IMPACT OF A NEAR IDEAL AMINO ACID PROFILE ON THE EFFICIENCY OF NITROGEN AND ENERGY UTILIZATION IN LACTATING SOWS

By

Sai Zhang

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Animal Science–Doctor of Philosophy

2019

ABSTRACT

IMPACT OF A NEAR IDEAL AMINO ACID PROFILE ON THE EFFICIENCY OF NITROGEN AND ENERGY UTILIZATION IN LACTATING SOWS

By

Sai Zhang

Improving dietary amino acid (AA) and energy efficiency in lactating sows is a potential nutritional approach to mitigate impacts of swine production on the environment. In addition, greater metabolic rate during lactation renders sows prone to heat stress (HS), therefore strategies to lessen metabolic heat production will improve sow welfare in particular given the foreseeable increase in global warming. The main hypothesis of this dissertation was that feeding a reduced protein diet with near ideal AA profile (NIAA) and a leucine:lysine of 1.14 improves the dietary essential AA (EAA) and energy utilization efficiency for lactation, and reduces the metabolic heat associated with lactation, compared to feeding diets containing leucine:lysine of 1.63. To test the hypothesis, three diets were formulated iso-calorically (2,580 kcal/kg net energy), including 1) control diet with a 1.63 leucine:lysine (CON; 18.75% CP), 2) reduced CP diet with 1.14 leucine:lysine referred to as optimal (OPT; 13.75% CP) and formulated to contain a NIAA by supplementation with the limiting AA in their crystalline form to meet their minimum requirements (i.e., L-Lysine (Lys), L-Valine (Val), L-Threonine (Thr), L-Phenylalanine (Phe), DL-Methionine (Met), L-Isoleucine (Ile), L-Histidine (His), and L-Tryptophan (Trp); and 3) OPT diet with L-Leucine (Leu) supplementation to achieve CON Leu:Lys of 1.63 (OPTLEU; 14.25% CP). The overall objective was to determine the efficiency of individual EAA and energy for lactation in sows fed CON, OPT and OPTLEU, and quantify the metabolic heat production of lactating sows fed CON and OPT. Three studies were conducted to address the following aims: 1) to estimate maximal biological efficiency value (MBEV) of EAA in lactating sows fed CON, OPT and

OPTLEU diets; 2) to estimate dietary energetic efficiency, energy partitioning and heat production in lactating sows fed CON, OPT and OPTLEU diets; and 3) to measure heat production in lactating sows fed CON and OPT diets and exposed to thermal neutral and HS environments. The first study showed that feeding OPT diet improved utilization efficiency of nitrogen (N) (79.1%), arginine (61.1%), His (78.3%), Ile (65.4%), Leu (75.1%), Met + Cys (78.2%), Phe (53.4%), Phe + Tyr (69.5%) and Trp (70.1%) and maximized the efficiency of Lys (63.2%), Met (67.9%), Thr (71.0%) and Val (57.0%) for milk production over a 21-day lactation period. Leucine reduced Met utilization but did not affect that of N and other EAA. The second experiment demonstrated that feeding OPT led to greater energy utilization for lactation due to less urinary energy and metabolic heat loss, and triggered dietary energy deposition into milk at the expense of maternal lipid mobilization. A Leu:Lys of 1.63 compared to 1.14 reduced dietary energy utilization for lactation by directing dietary energy away from the mammary gland and towards maternal pool, in part explaining the efficacy of a NIAA diet over CON. Sows fed OPT diet produced less metabolic heat and had lower body temperature when exposed to HS conditions compared to CON fed sows. In conclusion, feeding a diet with NIAA profile containing Leu:Lys of 1.14 improves dietary EAA and energy utilization efficiency for lactation, and reduces the metabolic heat associated with lactation compared to feeding a diet with Leu:Lys of 1.63 and meeting SID Lys requirement with feed ingredients as the sole source of Lys. This improvement is in part due to a lower dietary Leu:Lys. Feeding lactating sows with reduced CP diets with crystalline AA supplementation to attain NIAA profile is a feasible strategy to improve efficiency of N and energy utilization, and to mitigate the impacts of HS on lactating sows and of swine production on the environment.

ACKNOWLEDGEMENTS

I would like to express my greatest appreciation to my advisor, Dr. Nathalie L. Trottier to have given me the opportunity of pursuing my Ph.D. degree and begin a new chapter of my life in the United States. Dr. Trottier has been always supportive of my career development and offered me abundant academic guidance. Thanks to the selfless support from Dr. Trottier, I traveled and visited (for conferences, research and training programs) more than 15 states across North America, as well as 2 provinces of Canada, from which I furthered my life experience and friendships. I would also like to thank the other members of my dissertation committee, Dr. Juan C. Marini, Dr. Adam Moeser, Dr. Dale Rozeboom and Dr. Mike VandeHaar, for their counsel throughout the past 4 years. In addition, I would like to extend my gratitude and deep appreciation to Dr. Jay S. Johnson and Dr. Juan C. Marini, for their support and critical contributions to my Ph.D. program, training and research.

Starting a new life in a foreign country without acquaintance was very challenging, but fortunately I developed friendship here that I will treasure forever. Before I came to the United States, I had not realized how deeply difficult life would be without friends. I would like to extend my thanks in particular to Mu Qiao who was so supportive and helpful during my second year at Michigan State University, and to Yanjin Zhu at Purdue University while conducting part of my research under the guidance of our collaborator Dr. Johnson during the third year of my program. It would have been impossible to complete the intense experiments without their generous support. Also, there are countless undergraduate students who have been there to help me throughout my work at Michigan State University, i.e., Katherine Koebel, Olivia Stubbins, Jacquelyn Babcock, Mina Deboer, Lea Deming, Kaylee Mullennix, Kali Young, Michaela Conrad, and Hanna Martinez, and staffs and students at Purdue University/USDA-ARS, i.e., Jacob Maskal, Torey

Raber, Katelin Ade, Patricia Jaynes, Samantha and Jordan. At last but not least, I would like to thank the farm staff, i.e., Kevin Turner and Christopher Rozeboom, the MSU veterinarians Dr. Vengai Mavangira and Andrew Claude for their help in developing a dual ear vein catheterization technique, the lab staffs, i.e., Dave Main, Jim Liesman, Julie Moore and Jane Link, and the office staffs, in particular Debbie Roman and Barbara Sweeney. I would also like to acknowledge the incredible support I received from the graduate program coordinators, Dr. Steve Bursian and Dr. Cathy Ernst.

Finally, I would like to thank all my family members especially my amazing parents, Jianlong Zhang and Ping Sheng who live in China and offered me forever understanding and support for my personal life and choice, studying abroad and constant encouragement whenever I felt depressed. I would not have been able to accomplish my goals without your support!

TABLE OF CONTENTS

LIST OF TABLES ix

LIST OF FIGURES xiii

KEY TO ABBREVIATIONS........ xv

INTRODUCTION 1

CHAPTER 1 LITERATURE REVIEW 4
- Summary of the Current Challenge 4
  - Contribution of Lactating Sows to Nitrogen Excretion 4
  - Effect of Heat Stress on Lactating Sow Performance and Welfare 6
- Effect of Improving Amino Acid Balance on Nitrogen Utilization 7
- Amino Acid Utilization Efficiency for Lactation 9
  - Definition of Utilization Efficiency Value for Amino Acids 9
  - Interaction Between Amino Acid Utilization Efficiency........ 12
- Energy Utilization Efficiency for Lactation 14
  - Theoretical Estimation of Heat Production Arising from Amino Acid Oxidation and Ammonia Excretion 15

CHAPTER 2 FEEDING A REDUCED PROTEIN DIET WITH A NEAR IDEAL AMINO ACID PROFILE IMPROVES AMINO ACID EFFICIENCY AND NITROGEN UTILIZATION FOR MILK PRODUCTION IN SOWS 30
- ABSTRACT........ 30
- INTRODUCTION 31
- MATERIALS AND METHODS........ 32
  - Animals, Feeding and Experimental Design 32
  - Dietary Treatment 33
  - Nitrogen Balance 34
  - Milk Sampling 35
  - Nutrient and Titanium Analyses 36
  - Calculations........ 36
  - Statistical Analysis........ 39
- RESULTS 40

    - Dietary Amino Acid Analyses 40
    - Performance 41
    - Nitrogen Balance 42
    - True Nitrogen and Essential Amino Acid Efficiencies for Milk N and EAA Deposition.... 42
  - DISCUSSION 43
  - CONCLUSION 49

CHAPTER 3 REDUCED PROTEIN DIET WITH NEAR IDEAL AMINO ACID PROFILE IMPROVES ENERGY EFFICIENCY AND MITIGATE HEAT PRODUCTION ASSOCIATED WITH LACTATION IN SOWS 61

  - ABSTRACT 61
  - INTRODUCTION 62
  - MATERIALS AND METHODS 64
    - Animals and Feeding 64
    - Dietary Treatment 64
    - Energy Balance Procedure and Milk Sampling 66
    - Energy, Nutrient and Titanium Analysis 66
    - Calculations 66
    - Statistical Analysis 70
  - RESULTS 70
    - Experimental Diets 70
    - Body Protein and Lipid Mobilization 71
    - Energy Balance 71
    - Apparent Efficiency of Nitrogen and Energy 71
    - Dietary Energy Partitioning 72
    - Energy Efficiency and Estimated Heat Production Associated with Lactation 72
  - DISCUSSION 73
  - CONCLUSION 78

CHAPTER 4 EFFECT OF DIETARY NEAR IDEAL AMINO ACID PROFILE ON HEAT PRODUCTION IN LACTATING SOWS EXPOSED TO THERMAL NEUTRAL AND HEAT STRESS 92

  - ABSTRACT 92
  - INTRODUCTION 93
  - MATERIALS AND METHODS 94
    - Animals, Feeding and Experimental Design 94

    Dietary Treatment 95
    Environmental Control and Physiological Monitoring 96
    Indirect Calorimetry 97
    Nutrient Analysis for Diet and Milk 98
    Calculations 98
    Statistical Analysis 99
  RESULTS 102
    Experimental Diets 102
    Performance 102
    Body Lipid and Protein Mobilization 103
    Milk Yield and Composition 103
    Physiological Response to Ambient Temperature 104
    Heat Production 105
  DISCUSSION 106
  CONCLUSION 111

CHAPTER 5 SUMMARY AND CONCLUSIONS 126

APPENDICES 150
  APPENDIX A...... 131
  APPENDIX B........133
  APPENDIX C........150

LITERATURE CITED 169

LIST OF TABLES

Table 1.1. Performance of lactating sows fed diets reduced in crude protein (CP) concentration with supplemental crystalline amino acids over 21-d lactation 20

Table 1.2. Theoretical calculation of heat associated with dietary crude protein fed in excess ... 21

Table 2.1. Ingredient composition and nutrient content of experimental diets (as-fed) 51

Table 2.2.Analyzed and calculated concentration of nitrogen (N), total and free essential amino acids (EAA) in experimental diets (as-fed) 53

Table 2.3. Lactation performance of all sows fed Control (CON; 18.74 % CP), Optimal (OPT; 13.78% CP) or Optimal + Leucine (OPTLEU; 14.25% CP) over a 21-d lactation period........... 54

Table 2.4. Performance and milk nutrient composition and yield in early and peak lactation periods of sows selected for the N balance studies and fed Control (CON; 18.74 % CP), Optimal (OPT; 13.78% CP) or Optimal + Leucine (OPTLEU; 14.25% CP) diets 55

Table 2.5. Nitrogen utilization for milk in early and peak lactation periods in sows selected for the N balance studies and fed Control (CON; 18.74 % CP), Optimal (OPT; 13.78% CP) or Optimal + Leucine (OPTLEU; 14.25% CP) diets 57

Table 2.6. True dietary AA utilization efficiency estimated based on maternal N retention for milk protein production of sows fed Control (CON; 18.74 % CP), Optimal (OPT; 13.78% CP) or Optimal + Leucine (OPTLEU; 14.25% CP) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation).. 59

Table 3.1. Sow and litter growth performance of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets over a 21-d lactation period ... 80

Table 3.2. Energy balance of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation) 81

Table 3.3. Apparent utilization efficiency of nitrogen and energy of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation) 83

Table 3.4. Dietary energy partitioning of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation) 84

Table 3.5. The relative values between dietary gross energy (GE), digestible energy (DE), metabolizable energy (ME), and net energy (NE) of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation) 86

Table 3.6. True energy efficiency and heat production associated with milk production of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation) 88

Table 4.1. Ingredient composition and nutrient content of high crude protein (HCP) and low crude protein (LCP) diets (as-fed) 113

Table 4.2. Analyzed and calculated concentration of nitrogen (N), total and free essential amino acids in high crude protein (HCP) and low crude protein (LCP) diets (as-fed) 115

Table 4.3. Performance of litter and sow fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 116

Table 4.4. Body composition of sow fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 118

Table 4.5. Milk yield and composition of sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions........................... 120

Table 4.6. Physiological response of sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral (TN) and heat stress (HS) conditions 121

Table 4.7. Feed intake and metabolic total heat production (kcal$\cdot$d$^{-1}\cdot$BW$^{-0.75}$) of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions.. 122

Table 4.8. Metabolic total heat production (kcal$\cdot$d$^{-1}\cdot$BW$^{-0.75}$) during daytime of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 124

Table A1. Water balance in sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between day 4 and 8 of lactation (early lactation) and between day 14 and 18 of lactation (peak lactation) 132

Table B1. Metabolic oxygen (O_2) consumption ($L·d^{-1}·BW^{-0.75}$) of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 135

Table B2. Metabolic carbon dioxide (CO_2) production ($L·d^{-1}·BW^{-0.75}$) of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 136

Table B3. Respiratory quotient (RQ) of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 137

Table B4. Metabolic oxygen (O_2) consumption during daytime of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 138

Table B5. Metabolic carbon dioxide (CO_2) production ($L·d^{-1}·BW^{-0.75}$) during daytime of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 139

Table B6. Respiratory quotient (RQ) during daytime of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 140

Table B7. Metabolic total heat production ($kcal·d^{-1}·BW^{-0.75}$) of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 141

Table B8. Metabolic oxygen (O_2) consumption ($L·d^{-1}·BW^{-0.75}$) of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 142

Table B9. Metabolic carbon dioxide (CO_2) production ($L·d^{-1}·BW^{-0.75}$) of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 143

Table B10. Respiratory quotient (RQ) of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions ... 144

Table B11. Metabolic total heat production (kcal·d^{-1}·BW$^{-0.75}$) during daytime of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 145

Table B12. Metabolic oxygen (O_2) consumption during daytime of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 146

Table B13. Metabolic carbon dioxide (CO_2) production (L·d^{-1}·BW$^{-0.75}$) during daytime of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 147

Table B14. Respiratory quotient (RQ) during daytime of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 148

Table B15. Metabolic carbon dioxide (CO_2) production, oxygen (O_2) consumption, total heat production (THP) and respiratory quotient (RQ) of piglets from sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions 149

Table C1. Analyzed and calculated concentration of nitrogen (N), total and free essential amino acids in control (CON) and optimal (OPT) diets (as-fed) 160

Table C2. Lysine balance (g/d) of sows fed Control (CON; 18.4% CP) and Optimal (OPT; 14.0% CP) diets during peak lactation (day 14 to day 18) 161

Table C3. Body protein synthesis and breakdown of sows fed Control (CON; 18.4% CP) and Optimal (OPT; 14.0% CP) diets during peak lactation (day 14 to day 18) 162

LIST OF FIGURES

Figure 1.1. Urinary nitrogen excretion (g/d) from sows fed different dietary crude protein (CP) over 21-d lactation. Adapted from Chamberlin (2015a) and Huber et al. (2015). 22

Figure 1.2. Air ammonia production (g/d) in individual lactating sows and their litters. Sows were fed diets containing 17.55 (High) and 12.98% CP (Low) and housed under either a thermal neutral (TN) or heat stress (HS) environment. From Chamberlin et al. (2015b)......................... 23

Figure 1.3. Milk urea concentration (MUN conc., mg/kg) in sows fed different levels of dietary CP in early (d 4-8) and peak (d 14-18) lactation. Upper panel: control (CON, 17.55% CP), medium low crude protein (MCP, 15.25% CP) and low crude protein (LCP, 12.98% CP) (Adapted from Chamberlin, 2015a). Lower panel: high crude protein (HCP, 16.03% CP), medium high crude protein (MHCP, 15.70% CP), medium low crude protein (MLCP, 14.29% CP), low crude protein (LCP, 13.22% CP) (Adapted from Huber et al., 2015). 24

Figure 1.4. Milk urea nitrogen concentration (MUN conc., mg/kg) from sows exposed to thermo-neutral temperature (TN) and heat stress (HS) and fed a control diet (CON, 17.55% CP) or a low protein diet (OPT, 12.98% CP) during lactation. Adapted from Chamberlin et al. (2015b)....... 25

Figure 1.5. Plasma urea N concentration (PUN conc., µmol/L) of lactating sows fed control (CON, 17.55% CP), medium low crude protein (MCP, 15.25% CP) and low crude protein (LCP, 12.98% CP). Adapted from Chamberlin et al. (2015a)... 26

Figure 1.6. Relationship between estimated lysine in milk derived from SID lysine intake and estimated SID lysine intake for milk. The relationship is represented by the line and described as $y=0.6698x$ at zero intercept with r^2 of 0.925, where the slope of 0.6698 represents the efficiency of dietary lysine utilization into milk lysine (NRC, 2012). 27

Figure 1.7. Energy partitioning by pigs (Ewan et al., 2001).. 28

Figure 1.8. Partitioning of energy released from substrate oxidation and ATP synthesis and from energy utilization and ATP hydrolysis. Efficiency of ATP synthesis and hydrolysis are 50 and 67%, respectively, with the 50 and 33% of the energy lost as heat, respectively........................ 29

Figure 3.1. Dietary gross energy (GE) partitioning through digestible energy (DE), metabolizable energy (ME), heat increment (HI) towards lactation net energy ($NE_{lactation}$). 90

Figure 3.2.The partitioning of total heat production of sows fed control (CON), optimal (OPT) and optimal + leucine (OPTLEU) over a 21-day lactation period. Total heat production did not differ between diets. 91

Figure 4.1. Vaginal temperature of sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral (TN) and heat stress (HS) environments 125

Figure B1. Body protein and lipid tissue mobilization of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions. 134

Figure C1. Changes in plasma isotopic enrichment of Lys during peak lactation (between day 15 and 21) for sows fed Control (CON; 18.4% CP; n = 3) and Optimal (OPT; 14.0% CP; n = 5) diets. Plasma isotopic enrichment of [1-^{13}C]lysine differed between diets (*P* < 0.001) and time points (*P* < 0.01) with no interaction between diet and time (*P* = 0.477). Standard error of the mean, SEM = 0.53. 163

Figure C2. Milk isotopic enrichment of [1-^{13}C]lysine during peak lactation (between day 15 and 21) for sows fed Control (CON; 18.4% CP; n = 3) and Optimal (OPT; 14.0% CP; n = 5) diets. Milk isotopic enrichment of [1-^{13}C]lysine tended to differ between diets (*P* = 0.061) and did not differ between time points (*P* = 0.827), with no interaction between diet and time (*P* = 0.979). Standard error of the mean, SEM = 0.24. 164

Figure C3. Changes in plasma isotopic enrichment of 3-[methyl-$^{2}H_3$]histidine during peak lactation (day 15 to day 21) for sows fed Control (CON; 18.4% CP; n = 4) and Optimal (OPT; 14.0% CP; n = 4) diets. Plasma isotopic enrichment of 3-[methyl-$^{2}H_3$]histidine differed between diets (*P* < 0.001) and time points (*P* < 0.001), with no interaction between diet and time (*P* = 0.547). Standard error of the mean, SEM = 0.645. 165

Figure C4. Diagram of Lys balance of lactating sows at fed state 166

Figure C5. Timeline of isotope infusion and sampling........ 167

Figure C6. Two-pool model to estimate lysine oxidation........ 168

KEY TO ABBREVIATIONS

AA	amino acid
ADFI	average daily feed intake
ADG	average daily gain
Arg	arginine
BL	body lipid
BP	body protein
BW	body weight
$BW^{0.75}$	metabolic body weight
Ca	calcium
CON	control treatment
CP	crude protein
Cys	cysteine
d	day (s)
DE	digestible energy
DM	dry matter
h	hour (s)
HCP	high crude protein
His	histidine
HS	heat stress
Ile	isoleucine
kcal	kilocalories
LCP	low crude protein

Leu	leucine
LGR	litter growth rate
Lys	lysine
MBEV	maximal biological efficiency value
ME	metabolizable energy
Met	methionine
MUN	milk urea nitrogen
N	nitrogen
NE	net energy
NIAA	near ideal amino acid profile
OPT	optimal treatment
OPTLEU	optimal + leucine treatment
P	phosphorus
PFTN	pair fed thermal neutral
RR	respiration rate
SID	standardized ileal digestibility
STTD	stardardized total tract digestibility
THP	total heat production
Thr	threonine
TN	thermal neutral
Trp	tryptophan
Tyr	tyrosine
Val	valine

INTRODUCTION

A recent goal set by many swine producers in North America has been to attain a benchmark of 30 piglets weaned per sow per year (Gillespie, 2016). Thus lactation demand on sows is continually increasing in order to maintain piglet quality at weaning. These challenges are compounded by increasing environmental regulations to decrease carbon and ammonia emissions, and rising environmental temperatures.

Research in particular on growing-finishing pigs (Kerr et al., 2003; Otto et al., 2003a; Otto et al., 2003b; Madrid et al., 2013; Li et al., 2015) and a few in lactating sows (Manjarín et al., 2012; Huber et al., 2015; Chamberlin, 2017) has been conducted in recent years to improve the efficiency of nitrogen (N) utilization and mitigate N losses and ammonia emissions to the environment. These efforts have led to the development of diets with improved dietary amino acid (AA) balance. Such diets are formulated by lowering crude protein (CP) and meeting the minimum requirement of the limiting AA through supplementation of AA in their crystalline form. While the global efficiency of N is improved, knowledge of maximum biological efficiency values (MBEV) for individual essential AA (EAA) utilization into milk protein are needed for future model prediction of dietary EAA requirements. The NRC (2012) estimated a MBEV for Lys, and derived the dietary Lys requirement for lactating sows to maximize growth of the nursing pig using a factorial approach. This approach however remains limited due to lack of valid MBEV for the other EAA.

In growing-finishing pigs, lowering dietary CP improves energetic efficiency due to reduced urinary energy loss (Le Bellego et al., 2001) and heat loss (Le Bellego et al., 2001; Kerr et al., 2003). Thus improvement in energy utilization efficiency may due to reduced metabolic demand resulting from less AA destined to oxidation. In addition, based on previous work (Guan et al., 2002 and 2004; Manjarín et al., 2012), it appears that the relatively high Leu:Lys found in corn and soybean meal-based, non-reduced CP diets, may contribute to the relatively low efficiency of Lys utilization. Abatement of heat production through dietary manipulation may alleviate the impact of HS in lactating sows, which is of increasing concern given the rise in global warming and frequent heat waves throughout the summer season in the US.

Continued research on the impact of feeding diets with improved AA balance on sow performance, efficiency of EAA and energy utilization, and on metabolic heat production is needed to help in the sustainability of the swine industry. The overarching hypothesis of this dissertation was that feeding a reduced CP diet with near ideal amino acid profile (NIAA) and Leu:Lys of 1.14 improves the dietary EAA and energy utilization efficiency, and reduces metabolic heat associated with lactation in sows compared to feeding a non-reduced CP diet formulated to meet SID Lys with feed ingredients as the sole source of Lys. To test the hypothesis, three diets were designed: 1) a non-reduced CP diet with 18.75% CP and Leu:Lys of 1.63 (control or CON), 2) a reduced CP diet with 13.75% CP and Leu:Lys of 1.14 with a NIAA profile (optimal or OPT), and 3) a reduced CP with 13.75% CP with added Leu to achieve a Leu:Lys of 1.63 (optimal+Leu or OPTLEU). The OPTLEU was used to assess whether Leu plays a role in impacting Lys efficiency. Three specific aims were addressed and form the basis of the experiments presented in Chapters 2, 3 and 4. Chapter 2 addresses the first aim, i.e., to estimate efficiency value of EAA in lactating sows fed CON, OPT and OPTLEU diets. Chapter 3 addresses the second aim, i.e., to estimate dietary energetic efficiency, energy partitioning and heat production in lactating sows fed CON, OPT and OPTLEU diets. Chapter 4 addresses the third aim, i.e., to measure heat production in lactating sows fed CON and OPT diets and exposed to TN and HS environments. These chapters are preceded by a literature review presented in Chapter 1, integrating the updated knowledge of AA and energy metabolism, and utilization efficiency for lactating sows. The last chapter, Chapter 5, contains a summary of results and an overall conclusion.

CHAPTER 1

LITERATURE REVIEW

Summary of the Current Challenge

Lactation is nutrient and energy costly, and thus sows must rely on adequate consumption of feed to maximize milk production. Lactating sows commonly mobilize body lipid and protein (van den Brand et al., 2000) since voluntary feed intake is often limited (Eissen et al., 2000). Over the past decades, larger litter size at birth due to genetic selection have increased lactation demands (Strathe et al., 2016; Zhang et al., 2016). Achieving 30 piglets per sow per year has been set as a target in North America (Gillespie, 2016). Thus lactation demand on sows is continually increasing in order to maintain piglet quality at weaning. These challenges are compounded by increasing environmental regulations to decrease carbon and ammonia emissions, and rising environmental temperatures which impact sow welfare and performance.

Contribution of Lactating Sows to Nitrogen Excretion

Increasing environmental regulations have merged worldwide in the past decade to decrease carbon and ammonia emissions from the swine industry (Sommer et al., 2013). The emission of greenhouse gases, typified by carbon dioxide and methane from livestock production including the swine industry, is of massive concern to the environment and global warming (Philippe and Nicks, 2015). The carbon from undigested dietary proteins and carbohydrates serves as a major contributor of methane (Velthof et al., 2005), which can be mitigated by improving nutrient digestibility. Reduction of carbon dioxide emissions may be achieved by improvement of dietary caloric efficiency (Philippe and Nicks, 2015). In addition, wasted N via excretion is of significant environmental concern, with ammonia and urea the major forms of wasted N from livestock operations. Dietary proteins are not stored for body energy reserves, and the AA arising from their digestion are destined to deamination and oxidation if protein synthesis is limited, leading to N losses to the environment. The process of deamination generally occurs (Lewis, 2001; NRC 2012) when 1) excess amounts of protein have been ingested, or 2) insufficient energy from dietary lipids and carbohydrates are available to support bodily processes; or 3) dietary protein is deficient in one or more EAA, or there is a poor AA balance. Compared to carbohydrates and lipids, oxidation of AA is an inefficient biological process for supply of energy (Berg et al. 2015). Therefore minimizing AA oxidation may improve isocaloric efficiency.

Urea is hydrolyzed to ammonium upon contact with bacterial urease from fecal matter during manure storage (Le et al., 2005). Ammonium is oxidized to ammonia in the presence of low pH and high temperature, which poses health risk to animals and humans (Mackie et al., 1998; Schinasi et al., 2011). Ammonia also results in atmospheric ammonium sulfate, forming acid rain and acidifying the surface soil (Rideout et al., 2004). The breeding herd in the United States contributes to 11.8×10^6 metric tons of fresh manure annually in the United States (Koelsch et al., 2005). One lactating sow excretes on average of 1,150 g N over a 21-d lactation period or up to 2.6 kg per year during lactation, of which close to 70% is of urinary origin (Zhang et al., 2019). This figure translates into 19,000 metric tons of N yearly in the United States. Fecal N excretion is affected by dietary protein digestibility, and therefore is largely impacted by feed ingredient quality and processing. Significant progress has been made to minimize fecal N excretion in swine by processing feed ingredients and testing their AA digestibility. On the other hand, improving digestibility should be accompanied with ways of enhancing post-gut AA utilization.

Replacing a portion of protein-bound limiting AA with crystalline AA (CAA) in growing swine diets was initially used to optimize feed costs. As more CAA are becoming commercially available, aggressive reduction of CP with higher inclusion rates of AA is of increasing interest.

Urinary N excretion decreases with feeding less dietary CP (Figure 1.1) (Chamberlin et al., 2015a; Huber et al., 2015). Chamberlin (2015b) reported up to a 3-fold reduction in ammonia emissions (Figure 1.2.) in sows fed diets reduced in CP by 4.57 percentage units. Therefore, feeding reduced CP diets offers potential to improve N utilization efficiency and mitigate N loss through urinary excretion or ammonia emission. The global increase in N efficiency is due to an increase in efficiency of individual AA (Huber et al., 2015). The extent to which dietary CP can be reduced to maximize utilization efficiency of individual AA without affecting lactation performance remains to be determined.

Effect of Heat Stress on Lactating Sow Performance and Welfare

Heat stress negatively impacts animal health and welfare (Renaudeau et al., 2012). Seasonal HS is aggravated with longer time period of seasonal heat and higher average temperature in many parts of the world due to global warming. In 2003, it was estimated that HS cost to the swine industry was more than $360 million (St-Pierre et al., 2003), a figure that increased to $900 million in 2010 (Pollmann, 2010) and is predicted to continue increasing. Swine are naturally HS-sensitive due to a lack of functional sweat glands (Curtis, 1983) and the existence of a substantial subcutaneous fat layer (Qu et al., 2016). Newer genetic lines for greater lean yield have also contributed to an increase in metabolic heat production (Brown-Brandl et al., 2004 and 2014).

Sows are particularly prone to high ambient temperature because of lactation associated thermogenesis. Sows respond to HS by increasing rectal temperature and respiration rate (Lucy and Safranski, 2017). Heat stress also decreases voluntary feed intake (Pérez Laspiur and Trottier, 2001; Williams et al., 2013), milk production (Pérez Laspiur and Trottier, 2001; Renaudeau and Noblet, 2001; Chamberlin, 2017) and milk concentration of Arg, Lys, Val and Pro (Pérez Laspiur, 2001). Studies in which lactating sows were housed in TN conditions and pair-fed to sows under

HS conditions demonstrated that high ambient temperatures had a direct negative impact on milk yield, independent of the impact on feed intake (Mullan et al., 1992; Prunier et al., 1997). Heat stress directly affects post-absorptive protein catabolism with increased plasma concentration of markers of protein degradation including 3-methyl histidine, creatine and plasma urea N (Pearce, 2011). Aggravated protein catabolism due to HS is related to reproductive issues including anestrus, prolonged weaning to estrus interval, reduced farrowing rate and litter size (Nardone et al., 2006). Heat stress also increases embryonic mortality (Wildt et al., 1975) and the number of stillborn piglets (Wegner et al., 2016), and reduces the weight of neonates (Lucy et al., 2012). The long term effect of HS is less detectable (Lucy and Safranski, 2017) and *in utero* HS modifies nutrient partitioning to favor adipose deposition at the expense of skeletal muscle in finishing pigs (Johnson et al., 2015).

In the past decades, reduced CP diets with improved AA balance results in better utilization of dietary energy and lower metabolic heat production in growing pigs (Le Bellego et al., 2001; Kerr et al., 2003). Greater metabolic heat associated with lactation renders sows prone to HS (Renaudeau et al., 2012), and therefore an important research question is assessing whether reduced protein diets alleviate heat production during lactation.

Effect of Improving Amino Acid Balance on Nitrogen Utilization

Recent years have witnessed an increasing amount of research on reduced protein diets (Wang et al., 2018), with some limited studies in lactating sows. The increasing availability of CAA from the industry at competitive costs relative to feed ingredient proteins allows for reduction of excessive dietary protein, and adjustment of AA balance. Implementation of reduced CP diets with aggressive CAA supplementation is directly dependent on future research demonstrating their feasibility in lactating sows.

Research on growing-finishing pigs (Kerr et al., 2003; Otto et al., 2003a; Otto et al., 2003b; Madrid et al., 2013; Li et al., 2015) and lactating sows (Manjarín et al., 2012; Huber et al., 2015; Chamberlin, 2017) indicated that feeding reduced protein diets with improved AA balance improves the efficiency of N utilization and mitigates urinary N excretion and ammonia emission to the environment. The impact on growth or lactation performance remains unclear depending on the level of CP reduction and CAA supplementation.

When feeding sows with diets containing from 17.55 to 12.98% CP (Chamberlin, 2015a), milk urea-N (MUN) concentration decreased over 2 folds in early lactation and by more than 3 to 5 folds in peak lactation (Figure 1.2). Feeding a 16.03% CP with Val supplementation and graded reduction to 13.22% CP (Huber et al., 2015) also resulted in marked drop in MUN (Figure 1.3). Milk urea-N concentration from early to peak lactation (Figure 1.3) remained unchanged in sows fed the reduced CP diets, and nearly doubled for sows fed a non-reduced CP (control) diet (Chamberlin, 2015a; Huber et al., 2015). In a subsequent study, Chamberlin et al. (2015b) fed sows 17.55 to 12.98% CP and housed them in either TN or HS environments and observed the same responses (Figure 1.4). Therefore feeding low CP diets to lactating sows minimizes urinary excretion and MUN secretion.

Similarly, plasma urea-N of sows fed a low CP diet was nearly a half and up to a third that of control in early and peak lactation, respectively (Chamberlin et al., 2015a; Figure 1.5). Together, the MUN and plasma urea-N response indicate less AA catabolism and greater utilization of N compared to control-fed sows. These changes are equally reflected in urinary N excretion which are summarized across studies and depicted in Figure 1.1 (Chamberlin et al., 2015a; Huber et al., 2015). Additionally, reducing dietary CP by 4.57% decreases ammonia emission by 3 folds in lactating sows (Chamberlin, 2015b; Figure 1.2)

In all, literature data to date reveal the potential of dietary protein reduction to improve N utilization efficiency and mitigates urinary N excretion and ammonia emission. The impact of feeding low CP diet with a NIAA profile to lactating sows on the individual EAA and energy efficiency remains to be determined.

Amino Acid Utilization Efficiency for Lactation

Definition of Utilization Efficiency Value for Amino Acids

Knowledge of accurate efficiency values for individual EAA are needed for future model prediction of dietary AA requirements and feed formulation. Guan et al. (2002) estimated Val utilization efficiency by the porcine mammary gland for milk protein synthesis to be 56% using isotope tracer techniques. This value represents the net Val output to net Val uptake ratio by the mammary gland. The use of tracers in that study allowed for estimation of AA flux pathway and direct calculation of the true Val efficiency. The associated costs and labor demand however in lactating sows preclude from being widely used and consequently, very little progress has been made in generating true AA efficiency values for milk protein synthesis. Alternative approach to determine efficiency values has been used, however this approach yields an "apparent" efficiency value. The apparent efficiency value can be estimated as follows:

$$\text{Apparent AA utilization efficiency} = \frac{\text{Milk AA output (g/d)}}{\text{Dietary SID AA intake (g/d)}}$$

The caveat with the apparent efficiency is that it includes AA contribution from body protein mobilization, and therefore the numerator "milk AA output" is not "truly" originating from the diet per se. In addition, the denominator "dietary AA intake" is partitioned to both milk and maternal needs, and thus is not specific for milk. The NRC (2012) proposed a new approach to estimate a “true” efficiency by correcting the numerator and denominator to be specific for “milk AA output from diet” and “dietary SID AA intake for milk”, respectively, as follows:

$$\text{True AA utilization efficiency} = \frac{\text{Milk AA output from diet (g/d)}}{\text{Dietary SID AA intake for milk (g/d)}}$$

In the true utilization efficiency calculation, the numerator specifies “from diet” to indicate that AA contribution from body protein losses, if any, is corrected for, and the denominator specifies “for milk” to indicate that SID AA needed for maintenance is corrected for. Milk yield is estimated based on piglet ADG (NRC 2012). Thus,

$$\text{True AA utilization efficiency} \\ = \frac{\text{AA output in milk (g/d)} - \text{AA mobilized from body protein (g/d)}}{\text{SID AA intake (g/d)} - \text{AA for maintenance (g/d)}}$$

A unique true maximum biological efficiency value (MBEV) of 0.67 for Lys was first estimated by NRC (2012) using this approach. This value represents the slope of Lys output from diet regressed against SID Lys intake for milk (Figure 1.6). The data for the regression were mined from the literature using strict selection criteria to ensure validity of the estimate. The first criterion was that each selected study on Lys requirement for lactation needed to 1) be based on a minimum of 4 treatments and 2) attain significant convergence when submitted to a two-phase linear regression analysis. When dietary AA composition were presented on a total AA basis, they were recalculated using SID AA composition values in order to estimate post-gut (i.e. SID) Lys efficiency. Milk Lys output corresponding to the Lys requirement (i.e., at convergence) was calculated for each study and regressed against the corresponding SID requirement (at convergence) (Figure 1.6).

The regression however was done on single data points at Lys requirement, as shown in Figure 1.6. White et al. (2016) re-ran the regression with a more robust statistical approach by including all of the data points and accounting for the random effect of study and other factors. The same Lys efficiency value of 0.67 was confirmed but this time with a variance around the estimate (White et al., 2016). Given the paucity or lack of studies on the minimum requirement of the 8 remaining EAA, NRC (2012) instead had to recourse to approximation of their MBEV. A meta-analysis (White et al., 2016) was conducted to assess the actual efficiency values of these 8 AA based on the same studies used by NRC (2012) for estimation of Lys efficiency. Such assessment was needed to determine the degree of inefficiency as proxy of the current production systems and as such to set goals and assess the value of dietary AA balancing. The AA efficiency estimates are lower than that of Lys (i.e., 0.67) as follows: Arg= 0.42, His= 0.58, Ile= 0.53, Leu= 0.50, Met= 0.60, Phe= 0.43, Thr= 0.55 and Val= 0.55. Using these efficiency values in the factorial approach would overestimate the AA requirements. These data illustrate the large potential to improve N efficiency and the need to refine current dietary formulations in order to reduce N losses to the environment.

Given this high level of inefficiency for the majority of EAA, Huber et al. (2015) tested different concentrations of CP reduction with CAA supplementation to assess the efficacy of reducing CP and to arrive at MBEV. These MBEV are important because 1) they provide a bench mark for future implementation of low CP diets and 2) they are a key determinant for modeling of AA requirements. Across dietary CP concentrations and CAA inclusion rates, Huber et al. (2015) showed that AA efficiencies generally increase, and quite considerably for some AA (Arg, His, Ile and Leu) with improvement in dietary AA balance. Because NRC (2012) efficiency estimates were not systemically determined except for that of Lys, it is not surprising that many of the EAA efficiency values from NRC (2012) differ quite substantially from Huber et al. (2015). Relevant efficiency values for Arg and His remain debatable because of the *de novo* synthesis of Arg and the extensive recycling of 3-methyl-histidine between muscle protein and blood pools with possible milk secretion of His arising from mammary metabolism (Trottier et al., 1997). Given that the NRC (2012) does not provide solid MBEV estimates of EAA other than for Lys (White et al., 2016), MBEV generated by feeding a low protein diet with a NIAA profile would provide novel reference values of EAA efficiencies for prediction of AA requirements.

Interaction Between Amino Acid Utilization Efficiency

The mechanism by which AA efficiency increases with optimization of AA balance is not just due to the simple fact that less AA are available. There is a consistent increase in piglet litter gain and milk AA output, except under exposure to HS (Chamberlin et al., 2015b; Chamberlin, 2017), as shown in Table 1. There are likely interactions among AA at the mammary basolateral membrane interface that affect their efficiency of transport across the mammary cells and ultimately their utilization by the mammary gland (Guan et al., 2002; Guan et al., 2004; Manjarín et al., 2012).

Guan et al. (2004) and Huber et al. (2016) reported that the utilization efficiency of dietary Lys was reduced in sows fed a diet exceeding in CP. When below the CP requirement, the arterio-venous (AV) differences of AA across the mammary glands improved with increasing concentration of dietary CP, however the AV difference dropped when CP concentration was above the CP requirement (Guan et al., 2004). This suggested that when feeding excessive dietary CP, the mammary glands responded by decreasing transport of cationic (Lys and Arg) and other neutral limiting AA (Thr). Nevertheless, the response for Leu was remarkably different, whereby mammary uptake of Leu continued to increase when a diet containing as high 24% CP was fed, suggesting that high concentrations of Leu decreased net uptake of Lys.

In a subsequent study (Pérez Laspiur et al., 2009), feeding CP in excess of requirement (24 vs. 18%) decreased piglet average daily gain and reduced milk and casein yields. This change was associated with a reduction in gene expression of one of the Lys transporter (CAT-2b), suggesting a limitation in mammary protein synthesis as a result of decreasing cellular lysine uptake. Therefore cationic AA and the branched-chain AA (BCAA) likely interact for transport across the basolateral membrane of mammary epithelial cells.

Transcript abundance of several molecular entities involved in Lys uptake by porcine mammary tissue have been quantified (Pérez Laspiur et al., 2004, 2009; Manjarín et al., 2011). Transporters of the y^+ system (i.e. CAT-1 and CAT-2b), uniquely specific for transport of cationic AA (Lys and Arg) were found to be of low abundance while those responsible for uptake of neutral AA, in particular the large neutral AA (e.g. Leu) transporter $ATB^{0,+}$ of system $B^{0,+}$ were highly abundant (Manjarín et al., 2011). In this regard, Lys has been reported to be transported by shared systems with the large neutral AA (e.g. BCAA), such as system $B^{0,+}$, y^+L, and $b^{0,+}$. Manjarín et al. (2012) proposed that the greater blood BCAA to lysine ratio associated with feeding higher dietary CP levels may decrease the ability for cationic AA to compete with BCAA for mammary transport via $ATB^{0,+}$, resulting in efflux of Lys.

The notion that an interaction exists between cationic and BCAA for transport across the basolateral membrane of the mammary epithelial cell has been supported by some *ex vivo* and *in vivo* studies. Inhibited Lys uptake and increased Lys efflux in rat mammary explants was observed due to high concentrations of Leu (Shennan et al. 1994; Calvert and Shennan 1996). It has also been reported that Lys inhibited 67 % Val uptake by lactating sow mammary explants (Hurley et al. 2000). Although the mechanism of interactions between neutral and cationic AA in the mammary gland is unclear, some *in vivo* study confirms the *ex vivo* findings. Over-supplementation of dietary Lys in sow resulted in a decrease in Val utilization (Richert et al., 1997). Conversely, Guan et al. (2002) reported a decrease of Lys transport in the mammary gland by over-supplementation of crystalline Val for lactating sows by stimulating Lys outward movement. Therefore improvement in Lys utilization for milk production when dietary CP is reduced may be linked to a decrease in BCAA interaction with Lys at the mammary cell interface. Determining whether Leu affects Lys efficiency of utilization in practical diets remains to be further explored.

Energy Utilization Efficiency for Lactation

Lactation is an energetically costly process. Feed intake of lactating sows, in particular of primiparous sows, is often not sufficient to support nutrient demands of milk production required for large litters. Sows mobilize nutrients and energy from their body stores if greater energy requirement cannot be satisfied. The sow udder is a large organ where extensive protein turnover is taking place involving a variety of AA catabolic and anabolic processes, as reviewed by Trottier and Manjarín (2012). For instance, based on the A-V difference balance technique and tracer work, protein synthesis and breakdown rates were 975 and 400 g/d, respectively, within the lactating sow mammary gland (Guan et al., 2002). The net protein gain was 575 g/d, indicating that the efficiency of mammary protein synthesis was 59%. Such inefficiencies are energy costly. Both catabolic and anabolic processes lead to intense thermogenesis (Bender, 2012). Therefore, minimizing unnecessary heat production will improve energy utilization efficiency.

Excessive AA supply is generally regarded as one of the major reasons for additional thermogenesis (Kerr et al., 2003; Bender, 2012). Unlike fat and carbohydrate, surplus AA cannot be stored and are catabolized into ammonia and carbon skeleton, which will be further converted to urea and other form of nutrients (fatty acids and glucose), respectively. The carbon skeleton can also be oxidized when energy is needed. The processes of ammoniagenesis, urea synthesis, and gluconeogenesis or oxidation from AA carbon skeleton are all adding to thermogenesis. Although heat production is biologically significant for maintenance of body temperature in animals, this form of energy is not retained into animal products and thus contributes to energy inefficiency.

In addition to greater N utilization and lower N excretion and emissions, reduced protein diets improve dietary energy efficiency due to decreased metabolic heat and urinary energy loss. Hamilton (1939) was the first to demonstrate an association between feeding excessive proteins and an increase in heat production in young rats. Feeding low protein with increased dietary lipid to rainbow trout lowered the heat increment (LeGrow and Beamish, 1986). In that study, increased lipid availability as an energy source reduced the amount of AA deaminated and oxidized for energy, leaving more AA available for growth. Fuller et al. (1987) reported that the increase in heat production associated with protein accretion in growing pigs was less when dietary protein quality was improved compared to when dietary protein was high. Le Bellego et al. (2001) showed that replacing dietary CP with supplemental AA in growing pigs reduced urinary N loss and total heat production by up to 65 and 7.4%, respectively, and attenuated the negative effect of high ambient temperature on ADFI. Similarly, growing pigs fed a 12% CP diet with CAA to meet the minimum AA requirements produced 2.7% (4.5 $\text{kcal}\cdot\text{d}^{-1}\cdot\text{BW}^{-0.75}$) and 7.6% less heat (11.2 $\text{kcal}\cdot\text{d}^{-1}\cdot\text{BW}^{-0.75}$) under TN and HS, respectively, compared to those fed a 16% CP diet meeting Lys requirement (Kerr et al., 2003).

