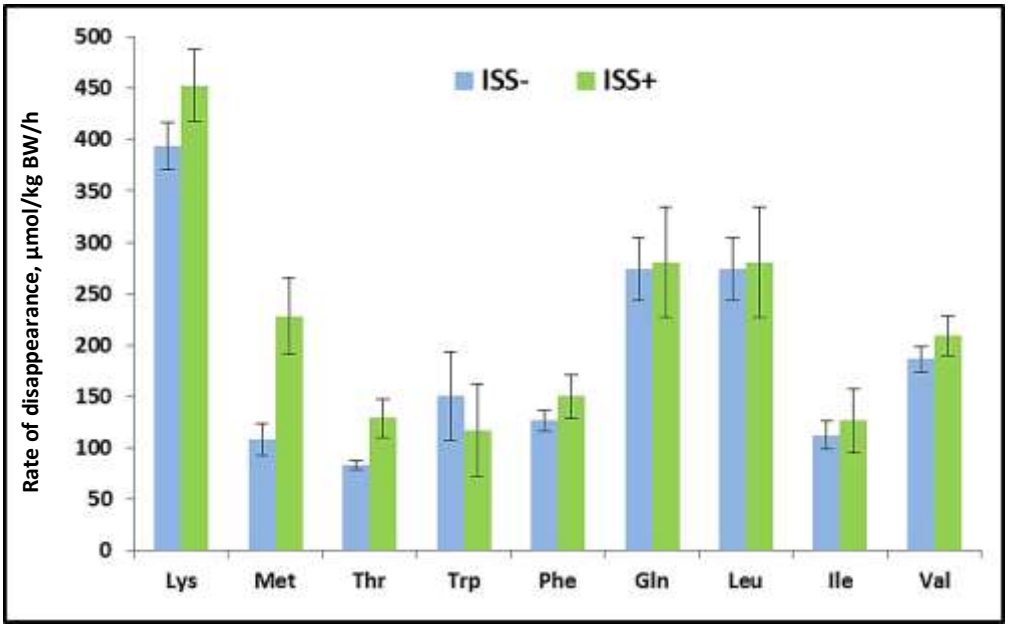


Figure 3. Impact of immune system stimulation (ISS) induced by porcine reproductive and respiratory syndrome virus (PRRSV) on rate of amino acid (AA) disappearance from plasma (i.e. AA flux). Data are least square mean ± standard error (SE) and corrected for AA intake. ISS was induced by intramuscular injection of live field PRRSV. Plasma AA flux: the sum of disappearance of AA from the plasma pool towards polypeptide (e.g. protein) synthesis and catabolism.

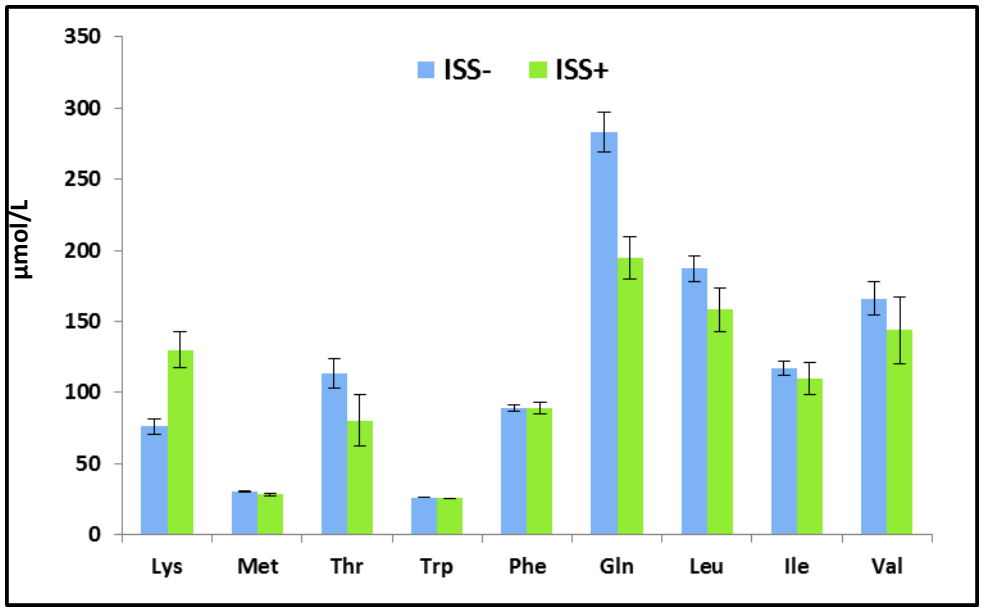


Figure 4. Impact of immune system stimulation (ISS) induced by porcine reproductive and respiratory syndrome virus (PRRSV) on plasma amino acid (AA) concentrations. Data are least square mean ± standard error (SE) and corrected for AA intake. ISS was induced by intramuscular injection of live field PRRSV.

Project was completed within the requested time frame. Three abstracts from the current project have been published in proceedings of two major international conferences and the fourth abstract has been submitted to be published in proceedings of The Obesity Society Annual Meeting (2015).
Manuscripts are under preparation for submission to prestigious (*J Nutr, and Brit J Nutr*) journals. In addition, one master student (Ms. Whitney D. Stuart), one scholar (Mr. Abbasali Gheisari) and one undergraduate student (Ms. Hailey Wooten) were trained using the current project.

W. Stuart, T.E. Burkey, N.K. Gabler, K. Schwartz, C.F.M. de Lange, and A. Rakhshandeh. (2015). Models of immune system stimulation in growing pigs: porcine reproductive and respiratory syndrome (PRRS) versus E.coli lipopolysaccharide (LPS). Proceedings of Midwest ASAS meeting (abstract # 11411)

Hailey Wooten, J.J. Mcglone, W. Stuart1, T.E. Burkey, N.K. Gabler, K. Schwartz, C.F.M. de Lange, and A. Rakhshandeh. (2015). Impact of porcine respiratory and reproductive virus on behavior and welfare of growing pigs. Proceedings of Midwest ASAS meeting (abstract # 432)

W. Stuart, T.E. Burkey, N.K. Gabler, K. Schwartz, T. Dinh, C.F.M. de Lange, D. Klein, J. A. Dawson, and A. Rakhshandeh. Infection with porcine reproductive and respiratory syndrome virus (PRRSV) affects body protein deposition and alters amino acid metabolism in growing pigs. (2015). Proceedings of 2015 ADSA-ASAS Joint Annual Meeting (abstract # 64649)

J. A. Dawson, W. Stuart, T.E. Burkey, N.K. Gabler, K. Schwartz, C.F.M. de Lange, and A. Rakhshandeh. Modeling plasma amino acid kinetics in growing pig. Proceedings of 2015the Obesity Society Annual Meeting (submitted).

Creative activities: An analytical technique was developed and validated to measure 20 isotopically-labeled amino acids in biological fluids simultaneously. This technique was developed in collaboration with Institute of Environmental and Human Health at Texas Tech University. Procedures will be disseminated in the form of a Technical Note in a refereed journal in 2015.