Theoretical Estimation of Heat Production Arising from Amino Acid Oxidation and Ammonia Excretion

Improvement of dietary energy efficiency is highly dependent on the utilization efficiency of the major nutrients, i.e., carbohydrates, fats and proteins, which serve as important carriers of energy. Compared to carbohydrates or fats, proteins have a considerable greater heat increment

(HI; Figure 1.7). Heat increment is the heat generated through 1) digestion or fermentation of nutrients in the intestinal tract, and 2) nutrient metabolism during the post-gut phase (Ewan, 2001; NRC 2012). In swine, fats, carbohydrates and proteins contribute to 9, 17, and 26% of the ME, respectively (Bondi, 1987). A recent study (Li et al., 2017) reported NE/ME of 76 to 78% (Figure 1.7). In other words, the HI/ME (Figure 1.7) ranges between 22 and 24% for mixed nutrients. Metabolic heat is produced during ATP turnover associated with post-gut catabolism of excess AA. Both production and consumption of ATP generate heat. Therefore, estimating heat production associated with excess N intake should be calculated separately for ATP production and consumption, rather than based on net ATP production. The amount of ATP synthesized varies depending on the different substrates and pathways (Bender, 2012) and on average 2 moles of ATP are formed per mole of N deaminated. Ammonium (NH_4^+), the product of deamination, is used to amidate glutamate (Glu) into glutamine (Gln) which is transported to the liver. The cost associated with the synthesis of each mole of Gln is 1 mole of ATP (Bender, 2012). The funneling of ammonia from AA into the urea cycle involves additional processes including synthesis of aspartic acid for donation of the second amino group. Bender (2012) detailed the possible routes and simplified that there is a cost of 4 moles of ATP equivalent and a yield of 2.5 moles of ATP equivalent for each molecule of urea produced in the urea cycle. Each mole of ATP hydrolyzed into ADP generate 7.3 kcal. This energy is not 100% utilized, and the remaining (~33%) is released in the form of heat (Figure 1.8) (de Meis et al., 1997). Therefore, 2.4 kcal/mole ATP (i.e., 7.3 kcal × 0.33) is lost as heat during ATP hydrolysis. Similarly, ATP production during cellular glucose oxidation for example also generates heat since the efficiency of ATP production is not 100% (Darnell et al., 1986; Tobin et al., 1997; Figure 1.8). Phosphorylation of ADP into ATP is only 50% efficient, hence approximately 50% of the energy can be trapped into ATP and 50% released as heat. Therefore the energy required to generate one mole of ATP is 14.6 kcal (i.e., 7.3 kcal/0.50), with 7.3 kcal lost as heat. The energy that is not captured into energy requiring processes or into ATP synthesis will add to thermogenesis.

In the following example, theoretical heat reduction associated with reduced dietary CP and improved AA balance is calculated, with the calculations depicted in Table 2. Assume a control diet containing 3% N (18.75% CP) compared to a reduced CP diet containing 2.2% N (13.75% CP) fed to a lactating sow with an average daily feed intake (ADFI) of 6 kg. The resulting reduction in N intake per day is 48 g or 3.43 moles of N. A reduction in N intake of 3.43 moles per day results in 58 kcal/d less heat associated with deamination-Gln formation and 48 kcal/d less heat associated with urea synthesis. Thus, the total reduction in heat production associated with removal of 3.43 moles of N (in excess) is 106 kcal/d.

The heat associated with digestion however is believed to represent the greatest portion of the total HI (NRC 2012), although quantification of this HI is difficult and lacking in the literature. Thus in this example, the HI associated with gastrointestinal metabolism due to excess AA indirectly is estimated indirectly by subtracting the heat associated with post-gut metabolism of excess AA (estimated above, i.e., 106 kcal/d) from the total HI.

First, HI was calculated based on ME and NE as follows (NRC, 2012):

$$\mathrm{HI}\ \left(\frac{\mathrm{kcal}}{\mathrm{kg}}\right) = \mathrm{ME}\ (\frac{\mathrm{kcal}}{\mathrm{kg}}) - \mathrm{NE}\ (\frac{\mathrm{kcal}}{\mathrm{kg}})$$

where prediction of NE content of diets for lactating sows is based on that for growing-finishing pigs (Noblet, 1994). Assuming a reduced dietary CP diet created by substituting soybean meal with corn, it was presumed here that the percentage decrease in dietary CP is accompanied by a corresponding percentage increase in dietary starch. The equation is as follows (with NE and ME as kcal/kg DM, and EE, starch, CP and ADF as g/kg DM):

$$\mathrm{NE}\ (\frac{\mathrm{kcal}}{\mathrm{kg}}) = \left(0.726 \times \mathrm{ME}\ (\frac{\mathrm{kcal}}{\mathrm{kg}})\right) + \left(1.33\ (\frac{\mathrm{kcal}}{\mathrm{g}}) \times \mathrm{EE}\right) + \left(0.39\ (\frac{\mathrm{kcal}}{\mathrm{g}}) \times \mathrm{starch}\right)$$

$$- \left(0.62\ (\frac{\mathrm{kcal}}{\mathrm{g}}) \times \mathrm{CP}\right) - \left(0.83\ (\frac{\mathrm{kcal}}{\mathrm{g}}) \times \mathrm{ADF}\right)$$

To simplify the calculation, 2 diets are assumed, a high dietary CP (HCP, 18.75% CP) and a low dietary CP (LCP, 13.75% CP) containing the same ME and 88% DM. The difference in HI between HCP, and LCP can be calculated as follows:

$$\mathrm{HI}_{\mathrm{HCP}}(\frac{\mathrm{kcal}}{\mathrm{kg}}) - \mathrm{HI}_{\mathrm{LCP}}(\frac{\mathrm{kcal}}{\mathrm{kg}}) = \mathrm{NE}_{\mathrm{LCP}}(\frac{\mathrm{kcal}}{\mathrm{kg}}) - \mathrm{NE}_{\mathrm{HCP}}(\frac{\mathrm{kcal}}{\mathrm{kg}})$$

$$= 0.39\ \left(\frac{\mathrm{kcal}}{\mathrm{g}}\right) \times (\mathrm{starch}_{\mathrm{LCP}} - \mathrm{starch}_{\mathrm{HCP}}) - 0.62(\frac{\mathrm{kcal}}{\mathrm{g}}) \times (\mathrm{CP}_{\mathrm{LCP}} - \mathrm{CP}_{\mathrm{HCP}})$$

$$57.4\ \left(\frac{\mathrm{kcal}}{\mathrm{kg}}\right) = 0.39\ (\frac{\mathrm{kcal}}{\mathrm{g}}) \times (\frac{187.5}{0.88} - \frac{137.5}{0.88}) \left(\frac{\mathrm{g}}{\mathrm{kg}}\right) - 0.62\ (\frac{\mathrm{kcal}}{\mathrm{g}}) \times (\frac{137.5}{0.88} - \frac{187.5}{0.88})(\frac{\mathrm{g}}{\mathrm{kg}})$$

$$344\ \left(\frac{\mathrm{kcal}}{\mathrm{d}}\right) = 57.4 \left(\frac{\mathrm{kcal}}{\mathrm{kg}}\right) \times 6\ (\frac{\mathrm{kg}}{\mathrm{d}})$$

For a sow consuming 6 kg/d, the theoretical decrease in HI is 344 kcal/d, with 211 kcal resulting from CP reduction, and 133 kcal resulting from starch increase. Therefore, the reduction of heat associated with digestion and absorption is estimated as follows:

$$\mathrm{Total}\ \Delta\mathrm{HI} \left(\frac{\mathrm{kcal}}{\mathrm{d}}\right) = \Delta\mathrm{HI}_{\mathrm{Pre\ gut}} \left(\frac{\mathrm{kcal}}{\mathrm{d}}\right) + \Delta\mathrm{HI}_{\mathrm{Post\ gut}}\ (\frac{\mathrm{kcal}}{\mathrm{d}})$$

$$344 \left(\frac{\mathrm{kcal}}{\mathrm{d}}\right) = \Delta\mathrm{HI}_{\mathrm{Pre\ gut}} + 106\ (\frac{\mathrm{kcal}}{\mathrm{d}})$$

Where $\mathrm{HI}_{\mathrm{Pre\ gut}}$ is 238 kcal/d. These values indicate that there is a lower impact on HI associated with post-gut metabolism compared to that of pre-gut when sows are fed this particular reduced

CP diet. Note that these are only theoretical estimates based growing-finishing pig NE values. A greater reduction of HI is expected in practice in lactating sows fed this reduced protein diet due to an improved efficiency of AA utilization at the mammary level.

High lactation demand on modern sows are compounded by increasing environmental regulations to decrease carbon and ammonia emissions, and rising environmental temperatures which impact sow welfare and performance. In the past decades, studies on reduced protein diet have been extensively conducted in growing-finishing pigs, and a few in lactating sows. These results suggest improvement of N utilization efficiency, and decrease in N excretion and ammonia emissions. However, there are still substantial gaps in knowledge of how reduced protein diet affects individual EAA and energy utilization efficiency, as well as metabolic heat production in lactating sows. This knowledge is critically needed, since 1) valid efficiency values of individual EAA (except Lys) are lacking, and these values are essential to predict EAA requirements and 2) higher metabolic rate due to lactation renders sows specifically prone to HS, which is of increasing concern. The following chapters present a series of studies focused on assessing the impact of dietary AA balance on utilization efficiency of individual EAA and energy, and heat production in lactating sows.

Table 1.1. Performance of lactating sows fed diets[1] reduced in crude protein (CP) concentration with supplemental crystalline amino acids over 21-d lactation

Study	CP (%)	Feed intake (kg/d)[2]	SID Lys (%)	SID Thr (%)	SID M+C (%)	SID Trp (%)	Litter gain (kg)	Piglet ADG (g/d)	Sow BW loss (g/d)	Sow P2 back fat Δ (mm)	Sow loin eye area Δ (cm^2)	Sow body protein Δ (g/d)[3]	Sow body lipid Δ (g/d)[4]
Manjarín et al., 2012	17.52	3.9	1.11	0.69	0.55	0.21	1.71	214	228	-	-	-	-
	13.53	3.9	0.85	0.53	0.42	0.16	2.26	282	232	-	-	-	-
Huber et al., 2015	17.62	5.1	0.74	0.59	0.50	0.18	1.86	186	414	-	-	-	-
	14.63	5.1	0.74	0.59	0.50	0.18	2.18	221	433	-	-	-	-
Huber et al., 2015	16.03	5.5	0.74	0.59	0.50	0.18	2.32	238	143	−0.1	+0.2	−22.9	−36.7
	15.70	5.7	0.74	0.59	0.50	0.18	2.53	256	176	−0.2	−0.8	−26.9	−50.0
	14.29	5.8	0.74	0.59	0.50	0.18	2.41	243	190	−0.1	−1.2	−30.9	−46.6
	13.22	5.7	0.74	0.59	0.50	0.18	2.60	260	285	−0.2	−2.7	−45.5	−73.1
Chamberlin et al., 2015a	17.16	5.8	0.78	0.53	0.48	0.18	2.53	262	270	-	-	-	-
	14.79	5.6	0.78	0.49	0.42	0.15	2.64	278	413	-	-	-	-
	12.56	5.7	0.78	0.49	0.41	0.15	2.56	258	358	-	-	-	-
Chamberlin et al., 2015b	17.16[5]	5.2	0.78	0.53	0.48	0.18	2.60	265	500	−1.4	-	−63.2	−194.8
	12.56[5]	5.5	0.78	0.49	0.41	0.15	2.80	279	300	−2.7	-	−8.4	−234.8
	17.16[6]	3.7	0.78	0.53	0.48	0.18	2.40	244	700	−3.2	-	−68.9	−351.3
	12.56[6]	4.3	0.78	0.49	0.41	0.15	2.30	238	800	−2.1	-	−103.4	−302.8

[1] NE=2,580 to 2,600 kcal/kg.

[2] Feed intake is an average value for a 21-d lactation period, and that of Manjarín et al. (2012) is for an 18-d lactation period.

[3] Maternal body lipid Δ (kg) = −26.4 + 0.212×maternal BW Δ (kg) + 1.331×backfat Δ (mm); NRC (2012).

[4] Maternal body protein Δ (kg) = 2.28 + 0.171×maternal BW Δ (kg) − 0.333×backfat Δ (mm); NRC (2012).

[5] Sows were housed under thermal neutral environmental temperature.

[6] Sows were housed under thermal heat stress environmental temperature.

Table 1.2. Theoretical calculation of heat associated with dietary crude protein fed in excess[1]

	ATP change (mole ATP/mole N)	Heat production (kcal/mole N)	Sum of heat production (kcal/mole N)	Heat production (kcal/d)
Deamination	+2.00	2.00 × 7.30 = 14.60	17.00[2]	58[4]
Glutamine synthesis	–1.00	1.00 × 2.40 = 2.40		
Urea synthesis	+1.25 –2.00	1.25 × 7.30 = 9.13 2.00 × 2.40 = 4.80	13.93[3]	48[5]
Total	-	-	30.93	106

[1] Assumes sows are consuming 48 g of CP in excess per day, corresponding to 3.43 moles of N per day.

[2] 14.6 kcal/mole N + 2.40 kcal/mole N.

[3] 9.13 kcal/mole N + 4.80 kcal/mole N.

[4] 17.00 kcal/mole N × 3.43 moles N.

[5] 13.93 kcal/mole N × 3.43 moles N.

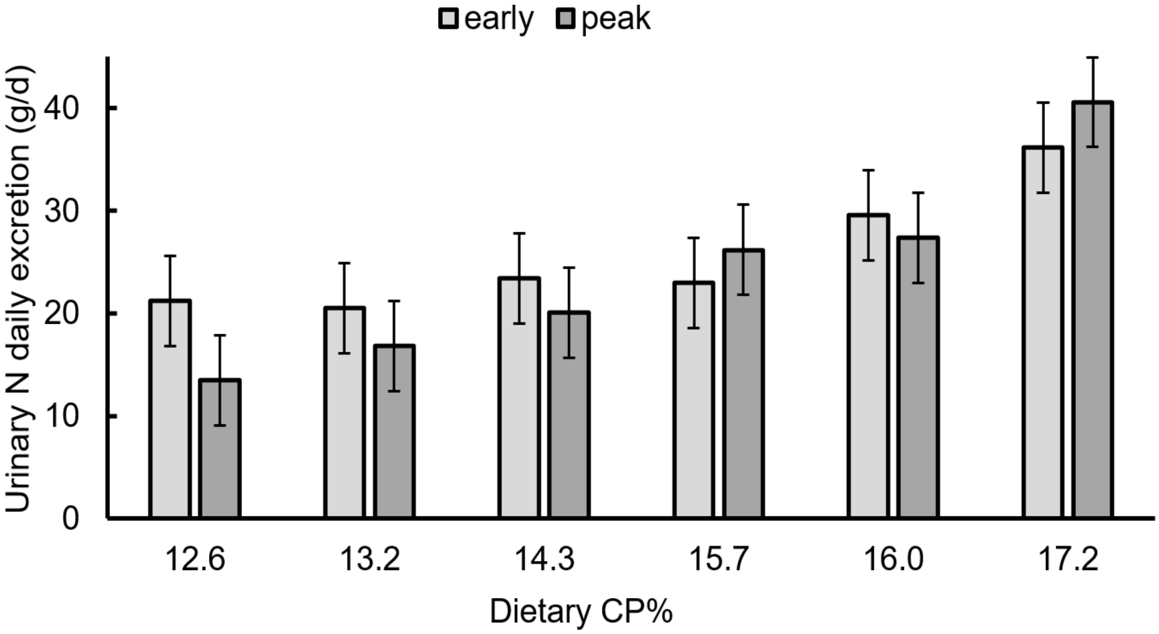

Figure 1.1. Urinary nitrogen excretion (g/d) from sows fed different dietary crude protein (CP) over 21-d lactation. Adapted from Chamberlin (2015a) and Huber et al. (2015).

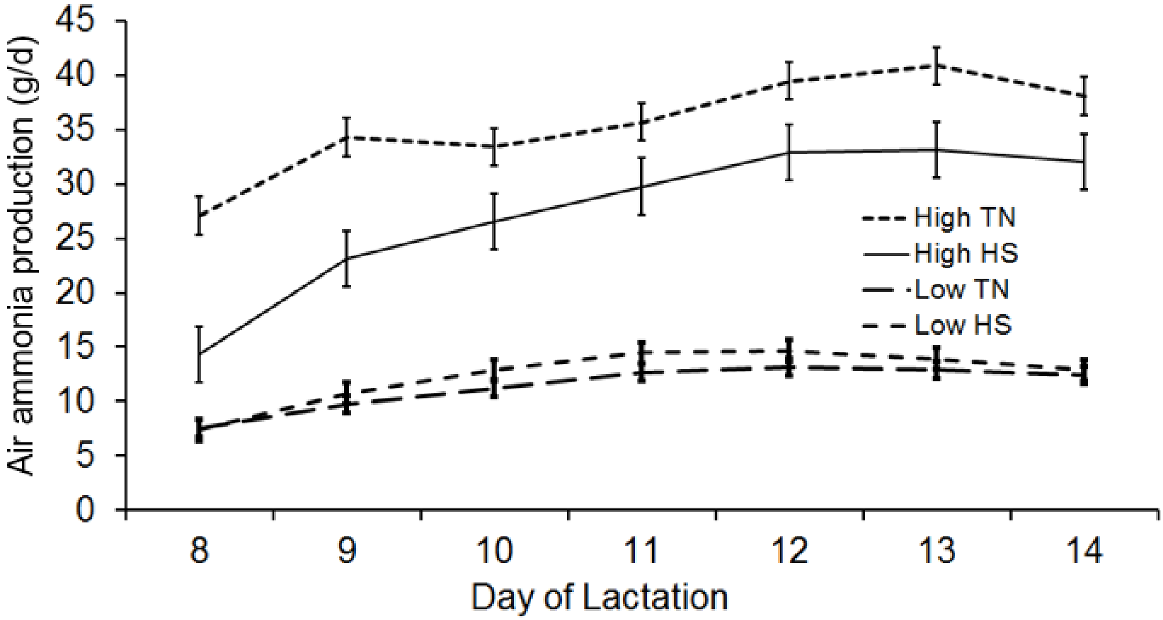

Figure 1.2. Air ammonia production (g/d) in individual lactating sows and their litters. Sows were fed diets containing 17.55 (High) and 12.98% CP (Low) and housed under either a thermal neutral (TN) or heat stress (HS) environment. From Chamberlin et al. (2015b).

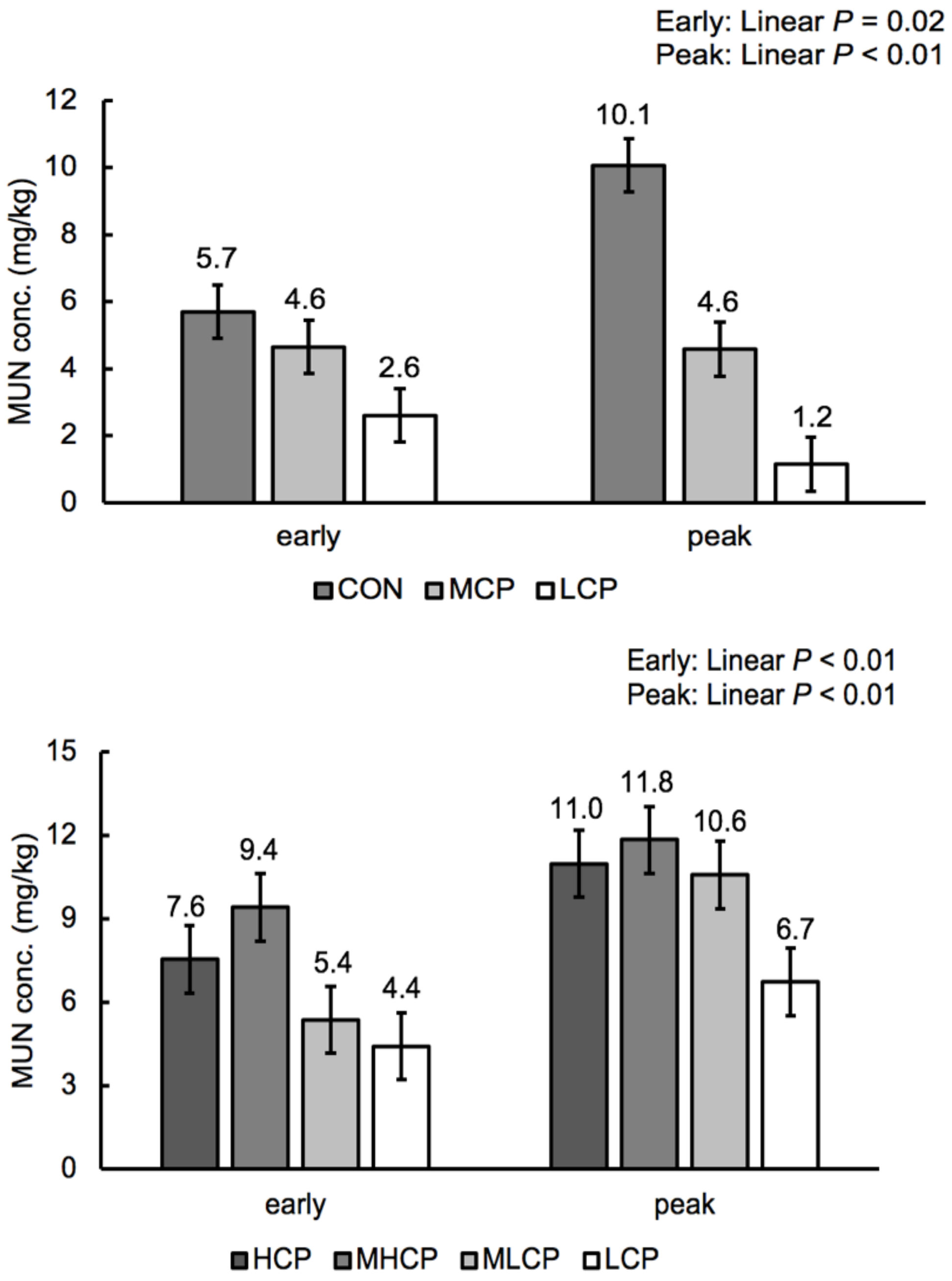

Figure 1.3. Milk urea concentration (MUN conc., mg/kg) in sows fed different levels of dietary CP in early (d 4-8) and peak (d 14-18) lactation. Upper panel: control (CON, 17.55% CP), medium low crude protein (MCP, 15.25% CP) and low crude protein (LCP, 12.98% CP) (Adapted from Chamberlin, 2015a). Lower panel: high crude protein (HCP, 16.03% CP), medium high crude protein (MHCP, 15.70% CP), medium low crude protein (MLCP, 14.29% CP), low crude protein (LCP, 13.22% CP) (Adapted from Huber et al., 2015).

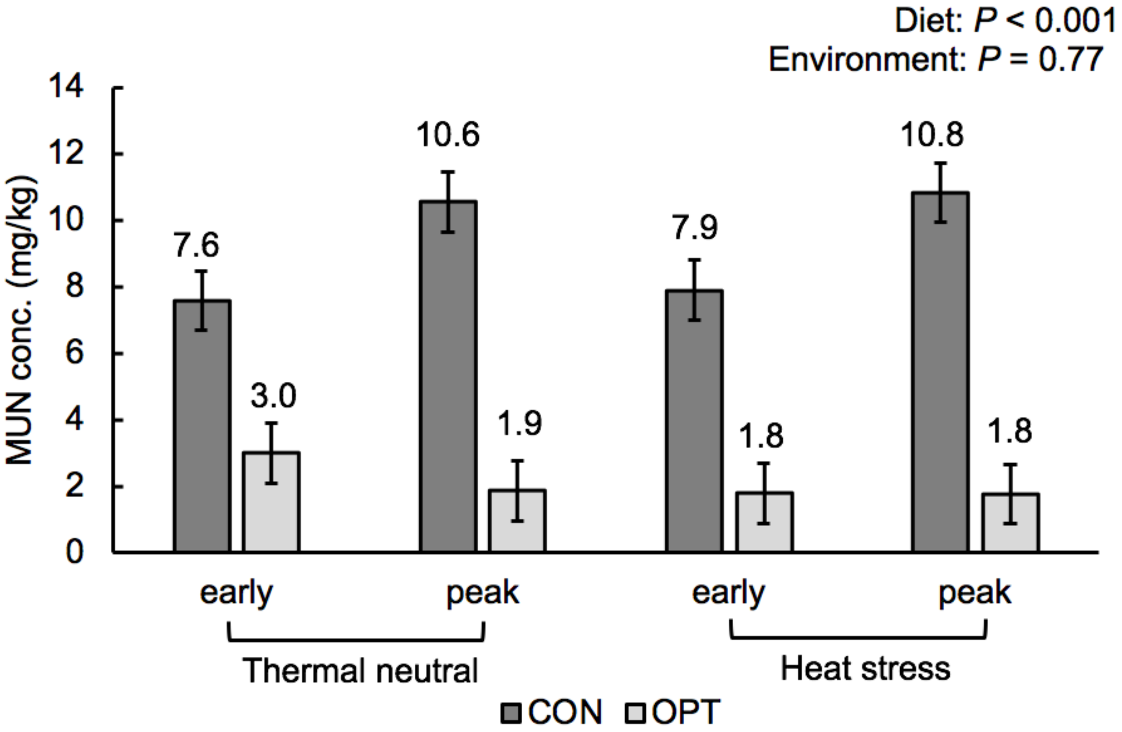

Figure 1.4. Milk urea nitrogen concentration (MUN conc., mg/kg) from sows exposed to thermo-neutral temperature (TN) and heat stress (HS) and fed a control diet (CON, 17.55% CP) or a low protein diet (OPT, 12.98% CP) during lactation. Adapted from Chamberlin et al. (2015b).

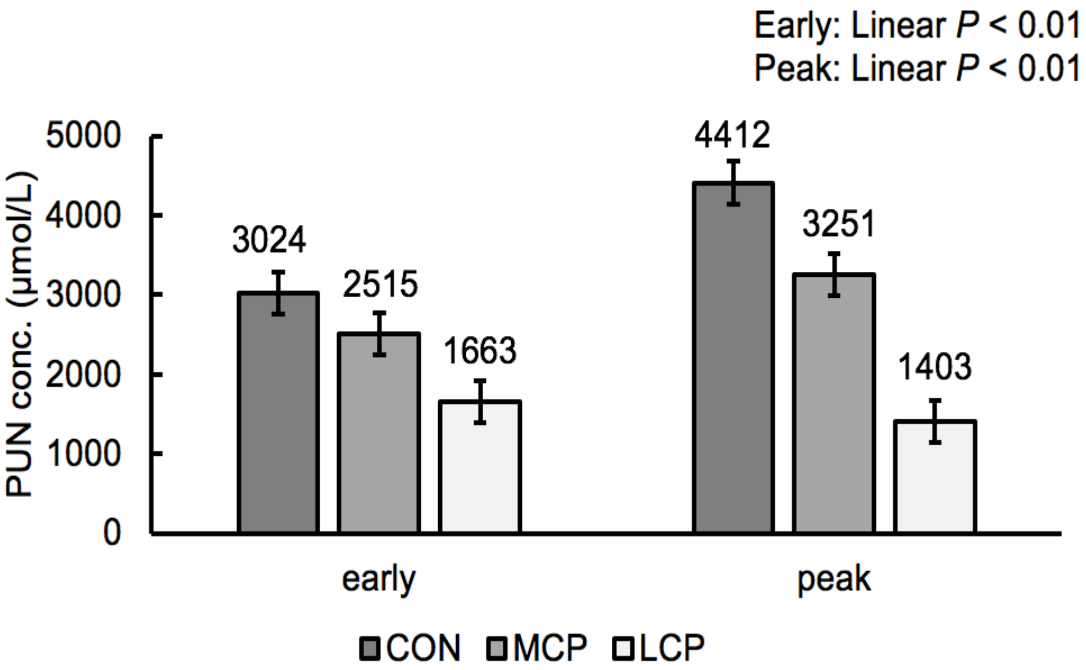

Figure 1.5. Plasma urea N concentration (PUN conc., µmol/L) of lactating sows fed control (CON, 17.55% CP), medium low crude protein (MCP, 15.25% CP) and low crude protein (LCP, 12.98% CP). Adapted from Chamberlin et al. (2015a).

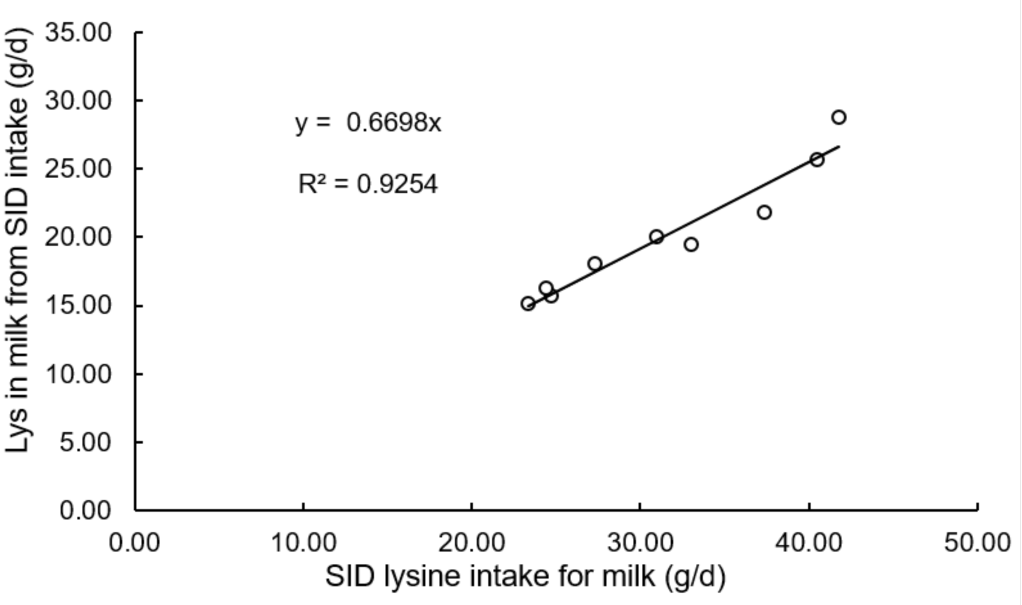

Figure 1.6. Relationship between estimated lysine in milk derived from SID lysine intake and estimated SID lysine intake for milk. The relationship is represented by the line and described as y=0.6698x at zero intercept with r^2 of 0.925, where the slope of 0.6698 represents the efficiency of dietary lysine utilization into milk lysine (NRC, 2012).

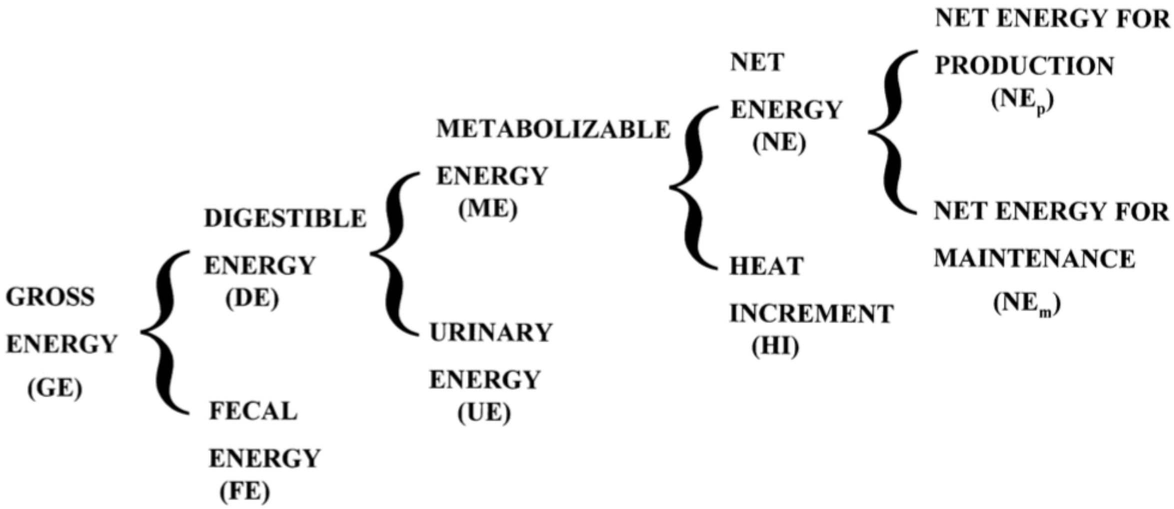

Figure 1.7. Energy partitioning by pigs (Ewan et al., 2001).

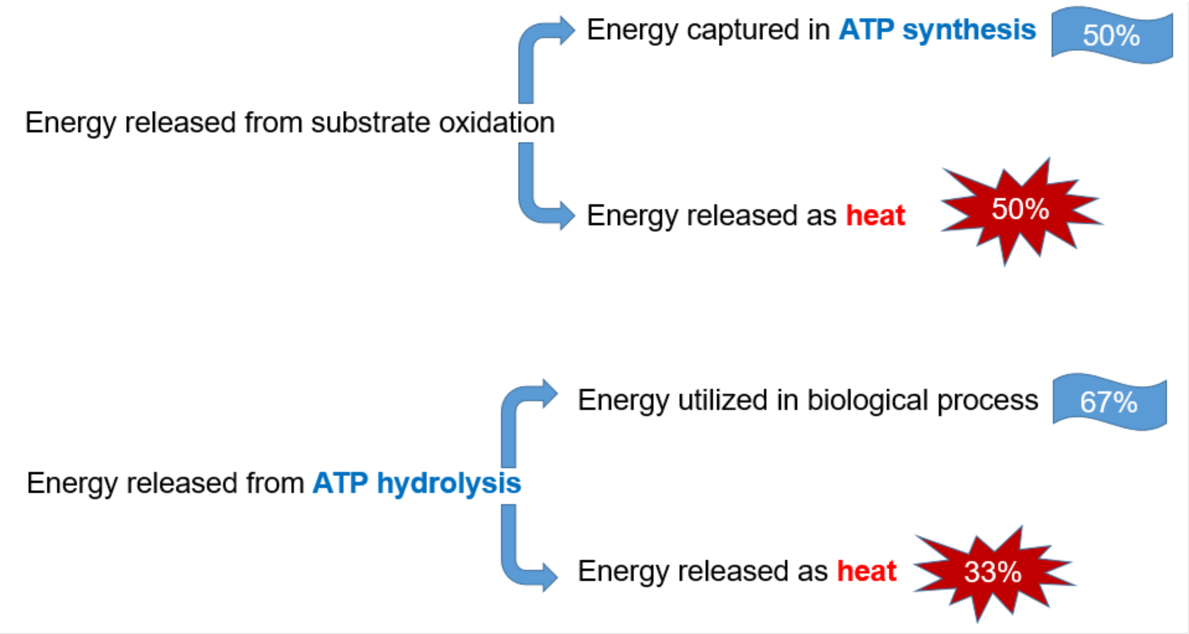

Figure 1.8. Partitioning of energy released from substrate oxidation and ATP synthesis and from energy utilization and ATP hydrolysis. Efficiency of ATP synthesis and hydrolysis are 50 and 67%, respectively, with the 50 and 33% of the energy lost as heat, respectively

CHAPTER 2

FEEDING A REDUCED PROTEIN DIET WITH A NEAR IDEAL AMINO ACID PROFILE IMPROVES AMINO ACID EFFICIENCY AND NITROGEN UTILIZATION FOR MILK PRODUCTION IN SOWS

ABSTRACT

Fifty-four lactating multiparous Yorkshire sows were used to test the hypothesis that feeding a reduced protein diet with a near ideal AA (NIAA) profile increases the biological utilization efficiency of nitrogen (N) and essential AA (EAA) for milk production in part as a result of reduced dietary Leu concentration. Sows were fed 1 of 3 isocaloric diets containing the following concentration of crude protein (CP % as fed, analyzed): 18.74 (Control: CON), 13.78 (Optimal: OPT), and 14.25 (Optimal+Leu: OPTLEU). The OPT and OPTLEU diets contained the same concentration of crystalline AA (CAA) to meet requirements of the limiting AA. Crystalline Leu was added to OPTLEU to contain the same standardized ileal digestible (SID) Leu concentration as that of CON. Sows were weighed on day 1 and 21 of lactation and piglets on day 1, 4, 8, 14, 18 and 21 of lactation. Nitrogen retention was measured for 48 or 72 h between day 4 and 8 (early) and day 14 and 18 (peak) of lactation. Sow body weight (BW) change and average daily feed intake (ADFI) did not differ between diets. Litter growth rate (LGR) during early lactation did not differ between diets. At peak lactation, LGR was higher in sows fed OPT compared to CON ($P < 0.05$) and lower in sows fed OPTLEU compared to OPT ($P < 0.05$). In early and peak lactation, total N retention and milk N output efficiency were greater in OPT ($P < 0.01$) and OPTLEU ($P < 0.05$) than CON. Compared to CON, overall biological efficiency of N, Arg, His, Ile, Leu, Phe and Trp were greater ($P < 0.05$) whereas those of Lys, Met, Thr and Val did not differ in sows fed OPT and OPTLEU, except for Leu which did not differ between OPTLEU and CON. Compared to OPT, only Leu and Met efficiency were lower ($P < 0.01$) and tended to be lower (*P* = 0.10), respectively, in sows fed OPTLEU. Reducing CP with a NIAA profile to attain the minimum Leu requirement maintained overall lactation performance, improved utilization efficiency of N, Arg, His, Ile, Leu, Phe+Tyr and Trp for milk production, and maximized efficiency of Ile, Leu, Lys, Met+Cys, Phe+Tyr, Thr, Trp and Val. Addition of Leu did not reduce N and EAA utilization efficiency. This study provides revised and novel maximum biological efficiency value (MBEV) for Ile (65.4), Leu (75.1), Lys (63.2), Met+Cys (78.2), Phe+Tyr (69.5), Thr (71.0), Trp (70.1) and Val (57.0). These MBEV can be used to more accurately predict requirement for those AA during lactation.

INTRODUCTION

The breeding herd contributes to as much as 11.8 × 10^6 metric tons of fresh manure produced annually in the United States (Koelsch et al., 2005). Therefore, small change in the efficiency of dietary N utilization in lactating sows can have major impacts on N excretion at the global scale. Determination of individual essential AA (EAA) biological efficiency value at near maximal biological potential is needed to accurately predict the requirement of each EAA. Underestimation of efficiency leads to overestimation of requirement and increase N losses to the environment. Except for Lys, maximum biological efficiency value (MBEV) of individual EAA reported by NRC (2012) were not empirically determined, nor have been validated. Furthermore, it is unclear why feeding individual EAA at or near minimum requirement in a low CP diet improves efficiency. It may be due to reduction in intake of the said EAA alone or in competitive inhibition with other AA present in excess of requirements. Previous work from the same lab (Guan et al., 2004; Manjarín et al., 2012) suggested that there is competition among AA, in particular between Leu and Lys utilization for milk production. Thus, Lys utilization even when present at its minimum requirement may not be maximized in the presence of excessive concentration of N or other specific EAA.

It is hypothesized that reducing CP to meet the minimum SID Leu requirement increases efficiency of individual EAA. It is further hypothesized that the relatively low Leu:Lys in a reduced CP diet (1.14:1) meeting minimum SID Leu requirement compared to a conventional corn-soybean meal-based diet (1.63:1) improves Lys efficiency for milk protein production. The objectives were to 1) estimate efficiency values of EAA in lactating sows and 2) determine if the corresponding decrease in Leu concentration in reduced CP diet affects Lys efficiency.

MATERIALS AND METHODS

Animals, Feeding and Experimental Design

The study was conducted at the Michigan State University Swine Teaching and Research Center, using 54 purebred multiparous (parity 2+) Yorkshire sows. Sows were moved to conventional farrowing crates between day 105 and 107 of gestation, grouped by parity and randomly assigned to 1 of 3 dietary treatments within parity groups (Control, n = 18; Optimal, n = 19; Optimal + Leu, n = 17). The study was conducted over 4 blocks of time, with 12 to 18 sows per block. Litters were standardized to 11 piglets within the first 24 h after farrowing with the aim of weaning 10 piglets per sow. Sows were adapted to the experimental diets (2.2 kg/d) 4 to 6 days before expected farrowing date. After farrowing, sows feed allowance was progressively increased from 1.88 kg/d at day 1 to 7.44 kg/d at day 21 of lactation, according to the NRC (2012) model, with targeted ADFI of 6.0 kg/d during the whole lactation period. Feed was provided daily in 3 equal meals (0700, 1300 and 1900) with feed intake and refusal recorded daily before the morning meal. Water was freely accessible to sows and piglets. Injection of iron and surgical castration were conducted on day 1 and 7, respectively. No creep feed was supplied to the piglets.

Sows and piglets were weighed on day 1 (i.e., 24 h postpartum) after standardization of litter size and day 21. Sow BW was only recorded on day 1 and 21 due to high variablility and labor intensive between short period of time. Sow back fat thickness was measured (Lean-meater®, series 12, Renco Corp., Golden Valley, MN, USA) on day 1 and 21. Corn oil was applied as an ultrasound enhancing agent and the probe was placed perpendicularly on the back 6-8 cm from the midline at the last rib. Two separate measurements were taken on each side of the midline and averaged. Litters were also weighed on day 4, 8, 14 and 18 of lactation to estimate milk yield (Theil et al., 2002) between day 4 and 8 and day 14 and 18, representing early and peak lactation periods, respectively.

Dietary Treatment

Ingredient and calculated nutrient composition of the diets are presented in Table 2.1. Analyzed total (hydrolysate) and free AA of the diets are presented in Table 2.2. The NRC (2012) model was used to estimate requirements for AA, NE, Ca and P for sows. The requirements were based on the swine herd performance at the Michigan State University Swine Teaching and Research Center, including sow BW of 210 kg, sow parity number of 2 and above, sow ADFI of 6 kg/d, litter size of 10, piglet BW gain of 280 g/d over a 21-d lactation period, and ambient temperature of 20 °C. The model predicted a minimum sow BW loss of 7.5 kg and the protein: lipid was adjusted to the minimum allowable value of near zero. All diets were formulated to contain the same SID Lys (0.90%) and NE (2,580 kcal/kg) concentrations. The control diet (CON) was formulated using corn and soybean meal as the only sources of Lys to meet NRC (2012) SID Lys requirement (0.90%) and consequently contained 18.74% CP. Valine met near SID requirement (NRC, 2012) (0.77 vs. 0.79%). All other EAA SID concentrations were in excess relative to NRC (2012). A second diet balanced to reach a near ideal AA (NIAA) profile was formulated. In this paper, the term “near ideal AA profile” is used in lieu of the conventional “ideal AA profile” because the “ideal AA profile” is conceptual rather than biologically factual. The rationale is further based on the notions that an “ideal AA profile” 1) cannot be limited to the relative contribution of only two AA pools (i.e., milk and maintenance), 2) needs an accurate characterization of the maintenance AA pool for the lactating sow, and 3) should include AA for which dietary essentiality in known lactating sows (i.e., Arg and His). The NIAA diet was designed by reducing soybean meal relative to corn to meet the minimum SID Leu requirement, which corresponded to a CP concentration of 13.78%. Then, supplemental crystalline source of L-Lys, L-Val, L-Thr, L-Phe, DL-Met, L-Ile, L-His, and L-Trp were added to meet the minimum SID requirement for those AA in the NIAA diet. DL-Met was added to meet the requirement of Met + Cys. This diet is referred to as the optimal diet (OPT) throughout the remainder of the manuscript. A third diet was formulated to be the same as OPT with added crystalline L-Leu to equate the SID Leu concentration of CON and referred to as optimal + Leu diet (OPTLEU). Sugar food product (International Ingredient Corporation, St. Louis, MO) was included in all 3 diets at 5% to increase diet palatability. Titanium dioxide was included at 0.10 % as indigestible marker in all experimental diets.

Nitrogen Balance

For the N balance study, sows with an actual feed intake relative to predicted feed intake of 75% or above were used. Nitrogen balance was conducted during early lactation (between day 4 and 8) and peak lactation (between day 14 and 18) on a subset of sows from blocks 2 (n = 10), 3 (n = 12) and 4 (n = 12) for a total of 34 sows. During the N balance period, sow overall activity and appetite were carefully monitored, along with measurements of rectal temperature before the morning and afternoon feeding to ensure that sows were healthy with no signs of urinary tract infections. The urinary catheter was removed for any sows showing signs of depression or increase in rectal temperature. Urine collection was performed for a minimum of 48 h and a maximum of 72 h. Balance studies were conducted in either early lactation or late lactation to minimize urinary tract irritation and follow animal care guidelines, hence the number of sows in early and peak lactation differed. Total urine collection and fecal grab sampling methods were as described in Huber et al. (2015) and Möhn and de Lange (1998), respectively. Briefly, Foley urinary catheters (BARDEX® I.C., 2-way, 30cc balloon, 18FR, Bard Medical, Covington, GA) were aseptically inserted into the bladder before feeding in the morning at 0600. The distal end of the catheter was connected to a sterilized polyvinyl tubing secured with electrical tape, and long enough to reach a 5-gallon bucket set behind the sow and outside of the crate. The tubing was maintained in place through a rubber stopper inserted into the bucket cover. The urine collection bucket contained 30 mL of H_2SO_4 to acidify the urine and maintain pH of less than 3. Urine was removed and weighed daily at 0700, and 2 subsamples (45 mL) were collected and frozen at −20 °C. Urinary catheters were removed before feeding at 0700 on the last day of the N balance (either 48 or 72 h). Fresh feces were collected by rectal digital stimulation on day 10 and 11, pooled and frozen at −20 °C.

Milk Sampling

Milk was collected after each N balance (day 8 and 18). For milk collection, piglets were separated from the sows for approximately 1 h, and sows were administered 1 mL of oxytocin i.m. (20 IU/mL oxytocin, sodium chloride 0.9% w/v, and chlorobutanol 0.5% w/v, VetTek™, Blue Springs, MO). A total of 100 mL milk was manually collected across all glands and stored in 2 separate 50-mL tubes (polypropylene centrifuge tubes with screw cap, Denville Scientific®). Piglets were immediately returned to sows to complete nursing.

Nutrient and Titanium Analyses

Approximately 50 g of subsampled feed was ground using a commercial coffee grinder and sent to the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for AA analyses [AOAC Official Method 982.30 E (a,b,c), 45.3.05, 2006] to verify accuracy of feed mixing. Both hydrolysate and free AA concentrations were analyzed to verify the accuracy of crystalline AA (CAA) inclusion during feed mixing (Table 2.2). The DM content of diets was measured via oven drying at 135℃ for 2 h according to the AOAC (1997; Method 930.15). Fecal samples were homogenized, oven dried at 65℃ for 4 days and ground using a commercial coffee grinder. Feed, fecal and urinary N concentration was measured based on the Hach method (Hach et al., 1987). Milk samples were submitted to the Michigan Dairy Herd Improvement Association (NorthStar Cooperative, Lansing, MI) for analyses of fat, true protein, lactose and milk urea N using infrared spectroscopy. Titanium concentration in feed and feces were analyzed based on Myers et al. (2004). Absorbance of standards and samples were measured by spectrophotometry (Beckman DU-7400; Beckman Instruments, Inc., Fullerton, CA) at 408 nm.

Calculations

Sow milk yield was estimated based on piglet ADG (g/d) during early (day 4-8) and peak (day 14-18) lactation (Theil et al., 2002) as follows (Eq. 1 and 2, respectively):

$$\text{Daily milk yield (g/d, d } 4-8\text{)} = \text{Litter size} \times (317 + 1.168 \times \text{ADG} + 0.00425 \times \text{ADG}^2) \quad (1)$$

$$\text{Daily milk yield (g/d, d } 14-18\text{)} = \text{Litter size} \times (582 + 1.168 \times \text{ADG} + 0.00425 \times \text{ADG}^2) \quad (2)$$

For all calculations pertaining to the N balance, the analyzed N concentration in each respective diet and corresponding block was used to calculate N intake. Daily total N retention (N maternal retention + N milk) and N maternal retention were calculated as follows (Eq. 3 and 4, respectively):

$$\text{Total N retention (g/d)} = \text{N intake (g/d)} - [\text{fecal N output (g/d)} + \text{urinary N output(g/d)}] \quad (3)$$

$$\text{Maternal N Retention (g/d)} = \text{N intake (g/d)} - [\text{fecal N output (g/d)} + \text{urinary N output (g/d)} + \text{milk N output (g/d)}] \quad (4)$$

Actual daily feed intake and analyzed N concentration of the diets (Table 2.2) were used to calculate daily N intake in each respective block. Apparent total tract digestibility (ATTD) of N was estimated using analyzed titanium dioxide concentration in feed and feces (Eq. 5) according to Zhu et al. (2005), and fecal N output was calculated based on the estimated N digestibility and N intake, as follows (Eq. 6).

$$\text{Apparent total tract digestibility of N} = 1 - \frac{\text{TiO}_2\text{\% in feed} \times \text{N\% in feces}}{\text{TiO}_2\text{\% in feces} \times \text{N\% in feed}} \quad (5)$$

$$\text{Fecal N output (g/d)} = (1 - \text{ATTD of N}) \times \text{N intake (g/d)} \quad (6)$$

Daily urine weight and urinary N concentrations were used to calculate daily urinary N output. Daily milk N output was calculated based on the sum of analyzed milk true protein N and milk urea N concentrations multiplied by the predicted daily milk yield. Apparent efficiency of dietary N utilization was expressed as efficiency of total N retention (maternal + milk) and of N secreted in milk, relative to N intake or N absorbed, as follows (Eq. 7 and 8, respectively):

$$\text{Apparent efficiency of total N retention} = \frac{\text{Total N retention (g/d)}}{\text{N intake or N absorbed (g/d)}} \times 100\% \quad (7)$$

$$\text{Apparent efficiency of N secreted in milk} = \frac{\text{N secreted in milk (g/d)}}{\text{N intake or N absorbed (g/d)}} \times 100\% \quad (8)$$

For calculations pertaining to true efficiency estimation of N and individual EAA utilization, an adjustment was made to account for any discrepancy between the analyzed and calculated dietary AA concentrations. Relying on calculated SID N and EAA intake alone may either underestimate or overestimate true efficiency values. Therefore, the SID N or individual SID EAA concentrations were adjusted by multiplying the calculated SID N or SID EAA concentration with the ratio of analyzed to calculated N or EAA concentrations (as fed basis) in each of the respective block (Eq. 9):

$$\text{Adjusted SID N or EAA concentration} = \text{Calculated SID N or EAA (\%)} \times \frac{\text{Analyzed N or EAA (\%,as fed)}}{\text{Calculated N or EAA (\%,as fed)}} \qquad (9)$$

Daily individual SID N or EAA intake was then calculated from the actual sow ADFI and adjusted dietary SID N or EAA as follows (Eq. 10):

$$\text{SID N or EAA intake (g/d)} = \text{Sow feed intake (g/d)} \times \text{ adjusted SID N or EAA (g/100 g)} \qquad (10)$$

True efficiency values of N and individual EAA secreted in milk were determined by correcting for N or EAA mobilized from body protein and used for maintenance, as follows (Eq. 11):

$$\text{True efficiency of N or EAA secretion in milk} = \frac{\text{N or EAA ouput in milk (g/d)} - \text{N or EAA mobilized from body protein (g/d)}}{\text{SID N or EAA intake (g/d)} - \text{N or EAA for maintenance (g/d)}} \qquad (11)$$

Where the N or EAA output in milk in early and peak lactation periods were calculated from estimated milk yield (Theil et al., 2002) for early lactation and peak lactation period, respectively, and the average N or EAA concentration in mature milk protein (NRC, 2012), as follows (Eq. 12):

$$\text{N or EAA output in milk (g/d)} = \text{Milk yield (g/d)} \times \text{N or EAA in milk protein (g/100 g)} \qquad (12)$$

Daily N mobilized from body protein and partitioned to milk was estimated by multiplying the negative maternal N retention with the efficiency of N secretion in milk from mobilized body N of 0.87 (NRC, 2012), as follows (Eq. 13):

$$\text{N mobilized (g/d)} = \text{Maternal N retention (g/d)} \times \text{Efficiency of N mobilization to milk N secretion (0.87)} \tag{13}$$

Daily individual EAA mobilized from body protein and partitioned to milk was estimated from the product of the negative maternal N retention and the EAA concentration in body protein (NRC 2012), multiplied by the efficiency of N secretion in milk from mobilized body N of 0.87, as follows (Eq. 14):

$$\text{EAA mobilized (g/d)} = \text{Maternal N retention (g/d)} \times 6.25 \times \text{EAA in body protein (g/100g)} \times \text{Efficiency of body N mobilization to milk N depostition (0.87)} \tag{14}$$

Daily SID N and SID EAA was calculated as described above in Eq. 9.

Maintenance requirement for N or individual EAA was calculated as the sum of basal endogenous gastrointestinal tract (GIT) and integumental N or EAA losses (NRC, 2012), and the efficiency of N or EAA utilization for maintenance (NRC, 2012), as follows (Eq. 15):

$$\text{N or EAA for maintenance} = \frac{\text{Basal endogenous GIT N or EAA loss (g/d)} + \text{ integumental N or EAA loss(g/d)}}{\text{N or EAA efficiency for maintenance}} \tag{15}$$

Statistical Analysis

Statistical analyses were conducted using SAS 9.4 (SAS Inst. Inc., Cary, NC). The homogeneity of residual variance among dietary treatments (Minimum *P* = 0.088 for milk protein output), and normality of residuals was confirmed by using Mixed Procedures and Univariate Procedures, respectively. Data were analyzed by ANOVA using the Glimmix procedures model as follows:

Response = diet + parity + period + *block* + $sow_{\text{diet×block}}$+ diet × parity + diet × period + *diet* × *block* + *e*

The response of sow depended on the fixed effects of **diet** (CON, OPT, and OPTLEU),

parity (early [P 2-3] and late [P 4-6]), and lactation **period** (early [d 4-8] vs. peak [d 14-18]). The random effects included ***block***, and ***sow*** nested within diet and block. The interactive effects of **diet** × **parity**, **diet** × **period**, and ***diet*** × ***block*** were also included. When appropriate, a reduced model was used. Specifically, effects of parity and parity × treatment were not significant (minimum $P = 0.18$ and $P = 0.13$, respectively) and therefore were excluded in the reduced model for analyses of all lactation performance and N balance data, and individual EAA efficiency values. Pairwise comparisons (OPT vs. CON, OPTLEU vs. CON, and OPTLEU vs. OPT) were carried out for different period of lactation (early, peak and 21-d overall lactation) using the slice option in SAS and Tukey adjustment. Effects were declared significant at $P \leq 0.05$, and tendencies at $0.05 \leq P \leq 0.10$.

RESULTS

Dietary Amino Acid Analyses

Analyzed N and individual EAA concentration values agreed closely with their calculated values derived from selected NRC (2012) feed ingredients (Table 2.2). Analyzed values were within a minimum of 96% of the expected calculated values. Of note however was Met, with analyzed to calculated values of 87, 82 and 94% in CON, OPT and OPTLEU diets, respectively. The discrepancy between calculated and analyzed values of Met was attributed to the omission of supplemental DL-Met in block 2 of the nitrogen balance studies, as revealed from the free AA analysis report (see Table 2.2 footnote). In addition, the lower analyzed relative to calculated Met concentration value in the CON diet may have been attributed to a lower Met concentration in soybean meal in NRC (2012) than that of the actual concentration in soybean meal used for this study. As described above in methods, because individual SID EAA intake was calculated with an adjustment to account for any discrepancy between analyzed and calculated EAA concentrations, albeit very small for the majority of EAA, there was no difference in Met efficiency between blocks.

Performance

Lactation performance data of all sows are presented in Table 2.3. Sow feed intake, BW and back fat loss did not differ between dietary treatments. Sow BW and back fat loss differed from zero ($P = 0.025$) for sows fed OPT and did not differ from zero in sows fed CON and OPTLEU. The interaction between dietary treatments and lactation period for litter growth rate (LGR) and ADG was significant ($P < 0.05$). Litter growth rate during early lactation period and over the 21 days of lactation period did not differ across dietary treatments. At peak lactation, compared to CON, LGR of sows fed OPT was greater ($P < 0.05$) and that of sows fed OPTLEU did not differ. Compared to OPT, sows fed OPTLEU had lower LGR ($P < 0.05$).

Lactation performance, and milk nutrient concentration and output are presented in Table 2.4. In early lactation, piglet ADG, estimated daily milk yield, milk true protein, lactose and fat concentration and output did not differ between diets. At peak lactation, piglet ADG of sows fed OPTLEU was lower ($P < 0.05$) compared with that of sows fed OPT. Estimated daily milk yield of sows fed OPT tended to be greater than CON ($P = 0.06$) and that of OPTLEU did not differ from CON and was lower ($P < 0.05$) than OPT. Milk true protein and lactose concentration did not differ between dietary treatments. Sows fed OPT tended to have higher ($P = 0.08$) milk fat concentration than CON, and those fed OPTLEU did not differ from CON or OPT. Milk true protein output did not differ between dietary treatments. Lactose output of sows fed OPT tended to be greater ($P = 0.107$) than that of CON, but did not differ between OPTLEU and CON, and was lower ($P < 0.05$) in sows fed OPTLEU compared to OPT. Milk fat output of sows fed OPT was higher ($P < 0.05$) than CON and did not differ for sows fed OPTLEU when compared to CON or OPT. In both early and peak lactation periods, milk urea N of sows fed OPT and OPTLEU was lower ($P < 0.01$) compared to CON and did not differ between OPTLEU and OPT.

Nitrogen Balance

Nitrogen absorption, retention and utilization efficiency are presented in Table 2.5.

Early Lactation. Milk N excretion did not differ between sows fed OPT and CON, as well as between OPTLEU and OPT. Compared to sows fed CON, urine output was lower ($P < 0.05$) in OPT and tended to be lower ($P = 0.10$) in OPTLEU. Maternal N retention was positive ($P < 0.05$) and did not differ between diets.

Peak Lactation. Milk N excretion of sows fed OPT tended to be greater ($P = 0.06$) than those fed CON, and did not differ between OPTLEU and OPT. Sows fed OPT and OPTLEU had lower ($P < 0.01$) maternal N retention compared with those fed CON.

Early and Peak Lactation. Nitrogen intake, N absorbed, urinary N excretion and total N retention were lower ($P < 0.05$), and apparent efficiency of N utilization for milk N secretion was greater ($P < 0.05$) in sows fed OPT and OPTLEU compared to sows fed CON, and did not differ between OPTLEU and OPT.

True Nitrogen and Essential Amino Acid Efficiencies for Milk N and EAA Deposition

True dietary N and EAA efficiency for milk production are presented in Table 2.6. Individual EAA efficiency did not differ between early and peak lactation periods. In early, peak and overall lactation period, compared to CON, N, Arg, His, Ile, Leu, Phe and Trp efficiency were greater ($P < 0.05$) and those of Lys, Met and Val did not differ in sows fed OPT or OPTLEU. In early lactation, compared to CON, Thr efficiency in sows fed OPT or OPTLEU did not differ. At peak lactation, compared to CON, Thr efficiency tended to be greater ($P = 0.054$) in sows fed OPT, but did not differ in sows fed OPTLEU. Individual EAA efficiency did not differ between

OPTLEU and OPT, except for that of Leu and Met. Utilization efficiency of Leu in sows fed OPTLEU was lower ($P < 0.01$) compared to sows fed OPT and did not differ from that of sows fed CON. Utilization efficiency of Met was lower ($P < 0.05$) and tended to be lower ($P = 0.10$) in sows fed OPTLEU compared to those fed OPT during peak and overall lactation period, respectively.

DISCUSSION

The goal of the study was in part to determine the MBEV of N and EAA in lactating sows by feeding a diet containing a NIAA profile. A diet limiting in all EAA down to the minimum SID Leu requirement was first formulated. Because Arg is synthesized *de novo*, and its essentiality has not been characterized for the lactating sow, it was not possible to create a practical diet limiting in Arg, and therefore MBEV for Arg was not determined. To generate MBEV biologically relevant for practical prediction of EAA requirement, each limiting EAA was supplemented in their crystalline form to meet their minimum SID requirement (NRC, 2012) and to attain a NIAA profile. Several previous studies reported that similar dietary strategies to the current work either maintained or increased milk yield, casein yield and LGR (Manjarín et al., 2012; Chamberlin et al., 2015a, b; Huber et al., 2015). In the current study, the overall lactation performance was unaffected however sow fed OPT had greater BW and back fat loss. In contrast, at peak lactation, sows fed OPT had greater LGR and milk fat output and tended to have greater milk yield. The results corroborate with those of Huber et al. (2015) who suggested that ameliorating dietary AA balance may facilitate nutrient partitioning toward milk protein synthesis. Although sows fed a NIAA profile diet had greater milk N production at peak lactation, neither milk true protein concentration nor true protein yield differed. What was noticeably greater was the milk fat yield. Estimation of body lipid mobilization is determined in the following chapter (Chapter 3) in order to further understand the potential impact of feeding a NIAA profile on nutrient partitioning. A second objective was to determine whether the corresponding decrease in Leu concentration in reduced CP diet (OPT) impacts the efficiency of Lys utilization. The only difference between OPT and OPTLEU was the additional LEU in the OPTLEU diet whereby SID Leu:SID Lys was 1.14:1 and 1.63:1, respectively. The SID Leu:SID Lys was identical between OPTLEU and CON. As initially hypothesized, addition of Leu to the OPT diet reduced milk yield at peak lactation to similar level as that of CON, potentially indicating an AA imbalance and interaction between Leu and other EAA utilization for milk production. Sows fed OPTLEU and CON did not lose appreciable BW and were in positive maternal N balance. Supplementary Leu has been reported to improve muscle (Escobar et al., 2006) and visceral (Torrazza et al., 2010) protein synthesis in piglets, thus Leu in CON and OPTLEU may have played a role in nutrients partitioning away from the mammary gland and towards maternal body.

It is clear that the reduced CP diets not only maintain lactation performances compared to non-reduced CP diets, but greatly improve the global efficiency of N utilization. Feeding either OPT or OPTLEU diets led to dramatic decrease in urinary N excretion and increase in overall apparent N utilization efficiency for milk N production up to 73% and true N utilization efficiency of up to 82.7%. Urine weight decreased by 58% and urinary N excretion by up to 60%. Difference in daily quantitative urinary N excretion between CON and low protein diets (OPT or OPTLEU) was attributed in this study to both urine volume and urinary N concentration. Others have also reported that reducing dietary CP concentrations can lead to lower urine volume in horses (Wickens, 2003), lactating sows (Huber et al., 2015) and growing pigs (Shaw et al., 2006). Additionally, the lower milk urea N secretion parallels the urinary N excretion, suggesting less AA catabolism in OPT than CON diets (Huber et al., 2015). Across diets, sows were in a positive maternal N balance in early lactation, whereas sows fed OPT ended up at maternal N equilibrium during peak lactation. The apparent discrepancy between average maternal N retention (17 ± 8 g/d, Table 2.5) and BW loss (400 ± 143 g/d, Table 2.3) of OPT fed sows may be explained in part by the contribution from fat loss rather than from body protein loss. Furthermore, 400 g BW loss per day translates into 2 ± 3 g N/d when accounting for water and protein mass (NRC, 2012). In addition, there may have been some degree of overestimation of N retention (MacRae et al., 1993).

Level of dietary CP reduction and CAA inclusion, and the practical implementation of thereof for lactating sows is dependent on feed and AA costs, and whether environmental constraints are in place. A major focus of the current study was to determine MBEV for individual EAA and to assess whether Leu impacts efficiency of EAA. Accurate prediction of dietary AA requirement using the factorial approach is directly dependent on MBEV, a fundamental focus of the modeling approach employed by NRC (2012). The reported MBEV in NRC (2012) however were not experimentally determined except for that of Lys, which was later validated by Huber et al. (2015). Thus, reduced CP diets with a NIAA profile is a powerful tool to experimentally generate MBEV of EAA. In the study reported by Huber et al. (2016), MBEV was only estimated for Lys because all other EAA were present in excess of requirement in the reduced CP diets. When predicting efficiency of EAA using the available literature data (White et al., 2016), the majority of efficiency of EAA are grossly underestimated relative to those of NRC (2012). The majority of available studies have focused on assessing the minimum requirement for Lys which corresponds to the point of near maximum biological utilization. Therefore, Lys is the only EAA for which reliable efficiency value can be predicted (NRC, 2012; White et al., 2016) and a close estimation of Lys requirement for milk production exist.

This study aimed at assessing whether MBEV of Lys is independent from N and EAA concentration because NRC (2012) estimated MBEV of Lys in diets containing N and all of the other AA in excess of their requirements. Similarly, Huber et al. (2015) validated MBEV of Lys in sows fed reduced CP diet and containing the other EAA in excess of their requirement. Utilization efficiency of 66.2% for Lys at peak lactation in the present study was similar to that of Huber et al. (2016) at 67.6% and NRC (2012) at 67.0 %. As mentioned earlier, separate MBEV for early and peak lactation may be potentially relevant if phase feeding is implemented in lactation. Both Huber et al. (2016) and NRC (2012) used calculated SID Lys values to estimate efficiency. Here, if calculated values are used, overall lactation Lys MBEV (data not shown) aligns perfectly with that of NRC (2012). Instead, the calculated AA values were adjusted based on the analyzed values to account for discrepancy, because a minor discrepancy can have a large impact on efficiency estimation.

In the current study, the OPT diet formulated to contain a NIAA profile was used to estimate MBEV of individual EAA. There were noticeable changes in efficiency values from early to peak lactation between diets; however, the limited number of sows and the relatively high SEM precluded drawing strong conclusions pertaining to the impact of lactation stage. Nonetheless, because trends were very consistent for each individual EAA, N and averaged EAA, additional work is clearly warranted to ascertain the individual MBEV of EAA in early and peak lactation with a larger number of sows. Results herein are pointing to possible larger differences in EAA requirements between early and peak lactation, which is not captured in the current NRC (2012) because only one MBEV was estimated for the entire lactation period.

Consistent and significantly greater efficiency of use for Arg, His, Ile, Leu, Met + Cys, Phe, Phe + Tyr and Trp in sows fed OPT relative to those fed CON indicate that these AA were in excess of requirements in the CON diet during both early and peak lactation. As well, except for

Arg, these EAA reached their MBEV in the OPT diet because this diet was definitively limiting in Ile, Leu, Met + Cys, Phe, Phe + Tyr and Trp. For Thr, the noticeable trend from early to peak lactation between diets is indicative that Thr was in excess of requirement in early lactation and near requirement in the OPT diet at peak lactation. On the other hand, efficiency values of Val and Met did not differ between OPT and CON. It is therefore likely that both Met and Val were near their minimum requirement and were at maximum biological efficiency in the CON diet. Such low MBEV for Val is supported by several studies, as previously mentioned in Chapter 1. For instance, Val uptake by the sow mammary gland relative to its output in milk is the largest amongst the EAA (Trottier et al., 1997; Lei et al., 2012). Previous *in vivo* isotope tracer research conducted in our lab (Guan et al., 2002) showed that the net Val output to net Val uptake ratio by mammary gland was 0.56 in sows fed a diet with Val: Lys of 1.04, and 0.45 in sows fed a diet with Val to Lys ratio of 1.37. In this study, Val MBEV was 57% in sows fed Val: Lys of 0.88 (OPT), closely agreeing with Guan et al. (2002). The net Val output:net Val uptake determined with tracer approach is essentially a true mammary efficiency value because it is independent from Val used for maintenance and Val from body protein mobilization. Therefore, the study by Guan et al. (2002) validates the calculations used herein and by others (NRC, 2012; Huber et al., 2016) for estimating efficiency of EAA utilization. It is proposed herein to adopt the word "true" when estimating efficiency using such approach. Moreover, Val requirement for swine lactation has been reported as 44.3 g/d by Guan et al. (2004) based on maximal mammary uptake of EAA, which is higher than a predicted 38.5 g/d based on NRC (2012) model. Xu et al. (2016) suggested a higher Val: Lys requirement ratio (88-113%) than 85% previously reported by NRC (2012) based on minimum back fat loss and maximum piglet growth rate, suggesting that Val MBEV from NRC (2012) may be slightly overestimated, and as such, underestimating Val requirement. Metabolic pathways of

Val utilization in mammary gland are unknown. Trottier (1995) proposed that Val is retained by the mammary gland for re-modelling of *in situ* mammary proteins. Valine was also reported to be used for the synthesis of glutamate AA family (Li et al., 2009). The data is this study point to Val among the top 4 limiting EAA, as previously suggested by others (Kim et al., 2001; Xu et al., 2016).

For several EAA, the overall MBEV derived from the OPT diet agree with those of NRC (2012), except for Arg and Phe. Estimated efficiency values for Arg and Phe were noticeably lower than those reported in NRC (2012), i.e., 61.1 vs. 81.6% and 53.4 vs. 73.3%, respectively. The amount of Arg taken up by the mammary glands greatly exceeds Arg output in milk (Trottier et al., 1997; O'Quinn et al. 2002), therefore its efficiency of use for milk protein we be expected to be relatively low. Furthermore, since Arg is synthesized *de novo* via the intestinal-renal axis (Tomlinson et al., 2011; Marini et al., 2017), it is recognized as a conditionally essential AA (NRC, 2012). It is likely that the NRC (2012) reported value of 81.6% is a gross overestimation and a true MBEV for Arg may not be estimable. In regard to Phe, it is unknown whether its low efficiency is indicative that Phe was in excess in the OPT diet. On the other hand, mammary metabolic pathways for Phe are unknown but it is possible that there is a high rate of Phe hydroxylation to Tyr in mammary tissue. For instance, total aromatic EAA efficiency value from OPT compared to NRC (2012) was very close, i.e., 69.5 vs. 70.5%. Threonine MBEV between the current study and NRC (2012) was lower than expected with 71.0 vs 76.4%. As observed for Lys, Thr MBEV at peak lactation was 74.5% which is in closer agreement with that of NRC (2012) value for the overall lactation of 76.4%.

In Chapter 1, presence of competitive inhibition between AA for their utilization by the mammary gland, potentially between Lys and Leu was reviewed (Guan et al., 2004; Manjarín et al., 2012). High concentration of Leu was reported to inhibit Lys uptake in rat mammary explants (Shennan et al., 1994; Calvert and Shennan, 1996). Reduced CP diet with CAA inclusion increased mammary extraction efficiency of Lys and Arg (Manjarín et al., 2012). Thus in this study, it was questioned whether an increase in efficiency of EAA in a reduced CP diet was related in part to a reduction in Leu. Addition of Leu to the OPT did not impact efficiency of Lys or the majority of EAA, but reduced efficiency of Met. This response was unexpected but offers an insight into potential interaction between crystalline Leu and Met utilization by the mammary gland via common transporter systems (Manjarín et al., 2014).

CONCLUSION

The MBEV for individual EAA were estimated for Ile, Leu, Lys, Met, Met + Cys, Phe + Tyr, Thr, Trp and Val by feeding a diet that met the minimum SID requirement for Leu. Generating efficiency estimates for Arg and potentially His may not be biologically relevant given *de novo* synthesis of Arg and possible mammary excretion of His (Trottier et al., 1997). Valine MBEV is low relative to other EAA and agrees with that of NRC (2012) and previous work which supports a low efficiency of Val utilization for milk production. Nonetheless, testing OPT diets with limiting Val as low as 50% of NRC (2012) are critically needed to further validate this low efficiency value. In addition, the MBEV of other EAA, in particular Thr and Phe should be validated using the same approach but with graded levels of inclusion from 30% below to 30% above NRC (2012) requirements using a similar OPT diet as used in this study. Leucine did not reduce efficiency of N, Lys and other EAA utilization, therefore Leu concentration in conventional diets is unlikely to be directly affecting the global utilization of N as proposed in earlier work. Feeding a NIAA diet not only maintained overall milk production and litter growth, but increased litter growth between d 14 and 18 of lactation, corroborating results from previous studies. The increase in performance was accompanied by greater milk fat yield and a tendency to increase milk production, reduction in maternal N retention and lost in BW and back fat, indicating possible nutrient repartitioning towards the mammary glands. Leucine therefore may be playing a role in maternal N retention and sow body condition during lactation rather than interacting with Lys utilization for milk production, as initially hypothesized. In fact, it is unknown whether feeding reduced CP diets to lactating sows over multiple lactations affect sow body condition and longevity. As mentioned earlier, practical implementation of such diets will depend on feed and CAA availability and costs, and on environmental constraints. Continued testing of such diets to generate and validate the MBEV of EAA is critical to refine future models for prediction of EAA requirements. The increase in several EAA efficiency with reduction in dietary protein and improvement of AA balance suggest a need to establish a dynamic model to predict EAA requirement under different scenarios of dietary protein concentrations and crystalline AA inclusion rates. In the next chapter, the impact of NIAA and Leu supplementation on the efficiency of energy partitioning and utilization is addressed.

Table 2.1. Ingredient composition and nutrient content of experimental diets (as-fed)

	Control	Optimal	Optimal + Leu
Ingredient composition, %			
Corn, yellow dent	59.17	61.45	61.21
Soybean meal, 48 % CP	30.00	14.00	14.00
Soy hulls	0	10.57	10.57
Sugar food product[1]	5.00	5.00	5.00
Beef tallow	3.35	5.02	4.81
L-Lys·HCl	0	0.47	0.47
L-Val	0	0.29	0.29
L-Thr	0	0.20	0.20
L-Phe	0	0.13	0.13
DL-Met	0	0.11	0.11
L-Ile	0	0.08	0.08
L-His	0	0.07	0.07
L-Trp	0	0.05	0.05
L-Leu	0	0	0.45
Limestone	1.18	0.93	0.93
Dicalcium phosphate	0.45	0.78	0.78
Sodium chloride	0.50	0.50	0.50
Vitamin and mineral premix[2]	0.25	0.25	0.25
Titanium dioxide	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated nutrient concentration[3]			
NE, kcal/kg	2,580	2,580	2,580
CP, %	19.24	14.00	14.34
Fermentable fiber, %	11.58	11.58	11.57
SID[4] AA, %			
Arg	1.17	0.71	0.71
His	0.47	0.37	0.37
Ile	0.71	0.52	0.52
Leu	1.47	1.03	1.47
Lys	0.90	0.90	0.90
Met[5]	0.27	0.30	0.30
Met + Cys	0.54	0.49	0.49
Phe	0.84	0.67	0.67
Phe + Tyr	1.38	1.03	1.03
Thr	0.61	0.58	0.58
Trp	0.21	0.17	0.17
Val	0.77	0.79	0.79

Table 2.1. (cont'd)

N	2.63	1.88	1.93
Total Ca, %[6]	0.65	0.65	0.65
STTD P, %[6]	0.23	0.23	0.23

[1] Supplied per kg: NE 2,842 kcal; fermentable fiber 0.05 %; CP 1.00 % (International Ingredient Corporation, St. Louis, MO).

[2] Sow micro 5 and Se-yeast PIDX15 (Provimi North America, Inc. Brookville, OH).

[3] Based on nutrient concentrations in feed ingredients according to NRC (2012).

[4] SID = standardized ileal digestible (NRC, 2012).

[5] Met concentration in OPT and OPTLEU is higher than CON because Met was added to meet Cys requirement (Met + Cys).

[6] Concentrations of Ca and P were based on phytase activity from the premix.

Table 2.2. Analyzed and calculated concentration of nitrogen (N), total and free essential amino acids (EAA) in experimental diets[1] (as-fed)

	Control		Optimal		Optimal + Leu	
	Analyzed	Calculated[2]	Analyzed	Calculated	Analyzed	Calculated
Total, %						
DM	88.76	-	88.95	-	89.15	-
N	3.00	3.08	2.20	2.24	2.28	2.29
Arg	1.23	1.26	0.75	0.78	0.80	0.78
His	0.49	0.53	0.39	0.43	0.40	0.43
Ile	0.85	0.81	0.61	0.60	0.64	0.60
Leu	1.65	1.67	1.14	1.19	1.59	1.64
Lys	1.11	1.04	1.08	1.01	1.11	1.01
Met	0.27	0.31	0.27	0.33	0.31	0.33
Met + Cys	0.56	0.63	0.48	0.57	0.52	0.57
Phe	0.98	0.96	0.75	0.76	0.77	0.76
Phe + Tyr	1.60	1.59	1.19	1.20	1.23	1.20
Thr	0.72	0.73	0.64	0.68	0.66	0.68
Trp	0.25	0.23	0.18	0.19	0.18	0.19
Val	0.94	0.90	0.89	0.89	0.92	0.89
Free AA, %						
Arg	0.03	0.00	0.01	0.00	0.01	0.00
His	0.00	0.00	0.07	0.07	0.07	0.07
Ile	0.01	0.00	0.08	0.08	0.08	0.08
Leu	0.01	0.00	0.01	0.00	0.43	0.45
Lys	0.02	0.00	0.36	0.37	0.37	0.37
Met[3]	0.00	0.00	0.07	0.11	0.07	0.11
Met + Cys	0.00	0.00	0.07	0.11	0.07	0.11
Phe	0.00	0.00	0.12	0.13	0.12	0.13
Phe + Tyr	0.01	0.00	0.12	0.13	0.12	0.13
Thr	0.02	0.00	0.20	0.20	0.20	0.20
Trp[4]	-	0.00	-	0.05	-	0.05
Val	0.00	0.00	0.27	0.29	0.27	0.29

[1] Analyzed values represent average across 3 blocks (feed mixes).

[2] Calculated values for the total AA are based on the AA concentration in feed ingredients according to NRC (2012), and calculated values for the free AA correspond to the dietary inclusion rate in crystalline form.

[3] Addition of DL-Met was omitted in one of the 3 blocks, thus reducing the overall free Met concentration across all 3 blocks. The average free Met concentration between blocks 1 and 3 was 0.11 and was zero in block 2. Therefore, across blocks 1, 2 and 3, average free Met was 0.07.

[4] Analysis of free Trp was not performed.

Table 2.3. Lactation performance of all sows fed Control (CON; 18.74 % CP), Optimal (OPT; 13.78% CP) or Optimal + Leucine (OPTLEU; 14.25% CP) over a 21-d lactation period

Item	Diet			SEM[1]	*P*-Value		
	CON	OPT	OPTLEU		OPT vs CON	OPTLEU vs. CON	OPTLEU vs. OPT
Number of sows	18	19	17				
Parity	3.4	3.5	3.3				
Sow ADFI, kg/d[2]							
Overall, day 1 to 21	5.30	5.18	5.23	0.22	0.809	0.923	0.970
Early, day 4 to 8	4.73	4.39	4.45	0.25	0.341	0.494	0.969
Peak, day 14 to 18	6.27	6.28	6.23	0.25	0.999	0.987	0.981
Sow initial BW, kg	246	249	252	7	0.921	0.787	0.953
Sow BW change[3], kg	-1.6	-8.4*	-0.6	3.0	0.282	0.969	0.216
Sow initial back fat, mm	16.9	18.8	18.8	1.4	0.432	0.445	1.000
Sow back fat change[3], mm	-1.2	-3.6*	-1.6	0.9	0.188	0.932	0.310
Litter size							
day 1[4]	10.3	10.3	10.2	0.2			
day 21	9.6	10.0	9.9	0.3			
Litter growth rate, kg/d[2]							
Overall, day 1 to 21	2.45	2.59	2.35	0.13	0.541	0.700	0.208
Early, day 4 to 8	2.33	2.35	2.44	0.18	0.990	0.854	0.911
Peak, day 14 to 18[5]	2.71	3.28	2.65	0.18	0.026	0.963	0.016
Piglet ADG, g/d[2]							
Overall, day 1 to 21	253	259	237	9	0.896	0.485	0.291
Early, day 4 to 8	233	234	244	15	1.000	0.877	0.885
Peak, day 14 to 18[5]	278	329	264	16	0.047	0.797	0.011

[1] Maximum value of the standard error of the least squares means.

[2] The main effect of period (early vs. peak) was significant ($P < 0.01$) for feed intake, LGR, and ADG. Interaction of treatment × period for LGR ($P = 0.035$) and ADG ($P = 0.033$). LGR = litter growth rate.

[3,*] Body weight and back fat change were different from 0 ($P = 0.025$ and $P = 0.005$, respectively).

[4] Litter size after standardization (within 24 h after parturition).

[5] One litter (OPTLEU) was excluded for LGR and ADG due to a negative growth rate.

Table 2.4. Performance and milk nutrient composition and yield in early and peak lactation periods of sows selected for the N balance studies and fed Control (CON; 18.74 % CP), Optimal (OPT; 13.78% CP) or Optimal + Leucine (OPTLEU; 14.25% CP) diets

Item	Diet			SEM[1]	*P*-Value		
	CON	OPT	OPTLEU		OPT vs CON	OPTLEU vs. CON	OPTLEU vs. OPT
Early Lactation (day 4-8)[2]							
Number of sows	12	11	11				
Sow ADFI, kg/d	4.93	4.64	4.58	0.23	0.390	0.268	0.957
Litter size	10.3	10.3	10.2	0.3			
Piglet ADG, g/d	248	248	255	21	1.000	0.962	0.957
Estimated milk yield, kg/d[3]	8.76	8.84	8.79	0.94	0.996	0.999	0.999
Milk nutrient concentration							
True protein, %	4.49	4.25	4.25	0.14	0.315	0.335	1.000
Urea nitrogen, mg/dL	12.30	3.81	3.51	0.82	< 0.001	< 0.001	0.949
Lactose, %	5.52	5.49	5.60	0.20	0.952	0.738	0.560
Fat, %	6.93	7.89	6.97	0.50	0.342	0.998	0.378
Milk nutrient output, g/d							
True protein output	390.5	375.3	387.4	39.0	0.954	0.998	0.971
Lactose output	484.6	486.7	494.5	53.4	0.999	0.981	0.988
Fat output	606.3	701.4	621.0	89.4	0.730	0.993	0.800
Peak lactation (day 14-18)[2]							
Number of sows	11	11	11				
Sow ADFI, kg/d	6.83	6.65	6.38	0.23	0.722	0.125	0.422
Litter size	9.9	10.2	9.9	0.3			
Piglet ADG, g/d	262	311	238	22	0.173	0.648	0.031
Estimated milk yield, kg/d[3]	11.62	13.90	11.01	0.98	0.059	0.809	0.016
Milk nutrient concentration							
True protein, %	4.41	4.35	4.39	0.14	0.934	0.994	0.966
Urea nitrogen, mg/dL	15.51	4.84	5.85	0.82	< 0.001	< 0.001	0.572
Lactose, %	5.65	5.69	5.62	0.20	0.888	0.965	0.755
Fat, %	6.23	7.76	7.00	0.50	0.083	0.510	0.510
Milk nutrient output, g/d							

Table 2.4. (cont’d)

True protein output	512.3	607.3	530.7	39.8	0.195	0.935	0.333
Lactose output	655.5	767.2	619.8	55.0	0.107	0.793	0.030
Fat output	725.9	1077.7	841.2	90.0	0.026	0.637	0.165

[1] Maximum value of the standard error of the least squares means.

[2] The main effect of period (early vs. peak) was significant except for ADG, milk fat, protein, lactose, and, milk N output/N intake.

[3] Estimated milk yield was based on piglet ADG.

Table 2.5. Nitrogen utilization for milk in early and peak lactation periods in sows selected for the N balance studies and fed Control (CON; 18.74 % CP), Optimal (OPT; 13.78% CP) or Optimal + Leucine (OPTLEU; 14.25% CP) diets[1]

Item	Diet			SEM[2]	*P*-Value		
	CON	OPT	OPTLEU		OPT vs CON	OPTLEU vs. CON	OPTLEU vs. OPT
Early lactation (day 4-8)[3]							
Number of sows	12	11	11				
Body weight, kg[4]	245.4	255.8	246.3	7.4	0.440	0.994	0.493
N intake, g/d	152.1	112.9	106.0	4.7	< 0.001	< 0.001	0.482
N absorbed, g/d	137.4	93.8	95.7	3.8	< 0.001	< 0.001	0.936
Dry fecal output, kg/d	0.523	0.538	0.586	0.057	0.979	0.713	0.826
Urine weight, kg/d	10.1	4.7	5.5	1.5	0.047	0.103	0.920
Urinary N, g/kg	4.91	3.22	3.23	0.64	0.161	0.166	0.999
N excretion, g/d							
Fecal N	14.6	15.5	16.6	1.5	0.909	0.625	0.864
Urinary N	37.8	14.3	14.9	4.4	< 0.001	< 0.001	0.993
Milk N	61.7	59.0	62.1	5.4	0.927	0.999	0.909
Total N retention, g/d	99.6	79.6	80.1	5.1	0.009	0.011	0.948
Maternal N retention, g/d[5]	37.8*	20.7*	20.0*	8.2	0.308	0.286	0.998
Apparent N utilization efficiency							
Total N retention, % of N intake	65.5	72.3	75.8	3.0	0.050	0.005	0.315
Total N retention, % of N absorbed	72.4	84.6	84.1	3.0	0.002	0.003	0.882
Milk N output, % of N intake	41.1	54.7	58.3	3.2	0.005	< 0.001	0.417
Milk N output, % of N absorbed	45.4	63.6	66.1	3.8	0.006	0.002	0.890
Peak lactation (day 14-18)[3]							
Number of sows	11	11	11				
Body weight, kg[4]	249.4	249.3	250.0	7.5	0.999	0.998	0.996
N intake, g/d	210.0	151.5	145.7	4.4	< 0.001	< 0.001	0.565
N absorbed, g/d	189.3	130.3	122.3	3.8	< 0.001	< 0.001	0.311
Dry fecal output, kg/d	0.72	0.74	0.81	0.06	0.971	0.461	0.600
Urine weight, kg/d	13.2	5.6	6.1	1.5	0.005	0.009	0.969

Table 2.5. (cont'd)

Urinary N, g/kg	4.06	3.30	3.17	0.64	0.683	0.593	0.988
N excretion, g/d							
Fecal N	20.3	21.2	22.9	1.5	0.901	0.429	0.689
Urinary N	36.9	17.7	18.6	4.5	0.006	0.008	0.984
Milk N	81.7	99.4	85.5	5.4	0.064	0.871	0.168
Total N retention, g/d	149.8	112.7	109.6	5.2	< 0.001	< 0.001	0.671
Maternal N retention, g/d	68.3[*]	13.4	17.8[*]	8.2	< 0.001	< 0.001	0.922
Apparent N utilization efficiency							
Total N retention, % of intake	71.4	74.5	73.4	3.0	0.363	0.556	0.756
Total N retention, % of absorbed	79.2	86.6	87.2	3.0	0.050	0.037	0.862
Milk N output, % of N intake	39.5	62.9	58.4	3.6	< 0.001	< 0.001	0.328
Milk N output, % of N absorbed	43.9	73.2	69.5	3.8	< 0.001	< 0.001	0.780

[1] Nitrogen balance was conducted between day 4 and day 8 or day 14 and day 18 for either 48 h or 72 h.

[2] Maximum value of the standard error of the least squares means.

[3] The main effect of period was significant for all variables, except BW, UN output, maternal N retention, NB/N intake, milk N/N intake, milk N/N absorb.

[4] Body weight of day 1 and day 21 were used as reference for early and peak lactation.

[*] Maternal N retention was different from 0 ($P < 0.05$).

Table 2.6. True dietary AA utilization efficiency estimated based on maternal N retention for milk protein production of sows fed Control (CON; 18.74 % CP), Optimal (OPT; 13.78% CP) or Optimal + Leucine (OPTLEU; 14.25% CP) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation)

Item	Diet			NRC 2012[1]	SEM[2]	*P*-value		
	CON	OPT	OPTLEU			OPT vs CON	OPTLEU vs. CON	OPTLEU vs. OPT
Early Lactation (day 4 - 8)[3]								
Number of sows[4]	12	10	11					
Arg	32.8	58.4	54.9	-	3.7	< 0.001	< 0.001	0.728
His	54.1	74.3	72.5	-	5.1	0.002	0.004	0.755
Ile	41.7	61.9	59.4	-	4.5	0.001	0.002	0.853
Leu	45.2	71.2	48.1	-	3.5	< 0.001	0.491	< 0.001
Lys	57.3	60.1	58.5	-	3.6	0.823	0.960	0.944
Met	62.4	64.6	56.0	-	4.3	0.885	0.368	0.197
Met+Cys	59.8	74.3	68.2	-	5.6	0.035	0.274	0.520
Phe	36.9	50.6	49.5	-	3.7	0.006	0.010	0.955
Phe+Tyr	45.8	65.8	63.9	-	4.4	0.002	0.004	0.926
Thr	58.7	67.4	66.4	-	4.6	0.252	0.314	0.984
Trp	44.5	66.1	66.7	-	6.0	0.010	0.008	0.996
Val	50.4	54.2	52.7	-	3.9	0.645	0.846	0.934
N	50.7	75.4	72.7	-	4.1	< 0.001	0.002	0.882
EAA[5]	50.1	63.4	58.8	-	4.2	0.026	0.167	0.607
Peak lactation (day 14-18)[3]								
Number of sows[4]	9	10	9					
Arg	33.8	63.8	57.5	-	3.8	< 0.001	< 0.001	0.399
His	55.7	82.2	75.9	-	5.3	< 0.001	0.004	0.308
Ile	42.9	68.8	62.3	-	4.6	< 0.001	0.003	0.391
Leu	46.3	79.1	50.7	-	3.7	< 0.001	0.366	< 0.001
Lys	58.9	66.2	61.3	-	3.8	0.325	0.893	0.581
Met	64.5	71.3	58.3	-	4.5	0.375	0.460	0.041
Met+Cys	61.8	82.2	71.0	-	5.8	0.005	0.287	0.148

Table 2.6. (cont'd)

Phe	38.1	56.3	51.8	-	3.8	< 0.001	0.011	0.542
Phe+Tyr	47.0	73.2	67.1	-	4.6	< 0.001	0.004	0.497
Thr	60.5	74.5	69.3	-	4.7	0.054	0.302	0.628
Trp	45.7	74.2	69.6	-	6.1	0.001	0.007	0.792
Val	51.8	59.9	55.0	-	4.1	0.191	0.764	0.531
N	51.9	82.7	75.9	-	4.3	< 0.001	0.002	0.497
EAA[5]	51.6	70.2	61.6	-	4.4	0.003	0.149	0.212
Overall lactation[6]								
Arg	33.3	61.1	56.2	81.6	3.2	0.004	0.008	0.469
His	54.9	78.3	74.2	72.2	4.4	0.016	0.030	0.679
Ile	42.3	65.4	60.8	69.8	4.0	0.007	0.016	0.500
Leu	45.7	75.1	49.4	72.3	2.9	0.002	0.574	0.004
Lys	58.1	63.2	59.9	67.0	2.9	0.451	0.889	0.688
Met	63.4	67.9	57.2	67.5	3.8	0.523	0.333	0.100
Met+Cys	60.8	78.2	69.6	66.2	5.1	0.034	0.224	0.230
Phe	37.5	53.4	50.6	73.3	3.2	0.016	0.030	0.677
Phe+Tyr	46.4	69.5	65.5	70.5	3.7	0.010	0.021	0.620
Thr	59.6	71.0	67.9	76.4	3.9	0.123	0.254	0.773
Trp	45.0	70.1	68.1	67.4	5.5	0.029	0.038	0.941
Val	51.1	57.0	53.8	58.3	3.4	0.297	0.719	0.650
N	51.3	79.1	74.3	75.9	3.3	0.009	0.017	0.600
EAA[5]	50.8	66.8	60.2	-	3.6	0.029	0.141	0.293

[1] Efficiency values of AA for lactation were reported by NRC (2012) only for the whole lactation period.

[2] Maximum value of the standard error of the least squares means.

[3] The main effect of period was not significant (EAA period effect: CON, *P* = 0.740; OPT, *P* = 0.128; OPTLEU, *P* = 0.537).

[4] Only sows consuming at least 75% of the predicted feed intake over the entire 4 day periods (i.e., 4-8 day and 14-18 day) were included in the estimation of efficiency values.

[5] EAA is the average efficiency values of all the EAA listed above excluding Arg.

[6] Mean values between early and peak.

CHAPTER 3

REDUCED PROTEIN DIET WITH NEAR IDEAL AMINO ACID PROFILE IMPROVES ENERGY EFFICIENCY AND MITIGATE HEAT PRODUCTION ASSOCIATED WITH LACTATION IN SOWS

ABSTRACT

The study objective was to test the hypothesis that 1) lowering dietary crude protein (CP) increases dietary energetic efficiency and reduces metabolic heat associated with lactation, and 2) excessive dietary leucine (Leu) supplementation in a low CP diet decreases dietary energetic efficiency and increases metabolic heat associated with lactation. Fifty-four lactating multiparous Yorkshire sows were allotted to 1 of 3 isocaloric diets (2,580 kcal/kg net energy): 1) Control (CON; 18.75% CP), 2) reduced CP with a near ideal or optimal AA profile (OPT; 13.75% CP) and 3) diet OPT with excessive Leu (OPTLEU; 14.25% CP). Sow body weight and backfat were recorded on day 1 and 21 of lactation and piglets were weighed on day 1, 4, 8, 14, 18, and 21 of lactation. Energy balance was measured on sows during early (day 4 - 8) and peak (day 14 -18) lactation, and milk was sampled on day 8 and 18. Over 21-day lactation, sows fed OPT lost body weight and body lipid ($P < 0.05$). In peak lactation, sows fed OPT had higher milk energy output ($P < 0.05$) than CON. Sows fed OPTLEU tended ($P = 0.07$) to have less milk energy output than OPT and did not differ from CON. Maternal energy retention was lower ($P < 0.05$) in OPT and OPTLEU compared to CON sows, and did not differ between OPTLEU and OPT sows. Milk nitrogen output relative to metabolizable energy intake tended to be higher ($P = 0.088$) for sows fed OPT than CON. Sows fed OPT had higher ($P < 0.05$) apparent energy efficiency for milk production compared to CON. Heat production associated with lactation was lower ($P < 0.05$) or tended to be lower ($P = 0.082$), respectively, in OPT and OPTLEU compared to CON sows. To summarize, the OPT diet, in peak lactation, improved dietary energy utilization for lactation due to less urinary

energy and metabolic heat loss, and triggered dietary energy deposition into milk at the expense of maternal lipid mobilization. Leucine supplementation above requirement, in peak lactation, may reduce dietary energy utilization for lactation by decreasing the energy partitioning towards milk, partially explaining the effectiveness of OPT diet over CON diets.

INTRODUCTION

Lactation is an energetically costly process that depends on the sow's ability to consume enough energy to sustain milk production. Voluntary feed intake however is biologically limiting (Eissen et al., 2000) and the sow must rely of her body fat and protein when milk energy demand exceeds energy intake. Over the past decades, larger litter size at birth due to genetic selection have increased lactation demands (Strathe et al., 2016; Zhang et al., 2016). Strategies to improve the efficiency of dietary energy utilization are needed to sustain greater levels of milk production.

Lowering dietary crude protein (CP) in growing-finishing pigs improves energetic efficiency (i.e., retained tissue net energy:gross energy intake) due to reduced heat and urinary energy loss (Le Bellego et al., 2001; Kerr et al., 2003). Feeding diets with reduced CP concentrations and improved amino acid (AA) balance to lactating sows improve the efficiency of N and essential amino acid (EAA) utilization (Huber et al., 2015; Huber et al., 2016). In Chapter 2 (Zhang et al., 2019), feeding a diet with NIAA profile maximized efficiency of utilization for several EAA and reduced urinary N excretion and appeared to increase nutrient partitioning towards mammary metabolism. Therefore, in this chapter, the impact of feeding such diet on energy partitioning and efficiency is examined. In addition, NIAA profile may also reduce heat production due to changes in metabolic demand resulting from less AA destined to oxidation.

In Chapter 2 (Zhang et al., 2019), it was hypothesized that the improved AA utilization efficiency from feeding reduced CP diets may be associated with lower intake of leucine (Leu). The premise was based on the notion that high Leu concentrations inhibit lysine (Lys) uptake in rat mammary explants (Shennan et al., 1994; Calvert and Shennan, 1996), and that potential competitive inhibition exists between Lys and Leu utilization by the mammary gland (Guan et al., 2004; Manjarín et al., 2012), as reviewed in Chapter 1. Addition of Leu to a reduced CP diet however did not have noticeable impact on Lys efficiency, but milk yield in peak lactation was reduced and similar to that of sows fed a conventional diet, indicating some energy partitioning away from the mammary gland. In contrast, the reduced CP diet without added Leu led to greater milk yield, milk fat and lactose output and litter growth rate, but also resulted in body weight (BW) and back fat losses during peak lactation. What was noticeably greater was the milk fat concentration and milk fat yield in sows fed the reduced CP diet. Estimation of body lipid mobilization is needed to further understand the potential impact of feeding an improved AA profile on energy partitioning.

The study objective was to estimate dietary energetic efficiency, energy partitioning and heat production for lactation in sows fed the same diets as presented in Chapter 2: a conventional diet with Leu:Lys of 1.63 (control), a reduced CP diet meeting the minimum standardized ileal digestibility (SID) requirement for Leu (NRC, 2012) and with Leu:Lys of 1.14 (optimal), and a reduced CP diet with a SID Leu concentration and ratio to Lys to be the as that of control (i.e., 1.63) (optimal + Leu). It is hypothesized that 1) lowering CP to meet the minimum SID Leu requirement and Leu:Lys of 1.14, increases dietary energetic efficiency for lactation and reduces heat production associated with lactation compared to a non-reduced CP diet with Leu:Lys of 1.63, and 2) supplementation of Leu to the reduced CP diet to meet Leu:Lys of 1.63 reduces dietary energy partitioning towards milk compared to the reduced CP diet with Leu:Lys of 1.14.

MATERIALS AND METHODS

Animals and Feeding

Fifty-four purebred multiparous (parity 3.4 ± 0.6) Yorkshire sows were selected at day 105 of gestation, balanced by parity and randomly assigned to 1 of 3 dietary treatments [control (CON), n = 18; Optimal (OPT), n = 19; Optimal + Leu (OPTLEU), n = 17)]. Sows were moved to conventional farrowing crates and accustomed to their experimental diets beginning at day 105 of gestation. Within the first 24 h of farrowing, litters were equalized to 11 piglets with the objective of weaning 10 piglets per sow. Sows were gradually fed 1.88 kg/d on day 1 to reach 7.44 kg/d on day 21 of lactation according to the NRC model (2012), corresponding to an average daily feed intake of 6 kg/d. Sows were provided 3 meals (0700, 1300, 1700 h) daily with actual feed intake and feed refusal recorded before each morning meal. Fresh water was available freely for all sows and piglets. Iron injection and surgical castration were conducted on day 1 and 7 post-farrowing, respectively, according to farm protocol. Piglets were not supplied with creep feed. The BW and backfat thickness of sows were recorded on day 1 and 21, and litter weights were recorded on day 1, 4, 8, 14, 18, and 21. Milk yield was estimated for early (between day 4 and 8) and peak lactation (between day 14 and 18) according to Zhang et al. (2019).

Dietary Treatment

Ingredients and calculated nutrient composition of the diets are presented in Table 2.1. Analyzed total (hydrolysate) and free AA of the diets are presented in Table 2.2. The NRC (2012) model was used to estimate requirements for AA, net energy (NE), calcium (Ca) and phosphorus (P). The requirements were predicted based on the swine herd performance at the Michigan State University Swine Teaching and Research Center, as follows: sow BW of 210 kg, parity number of 2 and above, and daily intake of 6 kg/day, litter size of 10, piglet BW gain of 280 g/day over a 21-

day lactation period, and an ambient temperature of 20 °C. The model predicted a minimum sow BW loss of 7.5 kg and the protein:lipid in the model was adjusted to the minimum allowable value of near zero. All diets were formulated to contain the same SID Lys (0.9%) and NE (2,580 kcal/kg) concentrations.

The control diet (CON) was formulated using corn and soybean meal as the only sources of Lys to meet NRC (2012) SID Lys requirement (0.9%) and consequently contained 18.75% CP. Valine met near SID requirement of 0.77% (vs. 0.79%) (NRC, 2012). All other EAA SID concentrations were in excess relative to NRC (2012).

A second diet balanced to reach a near ideal AA (NIAA) profile was formulated. In the present study, the term "near ideal AA profile" was chosen in lieu of the conventional "ideal AA profile" because the "ideal AA profile" is conceptual rather than biologically factual. The rationale is further based on the notions that an "ideal AA profile" 1) cannot be limited to the relative contribution of only two AA pools (i.e., milk and maintenance), 2) needs accurate characterization of the maintenance AA pool for the lactating sow, and 3) should include AA for which dietary essentiality is known for lactating sow (i.e., arginine and histidine). The NIAA diet was designed by reducing soybean meal relative to corn to meet the minimum SID Leu requirement, which corresponded to a CP concentration of 13.75%. Then, supplemental crystalline source of L-histidine (His), L-isoleucine (Ile), L-lysine, DL-methionine (Met), L-phenylalanine (Phe), L-threonine (Thr), L-tryptophan (Trp) and L-valine (Val) and were added to meet the minimum SID requirement for those AA in the NIAA diet. Crystalline DL-methionine was added to meet the requirement of Met + cysteine (Cys). This diet is referred to as the optimal diet (OPT) throughout the remainder of the manuscript.

A third diet was formulated to be the same as OPT with added crystalline L-leucine to equate the SID Leu concentration of CON and referred to as optimal + Leu diet (OPTLEU). Sugar food product (International Ingredient Corporation, St. Louis, MO) was included in all 3 diets at 5% to increase diet palatability. Titanium dioxide was included at 0.1% as an indigestible marker in all diets.

Energy Balance Procedure and Milk Sampling

Energy balance was performed during early lactation (between day 4 and 8) and peak lactation (between day 14 and 18) on a total of 33 sows. Urinary catheter insertion, urine collection and sow milk sampling were carried out according to Chapter 2 (Zhang et al., 2019).

Energy, Nutrient and Titanium Analysis

Feed, fecal and urinary samples were analyzed for gross energy (GE) by bomb calorimetry according to the manufacturer's instructions (Parr Instrument Inc., Moline, IL). Dry matter, N and titanium in feed and fecal samples were analyzed according to Zhang et al. (2019). Dietary AA analysis [AOAC Official Method 982.30 E (a,b,c), 45.3.05, 2006] was performed by the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) as outlined in Zhang et al (2019).

Whole milk samples were analyzed for fat, true protein, lactose, and milk urea N (MUN) with infrared spectroscopy by the Michigan Dairy Herd Improvement Association (NorthStar Cooperative®, Lansing, MI) (Zhang et al., 2019).

Calculations

Calculation of body protein (BP; Eq. 3, 4, and 5) and lipid (BL; Eq. 2, 4, and 5) composition were predicted by empty body weight (EBW; Eq. 1) and backfat (NRC, 2012) using the following equations:

$$\text{EBW (kg)} = 0.96 \times \text{maternal BW (kg)} \tag{1}$$

$$\text{Maternal BL (kg)} = -26.4\ \text{(kg)} + 0.221 \times \text{maternal EBW (kg)} + 1.331 \left(\frac{\text{kg}}{\text{mm}}\right) \times \text{P2 backfat (mm)} \tag{2}$$

$$\text{Maternal BP (kg)} = 2.28\ \text{(kg)} + 0.178 \times \text{maternal EBW (kg)} - 0.333 \left(\frac{\text{kg}}{\text{mm}}\right) \times \text{P2 backfat (mm)} \tag{3}$$

$$\text{Maternal BL or BP change (kg)} = \text{d 21 of maternal BL or BP (kg)} - \text{d 1 of maternal BL or BP (kg)} \tag{4}$$

$$\text{Maternal BP or BL Composition (\%)} = \frac{\text{Maternal BP or BL (kg)}}{\text{EBW (kg)}} \times 100\% \tag{5}$$

Calculation of total (Eq. 6) and maternal (Eq. 7) energy retention were performed as follows:

$$\text{Total energy retention} \left(\frac{\text{kcal}}{\text{d}}\right) = \text{energy intake} \left(\frac{\text{kcal}}{\text{d}}\right) - \text{fecal energy output} \left(\frac{\text{kcal}}{\text{d}}\right) - \text{urinary energy output} \left(\frac{\text{kcal}}{\text{d}}\right) - \text{energy for maintenance} (\frac{\text{kcal}}{\text{d}}) \tag{6}$$

$$\text{Maternal energy retention} \left(\frac{\text{kcal}}{\text{d}}\right) = \text{energy intake} \left(\frac{\text{kcal}}{\text{d}}\right) - \text{fecal energy output} \left(\frac{\text{kcal}}{\text{d}}\right) - \text{urinary energy output} \left(\frac{\text{kcal}}{\text{d}}\right) - \text{energy for maintenance} \left(\frac{\text{kcal}}{\text{d}}\right) - \text{milk energy output} \left(\frac{\text{kcal}}{\text{d}}\right) \tag{7}$$

Metabolizable energy (ME) value of diets for maintenance (kcal/kg feed; Eq. 8) and ME requirement per day (kcal/day; Eq. 9) was calculated based on metabolic body weight ($BW^{0.75}$) as follows:

$$\text{ME for maintenance} \left(\frac{\text{kcal}}{\text{kg feed}}\right) = \frac{\text{Daily ME for mainteance}(\frac{\text{kcal}}{\text{d}})}{\text{Daily intake } (\frac{\text{kg}}{\text{d}})} \tag{8}$$

$$\text{Daily ME for maintenance}\left(\frac{\text{kcal}}{\text{d}}\right) = 100 \times \text{BW}^{0.75} \tag{9}$$

The net energy (NE) value of diets for lactation was calculated as follows (Eq. 10):

$$\text{Dietary NE for lactation }\left(\frac{\text{kcal}}{\text{kg feed}}\right) = \text{NE in milk }\left(\frac{\text{kcal}}{\text{kg feed}}\right) - \text{NE mobilized }\left(\frac{\text{kcal}}{\text{kg feed}}\right) \tag{10}$$

where,

$$\text{NE in milk (kcal/kg feed)} = \frac{\text{Daily energy output in milk}\left(\frac{\text{kcal}}{\text{d}}\right)}{\text{Daily intake}\left(\frac{\text{kg}}{\text{d}}\right)} \tag{11}$$

$$\text{NE mobilized (kcal/kg feed)} = \frac{\text{Daily energy mobilized}\left(\frac{\text{kcal}}{\text{d}}\right)}{\text{Daily intake}\left(\frac{\text{kg}}{\text{d}}\right)} \tag{12}$$

Apparent energy efficiency for milk was calculated as follows (Eq. 13):

$$\text{Apparent energy efficiency(\%)} = \frac{\text{Milk energy output }(\frac{\text{kcal}}{\text{d}})}{\text{Energy intake or absorbed }(\frac{\text{kcal}}{\text{d}})} \times 100\% \tag{13}$$

Apparent energy efficiency does not account for the milk energy originating from mobilized body pool and energy lost in urine. To determine true energy efficiency for milk (Eq. 14), energy mobilized from the body was removed from the daily energy in milk (Eq. 15), and energy for maintenance was removed from ME intake (Eq. 16) as follows:

$$\text{True energy efficiency(\%)} = \frac{\text{Daily dietary energy in milk}\left(\frac{\text{kcal}}{\text{d}}\right)}{\text{Daily dietary ME for milk}\left(\frac{\text{kcal}}{\text{d}}\right)} \times 100\% \tag{14}$$

Where,

$$\text{Daily dietary energy in milk}\left(\frac{\text{kcal}}{\text{d}}\right) = \text{Daily energy in milk}\left(\frac{\text{kcal}}{\text{d}}\right) - \text{daily milk energy mobilized from body }(\frac{\text{kcal}}{\text{d}}) \tag{15}$$

$$\text{Daily dietary ME for milk}\left(\frac{\text{kcal}}{\text{d}}\right) = \text{Daily ME intake}\left(\frac{\text{kcal}}{\text{d}}\right) - \text{daily ME for maintenance}\left(\frac{\text{kcal}}{\text{d}}\right) \tag{16}$$

Energy in milk was calculated by summing energy in milk protein (5.7 kcal/g), fat (9.5

kcal/g) and lactose (3.95 kcal/g), respectively (Weast et al., 1984). Energy mobilized from the maternal body was calculated based on change in body protein (△BP) and change in body lipid (△BL) multiplied by 5.6 kcal/g protein and 9.4 kcal/g fat (Ewan, 2001; Eq. 17), respectively, with an efficiency of body energy mobilization to milk of 0.87 (NRC 2012), as follows:

$$\text{Mobilized energy}\ (\frac{\text{kcal}}{\text{d}}) = -(\Delta\text{BP} \times 5.7\ \frac{\text{kcal}}{\text{g}} + \Delta\text{BL} \times 9.4\ \frac{\text{kcal}}{\text{g}}) \times 0.87 \quad (17)$$

A value of 0 was used for mobilized energy when sow body protein and fat depositions were null or positive.

The ME for maintenance was calculated based on NRC (2012) as follows (Eq. 18):

$$\text{ME}_{\text{maintenance}}\ (\frac{\text{kcal}}{\text{d}}) = 100 \times \text{BW}^{0.75} \quad (18)$$

The NE for maintenance was assumed to be equal to ME for maintenance (Figure 3.1; Eq. 19)

$$\text{NE}_{\text{maintenance}} \left(\frac{\text{kcal}}{\text{d}}\right) = \text{ME}_{\text{maintenance}} \left(\frac{\text{kcal}}{\text{d}}\right) = 100 \times \text{BW}^{0.75} \quad (19)$$

The corrected dietary NE (NE$_c$) was calculated as follows (Pedersen et al., 2019; Eq. 20):

$$\text{NE}_{\text{c}}\ (\text{kcal/kg feed}\ ) = \frac{\text{NE}_{\text{maintenance}}\left(\frac{\text{kcal}}{\text{day}}\right) + \text{daily milk energy}\left(\frac{\text{kcal}}{\text{day}}\right) - \text{daily mobilized energy}\ (\frac{\text{kcal}}{\text{day}})}{\text{Daily feed intake}\ (\frac{\text{kg}}{\text{day}})} \quad (20)$$

Heat production associated with lactation was calculated as follows (Eq. 21):

$$\text{Heat Production}_{\text{lactation}} \left(\frac{\text{kcal}}{\text{d}\cdot\text{BW}^{0.75}}\right) = \frac{\text{Daily heat production}_{\text{lactation}}\ (\frac{\text{kcal}}{\text{d}})}{\text{Sow metabolic body weight}\ (\text{BW}^{0.75})} \quad (21)$$

Where,

$$\text{Daily Heat Production}_{\text{lactation}} \left(\frac{\text{kcal}}{\text{d}}\right) = \text{Daily dietary ME for milk} \left(\frac{\text{kcal}}{\text{d}}\right) -$$

$$\text{Daily dietary energy in milk} \left(\frac{\text{kcal}}{\text{d}}\right) \quad (22)$$

Statistical Analysis

Statistical analyses were conducted using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) according to the following model:

$\text{Response} = \text{diet} + \text{parity} + \text{period} + \mathit{block} + \mathit{sow}_{\text{diet}\times\text{block}} + \text{diet} \times \text{parity} + \text{diet} \times \text{period} + \mathit{diet} \times \mathit{block} + e$

The response of sow depended on the fixed effects of **diet** (CON, OPT, and OPTLEU), **parity** (early [P 2-3] and late [P 4-6]), and lactation **period** (early [d 4-8] vs. peak [d 14-18]). The random effects included ***block***, and ***sow*** nested within diet and block. The interactive effects of **diet** × **parity**, **diet** × **period**, and ***diet*** × ***block*** were also included. When appropriate, a reduced model was used. Specifically, parity and parity × treatment effects were not significant and therefore were not included in the reduced model for analyses of body tissue mobilization, energy balance, energy partitioning, estimated water output, energy efficiency and estimated total heat production. Pairwise comparisons were performed between diets (OPT vs. CON, OPTLEU vs. CON, and OPTLEU vs. OPT) for different periods of lactation (early, peak, and 21-d overall lactation) and between early and peak lactation for each diet using the slice option in SAS and Tukey adjustment. Simple t-test was conducted to compare the analyzed and calculated NE values. Effects were declared significant at $P \leq 0.05$, and tendencies were declared at $0.05 \leq P \leq 0.10$.

RESULTS

Experimental Diets

Diet composition and nutrient concentrations are presented in Table 2.1 and EAA concentrations are presented in Table 2.2, as described in Chapter 2 (Zhang et al., 2019).

Body Protein and Lipid Mobilization

The BP and BL mobilization over 21-day of lactation for all sows are presented in Table 3.1. Sow BW change, BP and BL mobilization did not differ between treatments. Body weight loss and BL mobilization differed from 0 ($P < 0.05$) in sows fed OPT.

Energy Balance

Energy balance results are presented in Table 3.2. In early lactation, urinary and milk energy concentration and output, and total and maternal energy retention did not differ across diets. In peak lactation, urinary energy concentration did not differ across diets. Sows fed OPT had lower urinary energy output ($P < 0.05$) than CON, while sows fed OPTLEU did not differ from either CON or OPT. Sows fed OPT had higher milk energy concentration ($P < 0.05$) and milk energy output ($P < 0.05$) than CON. Sows fed OPTLEU tended to ($P = 0.07$) have less milk energy output than OPT, and did not differ from CON in either milk energy concentration or output. Total energy retention did not differ across diets. Maternal energy retention was lower ($P < 0.05$) in sows fed low protein diets (OPT and OPTLEU) than those fed CON, and did not differ between OPTLEU and OPT.

Apparent Efficiency of Nitrogen and Energy

Apparent efficiency of N and energy utilization results are presented in Table 3.3. In early lactation, milk N output relative to ME or NE intake, and apparent energy efficiency for milk did not differ across diets. In peak lactation, milk N output relative to NE intake did not differ across diets. Milk N output relative to ME intake in OPT tended to be higher ($P = 0.088$) than CON, and those in OPTLEU did not differ from either CON or OPT. Sows fed OPT had higher ($P < 0.05$) apparent energy efficiency for milk compared to CON, and sows fed OPTLEU did not differ from either CON or OPT.

Dietary Energy Partitioning

Dietary energy partitioning is presented in Tables 3.4 and 3.5. In both early and peak lactation (Table 3.4), digestible energy (DE) value of low protein diets was lower (P < 0.01; OPTLEU) or tended to be lower (P = 0.06; OPT) than that of CON. The DE value of OPTLEU did not differ from OPT. The ME and $NE_{lactation}$ values of all diets did not differ. The analyzed $NE_{lactation}$ value was lower (P < 0.05) than the calculated NE value across all diets.

The energy values of NE, ME, DE expressed relative to ME, DE and GE, respectively, are presented in Table 3.6. In early lactation, the ME/DE, $NE_{lactation}$/ME, and NE_{milk}/ME did not differ across diets. In peak lactation, the ME/DE tended to be higher (P = 0.063) in OPT than CON. The ME/DE in OPTLEU did not differ from either CON or OPT. Compared to CON, the NE_{milk}/ME and $NE_{lactation}$ /ME was higher (P < 0.01) or tended to be higher (P = 0.092), respectively, in OPT. The NE_{milk}/ME and $NE_{lactation}$/ME in OPTLEU did not differ from either CON or OPT. In both early and peak lactation, the DE/GE did not differ between CON and OPT, and was lower (P < 0.01) in sows fed OPTLEU than those fed CON or OPT. The $NE_{lactation}$/ME did not differ across diets.

Energy Efficiency and Estimated Heat Production Associated with Lactation

True energy efficiency and estimated heat production associated with lactation are presented in Table 3.6. In early lactation, heat production did not differ across diets. In peak lactation, compared to CON, heat production was lower (P < 0.05) or tended to be lower (P = 0.082) in sows fed OPT and OPTLEU, respectively, and did not differ between OPT and OPTLEU. In both early and peak lactation, true milk energy efficiency did not differ across diets. Over 21-day lactation period, true milk energy efficiency and heat production did not differ across diets.

DISCUSSION

In Chapter 2, reducing dietary protein to meet the minimum SID Leu requirement increased utilization efficiency of N, arginine (Arg), His, Ile, Leu, Phe + tyrosine (Tyr) and Trp for milk yield while maintaining overall lactation performance. Supplementing Leu to the reduced CP diet did not impact the efficiency of EAA utilization but appeared to repartition nutrients away from the mammary gland. The current work aimed at determining dietary energetic efficiency, partitioning, and heat production associated with lactation in sows fed a reduced protein diet with a NIAA profile (OPT) and OPT diet with supplemental Leu (OPTLEU).

The loss of BW in sows fed OPT was mainly associated with BL rather than BP loss. Mobilization of BL, which is energy dense compared to protein (Ewan, 2001), is more efficient than mobilization of BP to satisfy the energy need for milk production. As reported in Chapter 2, milk fat content of sows fed OPT was greater, further supporting that the increased BW loss was associated mainly with BL for these sows. Sows generally lose more BL than BP throughout lactation (Strathe et al., 2017). Pedersen et al. (2019) reported the loss of BW in lactating sows fed diets containing CP from 14.6% to 18.6% was due to BL mobilization. On the other hand, Huber et al. (2015) reported that sows fed a similar low CP diet as this study lost BW over a 21-day lactation period, and indicated based on loin eye area measurements that the BW loss resulted from greater body protein as opposed to BL mobilization. The greater BP loss in that study may have been associated with feeding diets marginally deficient in Lys (Huber et al., 2015). In contrast, in Chapter 2, sows fed CON and OPTLEU lost a minimal amount of BW and were in a positive maternal N balance. This observation suggested that Leu to Lys of 1.63 may impact partitioning of DE by directing energy away from mammary gland and towards the maternal pool.

In this chapter, mobilization of BP and BL were quantified with BP values in the range reported by Pedersen et al. (2019) (i.e., 28 to 64 g/d vs. 20 to 40 g/day) for sows fed CP diets ranging from 14.6% to 18.6%, but those for BL were noticeably lower (i.e., 106 to 377 g/d vs. 800 to 820 g/day). It is unclear whether estimation of BL and BP mobilization by Pedersen et al. (2019) was associated with water or not. In this current study, BL and BP were quantified with or without water (Table 3.1). The other possible reason may be ascribed to a different prediction approach. Herein, BP and BL were predicted based on sow BW and P2 backfat thickness equations outlined in NRC (2012), while Pedersen et al. (2019) included D_2O space in addition to sow BW and P2 backfat thickness (Rozeboom et al., 1994). Earlier on, Pedersen et al. (2016) estimated BL and BP relative to BW. Their values were 15.7 and 26.8% for BP and BL, respectively, on day 3 of lactation, and 16.7 and 20.9% for BP and BL, respectively, on day 28 of lactation. In this study, on day 1 of lactation, BP and BL were 15.7 and 19.6%, respectively, for CON, and 15.5 and 20.6%, respectively, for OPT. On day 21 of lactation, BP and BL were 15.9 and 18.9%, respectively, for CON, and 15.9 and 18.6%, respectively, for OPT. Again, the predictions of BP % are fairly close between this study and those of Pedersen et al. (2016), but those of BL% are lower. It is possible that the approach of NRC (2012) may yield lower BL prediction than that of Rozeboom et al. (1994). Litter gain (22.4% and 23.4% greater) and therefore lactation energy demand was considerably greater in both studies by Pedersen et al. (2016 and 2019), compared to that of the current study. With the advancement of lactation, BL decreased by 5.9% (Pedersen et al., 2016) from day 3 to 28, and in this study, BL% decreased by 0.7 and 2% in CON and OPT, respectively, from day 1 to 21.

Feeding the OPT diet improved apparent energy utilization efficiency as well as milk N output efficiency relative to ME intake in peak lactation. Total energy retained was similar across diets, but sows fed OPT retained less maternal energy, suggesting that OPT diet resulted in more energy partitioning for milk production. Huber et al (2015) indicated that reduced protein diets favored partitioning of AA towards milk protein yield rather than maternal protein pool. This observation may be in part related to a reduced dietary Leu intake, because Leu stimulates maternal body protein gain (Norton et al., 2012; Wilkinson et al., 2013). The decreased milk energy output in OPTLEU compared to OPT during peak lactation combined with no differences in total energy retention across dietary treatments implies that additional Leu above requirement may reduce dietary energy partitioning towards milk. This observation is in line with N balance data presented in Chapter 2 (Zhang et al., 2019), where sows fed CON and OPTLEU did not lose as much BW as OPT and were in a positive maternal N balance.

The higher NE:ME and ME:DE in peak lactation for OPT fed sows aligns with their improved apparent energy efficiency in peak lactation compared to CON. In addition, the lack of difference in DE:GE in peak lactation indicates that the improvement in apparent energy efficiency in peak lactation likely occurred during the post-absorptive stage. By definition, urinary energy loss and heat increment represent the difference between “DE to ME” and “ME to NE” (Ewan, 2001), suggesting that the improved apparent energetic efficiency in OPT in peak lactation was due to less urinary energy and metabolic heat loss (Le Bellego et al., 2001; Pedersen et al., 2019). In fact, urinary energy loss and estimated heat production associated with lactation in the current study was lower in OPT than CON during the peak lactation period. Other studies on growing-finishing pigs (Le Bellego et al., 2001; Otto et al., 2003) and lactating sows (Huber et al., 2015; Zhang et al., 2019) showed that urinary N loss decreased by reducing dietary protein. Considering the major contributor of urinary energy is urinary N, primarily from urea (NRC, 2012), less urinary N loss also implies less urinary energy loss. Previous research in growing pigs also showed a 6.7% or 23.9 $\text{kcal}\cdot\text{d}^{-1}\cdot\text{BW}^{-0.65}$ decrease in heat production associated with feeding lower dietary CP (Le

Bellego et al., 2001). During the entire lactation period, the estimated heat associated with lactation was 69.1, 36.8, and 32.0 kcal·d^{-1}·BW$^{-0.75}$ for CON, OPT and OPTLEU, respectively, corresponding to a 46.7% or 32.3 kcal·d^{-1}·BW$^{-0.75}$ reduction in heat between CON and OPT. Note that the total heat production (maintenance + lactation (Figure 3.2) added up to be 169.7, 140.3 and 130.5 kcal·d^{-1}·BW$^{-0.75}$ for CON, OPT and OPTLEU, respectively. Those values fall within range of a previously reported value of 159.9 kcal·d^{-1}·BW$^{-0.75}$ measured by indirect calorimetry and respiratory quotient (RQ)-method to separate heat between sow and litter (Jakobsen et al., 2005). Recently, Pedersen et al. (2019) estimated heat production (maintenance + lactation) based on milk energy output and a constant lactation efficiency of 0.78 and reported values varying between 180.9 and 191.9 kcal·d^{-1}·BW$^{-0.75}$. In this study, the energy efficiency for lactation improved by decreasing dietary CP and with advancement of lactation. Pedersen et al. (2019) did not observe a clear trend of heat reduction as dietary CP content decreased, although the diets were all relatively high in CP (i.e., 14.6% to 18.6%). The results herein (Figure 3.2) also point to less lactation heat as percentage of total heat in OPT (26%) and OPTLEU (25%) compared to CON (41%). These values and those of Pedersen et al. (2019) are estimates and therefore further testing of the impact of dietary CP concentrations in lactating sows on heat production using indirect calorimetry is needed.

Sow milk energy is partially derived from the diet and partially from the maternal body pool. Dietary energy contribution to milk increased from 77% to 87% only in OPT diet as lactation progressed, indicating that the reduced dietary protein with NIAA profile may improve dietary energy partitioning towards milk with advancement of lactation. It is acknowledged that body mobilization was estimated over a 21-day lactation period, and it was assumed that mobilization rate (g/d) remained constant throughout lactation. Theil (2015) and Strathe et al. (2017) indicated that lactating sows mobilized greater amounts of body nutrients in early lactation compared to peak lactation. Similarly, in the present study, sows fed OPT had a negative maternal energy retention (−232 kcal/d for OPT and −437 kcal/d for OPTLEU) in early lactation only.

The true efficiency value for sows fed CON for a 21-day lactation period was 70.5%, which is fairly close to the estimated NRC (2012) value of 72% for sows fed conventional diets meeting the minimum SID Lys requirement. The true efficiency values of 82 and 83% for sows fed OPT and OPTLEU, respectively, did not differ statistically from CON value of 70.5%, presumably due to the variability associated with body weight loss. Nonetheless, future implementation of those values may impact prediction of energy requirement since the energy prediction model of NRC (2012) uses a value of 72%. Therefore additional work is needed with a higher number of animals to verify these values, and determine whether NIAA diet increases true energy efficiency. The efficiency value reported by Pedersen et al. (2019) is also higher than NRC (2012), with 78%. The decrease in true energy efficiency as lactation progressed for CON (79.9 to 65.2%) and OPTLEU (94 to 79.5%) albeit a tendency, suggests some potential negative effect of Leu on dietary energy partitioning towards milk, whereby Leu directs dietary energy away from the mammary gland and towards the maternal body. A true efficiency value of 94% for sows fed OPTLEU in early lactation is somewhat high and puzzling. Nonetheless, the true efficiency values reported herein for sows fed CON and OPT are within range of other reported values (NRC, 2012; Pedersen et al., 2019).

Despite that all three experimental diets were formulated iso-calorically based on the NE system (2,580 kcal/kg), the measured NE_c (maintenance + lactation) was higher than the calculated values (2,580 kcal/kg). The present study corrected the NE by excluding the milk energy mobilized from maternal body (Figure 3.1), since NE is the reflection of dietary energy only (NRC, 2012). Pedersen et al. (2019) estimated NE_c (maintenance + lactation), but the difference between calculated NE and measured NE_c was not statistically compared. A variation of NE_c between diets with graded levels of CP was observed and peaked at CP of 15.6% (Pedersen et al., 2019). Similarly, the measured NE_c in the current study was higher in OPT (13.8% CP) than CON (18.7% CP) during peak lactation. Note that the measured NE only for lactation ($NE_{lactation}$) in the present study were consistently lower than the calculated values (2,580 kcal/kg) across all diets. Also, $NE_{lactation}$ increased as lactation progressed only in the OPT diet, as reported by Pedersen et al. (2019) for NE_c. Such observation raises question regarding the adequacy of the book value of NE for lactating sows which were derived from growing-finishing pigs (NRC, 2012). In fact, sows utilize dietary energy more efficiently for lactation than growing pigs for retention (Pedersen et al., 2019). Whether the calculated NE (NRC, 2012) corresponded to the sum of maintenance and lactation or lactation alone is unclear and either of them differ from the calculated values. Current results also suggest that NE values for lactating sows are dynamic and dependent on diet (e.g. dietary CP level and AA balance) and stage of lactation of the sow, warranting the need for additional research on the NE system for lactation.

CONCLUSION

Feeding a NIAA diet improved the apparent dietary energy utilization due to less urinary energy and metabolic heat loss, a response that was associated with the peak stage of lactation. The estimated value for heat reduction was 36.8 $kcal \cdot d^{-1} \cdot BW^{-0.75}$ in sows fed a NIAA diet during peak lactation. Feeding a NIAA diet also triggered dietary energy deposition into milk at the expense of maternal mobilization. Leucine supplementation above requirement may reduce dietary energy utilization for lactation by directing dietary energy away from mammary gland and towards maternal pool, partially explaining the effectiveness of NIAA diet over non-reduced CP diets.

The estimated heat production values in this study need to be validated with indirect calorimetry, in addition to the response of feeding a NIAA under heat stress environment. The following chapter will specifically address heat production in lactating sows fed CON and OPT diets and exposed to TN and HS environments.

Table 3.1. Sow and litter growth performance of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets over a 21-d lactation period[1]

Item	Diet			SEM[2]	*P*-Value		
	CON	OPT	OPTLEU		OPT vs CON	OPT LEU vs. CON	OPTLEU vs. OPT
Number of sows	18	19	17				
Body protein day 1, kg	38.7	38.5	39.0	1.5	0.997	0.962	0.937
Body protein day 21, kg	38.7	38.3	39.4	1.4	0.952	0.876	0.719
Protein mobilization[3], g/day	5.5	-12.8	21.8	21.0	0.803	0.847	0.497
Protein tissue mobilization[4], g/day	27.5	-64.0	109.0	105.0	0.803	0.847	0.497
Body lipid day 1, kg	48.1	51.2	51.6	2.0	0.548	0.477	0.985
Body lipid day 21, kg	46.2	44.8	49.4	2.0	0.856	0.465	0.246
Lipid mobilization[3], g/day	-88.5	-314.1[*]	-113.9	74.6	0.143	0.968	0.207
Lipid tissue mobilization[4], g/day	-106.2	-376.9[*]	-136.7	89.5	0.143	0.968	0.207
Sow BW day 1, kg	246	249	252	7	0.921	0.787	0.953
Sow BW day 21, kg	244	241	251	7	0.931	0.724	0.518
Calculated BW change[5], kg	-1.6	-9.3	-0.6				
Actual BW change, kg	-1.6	-8.3[*]	-0.6	3.0	0.282	0.969	0.216

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Protein and lipid mobilization represent body protein and lipid loss without associated water, and the values were predicted based on sow body weight (BW) and backfat loss (NRC, 2012).

[4] Protein and lipid tissue mobilization represent body protein and lipid loss including the associated water as follows: 1 g of protein is associated with 4 g of water in 5 g of tissue and 1 g of fat is associated with 0.2 g of water in 1.2 g of tissue (Ewan, 2001).

[5] Calculated BW change (g) = (protein tissue mobilization + lipid tissue mobilization) × lactation length (21 day).

[*] BW change (*P* = 0.02) and lipid (tissue) mobilization (*P* < 0.01) differed from 0.

Table 3.2. Energy balance of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation)[1]

Item	Diet			SEM[2]	*P*-Value		
	CON	OPT	OPTLEU		OPT vs CON	OPT LEU vs. CON	OPTLEU vs. OPT
Early lactation (day 4-8)							
Number of sows	12	11	11				
Input							
Feed intake, kg/day	4.9	4.9	4.6	0.2	0.981	0.415	0.530
Energy intake, kcal/day	20,240	19,900	19,080	840	0.937	0.475	0.690
Energy absorbed, kcal/day	17,810	17,300	16,210	800	0.869	0.268	0.536
Output, kg/day							
Feces (Dry matter basis)	0.52	0.54	0.59	0.06	0.980	0.716	0.827
Urine (as-is)	10.68	4.66	5.49	1.68	0.041	0.087	0.930
Milk (as-is)	8.82	8.86	9.51	0.85	0.999	0.762	0.789
Energy concentration, kcal/kg							
Feces (Dry matter basis)	4,639	4,828	4,804	31	< 0.001	< 0.001	0.756
Urine (as-is)	53	62	62	12	0.766	0.791	0.999
Milk (as-is)	1,128	1,219	1,135	50	0.170	0.989	0.232
Energy output, kcal/day							
Feces	2,409	2,600	2,818	246	0.847	0.477	0.808
Urine	402	263	294	67	0.311	0.481	0.948
Milk	9,883	10,846	10,693	887	0.718	0.791	0.992
Energy for maintenance, kcal/day[3]	6,200	6,396	6,219	141	0.442	0.992	0.502
Total energy retention, kcal/day[4]	11,214	10,674	9,649	853	0.926	0.113	0.222
Maternal energy retention, kcal/day[5]	1,396	-232	-437	939	0.434	0.352	0.987
Peak lactation (day 14-18)							
Number of sows	11	11	11				
Input							
Feed intake, kg/day	6.8*	6.7*	6.3*	0.2	0.975	0.169	0.242
Energy intake, kcal/day	27,913*	27,474*	26,205*	837	0.898	0.216	0.418
Energy absorbed, kcal/day	24,579*	23,953*	22,247*	798	0.811	0.073	0.230

Table 3.2. (cont'd)

Output, kg/day							
Feces (Dry matter basis)	0.72[*]	0.74[*]	0.81[*]	0.06	0.969	0.463	0.605
Urine (as-is)	12.04[*]	5.64	6.14	1.68	0.029	0.047	0.974
Milk (as-is)	11.68[*]	13.93[*]	12.13[*]	0.85	0.077	0.893	0.178
Energy concentration, kcal/kg							
Feces (Dry matter basis)	4,639	4,828	4,804	31	< 0.001	< 0.001	0.756
Urine (as-is)	62	60	74	12	0.973	0.750	0.613
Milk (as-is)	1,064	1,202	1,150	50	0.027	0.213	0.562
Energy output, kcal/day							
Feces	3,310[*]	3,554[*]	3,903[*]	246	0.766	0.223	0.581
Urine	598[*]	308	423[†]	67	0.012	0.163	0.446
Milk	12,371[*]	16,781[*]	13,884[*]	891	0.005	0.461	0.072
Energy for maintenance, kcal/day[3]	6,276	6,276	6,288	141	0.999	0.997	0.996
Total energy retention, kcal/day[4]	17,722[*]	17,330[*]	15,550[*]	846	0.926	0.113	0.222
Maternal energy retention, kcal/day[5]	5,380[*]	540	1,685[†]	937	0.003	0.026	0.668

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Energy required for maintenance (kcal/day) was calculated as 100 $\text{kcal/kg}^{0.75}$ (NRC, 2012).

[4] Total energy retention= energy intake−fecal energy−urinary energy−maintenance energy.

[5] Maternal energy retention= energy intake−fecal energy−urinary energy−maintenance energy−milk energy.

[*] Main effect of period (early and late) was significant ($P < 0.05$).

[†] Main effect of period (early and late) tended to be significant: urinary energy output (OPTLEU $P = 0.054$); maternal energy retention (OPTLEU $P = 0.088$).

Table 3.3. Apparent utilization efficiency of nitrogen and energy of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation)[1]

Item	Diet			SEM[2]	*P*-Value		
	CON	OPT	OPTLEU		OPT vs CON	OPT LEU vs. CON	OPTLEU vs. OPT
Early lactation (day 4-8)							
Number of sows	12	11	11				
Nitrogen (N) utilization efficiency[3]							
Milk N output/ME intake, mg/kcal[4]	3.68	3.78	3.93	0.26	0.960	0.759	0.907
Milk N output/NE intake, mg/kcal[4]	4.91	4.95	5.19	0.34	0.997	0.824	0.869
Energy utilization efficiency							
Total energy retention, % of energy intake	55.1	53.3	50.8	1.6	0.703	0.163	0.537
Total energy retention, % of energy absorbed	62.6	61.5	59.6	1.6	0.847	0.298	0.606
Milk energy output, % of energy intake	49.5	55.2	54.6	3.7	0.529	0.599	0.993
Milk energy output, % of energy absorbed	56.2	63.4	63.6	4.4	0.461	0.442	0.999
Peak lactation (day 14-18)							
Number of sows	11	11	11				
Nitrogen (N) utilization efficiency[3]							
Milk N output/ME intake, mg/kcal[4]	3.58	4.40[*]	3.91	0.27	0.088	0.660	0.384
Milk N output/NE intake, mg/kcal[4]	4.78	5.79[*]	5.16	0.36	0.115	0.730	0.394
Energy utilization efficiency							
Total energy retention, % of intake	63.2[*]	62.8[*]	58.6[*]	1.6	0.986	0.140	0.187
Total energy retention, % of absorbed	71.8[*]	72.2[*]	69.1[*]	1.6	0.973	0.369	0.265
Milk energy output, % of energy intake	44.5	62.3	53.0	3.7	0.007	0.268	0.199
Milk energy output, % of energy absorbed	50.7	71.5	62.2	4.4	0.006	0.167	0.304

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Milk N = Milk true protein × 6.25 + milk urea N.

[4] The ME and NE intake were based on calculated values of ME and NE.

[*] Main effect of period (early and late) was significant ($P < 0.05$).

Table 3.4. Dietary energy partitioning of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation)[1]

Item	Diet			SEM[2]	*P*-value		
	CON	OPT	OPTLEU		OPT vs CON	OPT LEU vs. CON	OPTLEU vs. OPT
Early lactation (day 4-8)							
Number of sows	12	11	11				
Feed intake (kg/day)	4.9	4.9	4.6	0.10	0.899	0.102	0.135
Gross energy (GE), kcal/kg							
Analyzed	4,118	4,084	4,139	–	–	–	–
Calculated	4,114	4,199	4,197				
Digestible energy (DE), kcal/kg							
Analyzed	3,636	3,560	3,528	23	0.062	0.006	0.571
Calculated	3,591	3,511	3,513				
Metabolizable energy (ME), kcal/kg							
Analyzed	3,544	3,497	3,468	48	0.766	0.507	0.904
Calculated	3,449	3,405	3,407				
Corrected net energy (NE_c), kcal/kg[3]	3,093	3,059	3,360	163	0.989	0.474	0.405
$NE_{lactation}$[4]	1,827	1,740	2,047	169	0.928	0.625	0.417
$NE_{maintenance}$[5]	1,262	1,315	1,343	50	0.536	0.233	0.822
Calculated	2,580	2,580	2,580				
Peak lactation (day 14-18)							
No. of sows	11	11	11				
Feed intake (kg/day)	6.8	6.7	6.3	0.1	0.865	0.034	0.049
Gross energy (GE), kcal/kg							
Analyzed	4,118	4,084	4,139	–	–	–	–
Calculated	4,114	4,199	4,197				
Digestible energy (DE), kcal/kg							
Analyzed	3,636	3,560	3,528	22.7	0.062	0.006	0.571
Calculated	3,591	3,511	3,513				
Metabolizable energy (ME), kcal/kg							

Table 3.4. (cont'd)

Analyzed	3,537	3,505	3,452	47.7	0.887	0.427	0.709
Calculated	3,449	3,405	3,407				
Corrected net energy (NE_{c}), kcal/kg[3]	2,636*	3,155	2,954*	163	0.084	0.368	0.670
$\text{NE}_{\text{lactation}}$[4]	1,702	2,211*	1,941	170	0.105	0.584	0.506
$\text{NE}_{\text{maintenance}}$[5]	932*	946*	1,016*	50	0.952	0.221	0.349
Calculated	2,580	2,580	2,580				

[1] Data are least squares means; energy is presented as kcal/kg feed.

[2] Maximum value of the standard error of the means.

[3] $\text{NE}_{\text{c}}(\text{kcal/kg feed}) = \text{NE}_{\text{milk}}(\text{kcal/kg feed}) + \text{NE}_{\text{maintenance}}(\text{kcal/kg feed})$. NE was higher ($P < 0.05$) than calculated NE in each experimental diet during early lactation, and was higher in OPT and OPTLEU during peak lactation.

[4] $\text{NE}_{\text{lactation}}(\text{kcal/kg feed}) = \frac{\text{Milk energy output(kcal/day)} - \text{Milk energy from body (kcal/day)}}{\text{Daily feed intake(kg/day)}}$. $\text{NE}_{\text{lactation}}$ was lower than ($P < 0.01$) calculated NE in each experimental diet during both early and peak lactation.

[5] $\text{NE}_{\text{maintenane}}(\text{kcal/kg feed}) = \frac{100 \times \text{BW}^{0.75}(\text{kcal/day})}{\text{Daily feed intake(kg/day)}}$

* Main effect of period (early and late) was significant ($P < 0.05$).

Table 3.5. The relative values between dietary gross energy (GE), digestible energy (DE), metabolizable energy (ME), and net energy (NE) of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation)[1]

Item	Diet			SEM[2]	*P*-value		
	CON	OPT	OPTLEU		OPT vs CON	OPT LEU vs. CON	OPTLEU vs. OPT
Early lactation (day 4-8)							
Number of sows	12	11	11				
DE/GE, %							
Analyzed	88.3	87.2	85.2	0.4	0.162	< 0.01	0.007
Calculated	87.3	83.6	83.7				
ME/DE, %							
Analyzed	97.7	98.5	98.2	0.4	0.324	0.656	0.836
Calculated	96.0	97.0	97.0				
$NE_{lactation}$/ME, %[3]							
Analyzed	51.4	49.7	58.9	4.8	0.967	0.507	0.380
Calculated	74.8	75.8	75.7				
NE_{milk}/ME, %[4]	57.5	64.4	65.0	4.3	0.500	0.448	0.996
Peak lactation (day 14-18)							
Number of sows	11	11	11				
DE/GE, %							
Analyzed	88.3	87.2	85.2	0.4	0.162	< 0.01	0.007
Calculated	87.3	83.6	83.7				
ME/DE, %							
Analyzed	97.5	98.7	98.0	0.4	0.063	0.635	0.327
Calculated	96.0	97.0	97.0				
$NE_{lactation}$/ME, %[3]							
Analyzed	48.0	63.0*	56.2	4.8	0.092	0.468	0.584
Calculated	74.8	75.8	75.7				
NE_{milk}/ME, %[4]	51.9	72.4	63.5	4.4	0.008	0.167	0.339

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] $\mathrm{NE_{lactation}}(\mathrm{kcal/kg\ feed}) = \frac{\text{Milk energy output(kcal/day)} - \text{Milk energy frombody (kcal/day)}}{\text{Daily feed intake(kg/day)}}$

[4] $\mathrm{NE_{milk}}(\mathrm{kcal/kg\ feed}) = \frac{\text{Milk energy output(kcal/day)}}{\text{Daily feed intake(kg/day)}}$

[*] Main effect of period (early and late) was significant ($P < 0.05$).

Table 3.6. True energy efficiency and heat production associated with milk production of sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between d 4 and 8 of lactation (early lactation) and between d 14 and 18 of lactation (peak lactation)[1]

Item	Diet			SEM[2]	*P*-value		
	CON	OPT	OPTLEU		OPT vs CON	OPT LEU vs. CON	OPTLEU vs. OPT
Early lactation (day 4-8)							
Number of sows[3]	12	11	11				
ME_{milk}, kcal/day[4]	11,200	10,665	9,637	851	0.864	0.306	0.595
ME_{intake}	17,380	17,027	15,888	824	0.944	0.375	0.564
$ME_{maintenance}$	6,196	6,391	6,214	138	0.442	0.992	0.502
Milk energy output from diet, kcal/day[5]	8,934	8,577	9,808	1084	0.970	0.835	0.705
Milk energy output	9,876	10,840	10,686	887	0.718	0.791	0.992
Milk energy output from body	110	1,983	481	726	0.992	1.000	0.995
True energy efficiency, %[6]	79.9	78.8	94.0	7.0	0.993	0.333	0.293
Milk energy from diet[7]	89.8	77.2	90.6	4.7	0.167	0.992	0.132
Milk energy from body	10.2	22.8	9.4	4.7	0.167	0.992	0.132
Heat production associated with lactation[8], $kcal \cdot d^{-1} \cdot BW^{-0.75}$	38.32	31.48	7.13	15.22	0.944	0.319	0.504
Peak lactation (day 14-18)							
Number of sows[3]	11	11	11				
ME_{milk}, kcal/day[4]	17,706*	17,320*	15539*	851	0.928	0.114	0.222
ME_{intake}	23,956*	23,637*	21,810*	824	0.955	0.144	0.238
$ME_{maintenance}$	6,273	6,271	6,284	138	0.999	0.997	0.996
Milk energy output from diet, kcal/day[5]	11,461*	14,675*	12,502*	1084	0.112	0.780	0.347
Milk energy output	12,362*	16,769*	13,875*	891	0.005	0.461	0.072
Milk energy output from body	113.1	1969.5*	478.3	726	0.992	1.000	0.995
True energy efficiency, %[6]	65.2†	86.1	79.5†	7.0	0.106	0.329	0.786
Milk energy from diet[7]	90.6	86.8*	90.1	4.7	0.837	0.997	0.869
Milk energy from body	9.4	13.2*	9.9	4.7	0.837	0.997	0.869

Table 3.6. (cont'd)

Heat production associated with lactation[8], kcal·d^{-1}·$BW^{-0.75}$	98.8[*]	39.50	50.33[*]	15.22	0.028	0.082	0.870
Over-21 day lactation							
Number of sows[3]	11	9	9				
ME_{milk}, kcal/day[4]	14,519	14,128	12,481	879	0.924	0.229	0.349
ME_{intake}	20,735	20,516	18,723	864	0.978	0.264	0.335
$ME_{maintenance}$	6,227	6,386	6,199	152	0.670	0.987	0.617
Milk energy output from diet, kcal/day[5]	10,296	11,567	10,599	1314	0.736	0.982	0.843
Milk energy output	11,210	14,174	11,837	1172	0.153	0.873	0.272
Milk energy output from body	-149	2245	398	913	0.241	0.900	0.410
True energy efficiency, %[6]	70.5	82.2	83.2	6.3	0.439	0.390	0.993
Milk energy from diet[7]	91.6	81.6	88.3	5.3	0.425	0.898	0.668
Milk energy from body	8.5	18.4	11.7	5.3	0.425	0.898	0.668
Heat production associated with lactation[8], kcal·d^{-1}·$BW^{-0.75}$	68.95	36.76	31.99	14.25	0.321	0.248	0.970

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Sows with an actual feed intake as percentage of predicted > 75% during days 4-8 and days 14-18.

[4] Metabolizable energy (ME): $\mathrm{ME_{milk}(kcal/day)} = \mathrm{ME_{intake}(kcal/day)} - \mathrm{ME_{maintenance}(kcal/day)}$

[5] $\text{Milk energy output from diet (kcal/day)} = \text{Milk energy output (kcal/day)} - \text{Milk energy output from body (kcal/day)}$

[6] $\text{True energy efficiency}(\%) = \dfrac{\text{Milk energy output from diet (kcal/day)}}{\mathrm{ME_{milk}}\text{ (kcal/day)}} \times 100\%$

[7] $\text{Milk energy from diet}(\%) = \dfrac{\text{Milk energy output from diet (kcal/day)}}{\text{Milk energy (kcal/day)}}$

[8] $\text{Heat production associated with lactation}(\text{kcal}/(\text{day} \cdot \mathrm{BW}^{0.75}) = \dfrac{\mathrm{ME_{milk}}\left(\frac{\text{kcal}}{\text{day}}\right) - \text{milk energy output from diet (kcal/day)}}{\mathrm{BW}^{0.75}}$

[*] Main effect of period (early and late) was significant ($P < 0.05$).

[†] Main effect of period (early and late) tended to be significant for true energy efficiency (CON $P = 0.086$; OPTLEU $P = 0.100$).

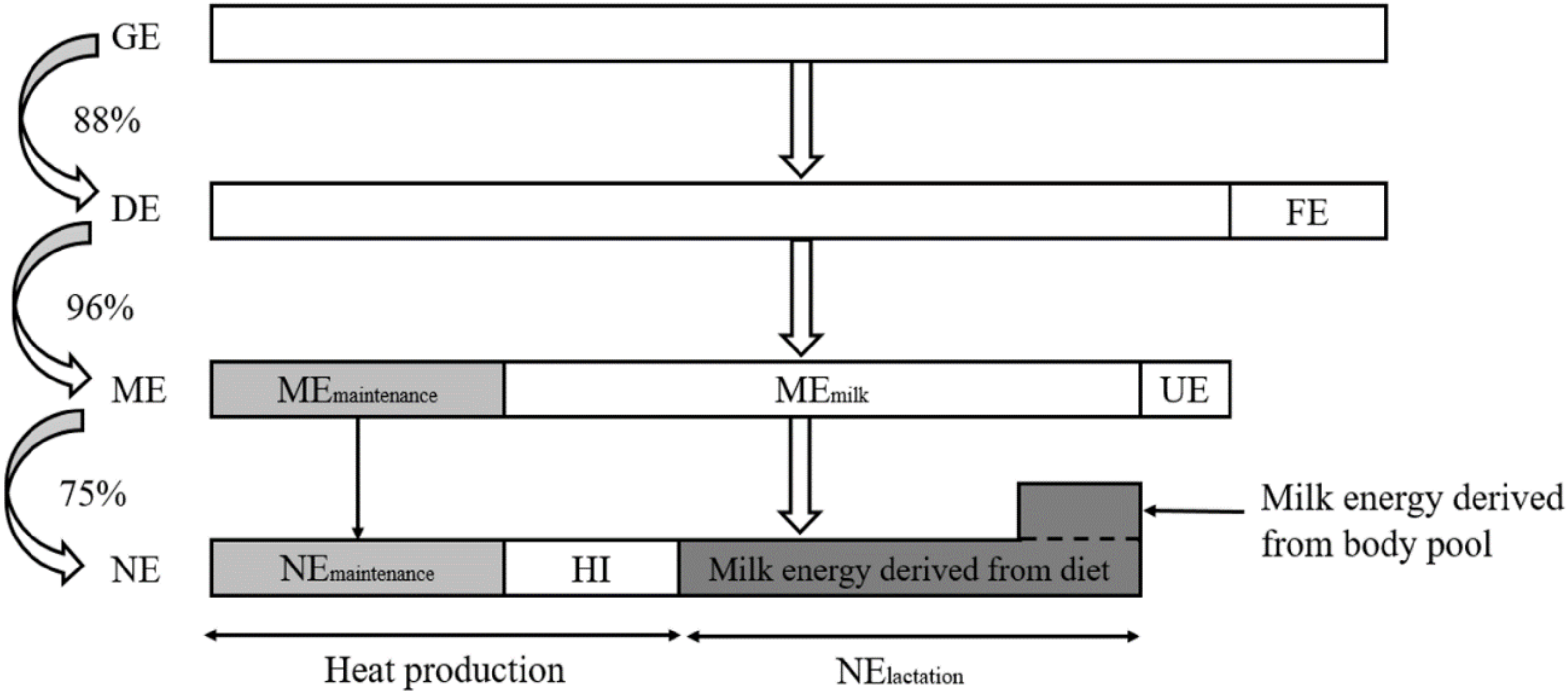

Figure 3.1. Dietary gross energy (GE) partitioning through digestible energy (DE), metabolizable energy (ME), heat increment (HI) towards lactation net energy ($NE_{lactation}$).

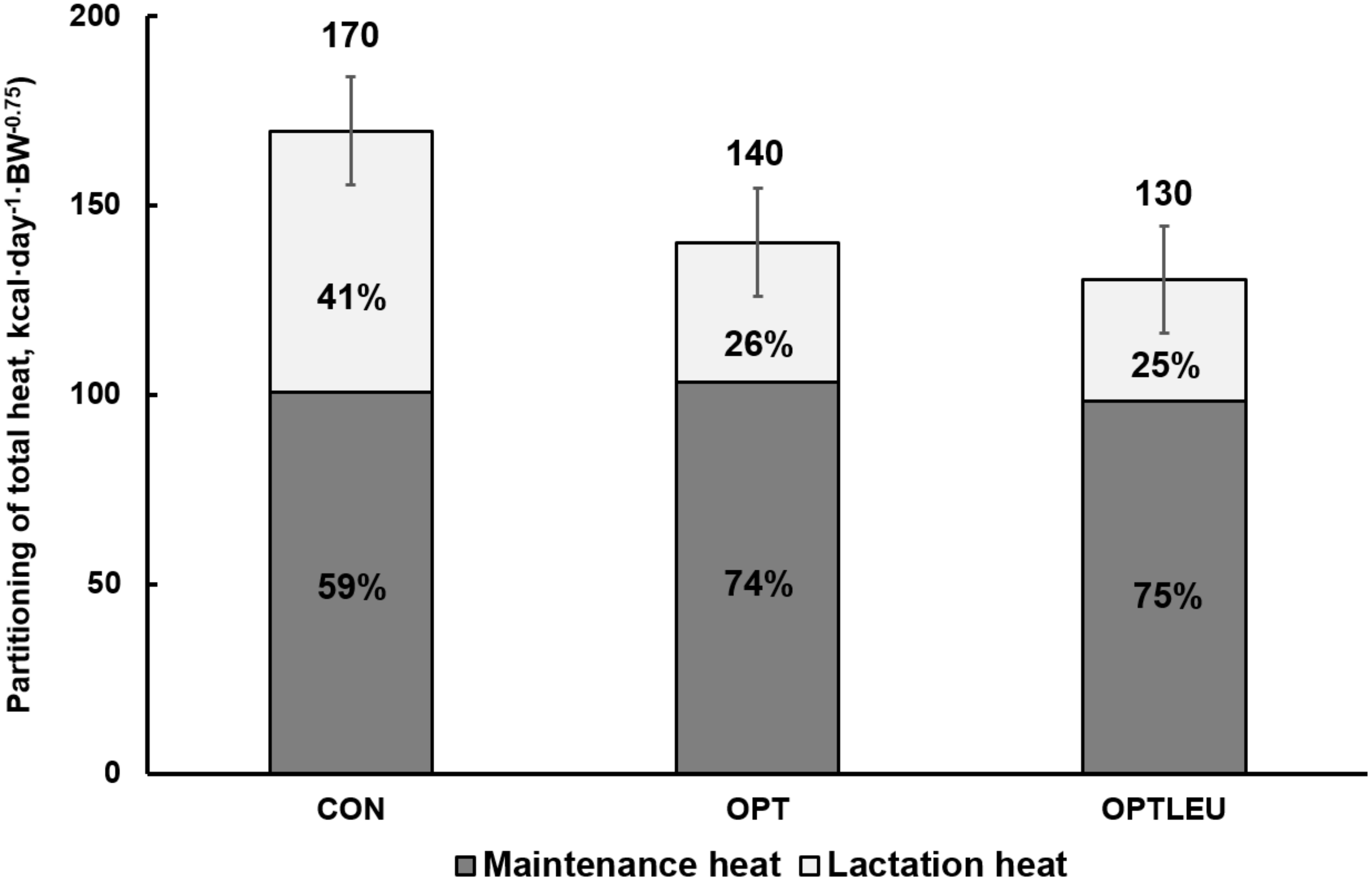

Figure 3.2. The partitioning of total heat production of sows fed control (CON), optimal (OPT) and optimal + leucine (OPTLEU) over a 21-day lactation period. Total heat production did not differ between diets.

CHAPTER 4

EFFECT OF DIETARY NEAR IDEAL AMINO ACID PROFILE ON HEAT PRODUCTION IN LACTATING SOWS EXPOSED TO THERMAL NEUTRAL AND HEAT STRESS

ABSTRACT

The hypothesis of this study was that lactating sows fed a low crude protein (LCP) diet with supplemental AA to improve AA balance have less total heat production (THP) compared to those fed a high crude protein (HCP) diet under both thermal neutral (TN) and heat stress (HS). Thirty-two lactating sows were allotted to HCP (19.3% CP) and LCP (14.0% CP) diets under thermal neutral (TN, 21±1.5°C) or cycling heat stress (HS, 32±1.5°C daytime and 24±1.5°C nighttime). Diets contained 0.90% SID Lys and 2,580 kcal/kg net energy. Positive pressure indirect calorimeters were used to measure gas exchange in individual sows with litters, and individual piglets on lactation days 4, 8, 14 and 18, and THP determined overnight (1900-0700) and during daytime (0700-1900). Sow and litter weights were recorded on days 1, 10 and 21. Sow THP was calculated by subtracting litter THP from sow + litter THP based on $BW^{0.75}$. Under HS, sows BW and body protein (BP) loss was greater for LCP diet compared to HCP diet in peak lactation ($P < 0.05$ and $P < 0.01$) and throughout the entire lactation period ($P < 0.05$ and $P = 0.056$). For the HCP diet, compared to TN, sows under HS had higher ($P < 0.05$) rectal temperature at 1300 ($P < 0.05$) and 1900 ($P < 0.01$), and higher respiration rate at 0700 ($P < 0.05$), 1300 ($P < 0.05$) and 1900 ($P < 0.05$). For the LCP diet, sows under HS tended to have higher ($P = 0.098$) rectal temperature at 1300, and had higher respiration rate at 0700 ($P < 0.05$), 1300 ($P < 0.05$) and 1900 ($P < 0.05$). The relationship between daily THP and days in lactation of sows fed LCP diet was quadratic ($P < 0.05$), with an ascending trend until day 14 and a descending trend from days 14 to 18. Under HS, compared to HCP diet, sows fed LCP diet had lower daily THP at day 18 ($P < 0.001$). To

conclude, feeding LCP reduced THP and this reduction was mainly associated with THP on day 18 of lactation under HS environment. Feeding LCP diet alleviated the increased body temperature in sows under HS throughout lactation, which was accompanied by a reduction in respiration rate. Total heat production is associated with days in lactation, in particular under HS conditions with THP appearing to peak between days 14 and 18.

INTRODUCTION

Despite various cooling strategies, swine production systems are suboptimal in the summer (St- Pierre et al., 2003). Heat stress (HS) causes a series of adaptive behavioral and metabolic changes (Bernabucci et al., 2010), including reduced voluntary feed intake (Pérez Laspiur and Trottier, 2001; Williams et al., 2013) and milk production in sows (Farmer and Prunier, 2002; Renaudeau et al., 2012), elevated respiration rate (RR) and body temperature (Johnson et al., 2013), and increased lipid tissue deposition in growing pigs (Brown-Brandl et al., 2004; Qu et al., 2016). Swine are naturally HS sensitive due to a lack of functional sweat glands (Curtis, 1983) and the existence of a substantial subcutaneous fat layer (Qu et al., 2016). Newer genetic lines for greater lean yield have also contributed to an increase in metabolic heat production (Brown-Brandl et al., 2004 and 2014). In 2003, St-Pierre et al. (2003) reported that HS contributed to $360 million in annual economic losses to the United States swine industry. This figure increased to $900 million in 2010 (Pollmann, 2010).

Greater metabolic rate during lactation due to the intense demand for milk production and litter-rearing (Johnson et al., 2019) increases heat sensitivity (Renaudeau et al., 2012) and HS risk to a larger extent than other production stages (Williams et al., 2013). Therefore reducing heat production in lactating sows exposed to high environmental temperature may improve production efficiency and welfare. Reducing dietary protein decreases metabolic heat production in growing-finishing pigs (Le Bellego et al., 2001; Kerr et al., 2003). In Chapter 3, estimated heat production at peak lactation was reduced from 69 to 37 $\text{kcal}\cdot\text{d}^{-1}\cdot\text{BW}^{0.75}$ in lactating sows housed under thermal neutral (TN) condition by lowering dietary CP from 18.7 to 13.8%. In this chapter, it is examined whether feeding reduced CP diets to lactating sows may be a nutritional strategy to mitigate heat production by using an indirect calorimetry approach. It was hypothesized that feeding a reduced CP diet formulated to contain a near ideal amino acid (NIAA) profile reduces total metabolic heat production in lactating sows under TN and HS conditions compared to feeding a non-reduced CP diet formulated to meet SID Lys requirement with feed ingredients as the sole source of Lys. The study objective was to use indirect calorimetry to measure heat production of lactating sows fed a diet containing 18.4% CP and a NIAA diet containing 13.6% CP and housed under TN or HS environments.

MATERIALS AND METHODS

Animals, Feeding and Experimental Design

The experiment was conducted at the USDA-ARS Livestock Behavior Research Unit (West Lafayette, IN) in four consecutive blocks. Thirty-two multiparous (parity 3.25 ± 0.54) lactating Yorkshire × Landrace sows were used, with 8 sows randomly assigned to 1 of 2 dietary treatments per block. In each block, sows were individually housed in farrowing stalls, with 6 located in chambers as described in Johnson et al. (2019), and 2 for backup substitutes outside of chambers. Sows were exposed to either TN environment (21.0±1.5°C and 41.8±6.5% relative humidity) in blocks 2 and 4, or cycling HS environment (24.0 and 32.0±1.5°C during nighttime and daytime, respectively, and 47.3±5.4% relative humidity) in blocks 1 and 3, described in further details below. All sows were acclimated to diets (2.2 kg/d) and ambient temperature 6 days prior to farrowing. After farrowing, HS sows in blocks 1 and 3 were provided *ad libitum* access to feed. Feed allowance of TN sows (i.e., blocks 2 and 4) was calculated based on feed intake of HS sows within the respective dietary treatments from the preceding block including the backup substitute sows. Feed was provided 3 times daily, and orts were weighed and discarded every other day to avoid interfering with calorimetry day and maintain protocol consistency. No creep feed was provided to piglets and all animals had free access to water. Tail docking, ear notching, teeth clipping, iron injection, and castration were performed according to farm protocol 24 h post birth. Sows were housed in farrowing crates, and litters were standardized to 11.5 ± 0.9 piglets within the first 24 h of birth. Sow and litter weights were recorded, and sow backfat was measured with a backfat scanner (Lean-meater®, series 12, Renco Corp., Golden Valley, MN, USA) on days 1, 10, and at weaning. Weaning varied between days 17 and 21 of lactation due to farrowing schedule and constraints of the breeding schedule. Two sows were weaned on days 15 and 16 and their performance data (feed intake, litter weight gain, piglet ADG for day 10 to weaning) were excluded from the analyses. Milk samples were obtained from all sows on days 6 and 16 to represent early and peak lactation, respectively.

Dietary Treatment

Ingredients and calculated nutrient composition of the diets are presented in Table 4.1. Analyzed total (hydrolysate) and free AA concentrations are presented in Table 4.2. The NRC (2012) model was used to estimate requirements for AA, net energy (NE), calcium (Ca) and phosphorus (P). The requirements were predicted based on the following parameters: sow BW of 210 kg, parity number of 2 and above, and daily intake of 6 kg/day, litter size of 10, piglet BW gain of 280 g/day over a 21-day lactation period. The model predicted a minimum sow BW loss of 7.5 kg and the protein:lipid in the model was adjusted to the minimum allowable value of near zero. All diets were formulated to contain the same SID Lys (0.90%) and NE (2,580 kcal/kg) concentrations.

The control diet was formulated using corn and soybean meal as the only sources of Lys to meet NRC (2012) SID Lys requirement (0.90%) and consequently contained 18.75% CP. Valine met near SID requirement of 0.77% (vs. 0.79%) (NRC, 2012). All other essential amino acid (EAA) SID concentrations were in excess relative to NRC (2012). This diet is referred to as the high crude protein (HCP) throughout the remainder of this chapter.

A second diet balanced to reach a near ideal AA (NIAA) profile was formulated as described in Chapter 2. Briefly, the NIAA diet was designed by reducing soybean meal relative to corn to meet the minimum SID Leu requirement 1.03%, which corresponded to a CP concentration of 13.75%. Then, supplemental crystalline source of L-histidine (His), L-isoleucine (Ile), L-lysine, DL-methionine (Met), L-phenylalanine (Phe), L-threonine (Thr), L-tryptophan (Trp) and L-valine (Val) were added to meet the minimum SID requirement for those AA. Crystalline DL-methionine was added to meet the requirement of Met + cysteine (Cys). This diet is referred to as the low crude protein (LCP) diet throughout the remainder of the manuscript.

Environmental Control and Physiological Monitoring

Under TN environment, ambient temperature was kept constant at 21°C, beginning 6 days prior to expected farrowing through weaning. Under HS environment, a cycling HS approach was used to simulate fluctuation in temperature over a 24-h period during the summer season. Sows were progressively adapted to increasing ambient temperature over a 6-day period prior to the expected farrowing date, with the basal temperature of 21.0°C increased by 1.8°C per day to a maximum of 32°C by day 7, which corresponded to day 114 of gestation. The nighttime temperature for HS was maintained at 24°C. By day 2, the temperature exceeded 24°C, therefore it was gradually decreased beginning at 1500 to reach 24°C by 1900. During lactation, the temperature was gradually increased every day from 24.0°C beginning at 0700 to 32.0°C at 1100, and thereafter the ambient temperature was maintained at 32.0°C until 1500. The temperature was gradually decreased beginning at 1500 to reach 24.0°C by 1900.

Physiological indicators of HS included body temperature (vaginal and rectal temperature) and RR. Vaginal temperature was recorded in 10 min intervals, 24 h per day starting at day 3 of lactation until weaning using vaginal implants as previously described (Johnson and Shade, 2017; Kpodo et al., 2019). Rectal temperature and RR were recorded daily at 0700, 1300, and 1900 starting at lactation day 1 until day of weaning. Respiration rate was measured by counting flank movement for 15 s and multiplying by 4 as previously described (Kpodo et al., 2019). Lights were automatically turned off and on at 2100 and 0600, respectively.

Indirect Calorimetry

In each block, six sows and their litters were housed in indirect calorimetry chambers and THP was determined on days 4-5, 8, 14-15 and 16-19 of lactation (corresponding to days 4, 8, 14 and 18, respectively, in the remainder of the chapter). Calorimetry was conducted in accordance with methods described in details in Johnson et al. (2019). One sow (LCP, block 2, TN) farrowed later than her expected due date and therefore did not participate in the last calorimetry measurement day (i.e., day 18) due to constraints of the breeding schedule. Another sow (LCP, block 1, HS) completed half of her last calorimetry day on day 16 also due to her late farrowing date relative to her expected day. These 2 sows were weaned on days 15 and 16, respectively. Within each indirect calorimetry testing day, total heat production was determined from 1900-0700 (overnight), 0700 (pre-feeding), 0800, 0900, 1000, 1100, 1300 (pre-feeding), 1500 and 1900 (pre-feeding). Indirect calorimetry was also conducted on sentinel piglets for their THP on days 4, 8, 14 and 18, and detailed in Johnson et al. (2019). The sentinel litter data were then used as a correction factor to estimate THP of the individual test sows.

Nutrient Analysis for Diet and Milk

Feed was subsampled and submitted to the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for AA analysis [AOAC Official Method 982.30 E (a,b,c), 45.3.05, 2006] to verify accuracy of feed mixing. Milk samples were submitted to the Michigan Dairy Herd Improvement Association (NorthStar Cooperative, Lansing, MI) for analyses of fat, true protein, lactose, total solids and milk urea N (MUN) using infrared spectroscopy.

Calculations

Milk N concentration

Milk N concentration was calculated based on milk true protein and milk MUN concentrations as follows (Eq. 1):

$$\text{Milk N concentration } (\%) = \text{milk true protein } (\%) \times 6.38 + \text{MUN } (\%) \tag{1}$$

Milk energy concentration

The milk energy content was calculated based on Weast et al. (1984) as follows (Eq. 2):

$$\text{Millk energy } (\text{kcal/g}) = \text{Fat } \% \times 9.5 + \text{protein } \% \times 5.7 + \text{lactose } \% \times 3.95 \tag{2}$$

Heat production

Heat production was calculated based on Brouwer (1965) as follows (Eq. 3):

$$\text{HP} = 3.87 \times O_2 + 1.20 \times CO_2 - 1.43 \times \text{urinary N} \tag{3}$$

Where,

HP = heat production (kcal), O_2 = oxygen consumption (L), CO_2 = carbon dioxide production (L) and urinary N excretion (g).

Based on the study by Chamberlin (2017), urinary N excretion accounts for only 0.24 - 0.64% of the total heat production in pigs, therefore it was not included in the calculation.

Sow metabolic CO_2 (Eq. 4), O_2 (Eq. 5) and THP (Eq. 6) was calculated by subtracting litter THP from sow + litter THP based on $BW^{0.75}$ of sow and litter, respectively.

$$\text{Sow metabolic } CO_2(\mathrm{L \cdot d^{-1} \cdot BW^{-0.75}}) =$$

$$\frac{\text{Sow and litter } CO_2 \text{ (L/d)} - \text{litter metabolic } CO_2 \left(\mathrm{kcal \cdot d^{-1} \cdot BW^{0.75}}\right) \times \mathrm{LW^{0.75}}}{\text{Sow } \mathrm{BW^{0.75}}} \tag{4}$$

$$\text{Sow metabolic } O_2(\mathrm{L \cdot d^{-1} \cdot BW^{-0.75}}) =$$

$$\frac{\text{Sow and litter } O_2 \text{ (L/d)}-\text{litter metabolic } O_2 \left(\mathrm{L \cdot d^{-1} \cdot BW^{0.75}}\right) \times \mathrm{LW^{0.75}}}{\text{Sow } \mathrm{BW^{0.75}}} \tag{5}$$

$$\text{Sow metabolic THP } (\mathrm{kcal \cdot d^{-1} \cdot BW^{0.75}}) =$$

$$\frac{\text{Sow and litter THP (kcal/d)} - \text{litter metabolic THP} \left(\mathrm{kcal \cdot d^{-1} \cdot BW^{0.75}}\right) \times \mathrm{LW^{0.75}}}{\text{Sow } \mathrm{BW^{0.75}}} \tag{6}$$

Litter weight (LW) could not be recorded on calorimetry days (days 4, 8, 14 and 18), therefore LW was estimated by assuming linear growth rate from days 1 to d 10 and from days 10 to wean day (Eq. 7-10).

$$\mathrm{LW_{d4}\ (kg) = LW_{d1}(kg) + \frac{LW_{d10}(kg) - LW_{d1}(kg)}{d10 - d1} \times (d4 - d1)} \tag{7}$$

$$\mathrm{LW_{d8}\ (kg) = LW_{d1}(kg) + \frac{LW_{d10}(kg) - LW_{d1}(kg)}{d10 - d1} \times (d8 - d1)} \tag{8}$$

$$\mathrm{LW_{d14}\ (kg) = LW_{d10}(kg) + \frac{LW_{wean}(kg) - LW_{d10}(kg)}{d_{wean} - d10} \times (d14 - d10)} \tag{9}$$

$$\mathrm{LW_{d_{wean}}\ (kg) = LW_{d10}\ (kg) + \frac{LW_{wean}\ (kg) - LW_{d10}\ (kg)}{d_{wean} - d10} \times (d_{wean} - d10)} \tag{10}$$

Statistical Analysis

Data were analyzed by ANOVA using the Mixed model procedures of SAS 9.4 (SAS

Inst. Inc., Cary, NC).

For the analysis of performance (Table 4.3), body composition (Table 4.4) and milk composition (Table 4.5) data, the following model was used:

Response = diet + environment + stage + $block_{environment}$ + $sow_{\mathrm{diet\times block}}$ + diet × environment + diet × stage + environment × stage + e

The **response** of sow depended on the fixed effects of **diet (**HCP vs. LCP**)**, **environment** (TN vs. HS), and lactation **stage** (early vs. peak lactation, if applicable). The random effects included ***block*** nested within the environment (TN and HS), individual ***sow*** nested within diet and block. The interactive effects of **diet × environment**, **diet × stage**, and **environment × stage** were also included.

For the analysis of physiological data, rectal temperature and RR was first averaged over the lactation period for each sow at each measurement time (0700, 1300 and 1900). (Table 4.6) and the following model was used:

Response = diet + environment + time + $block_{environment}$ + $sow_{\mathrm{diet\times block}}$ + diet × environment + diet × time + environment × time + e

The **response** of sow depended on the fixed effects of **diet (**HCP vs. LCP**)**, **environment** (TN vs. HS), and repeated measurements of **time** for body temperature and RR (0700, 1300 and 1900). The random effect included ***block*** nested within the environment (TN and HS), individual ***sow*** nested within diet and block. The interactive effect of **diet × environment**, **diet × time**, and **environment × time** were also included.

For the analysis of vaginal temperature (Figure 4.1), the following model was used:

Response = diet + environment + day + $block_{environment}$ + $sow_{\mathrm{diet\times block}}$ + diet × environment + diet × day + environment × day + e

The vaginal temperature (i.e., **response**) was averaged daily, and depended on the fixed effects of **diet (**HCP vs. LCP**)**, **environment** (TN vs. HS), and repeated measurement of **day** of lactation. The random effects included ***block*** nested within the environment (TN and HS), individual ***sow*** nested within diet and block. The interactive effects of **diet × environment**, **diet × day**, and **environment × day** were also included.

The THP on days 4, 8, 14 and 18 of lactation was analyzed to compare dietary effect (HCP vs. LCP) within each environment (HS or TN) (Table 4.7). Under HS, ME intake (MEI) between diets varied, thus the MEI was included as a covariable in the model as follows:

$$\text{Response} = \underline{\text{MEI}} + \text{diet} + \text{day} + \textit{block} + \textit{sow}_{\text{diet}\times\text{block}} + \text{diet} \times \text{day} + e$$

The **response** of sow corrected for MEI depended on the fixed effects of **diet (**HCP vs. LCP**)** and repeated measurements of each calorimetry **day** (days 4, 8, 14 and 18). The random effects included ***block***, individual ***sow*** nested within diet and block. The interactive effect of diet × day was also included. Under TN, sows were pair fed to HS counterparts, and therefore MEI was fixed. The MEI was not an independent and random variable, thus the model was the same as under HS except that the covariable MEI was not included.

The THP at different daytime points on days 4, 8, 14 and 18 of lactation was analyzed to compare dietary effect (HCP vs. LCP) within each environment (HS or TN) via double repeated measurements (day and sampling time) (Table 4.8). Under HS, MEI was included as a covariable in the model as follows:

$$\text{Response} = \underline{\text{MEI}} + \text{diet} + \text{day} + \text{sampling time} + \textit{block} + \textit{sow}_{\text{diet}\times\text{block}} + \text{diet} \times \text{day} + \text{diet} \times \text{sampling} + \text{day} \times \text{sampling} + e$$

The **response** of sow was corrected by MEI and depended on the fixed effect of **diet (**HCP vs. LCP**)**, and double repeated measurements of calorimetry **day** (days 4, 8, 14 and 18) and

sampling time (0700, 0800, 0900, 1000, 1100, 1300, 1500 and 1900) of CO_2 and O_2. The random effect included ***block***, individual ***sow*** nested within diet and block. The interactive effect of diet × day, diet × sampling time, and day × sampling time were also included. Under TN, the model was the same as under HS, except that the covariable was not included.

Effects were declared significant at $P \leq 0.05$ and tendency were declared at $0.05 < P \leq 0.10$.

RESULTS

Experimental Diets

Diet composition and nutrient concentrations are presented in Table 4.1 and EAA concentrations are presented in Table 4.2.

Performance

Sow and litter performances are presented in Table 4.3.

LCP vs. HCP. Under TN and HS, daily feed intake and backfat loss, and litter weight gain did not differ between sows fed LCP and HCP diets at any stages of lactation. Under TN, BW loss did not differ between diets. Under HS, BW loss was greater for sows fed LCP diet compared to HCP diet in peak lactation ($P < 0.05$) and throughout the entire lactation period ($P < 0.05$).

HS vs. TN. For HCP diet, daily feed intake, backfat loss, and litter weight gain did not differ between HS and TN at any stages of lactation, and compared to TN, sows under HS lost less BW ($P < 0.05$) during peak lactation. For LCP diet, daily feed intake, backfat loss, and litter weight gain did not differ between HS and TN at any stages of lactation. For LCP diet, compared to TN, sows under HS tended to lose more BW ($P = 0.052$) during peak lactation.

Body Lipid and Protein Mobilization

Body lipid and protein mobilization data are presented in Table 4.4 and illustrated in supplementary Figure B1.

LCP vs. HCP. Under TN, body lipid (tissue) and body protein (tissue) mobilization did not differ between sows fed LCP and HCP diets at any stages of lactation. Under HS, body lipid (tissue) mobilization did not differ between sows fed LCP and HCP diets at any stages of lactation. Under HS, compared to HCP diet, sows fed LCP diet mobilized and tended to mobilize more body protein (tissue) during peak ($P < 0.01$) and throughout the entire lactation periods ($P = 0.056$), respectively.

HS vs. TN. For the HCP diet, body lipid (tissue) mobilization did not differ between HS and TN at any stages of lactation. For sows fed HCP diet under HS, compared to TN, sows mobilized less ($P < 0.05$) body protein (tissue) during peak lactation. For sows fed the LCP diet, body lipid (tissue) mobilization did not differ between HS and TN at any stages of lactation. For sows fed the LCP diet under HS, compared to TN, sows mobilized more ($P < 0.05$) protein (tissue) during peak lactation, and tended to lose more ($P = 0.072$) protein (tissue) throughout the entire lactation period.

Milk Yield and Composition

Milk composition data are presented in Table 4.5.

LCP vs. HCP. Under TN, milk yield, and milk true protein, lactose, fat and energy concentrations did not differ between sows fed LCP and HCP diets at any stages of lactation. Under TN, compared to HCP diet, sows fed LCP diet had lower MUN during both early ($P < 0.01$) and peak ($P < 0.01$) lactation, and tended to have lower milk N concentration ($P = 0.098$). Under HS, milk yield, milk true protein and lactose did not differ between sows fed LCP and HCP diets at any stages of lactation. Under HS, compared to HCP diet, sows fed LCP diet had lower MUN during both early ($P < 0.01$) and peak ($P < 0.01$) lactation, and lower milk energy ($P < 0.05$), fat ($P < 0.05$), and tendency for lower milk N concentration ($P = 0.063$) during early lactation. Under HS, milk energy, fat, lactose and N concentrations did not differ between LCP and HCP during peak lactation.

HS vs. TN. Compared to TN, sows fed either HCP or LCP diets under HS did not differ in milk production, and milk true protein, MUN, N, energy, lactose and fat concentrations.

Physiological Response to Ambient Temperature

The rectal temperature and RR data are presented in Table 4.6. Vaginal temperature data are depicted in Figure 4.1.

HS vs. TN. For the HCP diet, compared to TN, sows under HS had higher ($P < 0.05$) rectal temperature at 1300 ($P < 0.05$) and 1900 ($P < 0.01$), and RR at 0700 ($P < 0.05$), 1300 ($P < 0.05$) and 1900 ($P < 0.05$). For the LCP diet, sows under HS tended to have higher ($P = 0.098$) rectal temperature at 1300, and RR at 0700 ($P < 0.05$), 1300 ($P < 0.05$) and 1900 ($P < 0.05$). For either HCP or LCP diets, compared to TN, sows under HS had higher ($P < 0.01$) vaginal temperature over 18 days of lactation period.

LCP vs. HCP. Under TN, sow rectal temperature and RR did not differ between LCP and HCP diets at 0700, 1300 and 1900. Under HS, compared to HCP diet, sows fed LCP diet had lower rectal temperature ($P < 0.05$) at 1900, lower RR at 0700 ($P < 0.05$) and tended to have lower RR at 1900 ($P = 0.085$). Under either TN or HS, compared to HCP diet, sows fed LCP diet had lower ($P < 0.01$) vaginal temperature over 18 days of lactation period.

Heat Production

Total heat production data are presented in Tables 4.7 and 4.8.

Nighttime. Under TN, compared to HCP diet, THP of sows fed LCP diet did not differ at days 4, 8, 14 and 18. Under HS, compared to HCP diet, sows fed LCP diet tended to have lower THP at day 4 ($P = 0.092$), and lower THP at day 18 ($P < 0.05$).

Daytime. Under TN, compared to HCP diet, THP of sows fed LCP diet did not differ at days 4, 8 and 18, and tended to have lower THP ($P = 0.093$) at day 14. Under HS, compared to HCP diet, sows fed LCP diet had lower THP ($P < 0.01$) at day 18.

24-hour period. Under TN, compared to HCP diet, THP of sows fed LCP diet did not differ at days 4, 8, 14 and 18. Under HS, compared to HCP diet, sows fed LCP diet had lower THP at day 18 ($P < 0.001$).

Over the course of lactation. The relationship between daily (overall 24 h) THP of sows fed LCP diet as lactation progressed was quadratic ($P < 0.05$) under HS, showing an ascending trend until day 14 and a descending trend from days 14 to 18. This relationship was also observed for sows fed LCP diet under HS environment during daytime (0700-1900) ($P < 0.05$) and nighttime (1900-0700) ($P < 0.05$). For sows fed HCP diet, this relationship was quadratic under TN during daytime (0700-1900) ($P < 0.05$). There was no relationship between THP and days in lactation for sows fed HCP during nighttime under TN.

Daytime time points. Under TN, compared to HCP diet, THP of sows fed LCP diet did not differ on days 4, 8 and 18 at any of the time points, and sows fed LCP on day 14 diet had lower ($P < 0.05$) THP at 0700, and did not differ at other time points.

Under HS, compared to HCP diet, THP of sows fed LCP diet did not differ on day 4 at any time points, and on day 8 tended to have lower THP at 0700 ($P = 0.061$) and 1500 ($P = 0.062$) and did not differ at other time points. On day 14, THP tended to be lower at 1000 ($P = 0.076$) and did not differ at other time points. On day 18, THP was lower at 0800 ($P < 0.01$), 0900 ($P = 0.08$), 1000 ($P < 0.01$), 1100 ($P < 0.01$), 1300 ($P < 0.01$) and 1500 ($P < 0.01$), and did not differ at 0700 (Table 4.8).

DISCUSSION

Daily metabolic O_2 consumption and CO_2 production values (supplementary Tables B1 and B2) were similar to those reported in growing pigs by Jaworski et al. (2016), ranging from 31.93 to 34.21 $L \cdot d^{-1} \cdot BW^{-0.75}$ and 30.99 to 32.42 $L \cdot d^{-1} \cdot BW^{-0.75}$ for metabolic CO_2 production and O_2 consumption, respectively. As well, daily THP were similar those reported by Jakobsen et al. (2005) who estimated an average THP of 164 $kcal \cdot d^{-1} \cdot BW^{-0.75}$ for individual lactating sows fed diets containing 18.8% CP by indirect calorimetry and double labeled water technique. Cabezón et al. (2017a) reported a model predicted-value of 178 $kcal \cdot d^{-1} \cdot BW^{-0.75}$ for parity 3-5 sows and assuming a BW of 250 kg. These findings are in line with the current results. Earlier on, Bond et al. (1959) measured THP of lactating sows, including their litters at 92 $kcal \cdot d^{-1} \cdot BW^{-0.75}$ using indirect calorimetry, reflecting lower lactation demand relative to this current study and others. Brown-Brandl et al. (2014) and Stinn and Xin (2014) reported THP values from 193 to 339 $kcal \cdot d^{-1} \cdot BW^{-0.75}$, and from 284 to 405 $kcal \cdot d^{-1} \cdot BW^{-0.75}$, respectively. In both of these studies, calorimetry was conducted at the facility level, hence the THP values include sows with their litters which are expected to be higher than for individual sows. In the current study, results of daily THP including sows and litters (Supplementary Table B7) were also higher than those of sows alone (Table 4.7).

Sows fed the LCP diet produced less daily metabolic heat than those fed the HCP diet throughout lactation, in particular on day 18 under HS environment. The lower MUN concentration for sows fed LCP diets under both TN and HS conditions resulted from less oxidation of excessive dietary AA and reduced urea synthesis as previously described (Kerr et al., 2003; Zhang et al., 2019; Zhang and Trottier, 2019). In Chapter 3, the estimated THP values of lactating sows based on energy balance were 170 and 140 kcal·d^{-1}·BW$^{-0.75}$ by decreasing dietary CP from 18.7 to 13.8%, respectively. In the present study, THP generated from indirect calorimetry decreased from 155 to 139 kcal·d^{-1}·BW$^{-0.75}$ under TN conditions, and 157 to 141 kcal·d^{-1}·BW$^{-0.75}$ under HS conditions by feeding the same diets. Thus, this study validates the estimated values presented in Chapter 3. In growing-finishing pigs, Kerr et al. (2003) reported that decreasing dietary protein from 16 to 12% reduced THP from 165 to 160 kcal·d^{-1}·BW$^{-0.75}$ under TN, and from 147 to 136 kcal·d^{-1}·BW$^{-0.75}$ under HS. Le Bellego et al. (2001) reported a reduction in THP from 357 to 333 kcal·d^{-1}·BW$^{-0.65}$ in response to decreasing dietary CP from 19 to 12%. In the present study, a reduction of total heat relative to dietary CP decrease were 2.97 and 3.22 kcal/g CP reduction under TN and HS, respectively. Such values for growing-finishing pigs were up to 1.8 and 4.9 kcal/g CP reduction under TN and HS conditions, respectively (Noblet et al., 1987; Le Bellego et al., 2001; Kerr et al., 2003). In the study by Kerr et al. (2003), pigs under HS has a lower feed intake than those under TN because they were not pair-fed. Thus it is possible that the difference in feed intake contributed to a larger reduction in heat (4.9 kcal/g CP) compared to reported values herein (3.22 kcal/g CP). Under either TN or HS, both daily feed intake and milk production did not differ between HCP and LCP diets, therefore the lower THP in sows fed LCP diet compared to HCP diet on lactation day 18 may be attributed to less oxidation of excessive dietary AA and reduced urea synthesis (Kerr et al., 2003; Zhang et al., 2019; Zhang and Trottier, 2019). In Chapter 2, the theoretical heat reduction associated with less AA intake was 344 kcal·d^{-1} (Zhang and Trottier, 2019) was reported based on the NE model for the growing-finishing pig, but excluded heat associated with mammary metabolism.

The relationship between THP and days in lactation in this study was previously reported by others (Brown-Brandl et al., 2014; Stinn and Xin, 2014), and followed a similar trend to that of milk production, piglet growth and nutrient demand (Chamberlin, 2017). Toner et al. (1996) described the milk production curve, composed of the colostral, ascending, plateau and descending phases, with duration of the ascending phase varying from day 14 to 28 of lactation, depending on breed, nutrition, and parity, and other factors (Elsley, 1971; Harkins et al., 1989). Hansen et al. (2012) reported a mean time to peak lactation of 18.7 days from a meta-analysis study. Increasing THP with progression of lactation followed by a descending trend reflects THP associated with lactation demand. The RQ (supplementary Tables B3 and B6) values in this study remained close to 1 throughout lactation, indicating that dietary carbohydrates were serving as primary oxidative substrate (Nienaber et al., 2009), and that sows were not in severe negative energy balance. A RQ close to 1 was also previously reported at fed state in growing pigs (Brown-Brandl et al., 2014; Jaworski et al., 2016; Li et al., 2017; Lyu et al., 2018), gestating sows (Stinn and Xin, 2014; Wang et al., 2019) and lactating sows (Stinn and Xin, 2014; Jakobsen et al., 2005).

The lower THP during nighttime compared to daytime, regardless of environmental conditions, was expected and similar to findings of Stinn and Xin (2014) and Brown-Brandl et al. (2014). This response was likely due to lower feed intake and activity level, as previously described (Pedersen and Rom, 2000) and to circadian rhythm differences between the daytime and the nighttime (Brown-Brandl et al., 2014). Reduction of THP between daytime and nighttime corresponded to a 19 and 16% decrease under TN and HS, respectively. Stinn and Xin (2014) reported a day to night THP reduction of 27 and 6% during late gestation and lactation, respectively, in sows housed at 20 °C.

Heat increment of lactating sows has not previously been reported. In this study, THP measured at different time points during the day was not affected by the feeding schedule (0700, 1300 and 1900), which was likely attributed to short duration of time between feedings. The longest time was 12 h, between the last evening feeding at 1900 and the morning feeding at 0700. In growing-finishing pigs, THP was reported to differ between pre- and post-feeding under feed restriction exceeding 30 h (Li et al., 2017; Lyu et al., 2018). In these studies, the RQ decreased to 0.8, suggesting oxidation of body protein and adipose tissues (Nienaber et al., 2009) and pointing to a fasted state (Labussière et al., 2008). Note that in this study, the RQ before the morning feeding was fairly close to 1 (see supplementary Table B6), suggesting the major substrate for oxidation was glucose, and that 12 h fasting overnight was not sufficient to elicit a fasting state despite the high metabolic demands of lactation.

Animals under high ambient temperature reduce their metabolic heat production and improve heat losses by latent and sensible pathways (Renaudeau et al., 2012). Thus, reduced feed intake, milk production or growth rate have been considered as adaptation mechanisms to high ambient temperature through mitigation of metabolic heat (Renaudeau et al., 2012). It was traditionally recognized that maintenance cost increases under HS in ruminants (Beede and Collier, 1986), rodents (Collins et al., 1980) and swine (Campos et al., 2014), as a results of greater energy associated with heat dissipation, such as sweating and panting. Conversely, Johnson et al. (2015) estimated that pigs exposed to HS requires 588 kcal/d less ME for maintenance than pigs raised under TN conditions. Yunianto et al. (1997) also reported lower heat production and reduced plasma triiodothyronine (T3), thyroxine (T4) in tube-fed broiler chickens under HS than TN. Lower THP under HS was also found in growing pigs (Collin et al., 2001; Kerr et al., 2003; Renaudeau et al., 2013). Heat reduction under HS may be related to reduction in visceral mass (Rinaldo and Le Dividich, 1991) or decreased feed intake (Collin et al., 2001). In lactating sows, in addition to milk nutrient synthesis the main contributor to THP is heat increment of feeding (NRC, 2012; Cabezón et al., 2017b). In this study, it was initially planned to pair feed the TN sows to preceding HS sows so that sows under HS has similar feed intake as sows under TN in order to compare THP under TN and HS. However, feed intake between diets varied within either TN or HS environment, thus MEI was included as a covariable under HS to adjust THP. The MEI under TN was fixed due to pair feeding, and was not an independent and random variable, thus the covariable MEI was not included under TN. In this sense, the THP under TN and HS was not compared since THP was analyzed by different model (i.e., TN without covariable MEI and HS with covariable MEI).

Sows fed LCP diet lost more BW than those fed HCP diet only under HS, which was attributed to greater body protein mobilization. Increase partitioning of AA towards mammary gland at the expense of maternal body reserves in sows fed a LCP diet has been suggested by Huber et al. (2015). Long term exposure to HS environment may further aggravate skeletal muscle catabolism (Wheelock et al., 2010; Pearce et al., 2013; Rhoads et al., 2013). The loss of BW and protein reserve is of potential concern for subsequent reproductive cycle (Bergsma et al., 2009) and therefore additional research is needed to evaluate the feasibility of feeding a LCP diet over several parities. Similar findings have been reported under TN condition (Chamberlin et al., 2015b; Huber et al., 2015; Zhang et al., 2019). On the other hand, others reported that sow BW loss did not differ between HCP and LCP diets under HS (Chamberlin et al., 2015a; Johnston et al., 1999). Of note, sows fed HCP diets lost less BW under HS compared to the pair fed TN (PFTN) counterparts in peak lactation, with similar results observed in gilts fed 17.5% CP (Pearce et al., 2013). Thus PFTN animals may be under greater physiological stress compared to their HS counterparts due to nutrient restriction (Pearce et al., 2013). In the latter, the greater BW loss of

PFTN counterparts fed 17.5% CP was also due to body protein loss. Conversely, when fed LCP diet, sows in the current study tended to lose more BW and body protein under HS compared to their PFTN counterparts, suggesting an interaction between diet and environment. It is possible that the LCP diet was limiting in certain AA under HS condition. For instance, AA oxidation increases due to greater maintenance cost under HS (Campos et al., 2014). In addition, lactating sows exposed to HS have reduced milk concentration of Arg, Lys, Val and Pro (Peréz Laspiur and Trottier, 2001). These observations (Peréz Laspiur and Trottier, 2001; Campos et al., 2014) suggest that HS may increase oxidation of certain AA and as a result may lead to AA imbalance.

CONCLUSION

Feeding reduced CP diet with a NIAA profile alleviated the increased body temperature of sows under HS environment which was accompanied by a reduction in respiration rate. Feeding LCP reduced daily THP by 10.3% over the lactation period, and this reduction was mainly associated with the THP response on day 18 of lactation. Sows fed LCP diet had 73% average reduction in MUN and maintained similar feed intake and lactation performance compared to sows fed HCP, suggesting that reduction of THP in sows fed LCP was attributed to less oxidation of excessive dietary AA and reduced urea synthesis. Total heat production is associated with days in lactation, in particular under HS conditions with THP appearing to peak between days 14 and 18.

Results suggest that sows under HS environment and fed reduced dietary CP with a NIAA balance demonstrated less physiological stress to heat. The reduction of THP also implies an increased dietary energy utilization efficiency for lactation during the later stage of lactation. Results presented in Chapter 3 also indicated the efficiency of energy utilization based on energy balance data and estimated heat production was greater in the peak stage of lactation in sows fed a NIAA profile diet. These results shed additional light on the potential benefits of feeding low protein diets, on a larger scale, including maximizing production efficiency, improving welfare of lactating sows under global warming and potentially mitigating the carbon footprint. Amino acid requirements of lactating sows exposed to HS will need to be re-evaluated in order to formulate diets with NIAA profile that maintain maternal body protein retention in order to implement such nutritional strategy over multiple parities.

Table 4.1. Ingredient composition and nutrient content of high crude protein (HCP) and low crude protein (LCP) diets (as-fed)

Item	HCP	LCP
Ingredient composition, %		
Corn, yellow dent	59.27	61.55
Soybean meal, 48 % CP	30.00	14.00
Soy hulls	0	10.57
Sugar food product[1]	5.00	5.00
Beef tallow	3.35	5.02
L-Lys·HCl	0	0.47
L-Val	0	0.29
L-Thr	0	0.20
L-Phe	0	0.13
DL-Met	0	0.11
L-Ile	0	0.08
L-His	0	0.07
L-Trp	0	0.05
L-Leu	0	0
Limestone	1.18	0.93
Dicalcium phosphate	0.45	0.78
Sodium chloride	0.50	0.50
Vitamin and mineral premix[2]	0.25	0.25
Total	100.00	100.00
Calculated nutrient concentration[3]		
NE, kcal/kg	2,580	2,580
CP, %	19.24	14.00
Fermentable fiber, %	11.58	11.58
SID[4] AA, %		
Arg	1.17	0.71
His	0.47	0.37
Ile	0.71	0.52
Leu	1.47	1.03
Lys	0.90	0.90
Met[5]	0.27	0.30
Met + Cys	0.54	0.49
Phe	0.84	0.67
Phe + Tyr	1.38	1.03
Thr	0.61	0.58
Trp	0.21	0.17
Val	0.77	0.79

Table 4.1. (cont'd)

N	2.63	1.88
Total Ca, %[5]	0.65	0.65
STTD P, %[5]	0.23	0.23

[1] Supplied per kg: NE 2,842 kcal; fermentable fiber 0.05 %; CP 1.00 % (International Ingredient Corporation, St. Louis, MO).

[2] Sow micro 5 and Se-yeast PIDX15 (Provimi North America, Inc. Brookville, Ohio).

[3] Based on nutrient concentrations in feed ingredients according to NRC (2012).

[4] SID: standardized ileal digestible (NRC, 2012).

[5] Concentration of Ca and P were based on phytase activity from the premix.

Table 4.2. Analyzed and calculated concentration of nitrogen (N), total and free essential amino acids in high crude protein (HCP) and low crude protein (LCP) diets (as-fed)

Item	HCP		LCP	
	Analyzed[1]	Calculated[2]	Analyzed[1]	Calculated[2]
Total, %				
N	2.94	3.08	2.17	2.24
Arg	1.20	1.26	0.69	0.78
His	0.49	0.53	0.39	0.43
Ile	0.81	0.81	0.56	0.60
Leu	1.58	1.67	1.06	1.19
Lys	1.06	1.04	0.96	1.01
Met	0.27	0.31	0.28	0.33
Met + Cys	0.57	0.63	0.48	0.57
Phe	0.93	0.96	0.69	0.76
Phe + Tyr	1.55	1.59	1.07	1.20
Thr	0.69	0.73	0.62	0.68
Trp[3]	0.22	0.23	0.16	0.19
Val	0.89	0.90	0.82	0.89
Free AA, %				
Arg	0.05	0.00	0.03	0.00
His	0.00	0.00	0.06	0.07
Ile	0.01	0.00	0.07	0.08
Leu	0.02	0.00	0.02	0.00
Lys	0.03	0.00	0.33	0.37
Met	0.00	0.00	0.10	0.11
Met + Cys	0.00	0.00	0.10	0.11
Phe	0.01	0.00	0.11	0.13
Phe + Tyr	0.02	0.00	0.12	0.13
Thr	0.01	0.00	0.18	0.20
Trp[3]	-	0.00	-	0.05
Val	0.00	0.00	0.24	0.29

[1] Analyzed values represents average across 4 blocks (feed mixes).

[2] Calculated values for the total AA are based on the AA concentration in feed ingredients according to NRC (2012), and calculated values for the free AA correspond to the dietary inclusion rate in crystalline form.

[3] Analysis of free Trp was not performed.

Table 4.3. Performance of litter and sow fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

Item	Thermal neutral				Heat Stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
No. of sows[3]	6	6			6	6		
Parity	3	3			3	4		
Wean day	19	18			19	17		
Sow ADFI[4], kg/d								
Overall	6.47	6.01	0.24	0.295	6.47	5.88	0.24	0.185
Early	5.66	5.20	0.24	0.308	5.73	5.43	0.24	0.505
Peak	7.36	6.93	0.24	0.347	7.36	6.83	0.24	0.252
Sow BW, kg								
Day 1	217.7	214.0	15.0	0.869	222.0	249.8	15.0	0.220
Day 10	220.2	211.4	13.7	0.669	223.7	247.5	13.7	0.253
Wean	209.7	206.7	14.5	0.878	221.4	237.2	14.5	0.422
Sow BW change[4], g/d								
Overall	-433.6	-426.8	188.5	0.982	-35.6	-790.6	188.5	0.023
Early	120.0	-154.6	128.6	0.177	83.9	-142.8	128.6	0.262
Peak	-553.7	-272.2	128.6	0.167	-119.5[*]	-647.8[†]	128.6	0.014
Sow back fat, mm								
Day 1	14.4	14.5	2.2	0.974	15.0	15.5	2.2	0.834
Day 10	13.7	13.4	1.8	0.892	14.7	14.6	1.8	0.964
Wean	12.6	11.3	2.0	0.496	13.5	13.8	2.0	0.898
Sow back fat change, mm/d								
Overall	-0.100	-0.191	0.038	0.246	-0.077	-0.102	0.038	0.749
Early	-0.043	-0.074	0.039	0.625	-0.015	-0.055	0.039	0.519
Peak	-0.057	-0.118	0.039	0.336	-0.062	-0.047	0.039	0.803
Litter size								
Day 1	12	11			12	11		
Day 10	11	11			11	11		
Wean	11	11			11	10		
Piglet daily gain, g/d								
Overall	259.7	255.2	34.3	0.849	220.3	230.0	34.3	0.683

Table 4.3. (cont'd)

Early	251.6	249.0	33.9	0.931	216.7	245.8	33.9	0.341
Peak	268.8	268.2	33.9	0.985	232.2	231.2	33.9	0.975
Litter weight gain, kg/d								
Overall	2.94	2.81	0.29	0.650	2.49	2.37	0.29	0.663
Early	2.91	2.74	0.29	0.631	2.56	2.57	0.29	0.970
Peak	2.98	2.96	0.29	0.962	2.49	2.35	0.29	0.686

[1] Data are least squares means. Overall: d 1-wean; early: d 1-10; peak: d 10-wean.

[2] Maximum value of the standard error of the means.

[3] Two sows were weaned on days 15 (LCP under TN) and 16 (LCP under HS) and their performance data (feed intake, litter weight gain, piglet ADG for day 10 to weaning) were excluded from the analyses.

[4] The main effect of lactation stage (early vs. peak) was significant for sow body weight (BW) change and average daily feed intake (ADFI).

[*] Within the same diet, environments differed ($P < 0.05$).

[†] Within the same diet, environments tended to differ for BW change at peak lactation ($P = 0.052$).

Table 4.4. Body composition of sow fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal neutral				Heat Stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
No. of sows[3]	6	6			6	6		
Parity	3	3			3	4		
Body protein, %								
D 1	16.7	16.6	0.3	0.841	16.5	16.6	0.3	0.877
D 10	16.8	16.7	0.3	0.849	16.6	16.7	0.3	0.639
Wean	16.9	17.1	0.3	0.565	16.7	16.8	0.3	0.863
Protein mobilization[4], g/d								
Overall	-38.7	-7.1	29.7	0.560	20.9	-87.5†	29.7	0.056
Early	74.8	-9.8	55.0	0.329	42.4	-10.4	55.0	0.540
Peak	-161.3	-22.2	55.0	0.116	2.2*	-267.9*	55.0	0.005
Protein tissue mobilization[4], g/d								
Overall	-193.5	-35.5	148.5	0.560	104.5	-437.5†	148.5	0.056
Early	374.0	-49	275.0	0.329	212.0	-52.0	275.0	0.540
Peak	-806.5	-111.0	275.0	0.116	11.0*	-1,339.5*	275.0	0.005
Body lipid, %								
D 1	18.0	18.2	1.8	0.926	19.0	19.7	1.8	0.729
D 10	17.8	17.7	1.5	0.925	18.9	19.1	1.5	0.897
Wean	16.8	15.9	1.7	0.572	18.1	18.4	1.7	0.845
Lipid mobilization[4], g/d								
Overall	-206.2	-337.9	64.1	0.296	-105.3	-276.7	64.1	0.179
Early	-52.5	-222.1	137.2	0.438	-3.8	-190.6	137.2	0.394
Peak	-415.9	-503.5	137.2	0.687	-232.2	-523.1	137.2	0.190
Lipid tissue mobilization[4], g/d								
Overall	-247.4	-405.5	76.9	0.296	-126.4	-332.0	76.9	0.179
Early	-63.0	-266.5	164.6	0.438	-4.6	-228.7	164.6	0.394
Peak	-499.1	-604.2	164.6	0.687	-278.6	-627.7	164.6	0.190

[1] Data are least squares means. Overall: d 1-wean; early: d 1-10; peak: d 10-wean.

[2] Maximum value of the standard error of the means.

[3] Two sows were weaned on days 15 (LCP under TN) and 16 (LCP under HS) and their performance data (feed intake, litter weight gain, piglet ADG for day 10 to weaning) were excluded from the analyses.

[4] The main effect of lactation stage (early vs. peak) was significant for sow body lipid (tissue) mobilization and body protein (tissue) mobilization.

[*] Within the same diet, environments differed ($P < 0.05$).

[†] Within the same diet, environments tended to differ for overall protein (tissue) mobilization ($P = 0.072$).

Table 4.5. Milk yield and composition of sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal neutral				Heat Stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
No. of sows	6	6			6	6		
Early lactation[3]								
Yield, kg/d	9.1	9.1	1.4	0.987	7.8	9.0	1.4	0.480
True protein, %	4.04	3.89	0.13	0.532	4.10	3.71	0.13	0.105
Urea-N, mg/dl	12.95	3.93	1.89	< 0.001	11.05	2.13	1.89	< 0.001
N, %	0.646	0.614	0.020	0.381	0.653	0.583	0.020	0.063
Energy,, kcal/g	110.2	117.5	6.3	0.257	105.1	119.7	6.3	0.032
Lactose, %	5.70	5.64	0.12	0.562	5.58	5.61	0.12	0.811
Fat, %	6.80	7.69	0.67	0.218	6.29	8.04	0.67	0.021
Peak lactation[3]								
Yield, kg/d	13.8	15.5	1.4	0.328	12.7	13.2	1.4	0.763
True protein, %	4.15	3.84	0.13	0.184	3.94	3.68	0.13	0.271
Urea-N, mg/dl	15.55	4.15	1.89	< 0.001	11.12	3.47	1.89	< 0.001
N, %	0.668	0.606	0.020	0.098	0.629	0.580	0.020	0.189
Energy, kcal/g	112.8	112.3	6.3	0.940	111.8	104.3	6.3	0.244
Lactose, %	5.82	5.86	0.12	0.728	5.62	5.66	0.12	0.732
Fat, %	6.95	7.09	0.67	0.851	7.07	6.41	0.67	0.358

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] The main effect of lactation stage (early vs. peak) was significant for milk yield.

Table 4.6. Physiological response of sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral (TN) and heat stress (HS) conditions[1]

	HCP				LCP			
	TN	HS	SEM	*P*-value	TN	HS	SEM	*P*-value
No. of sows	6	6			6	6		
Rectal body temp, °C								
0700	38.927	39.109	0.158	0.427	38.991	39.017	0.158	0.906
1300	39.229	39.818	0.158	0.012	39.279	39.653	0.158	0.098
1900	39.315	40.029	0.158	0.003	39.325	39.681*	0.158	0.115
Respiration rate, #/min								
0700	25	43	2	< 0.001	25	37*	2	< 0.001
1300	30	76	2	< 0.001	30	74	2	< 0.001
1900	28	55	2	< 0.001	29	51†	2	< 0.001

[1] Data are least squares means.

*Diets differed within the same environment (P < 0.05).

†Diets tended to differ within the same environment (P = 0.085).

Table 4.7. Feed intake and metabolic total heat production ($\text{kcal}\cdot\text{d}^{-1}\cdot\text{BW}^{-0.75}$) of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

Item	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Number of sows[3]	6	6			6	6		
Feed intake, kg/d[4]								
Day 4	4.96	4.46	-	-	5.23	4.83	0.46	0.536
Day 8	6.59	5.70	-	-	6.63	5.58	0.46	0.109
Day 14	6.93	6.57	-	-	6.97	6.56	0.46	0.524
Day 18[3]	7.26	7.80	-	-	7.58	6.93	0.46	0.406
Metabolic total heat production								
Nighttime (1900-0700)[5]								
Day 4	134.7	111.3	12.8	0.203	142.8	122.4	10.3	0.092
Day 8	135.8	127.4	12.8	0.645	141.0	128.8	9.9	0.263
Day 14	155.8	137.9	12.8	0.329	149.9	144.3	10.4	0.616
Day 18	146.3	118.0	14.0	0.145	137.1	109.3	9.8	0.013
Average	143.2	124.0	6.6	0.040	145.7	123.1	7.6	0.006
SEM[§2]	6.84	10.22			14.6	3.9		
Contrast[6]	-	-			-	Q^{*}, $D^{\dagger}$		
Daytime (0700-1900)[5]								
Day 4	150.0	147.9	12.3	0.873	162.0	161.3	7.2	0.940
Day 8	174.4	163.2	12.3	0.393	165.5	157.4	6.8	0.410
Day 14	186.4	164.1	12.3	0.093	166.7	160.6	6.7	0.529
Day 18	173.2	158.9	13.0	0.301	170.0	130.2	6.7	< 0.001
Average	171.0	158.6	9.54	0.065	169.4	149.5	3.5	0.009
SEM[§2]	6.29	11.71			4.11	5.3		
Contrast[6]	L^{*}, Q^{*}, $D^{\dagger}$	-			-	L^{*}, Q^{*}, D^{*}		
Overall 24 h								
Day 4	142.3	129.6	11.5	0.377	153.8	141.5	7.1	0.184
Day 8	155.1	145.3	11.5	0.494	153.0	142.8	6.8	0.259
Day 14	171.1	151.0	11.5	0.165	158.1	152.7	6.7	0.542
Day 18	159.8	138.6	12.4	0.164	153.3	119.5	6.7	< 0.001

Table 4.7. (cont’d)

Average	157.1	141.3	7.7	0.033	157.7	136.5	4.2	0.002
SEM§2	5.15	10.96			9.3	2.7		
Contrast[6]	-	-			-	Q*, D*		

---

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] One LCP sow under TN was missing for calorimetry day 18 and one LCP sow under HS completed calorimetry day 18 from 0700 until 1200.

[4] Feed intake under TN was fixed and pair fed to counterparts under HS, and thus no SEM and *P* value were included.

[5] Metabolic total heat production between nighttime and daytime differs under TN and HS conditions ($P < 0.01$).

[6] Linear, quadratic contrast and day effect on total heat production along lactation (d 4, 8, 14 and 18) was performed and represented as L, Q, and D, respectively.

[§] Standard error of the means for contrast over days 4, 8, 14 and 18.

[*] Within the same diet, environments differed ($P < 0.05$).

[†] Within the same diet, environments tended to differ ($0.05 < P \leq 0.10$).

Table 4.8. Metabolic total heat production (kcal·d^{-1}·BW$^{-0.75}$) during daytime of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Day 4								
0700[3]	149.0	145.8	12.0	0.840	167.3	153.7	11.3	0.380
0800	151.8	152.3	12.5	0.975	160.1	161.9	11.3	0.905
0900	138.2	137.7	12.0	0.976	161.5	171.1	11.3	0.534
1000	144.9	139.4	12.0	0.723	157.0	153.6	11.3	0.824
1100	152.3	145.8	12.0	0.677	135.0	151.9	12.3	0.296
1300	161.9	168.2	12.0	0.689	160.5	150.6	11.3	0.521
1500	156.4	149.9	12.0	0.677	167.3	146.6	12.4	0.201
1900	144.9	145.1	12.0	0.990	156.3	141.0	12.5	0.344
Day 8								
0700[3]	160.7	144.8	14.5	0.419	165.1	136.0	10.7	0.061
0800	179.0	173.6	14.5	0.784	177.8	162.4	10.7	0.317
0900	163.4	146.5	14.5	0.389	171.1	159.6	10.7	0.454
1000	175.4	159.7	14.5	0.422	161.0	146.4	10.7	0.345
1100	172.5	167.2	14.5	0.785	165.5	158.4	10.7	0.648
1300	180.4	173.2	14.5	0.716	168.8	144.2	10.7	0.113
1500	189.4	177.5	14.5	0.544	186.3	157.3	10.7	0.062
1900	174.2	163.1	14.5	0.570	156.6	170.0	10.7	0.381
Day 14								
0700[3]	194.8	157.4	13.5	0.042	178.9	166.3	12.1	0.456
0800	180.5	167.4	13.5	0.468	181.8	185.9	12.1	0.810
0900	175.8	157.5	13.5	0.311	176.2	158.8	12.1	0.307
1000	179.6	155.8	13.5	0.189	177.4	146.8	12.1	0.076
1100	176.8	165.9	13.5	0.546	181.2	167.6	12.1	0.422
1300	188.9	160.2	13.5	0.116	141.6	148.6	12.1	0.692
1500	205.7	189.6	13.5	0.375	173.5	168.7	12.1	0.780
1900	188.6	158.7	13.5	0.101	167.1	160.3	12.1	0.688
Day 18								
0700[3]	177.5	159.8	17.0	0.430	176.0	148.1	12.5	0.118
0800	191.9	165.9	17.0	0.246	182.9	132.0	12.5	0.005
0900	160.7	150.8	17.0	0.659	157.2	126.0	12.5	0.080
1000	152.6	131.7	17.0	0.350	155.2	108.6	12.5	0.010
1100	162.2	151.7	17.0	0.640	173.2	119.8	12.5	0.004
1300	175.5	157.5	17.0	0.421	203.1	132.6	13.6	0.001
1500	192.0	172.8	17.0	0.391	187.7	127.0	13.6	0.006
1900	173.3	149.1	17.0	0.279	164.0	139.6	13.6	0.227

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Total heat production before first morning meal.

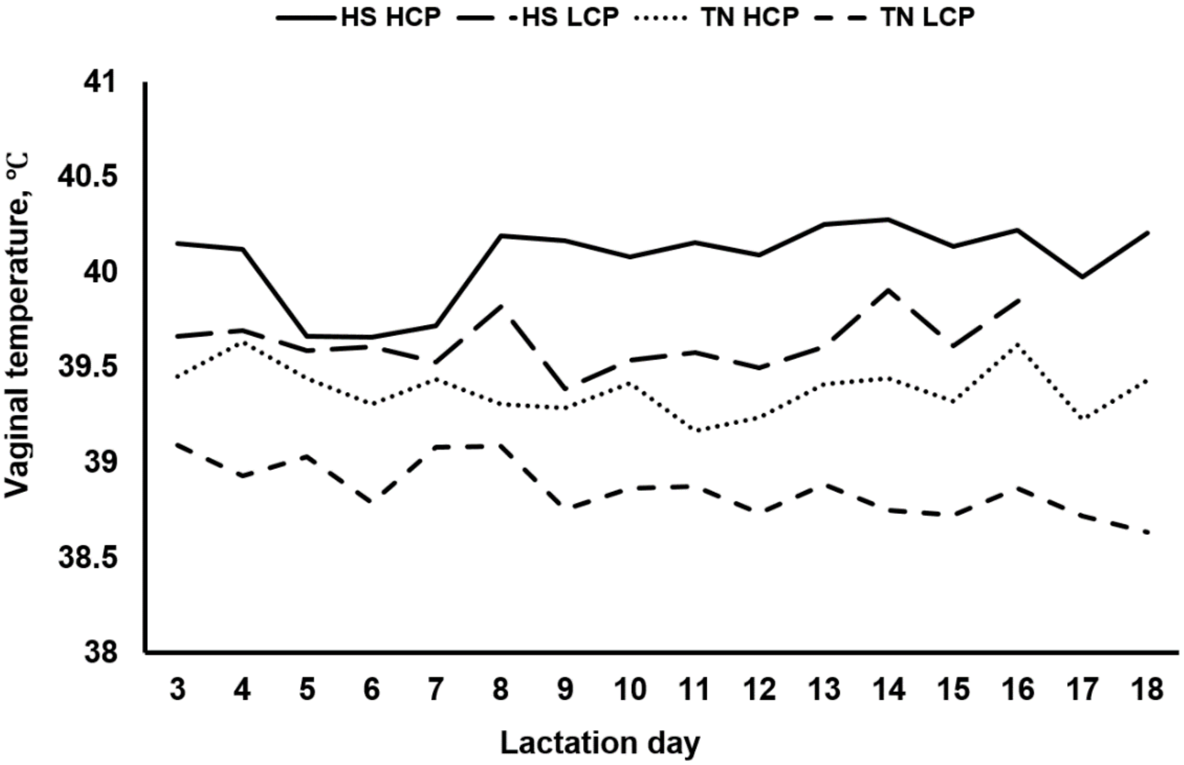

Figure 4.1. Vaginal temperature of sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral (TN) and heat stress (HS) environments. Within the same environment (TN or HS), diets (LCP vs. HCP) differed (*P* < 0.01). Within the same diet (HCP or LCP), environments (HS vs. TN) differed (*P* < 0.01). Standard error of the mean, SEM = 0.183.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Lactation demand on sows is continually increasing because of larger litter size at birth due to genetic selection. In addition, voluntary feed intake is limited relative to lactation demand. These challenges are compounded by increasing environmental regulations aimed at decreasing carbon and ammonia emissions, and rising environmental temperatures. Therefore efforts to improve N and energy utilization in lactating sows are of increasing importance. Prior work showed that feeding reduced protein with improved AA profile improves N utilization efficiency and mitigates urinary N excretion and ammonia emissions (Chamberlin et al., 2015; Huber et al., 2015). In this dissertation, a low CP diet was formulated to attain the minimum Leu requirement and a Leu:Lys of 1.14. To this, supplemental crystalline AA were added to create a NIAA profile. This diet was designed to estimate novel MBEV of individual EAA, assess the impact on energy efficiency and generate new energy efficiency estimates. Maximum biological efficiency values of individual EAA and associated energy efficiency are needed for future prediction of AA and energy requirements. In addition, two potential mechanisms behind the improvement in AA and energetic efficiency were addressed. First, whether the presence of high concentration of Leu relative to Lys (i.e., 1.63) in a typical corn and soybean meal-based, non-reduced CP diet, impacts Lys and energy efficiency. The premise of this first research question was based on previous work in our laboratory indicating that Leu affected Lys extraction by the mammary gland (Guan et al., 2002 and 2004; Manjarín et al., 2011 and 2012). Second, whether the presence of surplus or excess AA in a typical corn and soybean meal-based, non-reduced CP diet is associated with lower AA and energy efficiency due to heat production associated with deamination and N excretion. The premise for this second question was based on reported reduction in heat production in growing pigs fed reduced CP diets and theoretical estimates of heat associated with AA deamination, ammoniagenesis and urea synthesis (Zhang and Trottier, 2019).

The overarching hypothesis of this dissertation was that feeding a reduced CP diet with NIAA and Leu:Lys of 1.14 improves the dietary EAA and energy utilization efficiency, and reduces metabolic heat associated with lactation in sows, compared to feeding a non-reduced CP diet with Leu:Lys of 1.63, formulated to meet SID Lys with feed ingredients as the sole source of Lys. Thus, 3 diets were used to determine the efficiency of individual EAA and energy, and to measure the metabolic heat production of lactating sows: 1) a non-reduced CP diet containing 18.75% CP (CON), 2) a reduced CP diet containing 13.75% CP and NIAA profile (OPT) and 3) the same as OPT but with added Leu to mimic Leu:Lys in CON diet. In chapter 2, it was hypothesized that feeding a reduced CP diet with near ideal amino acid profile (NIAA) and Leu:Lys of 1.14 improves the dietary N and EAA utilization efficiency for milk production in part as a result of reduced dietary Leu concentration. Results indicated that reducing CP with a NIAA profile to attain the minimum Leu requirement (Leu:Lys = 1.14) maintained overall lactation performance, improved utilization efficiency of N (79.1%), Arg (61.1%), His (78.3%), Ile (65.4%), Leu (75.1%), Met + Cys (78.2%), Phe (53.4%), Phe + Tyr (69.5%) and Trp (70.1%) and maximized the efficiency of Lys (63.2%), Met (67.9%), Thr (71.0%) and Val (57.0%) for milk production over a 21-day lactation period. Adding Leu to the NIAA diet to mimic the Leu:Lys of 1.63 of the CON diet showed that Leu did not impact the efficiency of Lys or other EAA. This study provided revised and novel MBEV of EAA, which can be used to more accurately predict requirement for those AA during lactation. In Chapter 3, it was hypothesized that feeding a reduced CP diet with near ideal amino acid profile (NIAA) and Leu:Lys of 1.14 improves the dietary energy utilization efficiency, and reduces metabolic heat associated with lactation in part as a result of reduced dietary Leu concentration. Results indicated that feeding the same NIAA diet (Leu:Lys = 1.14) led to reduced urinary energy excretion and greater energy utilization. It was suggested that the greater energy utilization was due to less urinary energy and estimated metabolic heat loss associated with reduced AA oxidation. Results also indicated that the NIAA diet elicited greater energy deposition into milk at the expense of maternal lipid mobilization. Adding Leu to the NIAA diet to mimic CON Leu:Lys of 1.63reduced dietary energy utilization. The data point to a potential mechanism whereby supplemental Leu is directing dietary energy away from the mammary gland and towards maternal pool. Leucine is known to stimulate anabolic process of body protein (Norton et al., 2012; Wilkinson et al., 2013). In addition, the NIAA diet lowered the estimated heat production associated with lactation during peak lactation, suggesting the potential of alleviating HS by feeding NIAA diet. Therefore, Chapter 4 focused on indirect calorimetry measurement of total heat production in sows fed CON and NIAA diets exposed to TN and HS environments. Feeding NIAA diet alleviated the increased body temperature observed in sows under HS and the associated RR. The NIAA diet also reduced THP at day 18 of lactation, which is in the periphery of the peak lactation period, in sows housed under HS environment. Throughout the studies, the NIAA diet led to either higher BL loss under TN, or higher BP loss under HS. The former may be attributed to greater energy requirement for sows fed NIAA when housed under TN environment because of the potential for higher milk yield. Regarding the later, AA requirements of lactating sows exposed to HS should be re-evaluated. It is possible that HS increases muscle protein catabolism (Wheelock et al., 2010; Pearce et al., 2013; Rhoads et al., 2013) and AA oxidative processes (Campos et al., 2014), thus increasing AA requirements. Therefore it is possible that the NIAA diet formulated was limiting in one or several AA for maternal PB retention. The long term consequences of BL or BP losses over multiple parities is unknown. In the short term, diets with NIAA profile that maximize maternal body protein and lipid mobilization need to be designed and tested in order to implement such nutritional strategy over multiple parities.

Feeding lactating sows with a reduced CP diet and crystalline AA supplementation to attain NIAA profile improved efficiency of individual EAA and energy utilization, and mitigate the impacts of HS on lactating sows through less metabolic heat. This study provided revised and novel MBEV for individual EAA, which is the key to designing nutritional models for prediction of AA requirement. Results of this dissertation emphasize the potential benefits of feeding low protein diets, including maximizing production efficiency, improving welfare of lactating sows under global warming and potentially mitigating the carbon footprint.

APPENDICES

APPENDIX A

Table A1. Water balance in sows fed Control (CON; 18.74 %), Optimal (OPT; 13.78%) or Optimal + Leucine (OPTLEU; 14.25%) diets between day 4 and 8 of lactation (early lactation) and between day 14 and 18 of lactation (peak lactation)[1]

Item	Diet			SEM[2]	*P*-Value		
	CON	OPT	OPTLEU		OPT vs CON	OPT LEU vs. CON	OPTLEU vs. OPT
Early lactation (day 4-7)							
Number of sows	12	11	11				
Body weight, kg	245	256	246	7	0.440	0.994	0.493
Water intake from feed, kg/d	0.622	0.582	0.585	0.034	0.489	0.537	0.997
Water retained in the body, kg/d	0.112	-0.061	0.144	0.107	0.170	0.934	0.075
Water output in milk, kg/d	8.242	8.279	8.879	0.794	0.999	0.764	0.790
Water output in feces, kg/d	1.117	1.350	1.547	0.132	0.436	0.074	0.552
Water output in urine, kg/d	10.674	4.669	5.553	1.661	0.040	0.089	0.922
Estimated water intake, kg/d	19.177	13.861	15.651	1.871	0.126	0.384	0.772
Fecal DM, %[3]	32.15	28.48	27.96	0.67	0.001	< 0.001	0.839
Peak lactation (day 14-17)							
Number of sows	11	11	11				
Body weight, kg	249	249	250	7	0.999	0.998	0.996
Water intake from feed, kg/d	0.815[*]	0.794[*]	0.756[*]	0.034	0.831	0.234	0.525
Water retained in the body, kg/d	0.112	-0.061	0.144	0.107	0.170	0.934	0.075
Water output in milk, kg/d	10.907[*]	13.026[*]	11.319[*]	0.798	0.073	0.896	0.167
Water output in feces, kg/d	1.542[*]	1.859[*]	2.155[*]	0.132	0.228	0.008	0.268
Water output in urine, kg/d	13.100[*]	5.633	6.183	1.675	0.010	0.017	0.969
Estimated water intake, kg/d	24.563[*]	19.892[*]	18.722[*]	1.891	0.199	0.087	0.895
Fecal DM, %[3]	32.15	28.48	27.96	0.67	0.001	< 0.001	0.839

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Fecal samples were collected on day 10 of lactation.

[*] Main effect of period (early and late) was significant ($P < 0.05$).

APPENDIX B

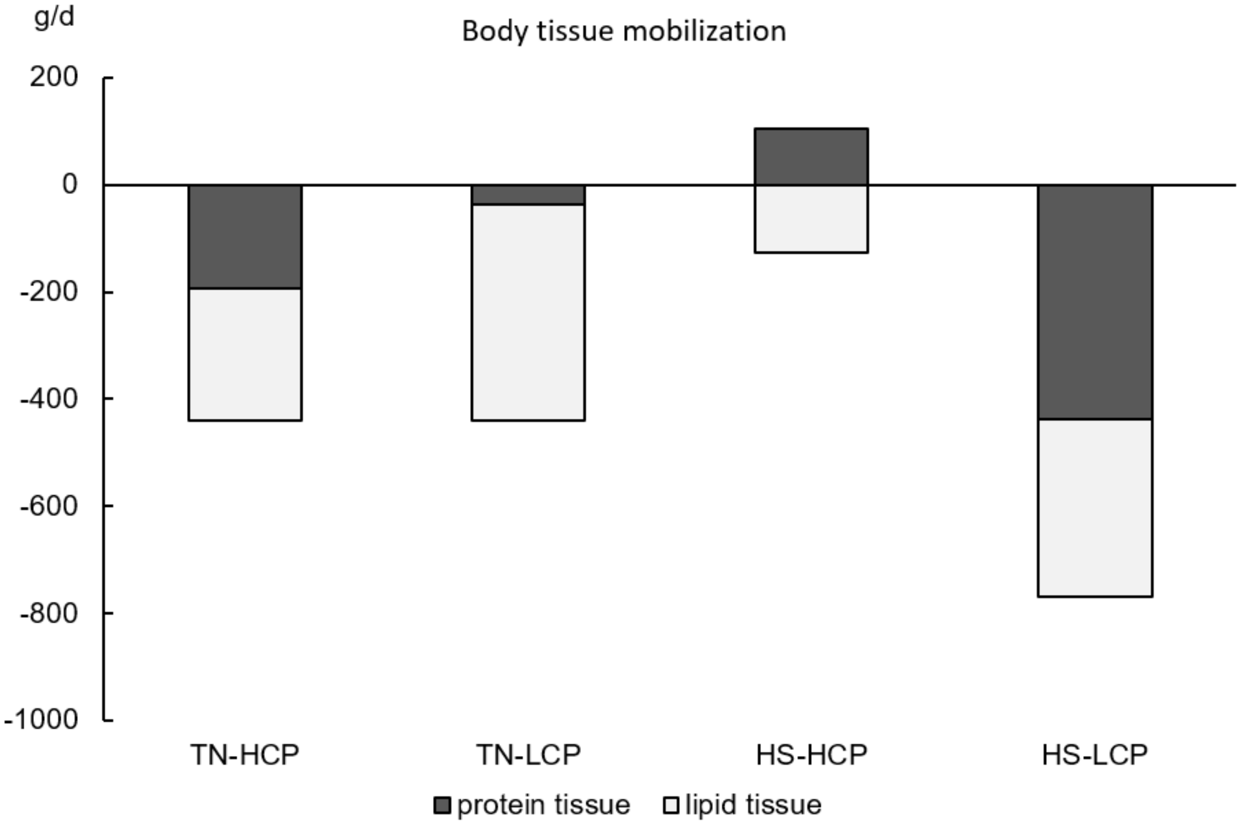

Figure B1. Body protein and lipid tissue mobilization of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions.

Table B1. Metabolic oxygen (O_2) consumption ($L \cdot d^{-1} \cdot BW^{-0.75}$) of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Nighttime[4]								
Day 4	25.49	20.02	2.77	0.171	27.89	25.09	2.05	0.254
Day 8	25.49	23.33	2.77	0.585	27.85	24.46	1.74	0.134
Day 14	29.81	25.49	2.77	0.277	28.44	26.70	1.77	0.444
Day 18	27.07	21.43	2.77	0.178	25.70	20.27	1.74	0.017
Daytime								
Day 4	29.64	29.38	2.33	0.910	31.73	31.56	1.49	0.932
Day 8	34.56	32.40	2.33	0.397	32.88	31.45	1.33	0.459
Day 14	37.32	31.80	2.33	0.036	32.73	31.26	1.36	0.436
Day 18	34.30	31.19	2.33	0.254	33.31	25.62	1.34	<0.001
24 h								
Day 4	27.48	24.62	2.25	0.321	30.12	28.26	1.46	0.319
Day 8	29.98	27.91	2.25	0.485	30.05	27.93	1.31	0.248
Day 14	33.52	28.68	2.25	0.095	30.65	29.28	1.34	0.440
Day 18	30.84	26.09	2.25	0.122	29.49	22.75	1.32	0.001

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

Table B2. Metabolic carbon dioxide (CO_2) production ($L \cdot d^{-1} \cdot BW^{-0.75}$) of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Nighttime[4]								
Day 4	29.52	28.23	2.98	0.748	28.70	21.68	3.63	0.046
Day 8	31.25	30.82	2.98	0.915	28.62	28.97	3.28	0.909
Day 14	33.84	32.55	2.98	0.748	32.81	34.69	3.32	0.556
Day 18	34.85	29.67	2.98	0.226	30.96	26.17	3.29	0.124
Daytime								
Day 4	29.66	28.94	2.90	0.805	32.61	32.75	1.72	0.948
Day 8	33.98	31.97	2.90	0.490	31.94	30.06	1.54	0.398
Day 14	35.57	34.13	2.90	0.621	32.97	32.25	1.57	0.743
Day 18	33.84	31.84	2.90	0.513	34.08	26.02	1.54	<0.001
24 h								
Day 4	29.38	28.51	2.69	0.783	31.02	27.50	1.92	0.113
Day 8	32.69	31.25	2.69	0.647	30.01	29.57	1.77	0.836
Day 14	34.71	33.26	2.69	0.647	32.89	33.00	1.80	0.960
Day 18	34.27	31.03	2.69	0.329	32.60	26.20	1.78	0.004

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

Table B3. Respiratory quotient (RQ) of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Nighttime								
Day 4	1.24	1.45	0.12	0.229	1.00	0.86	0.13	0.287
Day 8	1.29	1.36	0.12	0.709	1.04	1.22	0.11	0.156
Day 14	1.15	1.32	0.12	0.306	1.16	1.31	0.11	0.254
Day 18	1.30	1.41	0.12	0.525	1.20	1.34	0.11	0.247
Daytime								
Day 4	1.01	1.01	0.04	0.940	1.06	1.05	0.03	0.700
Day 8	0.98	1.02	0.04	0.443	0.97	0.96	0.03	0.688
Day 14	0.96	1.08	0.04	0.011	1.01	1.03	0.03	0.438
Day 18	1.00	1.03	0.04	0.673	1.03	1.03	0.03	0.921
24 h								
Day 4	1.12	1.23	0.06	0.218	1.05	0.96	0.05	0.185
Day 8	1.14	1.19	0.06	0.553	1.01	1.09	0.05	0.200
Day 14	1.05	1.20	0.06	0.081	1.08	1.14	0.05	0.365
Day 18	1.15	1.22	0.06	0.449	1.11	1.19	0.05	0.259

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

Table B4. Metabolic oxygen (O_2) consumption during daytime of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Day 4								
0700[3]	29.81	30.80	2.53	0.747	32.62	30.81	3.02	0.655
0800	29.66	31.31	2.53	0.595	31.18	29.93	3.02	0.757
0900	27.22	29.63	2.53	0.437	30.61	33.77	3.02	0.438
1000	28.66	29.56	2.53	0.771	31.03	31.08	3.02	0.991
1100	30.09	28.49	2.53	0.603	29.96	29.64	3.02	0.937
1300	31.82	35.35	2.53	0.258	30.74	26.66	3.02	0.316
1500	31.10	28.93	2.53	0.483	32.45	29.40	3.41	0.484
1900	28.51	29.44	2.70	0.774	21.77	28.57	3.41	0.123
Day 8								
0700[3]	32.40	27.93	3.03	0.262	33.28	26.69	2.24	0.045
0800	35.57	34.41	3.03	0.771	35.44	32.60	2.24	0.378
0900	31.97	28.23	3.03	0.346	34.15	31.01	2.24	0.332
1000	34.85	31.82	3.03	0.446	31.70	28.85	2.24	0.378
1100	34.13	33.26	3.03	0.827	32.85	31.44	2.24	0.662
1300	35.57	34.41	3.03	0.771	33.86	28.85	2.24	0.124
1500	37.58	36.14	3.03	0.717	37.03	31.59	2.24	0.096
1900	34.27	32.55	3.03	0.663	31.55	34.61	2.24	0.344
Day 14								
0700[3]	39.60	30.82	2.73	0.015	36.63	32.88	2.56	0.298
0800	35.71	32.98	2.73	0.437	35.92	36.90	2.56	0.783
0900	34.99	30.53	2.73	0.207	34.33	30.85	2.56	0.336
1000	35.71	29.95	2.73	0.105	35.34	28.69	2.56	0.069
1100	34.99	32.40	2.73	0.461	35.92	32.73	2.56	0.378
1300	38.02	31.54	2.73	0.069	27.85	29.46	2.56	0.665
1500	40.61	36.87	2.73	0.289	34.33	33.45	2.56	0.806
1900	37.58	30.09	2.73	0.037	32.89	31.29	2.56	0.657
Day 18								
0700[3]	36.29	32.25	3.63	0.390	35.42	29.51	2.61	0.115
0800	38.59	32.73	3.63	0.213	36.14	25.92	2.61	0.008
0900	31.25	28.79	3.63	0.600	30.66	25.34	2.61	0.154
1000	29.66	26.06	3.63	0.443	30.38	20.87	2.61	0.013
1100	31.54	30.52	3.63	0.828	33.98	23.61	2.61	0.007
1300	34.56	31.20	3.63	0.475	40.38	26.28	2.84	0.001
1500	38.45	34.64	3.63	0.417	36.94	24.88	2.85	0.009
1900	33.98	29.93	3.63	0.388	31.95	27.648	2.86	0.310

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Prior to morning feeding at 0700

Table B5. Metabolic carbon dioxide (CO_2) production ($L \cdot d^{-1} \cdot BW^{-0.75}$) during daytime of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Day 4								
0700[3]	28.66	27.10	2.75	0.626	32.92	30.13	2.34	0.379
0800	30.82	29.33	2.75	0.643	33.63	35.45	2.34	0.565
0900	27.36	29.50	2.75	0.506	33.09	36.18	2.34	0.333
1000	28.94	27.79	2.75	0.718	30.92	29.83	2.34	0.731
1100	30.39	29.18	2.75	0.706	32.26	31.42	2.53	0.799
1300	32.40	30.87	2.75	0.633	32.22	32.14	2.34	0.981
1500	30.53	30.40	2.75	0.968	33.44	29.98	2.55	0.299
1900	28.51	29.78	2.86	0.699	31.80	27.82	2.57	0.235
Day 8								
0700[3]	29.38	30.39	2.94	0.780	31.20	27.06	2.23	0.198
0800	34.71	33.12	2.94	0.661	35.09	30.08	2.23	0.121
0900	32.69	31.10	2.94	0.661	32.63	33.26	2.23	0.847
1000	33.98	30.82	2.94	0.382	32.20	28.94	2.23	0.308
1100	34.13	31.82	2.94	0.524	32.50	30.80	2.23	0.597
1300	36.14	33.41	2.94	0.450	31.92	26.48	2.23	0.093
1500	36.57	32.40	2.94	0.251	35.66	29.07	2.23	0.043
1900	34.99	30.96	2.94	0.267	29.47	30.52	2.23	0.743
Day 14								
0700[3]	35.42	31.68	2.93	0.302	32.58	32.70	2.26	0.969
0800	35.14	33.70	2.93	0.689	36.61	36.59	2.26	0.995
0900	33.98	33.41	2.93	0.873	36.32	32.56	2.26	0.239
1000	34.27	33.55	2.93	0.842	34.59	30.26	2.26	0.175
1100	34.27	34.27	2.93	1.000	35.46	34.15	2.26	0.678
1300	35.42	31.54	2.93	0.284	28.40	29.53	2.26	0.722
1500	40.03	39.60	2.93	0.904	34.74	33.13	2.26	0.613
1900	36.57	35.28	2.93	0.719	33.73	32.56	2.26	0.712
Day 18								
0700[3]	31.54	30.27	3.36	0.761	33.85	28.43	2.31	0.102
0800	35.71	33.85	3.36	0.653	36.59	27.13	2.31	0.005
0900	32.83	31.19	3.36	0.691	32.56	23.53	2.31	0.008
1000	31.68	27.57	3.36	0.323	31.69	23.24	2.31	0.012
1100	33.26	31.58	3.36	0.683	35.01	23.82	2.31	0.001
1300	34.56	32.44	3.36	0.610	38.02	27.03	2.50	0.004
1500	36.43	34.69	3.36	0.674	37.23	26.87	2.52	0.007
1900	34.85	29.19	3.36	0.175	33.69	28.50	2.52	0.167

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Prior to morning feeding at 0700

Table B6. Respiratory quotient (RQ) during daytime of lactating sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Day 4								
0700[3]	0.97	0.89	0.05	0.244	1.07	1.00	0.05	0.284
0800	1.04	0.93	0.05	0.120	1.08	1.15	0.05	0.277
0900	1.01	1.01	0.05	0.999	1.07	1.10	0.05	0.553
1000	1.02	0.95	0.05	0.331	1.00	1.01	0.05	0.945
1100	1.01	1.03	0.05	0.804	1.05	1.06	0.05	0.846
1300	1.02	0.88	0.05	0.046	1.04	1.11	0.05	0.230
1500	0.99	1.05	0.05	0.418	1.06	1.05	0.06	0.896
1900	0.99	1.00	0.06	0.962	1.02	1.00	0.07	0.732
Day 8								
0700[3]	0.89	1.21	0.09	0.014	0.94	1.02	0.04	0.131
0800	0.97	0.95	0.09	0.868	1.00	0.92	0.04	0.134
0900	1.01	1.25	0.09	0.064	0.96	1.06	0.04	0.073
1000	0.97	0.96	0.09	0.925	1.01	1.00	0.04	0.759
1100	1.00	0.95	0.09	0.661	0.98	0.97	0.04	0.787
1300	1.02	0.97	0.09	0.711	0.95	0.91	0.04	0.504
1500	0.97	0.90	0.09	0.593	0.96	0.92	0.04	0.524
1900	1.02	0.95	0.09	0.588	0.95	0.91	0.04	0.480
Day 14								
0700[3]	0.90	1.04	0.04	0.018	0.93	1.01	0.03	0.114
0800	0.98	1.04	0.04	0.287	1.02	1.00	0.03	0.675
0900	0.97	1.10	0.04	0.036	1.05	1.06	0.03	0.857
1000	0.95	1.13	0.04	0.004	0.99	1.06	0.03	0.128
1100	0.98	1.07	0.04	0.115	0.98	1.06	0.03	0.119
1300	0.94	1.03	0.04	0.101	1.02	1.01	0.03	0.821
1500	0.98	1.09	0.04	0.066	1.01	1.00	0.03	0.764
1900	0.98	1.18	0.04	0.001	1.02	1.06	0.03	0.486
Day 18								
0700[3]	0.97	0.94	0.06	0.735	0.97	0.96	0.04	0.853
0800	0.92	1.05	0.06	0.109	1.02	1.07	0.04	0.468
0900	1.05	1.06	0.06	0.843	1.07	0.96	0.04	0.088
1000	1.06	1.04	0.06	0.796	1.06	1.12	0.04	0.317
1100	1.05	1.03	0.06	0.836	1.04	1.02	0.04	0.690
1300	0.99	1.07	0.06	0.371	0.95	1.02	0.05	0.322
1500	0.95	0.99	0.06	0.670	1.00	1.12	0.05	0.131
1900	1.04	0.96	0.06	0.300	1.06	1.03	0.05	0.658

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Prior to morning feeding at 0700

Table B7. Metabolic total heat production (kcal·d^{-1}·BW$^{-0.75}$) of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Nighttime								
Day 4	160.8	138.0	12.5	0.176	166.1	139.7	11.0	0.033
Day 8	171.1	162.0	12.5	0.583	173.5	157.7	9.6	0.152
Day 14	199.0	185.0	12.5	0.403	195.1	183.8	9.8	0.322
Day 18	202.1	175.4	12.5	0.133	188.4	174.5	9.8	0.201
Daytime								
Day 4	174.7	172.8	12.2	0.859	185.5	175.4	7.4	0.314
Day 8	205.0	193.7	12.2	0.334	196.8	183.4	6.7	0.167
Day 14	224.6	207.4	12.2	0.144	209.8	198.2	6.7	0.227
Day 18	223.9	208.6	12.7	0.216	216.2	192.2	6.7	0.015
24 h								
Day 4	167.8	155.3	11.5	0.343	177.8	157.2	7.7	0.036
Day 8	188.2	177.8	11.5	0.435	185.0	170.2	7.0	0.117
Day 14	211.7	196.1	11.5	0.236	202.3	191.5	7.2	0.239
Day 18	213.1	192.2	12.2	0.134	202.3	183.1	7.0	0.044

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

Table B8. Metabolic oxygen (O_2) consumption ($L \cdot d^{-1} \cdot BW^{-0.75}$) of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Nighttime[4]								
Day 4	31.10	25.77	2.58	0.140	32.99	28.66	1.92	0.092
Day 8	32.98	30.39	2.58	0.468	34.12	30.23	1.82	0.098
Day 14	38.73	35.71	2.58	0.397	38.18	35.43	1.96	0.245
Day 18	39.03	33.60	2.81	0.152	36.61	33.96	1.80	0.242
Daytime								
Day 4	34.85	34.56	2.34	0.901	36.15	34.91	1.52	0.533
Day 8	40.89	38.59	2.34	0.321	39.17	36.83	1.35	0.238
Day 14	44.93	41.33	2.34	0.124	41.75	39.36	1.39	0.215
Day 18	44.93	41.45	2.45	0.156	43.09	38.47	1.36	0.020
24 h								
Day 4	32.83	30.09	2.28	0.302	34.69	31.58	1.56	0.125
Day 8	37.01	34.56	2.28	0.355	36.78	33.45	1.40	0.095
Day 14	41.76	38.73	2.28	0.255	39.85	37.68	1.43	0.260
Day 18	41.90	37.74	2.43	0.137	40.00	36.04	1.41	0.044

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

Table B9. Metabolic carbon dioxide (CO_2) production ($L \cdot d^{-1} \cdot BW^{-0.75}$) of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Nighttime[4]								
Day 4	33.71	32.11	2.73	0.659	31.88	23.98	3.08	0.014
Day 8	36.14	36.31	2.73	0.962	34.59	33.27	2.99	0.640
Day 14	40.88	38.29	2.73	0.475	40.18	39.48	3.11	0.807
Day 18	43.09	38.19	2.96	0.201	39.10	36.28	2.96	0.311
Daytime								
Day 4	33.91	32.29	2.70	0.534	35.07	34.40	1.55	0.747
Day 8	38.66	37.20	2.70	0.574	37.90	34.35	1.39	0.083
Day 14	42.32	39.67	2.70	0.313	40.29	37.39	1.42	0.146
Day 18	42.32	40.04	2.82	0.406	41.57	36.21	1.40	0.009
24 h								
Day 4	33.80	32.18	2.53	0.568	33.75	29.25	1.77	0.030
Day 8	37.39	36.76	2.53	0.822	36.19	33.83	1.63	0.230
Day 14	41.60	38.98	2.53	0.356	40.13	37.97	1.66	0.264
Day 18	42.70	39.13	2.68	0.233	40.28	36.23	1.64	0.040

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

Table B10. Respiratory quotient (RQ) of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Nighttime								
Day 4	1.14	1.25	0.07	0.260	0.95	0.83	0.08	0.217
Day 8	1.11	1.21	0.07	0.327	1.02	1.11	0.08	0.276
Day 14	1.06	1.08	0.07	0.774	1.06	1.12	0.08	0.506
Day 18	1.11	1.14	0.08	0.733	1.07	1.08	0.08	0.841
Daytime								
Day 4	0.98	0.93	0.02	0.124	0.96	1.00	0.02	0.131
Day 8	0.94	0.98	0.02	0.178	0.97	0.94	0.02	0.209
Day 14	0.94	0.96	0.02	0.444	0.96	0.95	0.02	0.464
Day 18	0.95	0.96	0.02	0.735	0.97	0.94	0.02	0.303
24 h								
Day 4	1.06	1.09	0.04	0.504	0.95	0.92	0.04	0.534
Day 8	1.02	1.09	0.04	0.180	0.99	1.03	0.03	0.415
Day 14	1.00	1.02	0.04	0.614	1.01	1.01	0.03	0.912
Day 18	1.03	1.05	0.04	0.671	1.01	1.01	0.03	0.978

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

Table B11. Metabolic total heat production (kcal·d^{-1}·BW$^{-0.75}$) during daytime of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Day 4								
0700[3]	173.8	169.4	12.1	0.757	187.9	168.2	10.6	0.175
0800	176.4	174.2	12.4	0.875	181.3	175.7	10.6	0.700
0900	164.1	173.3	12.4	0.523	182.7	184.0	10.6	0.927
1000	170.4	163.8	12.1	0.640	178.3	167.8	10.6	0.466
1100	177.0	169.5	12.1	0.595	172.4	166.3	12.7	0.704
1300	185.7	190.1	12.1	0.755	181.7	165.2	10.6	0.257
1500	180.7	173.3	12.1	0.598	187.3	161.5	11.5	0.091
1900	170.4	169.0	12.1	0.919	177.4	156.2	11.5	0.164
Day 8								
0700[3]	193.2	177.1	14.2	0.362	195.3	163.7	9.9	0.029
0800	209.0	202.8	14.2	0.724	207.1	187.7	9.9	0.174
0900	195.2	179.2	14.2	0.363	201.0	185.2	9.9	0.267
1000	206.0	190.6	14.2	0.380	192.0	173.5	9.9	0.194
1100	203.4	197.0	14.2	0.716	195.8	184.0	9.9	0.406
1300	210.4	202.9	14.2	0.672	198.7	171.4	9.9	0.058
1500	218.2	206.6	14.2	0.510	216.4	183.1	9.9	0.022
1900	205.0	193.6	14.2	0.519	195.4	194.3	9.9	0.940
Day 14								
0700[3]	231.6	201.4	13.1	0.063	220.5	203.7	10.9	0.276
0800	219.5	209.7	13.1	0.538	223.6	220.7	10.9	0.852
0900	215.8	201.9	13.1	0.385	218.5	197.0	10.9	0.164
1000	219.1	200.2	13.1	0.239	219.2	186.7	10.9	0.037
1100	216.5	208.9	13.1	0.633	223.0	204.3	10.9	0.226
1300	227.0	204.5	13.1	0.162	188.8	188.4	10.9	0.982
1500	240.8	229.4	13.1	0.476	216.5	205.6	10.9	0.476
1900	226.4	202.5	13.1	0.138	210.8	198.2	10.9	0.413
Day 18								
0700[3]	227.0	209.4	15.2	0.345	221.8	207.0	10.8	0.337
0800	238.6	214.2	15.2	0.192	227.3	193.7	10.8	0.033
0900	213.8	198.5	15.2	0.411	205.7	188.9	10.8	0.279
1000	207.4	186.5	15.2	0.263	204.0	174.1	10.8	0.056
1100	215.4	203.4	15.2	0.517	219.1	183.3	10.8	0.023
1300	225.8	207.7	15.2	0.333	244.8	193.9	11.7	0.004
1500	239.3	219.6	15.2	0.291	231.7	188.1	11.7	0.021
1900	223.6	200.4	15.2	0.214	212.3	200.2	11.7	0.486

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Total heat production before first morning meal.

Table B12. Metabolic oxygen (O_2) consumption during daytime of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Day 4								
0700[3]	34.71	34.41	2.48	0.921	37.64	33.64	2.41	0.224
0800	34.71	35.09	2.57	0.898	35.86	34.08	2.41	0.584
0900	32.40	34.60	2.57	0.462	36.34	36.09	2.41	0.940
1000	33.84	32.98	2.48	0.767	35.86	33.64	2.41	0.500
1100	35.14	33.84	2.48	0.657	27.70	32.92	2.41	0.115
1300	36.87	38.73	2.48	0.521	35.93	32.49	2.41	0.296
1500	35.86	34.27	2.48	0.587	37.23	32.20	2.63	0.147
1900	33.84	33.70	2.48	0.961	35.08	31.34	2.64	0.279
Day 8								
0700[3]	39.46	34.71	2.92	0.194	39.13	32.44	2.05	0.026
0800	41.90	40.61	2.92	0.722	41.01	37.91	2.05	0.294
0900	38.88	35.14	2.92	0.305	40.14	36.62	2.05	0.233
1000	41.47	38.16	2.92	0.364	37.83	34.60	2.05	0.273
1100	40.46	39.60	2.92	0.812	38.85	36.62	2.05	0.449
1300	41.76	40.61	2.92	0.751	39.56	34.74	2.05	0.105
1500	43.78	41.76	2.92	0.580	43.02	37.05	2.05	0.046
1900	40.75	38.59	2.92	0.553	39.13	39.64	2.05	0.863
Day 14								
0700[3]	46.94	40.46	2.61	0.044	44.73	40.87	2.28	0.233
0800	43.63	42.05	2.61	0.613	44.44	44.18	2.28	0.937
0900	43.20	39.89	2.61	0.293	43.14	39.29	2.28	0.233
1000	43.78	39.74	2.61	0.202	43.72	37.41	2.28	0.053
1100	43.35	41.47	2.61	0.551	44.30	40.73	2.28	0.269
1300	45.79	41.33	2.61	0.159	37.52	37.64	2.28	0.973
1500	48.10	45.51	2.61	0.410	43.00	41.30	2.28	0.598
1900	45.21	39.89	2.61	0.094	41.56	39.43	2.28	0.508
Day 18								
0700[3]	46.22	42.25	3.16	0.308	44.45	41.90	2.28	0.430
0800	48.24	42.65	3.16	0.153	45.32	38.59	2.28	0.041
0900	42.34	39.30	3.16	0.435	40.56	38.15	2.28	0.456
1000	41.04	37.13	3.16	0.315	40.43	34.56	2.28	0.073
1100	42.77	40.74	3.16	0.601	43.45	36.86	2.28	0.045
1300	45.07	41.45	3.16	0.352	49.23	38.86	2.46	0.006
1500	48.10	43.79	3.16	0.269	46.17	37.14	2.47	0.023
1900	44.35	40.11	3.16	0.276	42.16	40.14	2.47	0.579

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Prior to morning feeding at 0700

Table B13. Metabolic carbon dioxide (CO$_2$) production (L·d^{-1}·BW$^{-0.75}$) during daytime of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Day 4								
0700[3]	32.83	30.24	2.44	0.375	35.73	31.81	2.42	0.236
0800	34.85	31.89	2.49	0.320	36.00	36.86	2.42	0.794
0900	31.82	32.55	2.44	0.804	35.82	37.58	2.42	0.594
1000	33.26	30.67	2.44	0.375	33.70	31.24	2.42	0.454
1100	34.56	32.55	2.44	0.489	30.83	32.82	2.42	0.544
1300	36.57	33.98	2.44	0.375	35.13	33.69	2.42	0.661
1500	34.56	33.84	2.44	0.804	36.08	31.67	2.65	0.204
1900	32.69	32.25	2.44	0.882	34.67	29.65	2.66	0.150
Day 8								
0700[3]	34.85	35.86	2.72	0.764	36.68	31.48	2.04	0.080
0800	39.31	38.45	2.72	0.797	40.42	34.21	2.04	0.038
0900	37.30	36.57	2.72	0.830	38.55	37.24	2.04	0.655
1000	38.59	36.29	2.72	0.493	38.12	33.50	2.04	0.118
1100	38.73	37.15	2.72	0.637	37.97	34.79	2.04	0.279
1300	40.46	38.59	2.72	0.578	37.69	31.19	2.04	0.030
1500	40.89	37.87	2.72	0.370	41.58	33.50	2.04	0.008
1900	39.60	36.57	2.72	0.370	36.82	34.65	2.04	0.459
Day 14								
0700[3]	42.19	37.58	2.66	0.164	40.06	38.07	2.02	0.483
0800	41.90	39.46	2.66	0.455	43.37	41.24	2.02	0.452
0900	41.04	38.88	2.66	0.509	43.08	38.07	2.02	0.081
1000	41.33	39.17	2.66	0.509	41.50	35.62	2.02	0.042
1100	41.19	39.89	2.66	0.692	42.51	39.08	2.02	0.229
1300	42.19	37.73	2.66	0.178	36.46	34.90	2.02	0.582
1500	45.79	44.50	2.66	0.692	41.93	38.21	2.02	0.193
1900	43.05	40.46	2.66	0.429	40.92	38.07	2.02	0.316
Day 18								
0700[3]	40.32	38.37	2.93	0.586	41.38	37.99	2.00	0.237
0800	43.78	41.08	2.93	0.452	43.68	36.98	2.00	0.022
0900	41.62	38.98	2.93	0.461	40.37	33.96	2.00	0.028
1000	40.61	36.10	2.93	0.211	39.79	33.82	2.00	0.040
1100	41.90	39.35	2.93	0.476	42.38	34.39	2.00	0.007
1300	43.05	40.12	2.93	0.413	44.95	37.17	2.15	0.017
1500	44.35	41.71	2.93	0.461	44.49	36.79	2.16	0.018
1900	43.05	37.90	2.93	0.154	41.28	38.46	2.16	0.374

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Prior to morning feeding at 0700

Table B14. Respiratory quotient (RQ) during daytime of lactating sows with litters fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	Thermal Neutral				Heat stress			
	HCP	LCP	SEM[2]	*P*-value	HCP	LCP	SEM[2]	*P*-value
Day 4								
0700[3]	0.95	0.88	0.04	0.180	0.95	0.94	0.04	0.906
0800	1.01	0.91	0.04	0.071	0.99	1.07	0.04	0.135
0900	0.98	0.96	0.04	0.633	0.98	1.04	0.04	0.265
1000	0.99	0.94	0.04	0.394	0.94	0.95	0.04	0.812
1100	0.98	0.97	0.04	0.816	0.98	1.00	0.05	0.723
1300	0.99	0.88	0.04	0.037	0.98	1.04	0.04	0.253
1500	0.97	0.99	0.04	0.725	0.95	0.98	0.04	0.521
1900	0.97	0.95	0.04	0.752	0.97	0.94	0.04	0.613
Day 8								
0700[3]	0.87	1.10	0.05	0.003	0.95	0.98	0.03	0.484
0800	0.93	0.94	0.05	0.897	0.99	0.90	0.03	0.035
0900	0.96	1.10	0.05	0.056	0.96	1.01	0.03	0.247
1000	0.93	0.95	0.05	0.820	1.00	0.96	0.03	0.308
1100	0.96	0.94	0.05	0.838	0.98	0.94	0.03	0.376
1300	0.97	0.96	0.05	0.891	0.95	0.90	0.03	0.177
1500	0.93	0.90	0.05	0.700	0.96	0.91	0.03	0.188
1900	0.97	0.94	0.05	0.727	0.95	0.90	0.03	0.213
Day 14								
0700[3]	0.90	0.93	0.03	0.347	0.91	0.93	0.02	0.524
0800	0.95	0.94	0.03	0.641	0.98	0.93	0.02	0.150
0900	0.95	0.97	0.03	0.595	1.00	0.97	0.02	0.314
1000	0.94	0.99	0.03	0.166	0.95	0.96	0.02	0.831
1100	0.95	0.96	0.03	0.915	0.95	0.97	0.02	0.652
1300	0.93	0.93	0.03	0.981	0.97	0.93	0.02	0.198
1500	0.96	0.98	0.03	0.602	0.97	0.93	0.02	0.157
1900	0.95	1.02	0.03	0.061	0.98	0.96	0.02	0.626
Day 18								
0700[3]	0.92	0.91	0.04	0.977	0.93	0.91	0.03	0.542
0800	0.91	0.98	0.04	0.153	0.97	0.96	0.03	0.800
0900	0.98	0.99	0.04	0.866	0.99	0.90	0.03	0.017
1000	0.98	0.97	0.04	0.777	0.99	0.98	0.03	0.927
1100	0.98	0.97	0.04	0.796	0.98	0.94	0.03	0.265
1300	0.95	0.98	0.04	0.507	0.92	0.94	0.03	0.642
1500	0.92	0.95	0.04	0.624	0.96	0.98	0.03	0.589
1900	0.98	0.93	0.04	0.334	0.99	0.95	0.03	0.419

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] Prior to morning feeding at 0700

Table B15. Metabolic carbon dioxide (CO_2) production, oxygen (O_2) consumption, total heat production (THP) and respiratory quotient (RQ) of piglets from sows fed high crude protein (HCP) and low crude protein (LCP) diet and exposed to thermal neutral and heat stress conditions[1]

	BW, kg[2]	Heat stress			
		CO_2, $L \cdot d^{-1} \cdot BW^{-0.75}$	O_2, $L \cdot d^{-1} \cdot BW^{-0.75}$	THP, $kcal \cdot d^{-1} \cdot BW^{-0.75}$	RQ
HCP					
Day 4	2.20	31.28	41.30	196.99	0.76
Day 8 (9)	3.46	40.35	41.73	209.86	0.97
Day 14 (15)	5.47	41.99	47.43	233.59	0.89
Day 18 (19)	6.13	41.21	47.35	232.44	0.87
LCP					
Day 4	2.10	24.36	34.39	162.05	0.71
Day 8 (9)	4.50	33.96	39.66	194.04	0.86
Day 14 (15)	5.68	34.91	45.79	218.88	0.76
Day 18 (17)	5.65	47.87	56.42	275.45	0.85

	BW, kg[2]	Thermal neutral			
		CO_2, $L \cdot d^{-1} \cdot BW^{-0.75}$	O_2, $L \cdot d^{-1} \cdot BW^{-0.75}$	THP, $kcal \cdot d^{-1} \cdot BW^{-0.75}$	RQ
HCP					
Day 4	1.75	34.56	38.88	191.76	0.89
Day 8 (9)	3.47	35.42	43.20	207.84	0.82
Day 14 (13)	5.17	40.61	44.93	217.68	0.90
Day 18 (17)	6.10	43.20	49.25	240.72	0.88
LCP					
Day 4 (3)	2.19	31.97	39.74	192	0.80
Day 8 (9)	3.42	38.02	41.47	204.72	0.92
Day 14 (15)	5.18	36.29	48.38	229.92	0.75
Day 18	7.20	41.47	47.52	246.72	0.87

[1] Acual day of lactation is shown in parentheses.

[2] BW: body weight

APPENDIX C

EFFECTS OF A NEAR IDEAL AMINO ACID BALANCE DIET ON LYSINE MAMMARY UPTAKE, WHOLE BODY PROTEIN OXIDATION AND MUSCLE PROTEIN BREAKDOWN ON LACTATING SOWS

MATERIALS AND METHODS

Dietary Treatments

Ingredients and calculated nutrient composition of the diets are presented in Table 4.1. Analyzed total (hydrolysate) and free AA of the diets are presented in Table C1. The NRC (2012) model was used to estimate requirements for AA, net energy (NE), calcium (Ca) and phosphorus (P). The requirements were predicted based on the swine herd performance at the Michigan State University Swine Teaching and Research Center, as follows: sow BW of 210 kg, parity number of 2 and above, and daily intake of 6 kg/day, litter size of 10, piglet BW gain of 280 g/day over a 21-day lactation period, and an ambient temperature of 20 °C. The model predicted a minimum sow BW loss of 7.5 kg and the protein:lipid in the model was adjusted to the minimum allowable value of near zero. All diets were formulated to contain the same SID Lys (0.90%) and NE (2,580 kcal/kg) concentrations.

The control diet (CON) was formulated using corn and soybean meal as the only sources of Lys to meet NRC (2012) SID Lys requirement (0.90%) and consequently contained 18.75% CP. Valine met near SID requirement of 0.77% (vs. 0.79%) (NRC, 2012). All other EAA SID concentrations were in excess relative to NRC (2012). A second diet balanced to reach a near ideal AA (NIAA) profile was formulated, as described in Chapter 2 (Zhang et al., 2019) and is referred to as the optimal diet (OPT) throughout the remainder of the manuscript.

Animals and Feeding

The study was conducted at the Michigan State University Swine Teaching and Research Center. Ten purebred multiparous (parity 2+) Yorkshire sows were moved to conventional farrowing crates between days 105 and 107 of gestation, grouped by parity, and randomly assigned to 1 of 2 dietary treatments within parity groups (Control, n = 5; Optimal, n = 5). The study was conducted over 3 blocks of time, with 2 to 5 sows per block. Litters were standardized to 11 piglets within the first 24 h after farrowing with the aim of weaning 10 piglets per sow. Sows were adapted to the experimental diets (2.2 kg/d) 4 to 6 days before the expected farrowing date. After farrowing, sows feed allowance was progressively increased from 1.88 kg/d at day 1 to 7.44 kg/d at day 21 of lactation, according to the NRC (2012) model, with targeted ADFI of 6.0 kg/d during the whole lactation period. Feed was provided daily in 3 equal meals (0700, 1300, and 1900) with feed intake and refusal recorded daily before the morning meal. Water was freely accessible to sows and piglets. Injection of iron and surgical castration were conducted on days 1 and 7, respectively. No creep feed was supplied to the piglets. On infusion day, 2 meals (0700, 1300) were divided into 6 aliquots and supplied every 2 h from 0700 to 1700. The BW and backfat thickness of sows were recorded on days 1 and 21, and litter weights were recorded on days 1, 14, 18 and 21. Milk yield was estimated for peak lactation (between days 14 and 18) according to Zhang et al. (2019).

Ear Vein Catheterization

The sows were restrained with a rope snare and remained in their farrowing stall where sedation was induced. For sedation, Telazol was reconstituted with 2.5 mL of 100 mg/mL ketamine and 2.5 mL of 100 mg/mL xylazine to a volume of 5 mL. This sedative mixture was administered i.m. in the Brachiocephalicus muscle caudal the ear, at a dosage of 0.1 mL/10 lbs body weight. Sows were carefully assisted to facilitate laying down in ventral recumbence. Sedation lasted for 45 to 60 minutes. The depth of anesthesia was monitored by the degree of muscle relaxation and respiratory (i.e., 10 to 25 breaths/min).

The entire dorsal surface of both ears was prepared for aseptic placement of ear vein catheters (one for infusion, and the other for blood sampling). The skin was scrubbed gently with 10% betadine solution following with 70% isopropyl alcohol. The areas caudal to the ear and dorsal to the neck were clipped using a professional clipper to remove hair in and provide adhesion for the tape.

A pre-cut 61 cm, round tip, medical grade microbore intravascular tubing (1.65 mm o.d., 1.02 mm i.d.) with hydromer coating (Access Technology Corp., Skokie, IL) was prefilled at the time of catheterization with heparinized saline (30 IU/mL) before insertion. A hand tourniquet was applied at the base of the ear to distend the medial and lateral branches of the auricular vein. Either vein was used for catheterization.

A short-term stylet catheter (14G, 5.08 cm, Safety IV catheter; B. Braun Melsungen AG, Germany) was inserted into the vein with needle bevel facing up. Upon appearance of blood, the vein was occluded and the needle rotated 180° to angle the needle bevel facing down. The short-term catheter was inserted into the vein while holding the needle in place. The needle was then removed and the intravascular tubing was inserted through the short-term catheter and pushed for approximately 30 cm caudally to reach the external jugular vein. Small sections of tape (5.1 cm wide, ZONAS® porous tape, Johnson & Johnson Consumer Companies, Inc. Skillman, NJ) were affixed to the remaining section of intravascular tubing and used to suture the tubing to the skin. The catheter was sutured (monocryl, CP-1, 36 mm, 1/2c; Ethicon Inc. USA) to the ear at the entry point of the tubing and at approximately 5 cm away from the entry point. Gauze was placed to cover the suture sites and elastic adhesive tape was used to wrap the ear to protect and hold the catheter in position. A blunt-end needle adapter was placed onto the distal end of the catheter with an adaptor injection cap with male Luer lock. The same catheterization procedure was done for the other ear vein. Then both catheters were inserted through a small incision on the bottom of a denim protective purse glued with Livestock ID Tag Cement (W.J. Ruscoe Company, Akron, OH) on the dorsal region of the neck, caudally to the ears and cranial to the shoulders. The catheters were coiled and stored in the purse until used for infusion and blood sampling. The catheters were flushed with sterilized heparinized saline (30 IU/mL) twice per day to maintain patency.

Elastic adhesive tape (7.5 cm wide, 3M veterinary adhesive tape) was used tape each ear into a cone shape and also tape the tubing onto the skin from the ears up to the purse. Then, elastic bandage (15 cm wide, Novation®, Hartmann USA, Inc., Rock Hill, SC) was wrapped around the neck and upper body of the sow in the shape of a life vest (crisscross) to protect the protective purse from damage.

Following the termination of the infusion protocol, catheters were removed. The elastic adhesive tape was carefully pulled to expose the sutures. The sutures were cut with small surgical scissors. The catheters were gently pulled out of the ear veins, and gauze was held in place until the insertion site was coagulated. The remaining bandage and adhesive tape around the neck and thorax were then removed once the catheters were out of the ear veins. The sow health status (rectal temperature and feed intake) and potential infection were monitored daily from the day of catheterization and for 3 days following the removal of catheters.

Preparation of Isotope Solution and Infusion

Tracers were weighed, dissolved in saline and filtered through sterile millipore steriflip filters (0.22 μm). For each sow, 3-[methyl-$^2H_3$]histidine (183 μmol in 20 mL saline for bolus injection), [^{13}C]bicarbonate (368 μmol in 20 mL saline for prime; and 736 μmol in 30 mL saline for 2-h infusion), and [1-^{13}C]lysine (1.28 mmol in 30 mL saline for prime; and 9.00 mmol in 60 mL saline for 6-h infusion) were prepared. The solution of [^{13}C]bicarbonate was freshly prepared to minimize the loss of $^{13}CO_2$. Specifically, [^{13}C]bicarbonate was weighed and dissolved in 20 mL 3-[methyl-$^2H_3$]histidine solution in the morning of infusion day (Figure C5). Mixed 20 mL saline solution of 3-[methyl-$^2H_3$]histidine (183 μmol bolus injection) and [^{13}C]bicarbonate (368 μmol priming dose) was given through the infusion catheter 1 hour prior to the constant 2-hour [^{13}C]bicarbonate infusion (368 μmol/h) followed by a 6-hour primed constant [1-^{13}C]lysine infusion (1.50 mmol/h) (Figure C5).

Blood Sampling

For plasma 3-[methyl-$^{2}H_3$]histidine, blood samples were collected through sampling catheter at 0, 5, 10, 15, 30 and 45 min and 1, 2, 3, 4, 5, 6, 7, 8, 24, 34, 48, 58 and 72 h post bolus infusion, transferred into 500 μL BD microtainer tubes (K_2EDTA), centrifuged (1,500 × g at 4°C for 5 min) and transferred to 1.5-mL microcentrifuge tubes for analysis of plasma 3-[methyl-$^{2}H_3$]histidine.

For plasma [1-^{13}C]lysine, blood samples were collected prior to infusion for background enrichment and at 1, 2, 3, 4, 5 and 6 h from the start of [1-^{13}C]lysine infusion (Figure C5).

For blood CO_2, blood samples (2 mL) were collected prior to [^{13}C]bicarbonate-prime infusion for background, and at 1, 2, 3, 4, 5, 6, 7 and 8 h following prime infusion. Blood samples were injected into evacuated vacutainer tubes (Becton Dickinson, Plymouth, UK) previously prepared with 2 mL of phosphoric acid, immediately mixed, and cooled to room temperature. The CO_2 was then transferred from evacuated vacutainers to Exetainer tubes (Labco Breath Tube, UK) by using pure nitrogen (N) gas as medium for further analysis.

Milk Sampling

Milk was sampled before infusion for background enrichment, and at 1, 2, 3, 4, 5 and 6 h of primed constant infusion of Lys. Piglets were separated from the sows for approximately 1 h, and sows were administered 1 mL of oxytocin (20 IU/mL oxytocin, sodium chloride 0.9% w/v, and chlorobutanol 0.5% w/v, VetTek, Blue Springs, MO) through the sampling catheter, following blood sample. A total of 30-mL milk was manually collected across all glands and stored in 2 separate 15-mL tubes (polypropylene centrifuge tubes with screw cap, Denville Scientific). Piglets were immediately returned to sows to complete nursing and empty the mammary glands. Piglets were then removed from the sows immediately after nursing and kept separate from the sow until the next milk sampling time.

Isotope Analysis

[1-^{13}C]lysine and 3-[methyl-$^{2}H_3$]histidine in plasma and milk (after acid hydrolysis) were determined as their dansyl derivatives by HESI LC-MS as previously described (Marini, 2011). The following *m/z* transitions were monitored: 613→379 and 614→380 for [1-^{13}C]lysine and 403→124 and 406→127 for 3-[methyl-$^{2}H_3$]histidine. Determination of blood $^{13}CO_2$ enrichment was performed by IRMS (Delta+XL IRMS coupled with GasBench-II peripheral device, Thermo-Quest Finnigan, Bremen, Germany) as previously described (Verbruggen et al. 2009).

Nutrient Analysis

Feed samples were analyzed for gross energy (GE) by bomb calorimetry according to the manufacturer's instructions (Parr Instrument Inc., Moline, IL). Dry matter, N and in feed samples were analyzed as described in Chapter 2 (Zhang et al., 2019). Dietary AA analysis [AOAC Official Method 982.30 E (a,b,c), 45.3.05, 2006] was performed by the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) as outlined in Zhang et al (2019).

Whole milk samples were analyzed for fat, true protein, lactose, and milk urea N (MUN) with infrared spectroscopy by the Michigan Dairy Herd Improvement Association (NorthStar Cooperative®, Lansing, MI) (Zhang et al., 2019).

Calculations

Lysine oxidation

The enrichment of CO_2 during the period of primed-constant infusion of [^{13}C]bicarbonate was presented as follows (Eq. 1):

$$\mathrm{E}_{\mathrm{CO_2}}(\%) = \frac{\text{Infusion rate}_{\mathrm{H13CO_3^-}}\ (\mu\text{mol/h})}{\mathrm{Ra}_{\mathrm{HCO_3^-}}(\mu\text{mol/h})} \tag{1}$$

Where "infusion rate$_{\mathrm{H13CO3}}$" represented the infusion rate (368 μmol/h) of [^{13}C]bicarbonate, and "Ra$_{\mathrm{HCO3}}$" represented the rate of appearance of unlabeled bicarbonate (baseline) in the body.

The enrichment of CO_2 during the period of primed-constant infusion of [1-^{13}C]lysine was presented as follows (Eq. 2):

$$\mathrm{E}'_{\mathrm{CO_2}}(\%) = \frac{\mathrm{Ra}_{\mathrm{H13CO_3^-}}(\mu\text{mol/h})}{\mathrm{Ra}_{\mathrm{HCO_3^-}}(\mu\text{mol/h})} \tag{2}$$

Where "Ra$_{\mathrm{H13CO3}}$" represents the rate of appearance of labeled bicarbonate from [1-^{13}C]lysine oxidation, and "Ra$_{\mathrm{HCO3}}$" represents the rate of appearance of unlabeled bicarbonate (baseline) in the body as in Eq. 1.

The enrichment of lysine during the period of primed-constant infusion of [1-^{13}C]lysine was presented as follows (Eq. 3):

$$\mathrm{E}_{\mathrm{Lys}}(\%) = \frac{\text{Infusion rate}_{[1-^{13}\mathrm{C}]\mathrm{Lys}}\ (\text{mmol/h})}{\mathrm{Ra}_{\mathrm{Lys}}(\text{mmol/h})} = \frac{\mathrm{Ra}_{\mathrm{H13CO_3^-}}(\mu\text{mol/h})}{\mathrm{Ra}_{\mathrm{H13CO_3^-}\ \text{from Lys oxidation}}\ (\mu\text{mol/h})} \tag{3}$$

Where Ra$_{\mathrm{Lys}}$ represents the rate of appearance of unlabeled lysine in the body.

Lysine oxidation was estimated as follows (Eq. 4):

$$\text{Lys oxidation } (\mu\text{mol/h}) = \text{Ra}_{\text{H13CO}_3^- \text{ from Lys oxidation}}\ (\mu\text{mol/h}) = \frac{\text{E}'_{\text{CO}_2}(\%)}{\text{E}_{\text{Lys}}(\%)} \times \frac{\text{Infusion rate}_{\text{H13CO}_3^-}\ (\mu\text{mol/h})}{\text{E}_{\text{CO}_2}(\%)} \qquad (4)$$

<u>Whole body protein breakdown and synthesis</u>

Protein breakdown and synthesis were calculated as follows (Eq. 5 and 6):

$$\text{Protein breakdown (mmol/h)} = \text{Ra}_{\text{Lys}}\ \text{(mmol/h)} - \text{intake (mmol/h)} \times \text{SID (\%)} =$$

$$\frac{\text{Infusion rate}_{[1-{}^{13}\text{C}]\text{Lys}}\ \text{(mmol/h)}}{\text{E}_{\text{Lys}}(\%)} - \text{intake (mmol/h)} \times \text{SID (\%)} \qquad (5)$$

$$\text{Protein synthesis(mmol/h)} = \text{Ra}_{\text{Lys}}\ \text{(mmol/h)} - \text{Total Lys oxidation(mmol/h)} =$$

$$\frac{\text{Infusion rate}_{[1-{}^{13}\text{C}]\text{Lys}}\ \text{(mmol/h)}}{\text{E}_{\text{Lys}}(\%)} - \text{Total Lys oxidation(mmol/h)} \qquad (6)$$

<u>Lysine utilization efficiency for lactation</u>

Lysine utilization efficiency for lactation was calculated as follows (Eq. 7):

$$\text{Efficiency of lysine} = \frac{\text{Protein net synthesis (mmol/h)}}{\text{Ra}_{\text{Lys}}\ \text{(mmol/h)}} = \frac{\text{Protein synthesis (mmol/h)} - \text{protein breakdown(mmol/h)}}{\text{Ra}_{\text{Lys}}\ \text{(mmol/h)}} \qquad (7)$$

Statistical Analysis

Data were analyzed by ANOVA using the Mixed model procedures of SAS 9.4 (SAS Inst. Inc., Cary, NC).

For the analysis of lysine enrichment in plasma and milk, the following model was used:

Enrichment of lysine = diet + hour + *block* + *sow* + diet × hour + *e*

The **Enrichment of lysine** depended on the fixed effects of **diet (**CON vs. OPT**)**, and sampling **hour**, with hour as repeated measurement. The random effects included ***block*** and individual ***sow***. The interactive effect of **diet × hour** was also included.

For the analysis of lysine balance and body protein breakdown and synthesis, the following model was used:

Response = diet + *block*+ *sow* + *e*

The **Response** depended on the fixed effects of **diet (**CON vs. OPT**)**. The random effects included ***block*** and individual ***sow***.

For the analysis of dynamics of 3-methyl-histidine (3MH), the following model was used:

Enrichment of 3MH = diet + hour + *block* + *sow* + diet × hour + *e*

The **Enrichment of 3MH** depended on the fixed effects of **diet (**CON vs. OPT**)**, and sampling **hour**, with hour as repeated measurement. The random effects included ***block*** and individual ***sow***. The interactive effect of **diet × hour** was also included.

Table C1. Analyzed and calculated concentration of nitrogen (N), total and free essential amino acids in control (CON) and optimal (OPT) diets (as-fed)

	CON		OPT	
	Analyzed[1]	Calculated[2]	Analyzed[1]	Calculated[2]
Total, %				
N	2.95	3.08	2.24	2.24
Arg	1.18	1.26	0.70	0.78
His	0.51	0.53	0.40	0.43
Ile	0.84	0.81	0.60	0.60
Leu	1.60	1.67	1.10	1.19
Lys	1.06	1.04	1.03	1.01
Met	0.26	0.31	0.26	0.33
Met + Cys	0.55	0.63	0.47	0.57
Phe	0.95	0.96	0.73	0.76
Phe + Tyr	1.52	1.59	1.13	1.20
Thr	0.69	0.73	0.61	0.68
Trp[3]	0.22	0.23	0.17	0.19
Val	0.91	0.90	0.87	0.89
Free AA, %				
Arg	0.05	0.00	0.03	0.00
His	0.00	0.00	0.07	0.07
Ile	0.01	0.00	0.08	0.08
Leu	0.01	0.00	0.01	0.00
Lys	0.02	0.00	0.41	0.37
Met	0.00	0.00	0.10	0.11
Met + Cys	0.00	0.00	0.10	0.11
Phe	0.00	0.00	0.13	0.13
Phe + Tyr	0.01	0.00	0.15	0.13
Thr	0.02	0.00	0.21	0.20
Trp[3]	-	0.00	-	0.05
Val	0.01	0.00	0.27	0.29

[1] Analyzed values represents average across 3 blocks (feed mixes).

[2] Calculated values for the total AA are based on the AA concentration in feed ingredients according to NRC (2012), and calculated values for the free AA correspond to the dietary inclusion rate in crystalline form.

[3] Analysis of free Trp was not performed.

Table C2. Lysine balance (g/d) of sows fed Control (CON; 18.4% CP) and Optimal (OPT; 14.0% CP) diets during peak lactation (day 14 to day 18)[1]

Item	Diet		SEM[2]	*P*-value
	CON	OPT		
SID Lys intake	85.54	87.12	1.1	0.164
Lys oxidation	30.91	17.29	14.0	0.364
Lys flux	135.61	154.23	13.8	0.456
Lys from body protein breakdown	50.08	66.79	13.4	0.487
Lys for body protein synthesis	107.70	128.86	24.5	0.572

[1] Data are least squares means.
[2] Maximum value of the standard error of the means.

Table C3. Body protein synthesis and breakdown of sows fed Control (CON; 18.4% CP) and Optimal (OPT; 14.0% CP) diets during peak lactation (day 14 to day 18)[1]

Item	Diet		SEM[2]	*P*-value
	CON	OPT		
Body protein breakdown, g/d	743	991	256	0.487
Body protein synthesis, g/d	1,598	1,912	363	0.572
Body protein net synthesis, g/d	791	1,031	213	0.279
Body protein synthesis/ breakdown	2.32	2.51	0.71	0.834
Efficiency[3]	0.42	0.51	0.17	0.623

[1] Data are least squares means.

[2] Maximum value of the standard error of the means.

[3] $\text{Efficiency of lysine} = \frac{\text{Protein net synthesis (g/d)}}{\text{Lysine flux (g/d)}}$

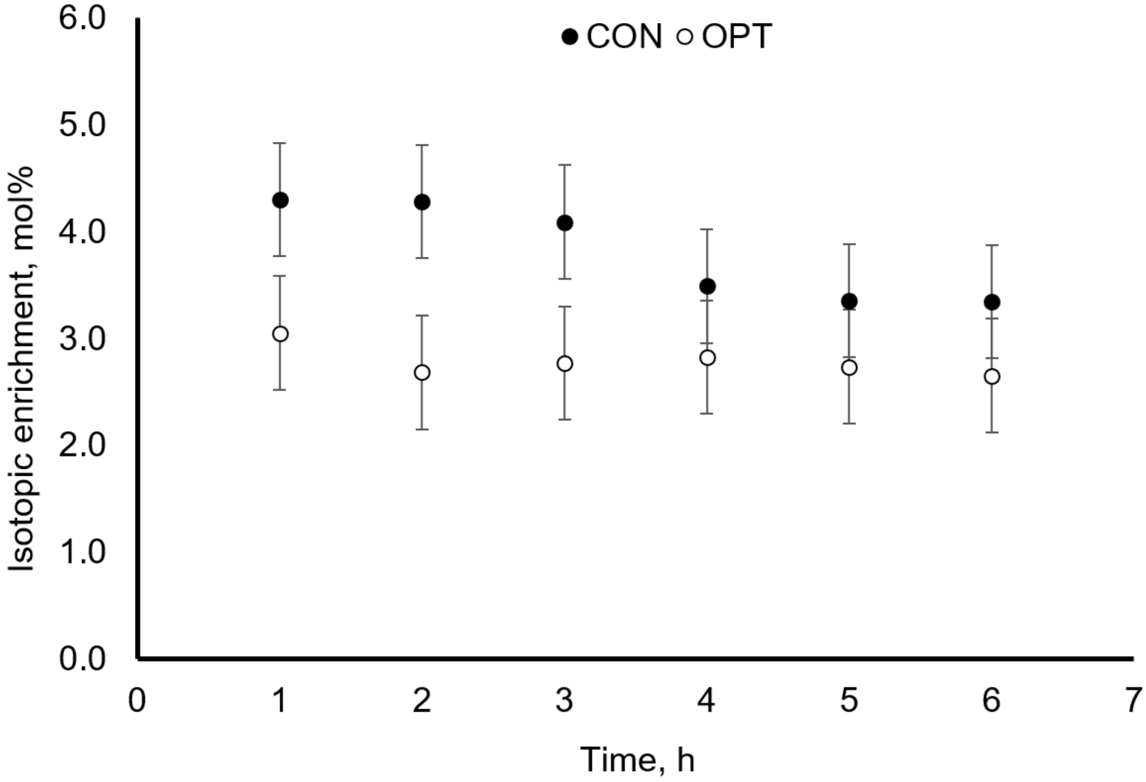

Figure C1. Changes in plasma isotopic enrichment of Lys during peak lactation (between day 15 and 21) for sows fed Control (CON; 18.4% CP; n = 3) and Optimal (OPT; 14.0% CP; n = 5) diets. Plasma isotopic enrichment of [1-^{13}C]lysine differed between diets (*P* < 0.001) and time points (*P* < 0.01) with no interaction between diet and time (*P* = 0.477). Standard error of the mean, SEM = 0.53.

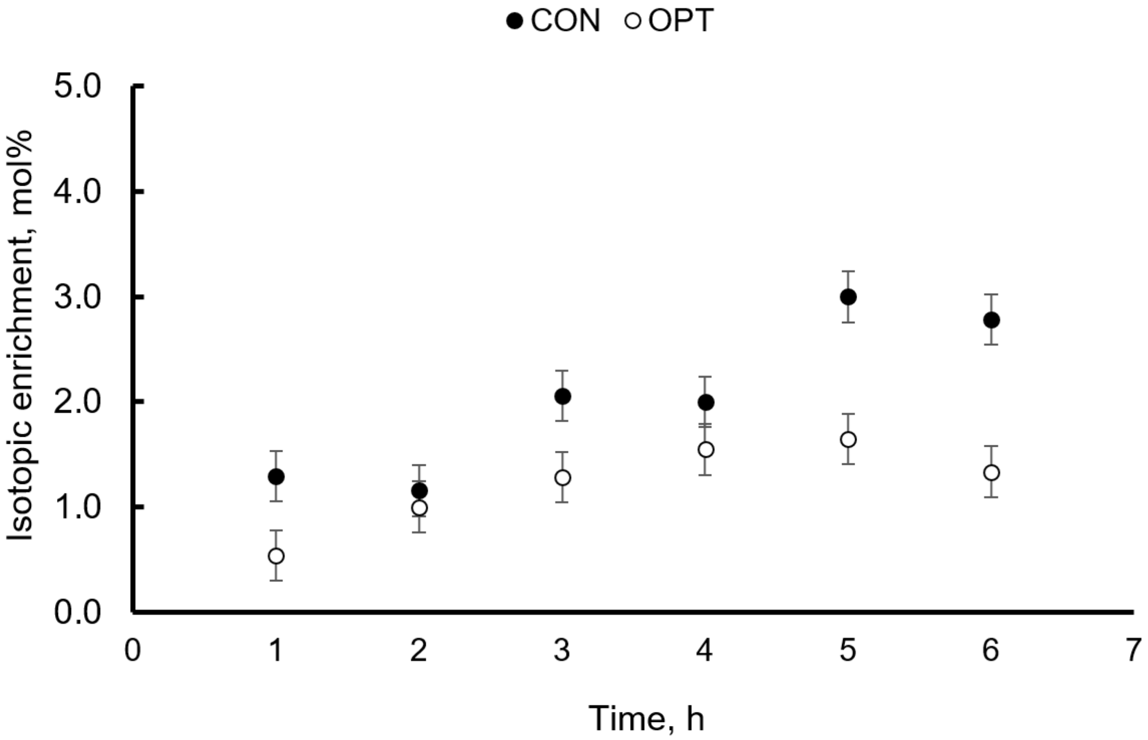

Figure C2. Milk isotopic enrichment of [1-^{13}C]lysine during peak lactation (between day 15 and 21) for sows fed Control (CON; 18.4% CP; n = 3) and Optimal (OPT; 14.0% CP; n = 5) diets. Milk isotopic enrichment of [1-^{13}C]lysine tended to differ between diets (P = 0.061) and did not differ between time points (P = 0.827), with no interaction between diet and time (P = 0.979). Standard error of the mean, SEM = 0.24.

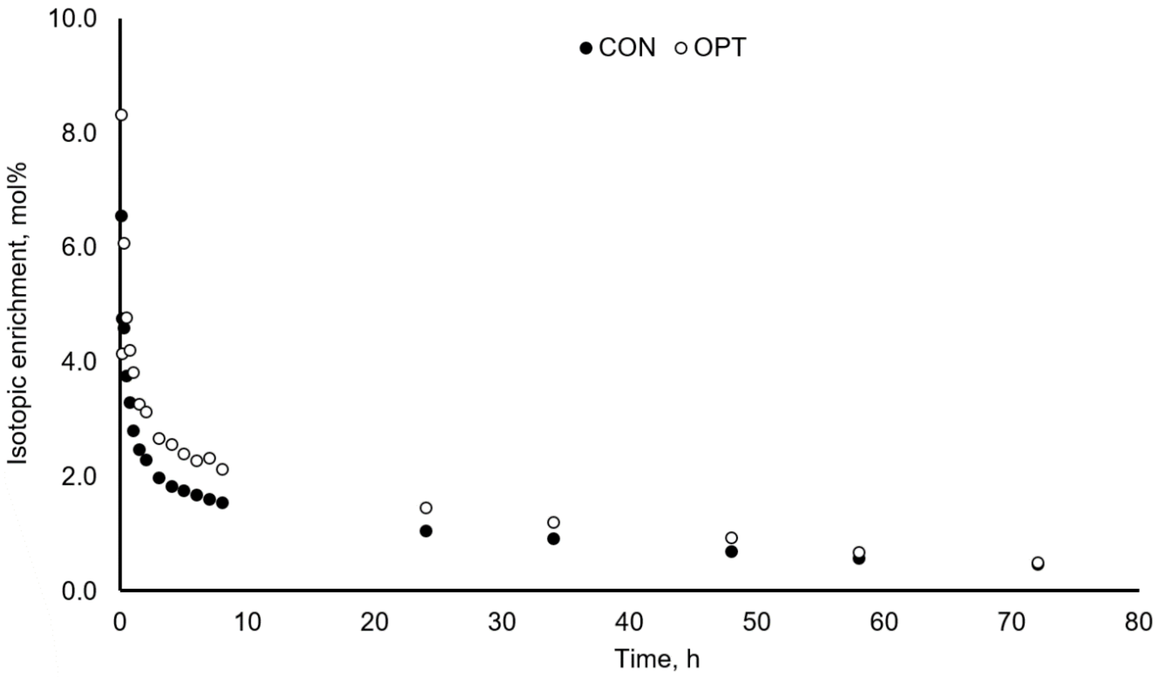

Figure C3. Changes in plasma isotopic enrichment of 3-[methyl-$^2H_3$]histidine during peak lactation (day 15 to day 21) for sows fed Control (CON; 18.4% CP; n = 4) and Optimal (OPT; 14.0% CP; n = 4) diets. Plasma isotopic enrichment of 3-[methyl-$^2H_3$]histidine differed between diets ($P < 0.001$) and time points ($P < 0.001$), with no interaction between diet and time ($P = 0.547$). Standard error of the mean, SEM = 0.645.

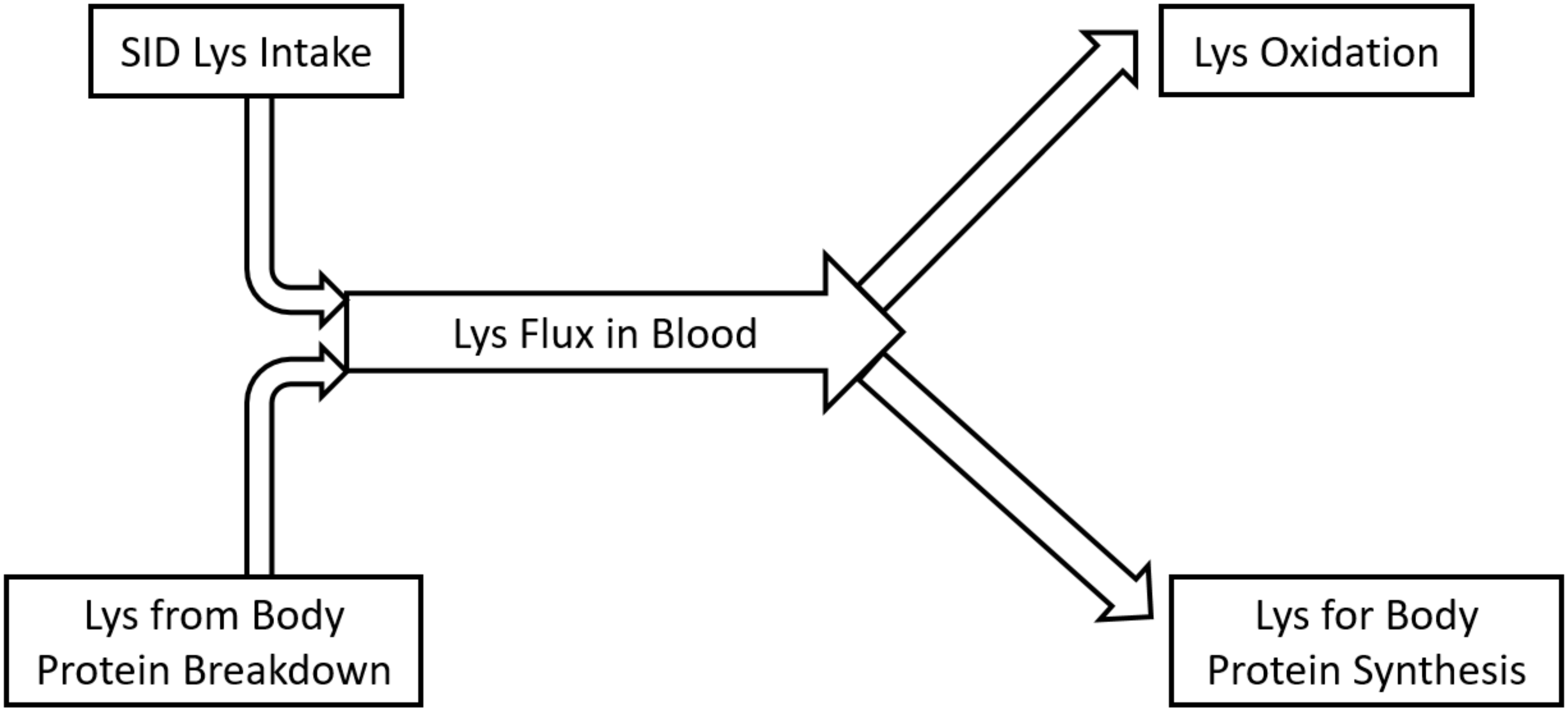

Figure C4. Diagram of Lys balance of lactating sows at fed state

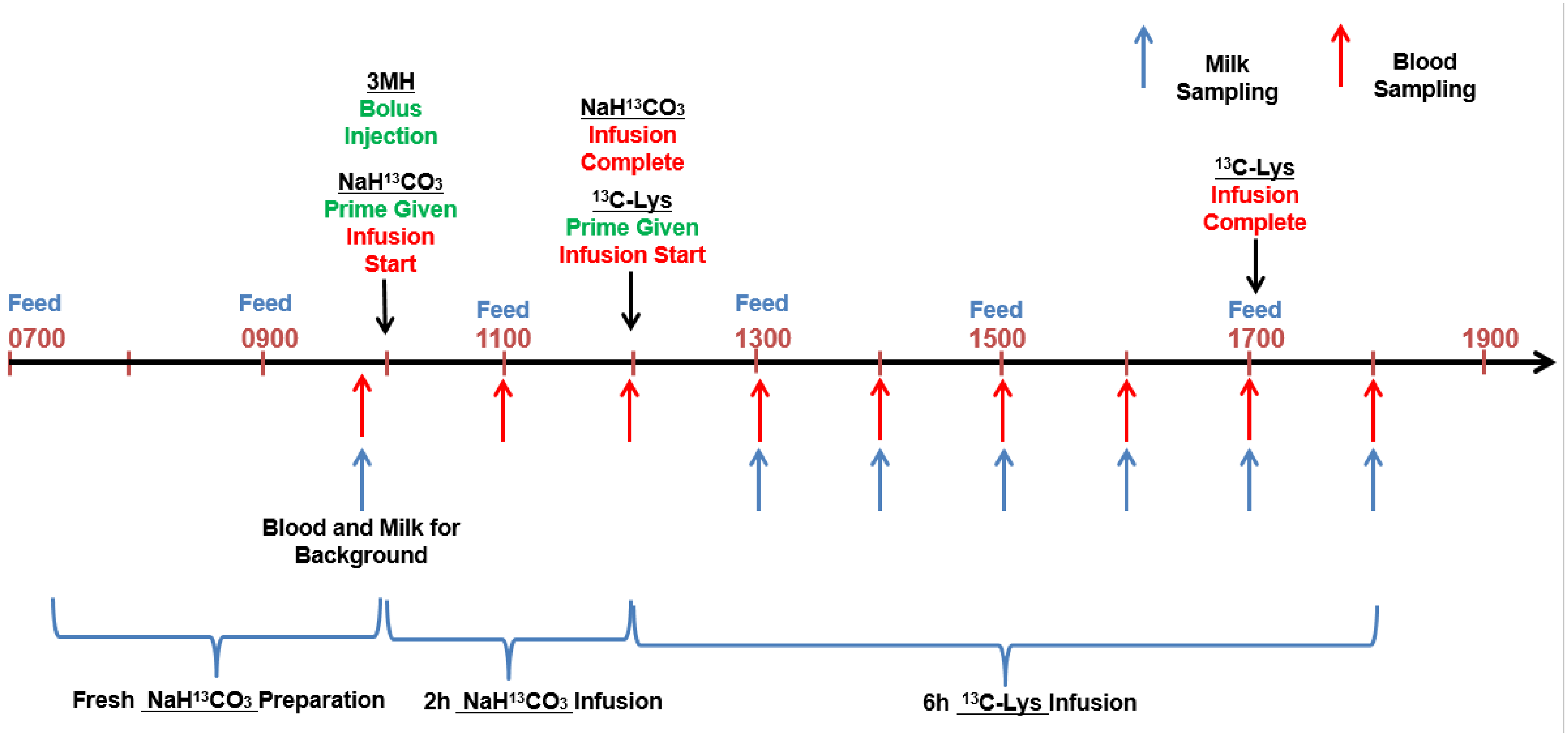

Figure C5. Timeline of isotope infusion and sampling

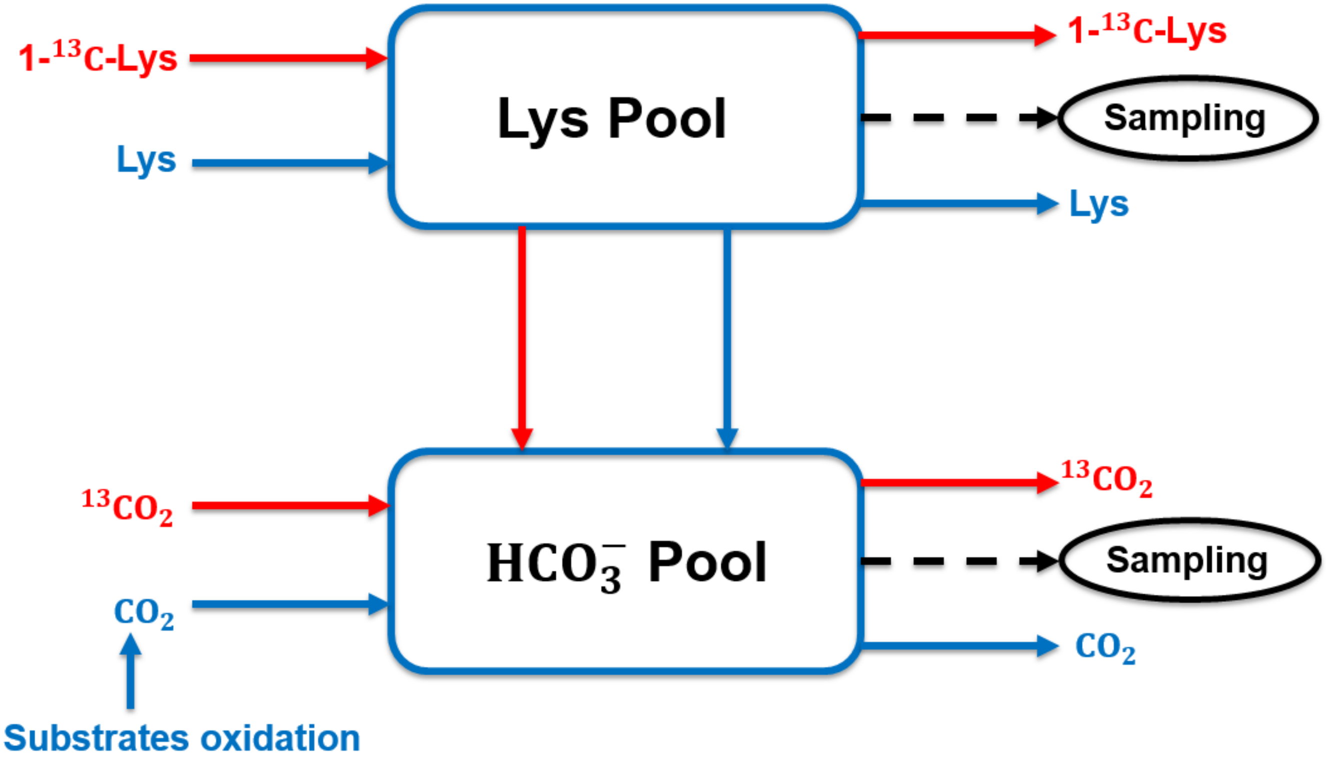

Figure C6. Two-pool model to estimate lysine oxidation

LITERATURE CITED

LITERATURE CITED

AOAC. 1997. Official methods of analysis. 16th ed. Assoc. Off. Anal. Chem., Washington, DC.

AOAC. 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Washington, DC

Beede, D. K., and R. J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim. Sci. 62:543–554.

Bender, D.A. 2012. The metabolism of “surplus” amino acids. Br. J. Nutr. 108: S113-S121.

Berg, J. M., J. L. Tymoczko, G. J. Gatto, and L. Stryer. 2015. Biochemistry 8th Ed. W. H. Freeman and Compnay, New York.

Bergsma, R., E. Kanis, M.W.A. Verstegen, C.M.C. van der Peet-Schwering, and E. F. Knol. 2009. Lactation efficiency as a result of body composition dynamics and feedintake in sows. Livest. Sci. 125:208–222.

Bernabucci, U., N. Lacetera, L. H. Baumgard, R. P. Rhoads, B. Ronchi and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal. 4:7. doi:10.1017/S175173111000090X

Bond, T. E., C. F. Kelly, and H. Heitman Jr. 1959. Hog house air conditioning and ventilation data. Trans. ASAE 2:1–4. doi:10.13031/2013.41147

Bondi, A. A. 1987. Animal Nutrition. John Wiley and Sons Ltd, Chichester, United Kingdom.

Brouwer, E. 1965. Report of sub-committee on constant and factors. In: Blaxter, K. L., editor. Energy metabolism. Vol. 11. Troon (Scotland): European Association for Animal Production; p. 441–443. doi:10.4236/ns.2011.312129

Brown-Brandl, T. M., J. A. Nienaber, H. Xin, R. S. Gates. 2004. A literature review of swine heat production. Trans. ASAE. 47:259-270.

Brown-Brandl, T. M., M. D. Hayes, H. Xin, J. A. Nienaber, and H. Li. 2014. Heat and moisture production of modern swine. ASHRAE Trans. 120:469–489.

Cabezón, F.A., Schinckel, A.P., Richert, B.T., Peralta, W.A., Gandarillas, M., 2017a.Development and application of a model of heat production for lactating sows. Prof. Anim. Sci. 33, 357–362.

Cabezón, F. A., A. P. Schinckel, A. J. Smith, J. N. Marchant-Forde, J. S. Johnson, and R. M. Stwalley. 2017b. Technical Note: Initial evaluation of floor cooling on lactating sows under acute heat stress. Prof. Anim. Sci. 33:254–260. doi:10.15232/pas.2016-01584

Calvert, D. T., and D. B. Shennan. 1996. Evidence for an interaction between cationic and neutral amino acids at the blood-facing aspect of the lactating rat mammary epithelium. J. Dairy Res. 63:25–33.

Campos, P. H., J. Noblet, Y. Jaguelin-Peyraud, H. Gilbert, P. Mormede, R. F. M. de Oliveira Donzele, J. L. Donzele, and D. Renaudeau. 2014. Thermoregulatory responses during thermal acclimation in pigs divergently selected for residual feed intake. Int. J. Biometeorol. 58:1545–1557.

Chamberlin, D. P., W. J. Powers, D. W. Rozeboom, T. M. Brown-Brandl, S. Erwin, C. Walker, and N. L. Trottier. 2015a. Impact of reduced dietary crude protein concentration with crystalline amino acid supplementation on lactation performance and ammonia emission of sows housed under thermo-neutral and thermal heat stress environments. J. Anim. Sci. 93 (E-Suppl. 2):67.

Chamberlin, D. P., D. W. Rozeboom, S. Erwin, and N. L. Trottier. 2015b. Lactation performance in sows fed diets with graded levels of crystalline amino acids as substitute for crude protein at lysine requirement. J. Anim. Sci. 93 (E-Suppl. 2):69. (Abstr.)

Chamberlin, D.P. 2017. Impacts of reducing dietary crude protein with crystalline amino acid supplementation on lactating sow performance, nitrogen utilization and heat production. MS. Thesis, Michigan State University, East Lansing.

Collin, A., J. van Milgen, S. Dubois, and J. Noblet. 2001. Effect of high temperature on feeding behavior and heat production in group-housed young pigs. Br. J. Nutr. 86:63–70.

Collins, F. G., F. A. Mitros, and J. L. Skibba. 1980. Effect of palmitate on hepatic biosynthetic functions at hyperthermic temperatures. Metabolism 29:524–531.

Curtis, S. 1983. Environmental Management in Animal Agriculture. Iowa State University Press. Ames, Iowa. pp 6-96.

Darnell, J., H. Lodish, and D. Baltimore. 1986. Molecular Cell Biology. Scientific American Books, Inc., New York.

de Meis, L., M. L. Bianconi, and V. A. Suzano. 1997. Control of energy fluxes by the sarcoplasmic reticulum Ca^{2+}-ATPase: ATP hydrolysis, ATP synthesis and heat production. FEBS Letters. 406: 201-204.

Eissen, J.J., E. Kanis, and B. Kemp. 2000. Sow factors affecting voluntary feed intake during lactation. Livst. Prod. Sci. 64: 147-165.

Elsley, F.W.H., 1971. Nutrition and lactation in the sow. In: Falconer, I. R. (ed.) Lactation. Butterworths, London, UK, pp: 393-411

Escobar, J. J. W. Frank, A. Suryawan, H. V. Nguyen, S. R. Kimball, L. S. Jefferson, and T. A. Davis. 2006. Regulation of cardiac and skeletal muscle protein synthesis by individual branched-chain amino acids in neonatal pigs. Am. J. Physiol. Endocrinol Metab. 290: E612–E621. doi:10.1152/ajpendo.00402.2005

Ewan, R.C. 2001. Energy utilization in swine nutrition. In: A.J. Lewis and L. L. Southern, editor, Swine Nutrition. 2nd ed. CRC Press, Washington, DC. p. 85–94.

Farmer, C. and Prunier, A., 2002. High ambient temperatures: how they affect sow lactation performance. Pig News and Information 23: 95N-102N.

Fuller, M.F., A. Cadenhead, G. Mollison, and B. Seve. 1987. Effects of the amount and quality of dietary protein on nitrogen metabolism and heat production in growing pigs. Br. J. Nutr. 58: 277-285.

Gillespie, T. 2016. Key production indicators: why are they so different between units? Benchmark. Spring edition: 6-7.

Guan, X., B. J. Bequette, G. Calder, P.K. Ku, K. N. Ames, and N. L. Trottier. 2002. Amino acid availability affects amino acid flux and protein metabolism in the porcine mammary gland. J. Nutr. 132: 1224–1234.

Guan X., J. E. Pettigrew, P. K. Ku, N. K. Ames, B. J. Bequette, and N. L. Trottier. 2004. Dietary protein concentration affects plasma arteriovenous difference of amino acids across the porcine mammary gland. J. Anim. Sci. 82: 2953–2963.

Hach, C. C., B. K. Bowden, A. B. Kopelove, and S. V. Brayton. 1987. More powerful peroxide Kjeldahl digestion method. J. AOAC Int. 70:783–787.

Hamilton, T. S. 1939. The growth, activity, and composition of rats fed diets balanced and unbalanced with respect to protein. Nutrition. 17: 565-582.

Hansen, A. V., A. B. Strathe, E. Kebreab, J. France, and P. K. Theil. Predicting milk yield and composition in lactating sows: A Bayesian approach. J. Anim. Sci. 2012.90:2285–2298. doi:10.2527/jas2011-4788

Harkins, M., R. D. Boyd and D. E. Bauman. Effect of recombinant porcine somatotropin on lactational performance and metabolite patterns in sows and growth of nursing pigs. J. Anim. Sci. 1989. 67:1997-2008. doi:10.2527/jas1989.6781997x

Huber, L., C.F.M. de Lange, U. Krogh, D. Chamberlin, and N.L. Trottier. 2015. Impact of feeding reduced crude protein diets to lactating sows on nitrogen utilization. J. Anim. Sci. 93:5254–5264.

Huber, L., C. F. de Lange, C. W. Ernst, U. Krogh, and N. L. Trottier. 2016. Impact of improving dietary amino acid balance for lactating sows on efficiency of dietary amino acid utilization and transcript abundance of genes encoding lysine transporters in mammary tissue. J. Anim. Sci. 94:4654–4665.

Hurley, W. L., H. Wang, J. M. Bryson, and D. B. Shennan. 2000. Lysine uptake by mammary gland tissue from lactating sows. J. Anim. Sci. 78:391–395.

Jakobsen, K., P.K. Theil, and H. Jørgensen. 2005. Methodological considerations as to quantify nutrient and energy metabolism in lactating sows. J. Anim. Feed Sci. 14, Suppl. 1, 31–47.

Jaworski, N. W., D. W. Liu, D. F. Li, and H. H. Stein. 2016. Wheat bran reduces concentrations of digestible, metabolizable, and net energy in diets fed to pigs, but energy values in wheat bran determined by the difference procedure are not different from values estimated from a linear regression procedure. J. Anim. Sci. 2016.94:3012–3021. doi:10.2527/jas2016-0352

Johnson, J. S., R. L. Boddicker, M. V. Sanz-Fernandez, J. W. Ross, J. T. Selsby, M. C. Lucy, T. J. Safranski, R. P. Rhoads, and L. H. Baumgard. 2013. Effects of mammalian in utero heat stress on adolescent body temperature. Int. J. Hyperthermia. 29: 696–702.

Johnson, J. S. M. V. Sanz Fernandez, N. A. Gutierrez, J. F. Patience, J. W. Ross, N. K. Gabler, M. C. Lucy, T. J. Safranski, R. P. Rhoads, and L. H. Baumgard. 2015. Effects of in utero heat stress on postnatal body composition in pigs: I: Growing phase. J. Anim. Sci.

Johnson, J. S., M. V. Sanz Fernandez, J. F. Patience, J. W. Ross, N. K. Gabler, M. C. Lucy, T. J. Safranski, R. P. Rhoads, and L. H. Baumgard. 2015. Effects of in utero heat stress on postnatal body composition in pigs: II. Finishing phase. J. Anim. Sci.93:82–92.

Johnson, J.S., and K. A. Shade. 2017. Characterizing body temperature and activity changes at the onset of estrus in replacement gilts. Livst. Sci. 199, 22-24.

Johnson, J. S., S. Zhang, G. M. Morello, J. M. Maskal, and N. L. Trottier. 2019. Technical note: development of an indirect calorimetry system to determine heat production in individual lactating sows. J. Anim. Sci. 2019.97:1609–1618. doi: 10.1093/jas/skz049

Johnston, L. J., M. Ellis, G. W. Libal, V. B. Mayrose, W. C. Weldon, and NCR-89 Committee on Swine Management. 1999. Effect of Room Temperature and Dietary Amino Acid Concentration on Performance of Lactating Sows. J. Anim. Sci. 77:1638–1644.

Kerr B. J., L. L. Southern, T. D. Bidner, K. G. Friesen, and R. A. Easter. 2003. Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing finishing pig performance and carcass composition. J. Anim. Sci. 81:3075–3087.

Kerr, B. J., J. T. Yen, J. A. Nienaber, and R. A. Easter. 2003. Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production of growing pigs. J. Anim. Sci., 81:1998-2007.

Kim, S. W., D. H. Baker, and R. A. Easter. 2001. Dynamic ideal protein and limiting amino acids for lactating sows: The impact of amino acid mobilization. J. Anim. Sci. 79:2356–2366.

Koelsch, R.K., W. Powers, and A.L. Sutton. 2005. Integrating Animal Feeding Strategies Into CNMP Processes: Role Of Updated ASAE Standard D384.2, Paper number 054074, 2005 American Society of Agricultural and Biological Engineers Annual Meeting, St. Joseph, Michigan, USA.

Kpodo, K.R., A.W. Duttlinger, and J.S. Johnson. 2019. Effects of pen location on thermoregulation and growth performance in grow-finish pigs during late summer. Trans. Anim. Sci. 3: txz033. doi: 10.1093/tas/txz033.

Labussière, E., S. Dubois, J. van Milgen, G. Bertrand and J. Noblet. 2008. Fasting heat production and energy cost of standing activity in veal calves. Br. J. Nutr. 100, 1315–1324. doi:10.1017/S0007114508980648

Le Bellego, L., J. van Milgen, S. Dubois, and J. Noblet. 2001. Energy utilization of low-protein diets in growing pigs. J. Anim. Sci., 79:1259-1271.

Le, P. D., A. J. Aarnink, N. W. Ogink, P. M. Becker, and M. W. Verstegen. 2005. Odor from animal production facilities: its relationship to diet. Nutr. Res. Rev. 181:3–30.

LeGrow, S.M., and F.W.H. Beamish. 1986. Influence of dietary protein and lipid on apparent heat increment of rainbow trout, *salmo gairdneri*. Can. J. Fish. Aquat. Sci. 43:19-25.

Lei, J., D. Feng, Y. Zhang, F. Q. Zhao, Z. Wu, A. San Gabriel, Y. Fujishima, H. Uneyama, and G. Wu. 2012. Nutritional and regulatory role of branched-chain amino acids in lactation. Front. Biosci. 17:2725–2739.

Lewis, A.J. 2001. Energy utilization in swine nutrition. In: A.J. Lewis and L. L. Southern, editor, Swine Nutrition. 2nd ed. CRC Press, Washington, DC. p. 142–161.

Li, P., D. A. Knabe, S. W. Kim, C. J. Lynch, S. M. Hutson, and G. Wu. 2009. Lactating porcine mammary tissue catabolizes branched-chain amino acids for glutamine and aspartate synthesis. J. Nutr. 139:1502–1509. doi:10.3945/jn.109.105957

Li, Z., Y. Li, Z. Lv, H. Liu, J. Zhao, J. Noblet, F. Wang, C. Lai, and D. Li. 2017. Net energy of corn, soybean meal and rapeseed meal in growing pigs. J. Anim. Sci. Biotechnol. 8: 44.

Li, Q. F., N. Trottier, and W. Powers. 2015. Feeding reduced crude protein diets with crystalline amino acids supplementation reduce air gas emissions from housing. J. Anim. Sci. 2015.93:721–730.

Lucy, M. C., T. J. Safranski, J. N. Rhoades, J. W. Ross, N. K. Gabler, R. P. Rhoads, and L. H. Baumgard. 2012. Litter characteristics and thermoregulatory behavior of first parity sows exposed to a controlled heat stress (HS) during gestation. J. Anim. Sci. 90(Suppl 3): 731–732(Abstract).

Lucy, M. C., and T. J. Safranski. 2017. Heat stress in pregnant sows: Thermal responses and subsequent performance of sows and their offspring. Mol. Reprod. Dev.84:946–956.

Lyu, Z., Y. Li, H. Liu, E. Li, P. Li, S. Zhang, F. Wang, and C. Lai. 2018. Net energy content of rice bran, defatted rice bran, corn gluten feed, and corn germ meal fed to growing pigs using indirect calorimetry. J. Anim. Sci. 96:1877–1888. doi: 10.1093/jas/sky098

Mackie, R. I., P. G. Stroot, and V. H. Varel. 1998. Biochemical identification and biological origin of key odor components in livestock waste. J. Anim. Sci. 76:1331–1342.

MacRae, J. C., A. Walker, D. Brown, and G. E. Lobley. 1993. Accretion of total protein and individual amino acids by organs and tissues of growing lambs and the ability of nitrogen balance techniques to quantitate protein retention. Anim. Sci. 57:237–245.

Madrid, J., S. Martínez, C. López, J. Orengo, M. J. López, and F. Hernández. 2013. Effects of low protein diets on growth performance, carcass traits and ammonia emissions of barrows and gilts. Anim. Prod. Sci. 53:146–153.

Manjarín, R., J. P. Steibel, V. Zamora, N. Am-in, R. N. Kirkwood, C. W. Ernst, P. S. Weber, N.P. Taylor, and N. L. Trottier. 2011. Transcript abundance of amino acid transporters, β-casein, and α-lactatlbumin in mammary tissue of periparturient, lactating, and postweaned sows. J. Dairy Sci. 94:3467-3476.

Manjarín, R., V. Zamora, G. Wu, J. P. Steibel, R. N. Kirkwood, N. P. Taylor, E. Wils-Plotz, K. Trifilo, and N. L. Trottier. 2012. Effect of amino acids supply in reduced crude protein diets on performance efficiency of mammary uptake, and transporter gene expression in lactating sows. J. Anim. Sci. 90:3088-3100.

Manjarín, R., B. J. Bequette, G. Wu, and N. L. Trottier. 2014. Linking our understanding of mammary gland metabolism to amino acid nutrition. Amino Acids 46:2447–2462. doi:10.1007/s00726-014-1818-8

Marini, J. C. 2011. Quantitative analysis of ^{15}N-labeled positional isomers of glutamine and citrulline via electrospray ionization tandem mass spectrometry of their dansyl derivatives. Rapid Commun. Mass Spectrom. 25: 1291–1296.

Marini, J. C., U. Agarwal, J. L. Robinson, Y. Yuan, I. C. Didelija, B. Stoll, and D. G. Burrin. 2017. The intestinal-renal axis for arginine synthesis is present and functional in the neonatal pig. Am. J. Physiol. Endocrinol. Metab. 313:E233–E242. doi:10.1152/ajpendo.00055.2017

Mohn, S., and C. F. 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. doi:10.2527/1998.761124x

Mullan, B.P., W. Brown, and M. Kerr. 1992. The response of the lactating sow to ambient temperature. Proceedings of the Nutrition Society of Australia 17, 215 (Abstr.).

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82:179–183. doi:10.2527/2004.821179x

Nardone, A., B. Ronchi, N. Lacetera, and U. Bernabucci. 2006. Climatic effects on productive traits in livestock. Vet. Res. Commun. 30(Suppl. 1), 75–81.

Nienaber, J. A., J. S. DeShazer, H. Xin, P. E. Hillman, and J. T. Yen. 2009. Measuring energetics of biological processes. In: DeShazer, J. S., editor. Livestock energetics and thermal environmental management. St. Joseph (MI):ASAE; p. 73–112. doi:10.13031/2013.28297

Noblet, J., Y. Henry, and S. Dubois. 1987. Effect of amino acid balance on nutrient utilization and carcass composition of growing swine. J. Anim. Sci. 65:717–726.

Noblet, J., H. Fortune, X. S. Shi, S. Dubois. 1994. Prediction of net energy value of feeds for growing pigs. J. Anim. Sci. 72:344–353.

Norton, L. E., G. J. Wilson, D. L. Layman, C. J. Moulton, and P. J. Garlick. 2012. Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats. Nutr. Metab. 9:67–75.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington DC.

O’Quinn, P. R., D. A. Knabe, and G. Wu. 2002. Arginine catabolism in lactating porcine mammary tissue. J. Anim. Sci. 80:467–474. doi:10.2527/2002.802467x

Otto, E. R., M. Yokoyama, P. K. Ku, N. K. Ames, and N. L. Trottier. 2003a. Nitrogen balance and ileal amino acid digestibility in growing pigs fed diets reduced in protein concentration. J. Anim. Sci. 81:1743-1753.

Otto, E. R., M. Yokoyama, S. Hengemuehle, R. D. von Bermuth, T. van Kempen, and N. L. Trottier. 2003b. Ammonia, volatile fatty acids, phenolics, and odor offensiveness in manure from growing pigs fed diets reduced in protein concentration. J. Anim. Sci. 81:1754-1763.

Pearce, S. 2011. The effects of heat stress and nutritional status on metabolism and intestinal integrity in growing pigs. PhD. Diss., Iowa State University, Ames.

Pearce, S.C., N.K. Gabler, J.W. Ross, J. Escobar, J.F. Patience, R.P. Rhoads, and L.H. Baumgard. 2013. The effects of heat stress and plane of nutrition on metabolism in growing pigs. J. Anim. Sci. 91: 2108-2118.

Pedersen, S., and H.B. Rom. 2000. Diurnal variation in heat production from pigs in relation to animal activity. European Agricultural Engineers.

Pedersen, T.F., T. S. Bruun, T. Feyera, U. K. Larsen, P. K. Theil. 2016. A two-diet feeding regime for lactating sows reduced nutrient deficiency in early lactation and improved milk yield. Livest. Sci. 191: 165-173.

Pedersen, T.F., C. Y. Chang, N. L. Trottier, T. S. Bruun, and P. K. Theil. 2019. Effect of dietary protein intake on energy utilization and feed efficiency of lactating sows. J. Anim. Sci.97:779–793.

Pérez Laspiur, J. 2001. Effect of dietary supplementation of L-Arginine to lactating sows under heat stress. PhD. Diss., Michigan State University, East Lansing.

Pérez Laspiur, J., and N. L. Trottier. 2001. Effect of dietary arginine supplementation and environmental temperature on sow lactation performance. Livst. Prod. Sci. 70:159-165.

Pérez Laspiur, J., J. L. Burton, P. S. D. Weber, R. N. Kirkwood, and N. L. Trottier. 2004. Short communication: amino acid transporters in porcine mammary gland during lactation. J. Dairy Sci. 87:3235-3237.

Pérez Laspiur, J., J. L. Burton, P. S. D. Weber, J. Moore, R. N. Kirkwood, and N. L. Trottier. 2009. Dietary protein intake and stage of lactation differentially modulate amino acid transporter mRNA abundance in porcine mammary tissue. J. Nutr. 139: 1677–1684.

Philippe, F.-X, and B. Nicks. 2015. Review on greenhouse gas emissions from pig houses: Production of carbon dioxide, methane and nitrous oxide by animals and manure. Agr. Ecosyst. Envron. 199: 10-25.

Pollmann, D. S. 2010. Seasonal Effects on Sow Herds: Industry experience and management strategies. Des Moines, IA: Midwest American Society of Animal Science.

Prunier, A., M.M. de Braganca, and J. L. Dividich. 1997. Influence of high ambient temperature on performance of reproductive sows. Livest. Prod. Sci. 52: 123-133.

Qu, H., H. Yan, H. Lu, S. S. Donkin, and K. M. Ajuwon. 2016. Heat stress in pigs is accompanied by adipose tissue–specific responses that favor increased triglyceride storage J. Anim. Sci.94:1884–1896.

Renaudeau, D., and J. Noblet. 2001. Effects of exposure to high ambient temperature and dietary protein level on sow milk production and performance of piglets. J. Anim. Sci. 79:1540–1548.

Renaudeau, D., A. Collin, S. Yahav, V. de Basilio, J. L. Gourdine, and R. J. Collier. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal. 6: 707–728

Renaudeau, D., G. Frances, S. Dubois, H. Gilbert, and J. Noblet. 2013. Effect of thermal heat stress on energy utilization in two lines of pigs divergently selected for residual feed intake. J. Anim. Sci. 91:1162–1175.

Rhoads, R. P., L. H. Baumgard, and J. K. Suagee. 2013. Metabolic priorities during heat stress with an emphasis on skeletal muscle. J. Anim. Sci. 2013.91:2492–2503. doi:10.2527/jas2012-6120

Richert, B. T., M. D. Tokach, R. D. Goodband, J. L. Nelssen, R. G. Campbell, and S. Kershaw. 1997. The effect of dietary lysine and valine fed during lactation on sows and litter performance. J. Anim. Sci. 75:1853-1860.

Rideout, T. C., and M. Z. Fan. 2004. Nutrient utilization in responses to chicory inulin supplementation in studies with pigs. J. Sci. Food Agri. 84:1005–1012.

Rinaldo, D., and J. Le Dividich. 1991. Effects of warm exposure on adipose tissue and muscle metabolism in growing pigs. Comp. Biochem. Physiol. A 100:995–1002.

Rozeboom, D. W., J. E. Pettigrew, R. L. Moser, S. G. Cornelius, and S. M. EL Kandelgy. 1994. In vivo estimation of body composition of mature gilts using live weight, backfat thickness, and deuterium oxide. J. Anim. Sci.

Schinasi, L., R. A. Horton, V. T. Guidry, S., Wang, S. W. Marshall, and K. B. Morland. 2011. Air pollution, lung function, and physical symptoms in communities near swine feeding operations. Epidemiology. 22:208–215.

Shaw, M. I., A. D. Beaulieu, and J. F. Patience. 2006. Effect of diet composition on water consumption in growing pigs. J. Anim. Sci. 84:3123–3132. doi:10.2527/jas.2005-690

Shennan, D. B., S. A. McNeillie, E. A. Jamieson, and D. T. Calvert. 1994. Lysine transport in lactating rat mammary tissue: Evidence for an interaction between cationic and neutral amino acids. Acta Physiol. Scand. 151:461–466.

Sommer, S. G., O. Oenema, T. Matsunaka, and L. S. Jensen. 2013. Regulations on animal manure management. In: S.G. Sommer, M. L. Christensen, T. Schmidt, and L. S. Jensen, editors, Animal Manure Recycling—Treatment and Management. John Wiley and Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom.

Stinn, J. P., and H. Xin. 2014. Heat and moisture production rates of a modern U.S. swine breeding, gestation, and farrowing facility. Trans. ASABE 57:1517–1528.

St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86: E52-E77.

Strathe, A. V., T. S. Bruun, J.-E. Zerrahn, A.-H. Tauson, and C. F. Hansen. 2016. The effect of increasing the dietary valine-to-lysine ratio on sow metabolism, milk production, and litter growth. J. Anim. Sci. 94, 155–164.

Strathe, A. V., T. S. Bruun, and C. F. Hansen. 2017. Sows with high milk production had both a high feed intake and high body mobilization. Animal. 11:1913–1921

Theil, P.K., T. T. Nielsen, N. B. Kristensen, R. Labouriau, V. Danielsen, C. Lauridsen, and K. Jakobsen. 2002. Estimation of milk production in lactating sows by determination of deuterated water turnover in three piglets per litter. Acta Agric. Scand. 52:221–232.

Theil, P. K. 2015. Transition feeding of sows. In: C. Farmer, editor, The Gestating and Lactating Sow. Wageningen Academic Publishers, Wageningen, The Netherlands. p. 147-172.

Tobin, A.J., R. E. Morel. 1997. Asking About Cells. 1st Edition. Harcourt Brace and Company, New York.

Tomlinson, C., M. Rafii, R. O. Ball, and P. Pencharz. 2011. Arginine synthesis from enteral glutamine in healthy adults in the fed state. Am. J. Physiol. Endocrinol. Metab. 301:E267–E273. doi:10.1152/ajpendo.00006.2011

Toner, M. S., R. H. King, F. R. Dunshea, H. Dove, and C. S. Atwood. The Effect of Exogenous Somatotropin on Lactation Performance of First-Litter Sows. J. Anim. Sci. 1996. 74:167–172. doi:10.2527/1996.741167x

Torrazza, R. M., A. Suryawan, M. C. Gazzaneo, R. A. Orellana, J. W. Frank, H. V. Nguyen, M. L. Fiorotto, S. El-Kadi, and T. A. Davis. 2010. Leucine supplementation of a low protein meal increases skeletal muscle and visceral tissue protein synthesis in neonatal pigs by stimulating mTOR-dependent translation initiation. J. Nutr. 140:2145–2152. doi:10.3945/jn.110.128421

Trottier, N. L. 1995. Protein metabolism in the lactating sow. PhD Diss. Univ. Illinois, Urbana–Champaign.

Trottier, N.L., C. F. Shipley, and R.A. Easter. 1997. Plasma amino acid uptake by the mammary gland of the lactating sow. J. Anim. Sci. 75:1266–1278.

Trottier, N. L., and R. Manjarín. 2012. Amino acid utilization. In: L. I. Chiba, editor, Sustainable Swine Nutrition. Wiley-Blackwell, Ames, Iowa, USA. p. 81-108.

van den Brand, H., M. J. Heetkamp, N. M. Soede, J. W. Schrama, and B. Kemp. 2000. Energy balance of lactating primiparous sows as affected by feeding level and dietary energy source. J. Anim. Sci. 78:1520–1528.

Velthof, G. L., J. A. Nelemans, O. Oenema, and P. J. Kuikman. 2005. Gaseous nitrogen and carbon losses from pig manure derived from different diets. J. Envion. Qual. 34:698–706.

Verbruggen, S., J. Sy, W. E. Gordon, J. Hsu, M. Wu, S. Chacko, D. Zurakowski, D. Burrin, and L. Castillo. 2009. Ontogeny of methionine utilization and splanchnic uptake in critically ill children. Am. J. Physiol. Endocrinol. Metab. 297: E1046–E1055.

Wang, Y., J. Zhou, G. Wang, S. Cai, X. Zeng, and S. Qiao. 2018. Advances in low-protein diets for swine. J. Anim. Sci. Biotechno. 9:60.

Wang, Z., Y. Chen, J. Ding, H. Liu, Z. Lyu, W. Dong, Z. Wang, S. Zhang, and F. Wang. 2019. Net energy content of five fiber-rich ingredients fed to pregnant sows. Anim. Sci. J. 00:1–9. doi:10.1111/asj.13211

Weast, R.C., M.J. Astle, and W.H. Beyer. 1984. CRC Handbook of Chemistry and Physics. CRC Press Inc., Boca Raton, FL.

Wegner, K., C. Lambertz, G. Das, G. Reiner, and M. Gauly. 2016. Effects of temperature and temperature-humidity index on the reproductive performance of sows during summer months under a temperate climate. Anim. Sci. J. 87, 1334–1339.

Wheelock, J. B., R. P. Rhoads, M. J. VanBaale, S. R. Sanders, and L. H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93:644–655.

White, R.R., S. Zhang, N. Regmi, and N. L. Trottier. 2016. Quantifying variable amino acid efficiencies in lactating sows. J. Anim. Sci. 94 (E-Suppl. 2):17.

Wickens, C. L. 2003. Dietary protein requirement of mature, moderately exercised horses. M.S. Thesis. Michigan State Univ., East Lansing, MI.

Wildt, D. E., G.D. Riegle, and W. R. Dukelow. 1975. Physiological temperature response and embryonic mortality in stressed swine. Am. J. Physiol. 229:1471–1475.

Wilkinson, D. J., T. Hossain, D. S. Hill, B. E. Phillips, H. Crossland, J. Williams, P. Loughna, T. A. Churchward-Venne, L. Breen, S. M. Phillips, T. Etheridge, J. A. Rathmacher, K. Smith, N. J. Szewczyk, and P. J. Atherton. 2013. Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. J. Physiol. 591:2911–2923.

Williams, A. M., T. J. Safranski, D. E. Spiers, P. A. Eichen, E. A. Coate, and M. C. Lucy. 2013. Effects of a controlled heat stress during late gestation, lactation, and after weaning on thermoregulation, metabolism, and reproduction of primiparous sows. J. Anim. Sci. 91:2700–2714.

Xu, Y., Z. Zeng, X. Xu, Q. Tian, X. Ma, S. Long, M. Piao, Z. Cheng, and X. Piao. 2017. Effects of the standardized ileal digestible valine: Lysine ratio on performance, milk composition and plasma indices of lactating sows. Anim. Sci. J. 88:1082–1092. doi:10.1111/asj.12753

Yunianto, V. D., K. Hayashit, S. Kaiwda, A. Ohtsuka, and Y. Tomita. 1997. Effect of environmental temperature on muscle protein turnover and heat production in tube-fed broiler chickens. Br. J. Nutr. 77: 897-909.

Zhang, T., L. Wang, H. Shi, H. Yan, L. Zhang, X. Liu, L. Pu, J. Liang, Y. Zhang, K. Zhao, and L. Wang. 2016. Heritabilities and genetic and phenotypic correlations of litter uniformity and litter size in Large White sows. J. Integr. Agric. 15, 848–854.

Zhang, S., M. Qiao, and N.L. Trottier. 2019. Feeding a reduced protein diet with a near ideal amino acid profile improves amino acid efficiency and nitrogen utilization for milk production in sows. J. Anim. Sci. In press. doi: 10.1093/jas/skz220

Zhang, S., and N. L. Trottier. 2019. Review: dietary protein reduction improves the energetic and amino acid efficiency in lactating sows. Anim. Prod. Sci. In press.

Zhu, C. L., M. Rademacher, and C. F. de Lange. 2005. Increasing dietary pectin level reduces utilization of digestible threonine intake, but not lysine intake, for body protein deposition in growing pigs. J. Anim. Sci. 83:1044–1053. doi:10.2527/2005.8351044x