UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS

CÂMPUS DE JABOTICABAL

The ideal protein profile for growing-finishing pigs in precision feeding systems: threonine

Aline Remus

Mestre em Zootecnia

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Orientadores: Prof. Dr. Luciano Hauschild e

Profª. Dra. Marie-Pierre Létourneau Montminy

Tese em co-tutela apresentada à Université Laval e à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Philosophiae doctor (Ph.D.) e de doutora em Zootecnia, respectivamente.

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TÍTULO DA TESE: THE IDEAL PROTEIN PROFILE FOR GROWING-FINISHING PIGS IN PRECISION FEEDING SYSTEMS: THREONINE

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Jaboticabal, 28 de março de 2018

DADOS CURRICULARES DO AUTOR

ALINE REMUS – nascida em São Valentim – RS, no dia 28 de abril de 1989. Técnica em Agropecuária com ênfase em Zootecnia pela Escola Agrotécnica Federal de Sertão (atual IFFRS-Campus de Sertão) obtendo esse título em Julho de 2007. Mesmo mês em que ingressou no curso de Zootecnia – Ênfase em sistemas orgânicos de produção animal, do Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina, Chapecó (CEO – UDESC), transferindo a graduação para Universidade Federal de Santa Maria (UFSM) ingressando no curso de Zootecnia em março de 2010 e concluindo em dezembro de 2012. Durante a graduação foi bolsista de extensão (FAPERGS) e iniciação científica (CNPq) no Grupo de Modelagem animal da UFSM sendo orientada do Prof. Dr. Paulo Alberto Lovatto (*In memorian*).Em março de 2013 iniciou o curso de Mestrado pelo Programa de Pós Graduação em Zootecnia, na Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho" (FCAV–UNESP), Campus de Jaboticabal, como bolsista da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) realizando parte do mestrado no Agriculture and Agri-Food Canada sob supervisão do Dr. Candido Pomar. Obteve o título de Mestre em Zootecnia em janeiro de 2015 sob a orientação do Prof. Dr. Luciano Hauschild. Mesmo ano que iniciou o doutoramento co-tutela entre a FCAV-UNESP e a Université Laval (Québec, QC, CA) obtendo os títulos de Ph.D. en Sciences Animales (Université Laval) e doutora em Zootecnia (FCAV-UNESP) em março de 2018, tendo sido bolsista FAPESP.

“We must not forget that when radium was discovered no one knew that it would prove useful in hospitals. The work was one of pure science. And this is a proof that scientific work must not be considered from the point of view of the direct usefulness of it. It must be done for itself, for the beauty of science, and then there is always the chance that a scientific discovery may become like the radium a benefit for humanity.”

(Marie Curie, Lecture at Vassar College, Poughkeepsie, NY, USA (14 May 1921), in Cambridge Editorial Partnership, Speeches that Changed the World, page 53)

DEDICATION

To Daniel, my partner and my friend

ACKNOWLEDGEMENTS

To my research advisor: Candido. He taught me that to be a good scientist you need to be honest: honest to yourself, to your limitations, to your team, to your data and especially with the people that surround you. He taught me that is fine to be down sometimes, but you cannot be down too long, just time enough to see the situation from the bright side. And he taught me one of the most important things someone can: if your data is different does not mean your work is wrong, trust yourself! Candido, Gracias!

To my Brazilian advisor Luciano, whom I am proud to call my friend as well. We went through so much together since my undergraduate course, up to so many fights you had to put against bureaucracy to get me in this dual-doctoral program. You taught me that to make people do what you need them to do, or to respect you it is not necessary to scream, or threaten people. But instead of it, asking nicely and show them an example of hard work and dedication will be enough to make them respect you. Thank you so much for this amazing opportunity you gave me and for everything you shared and taught me, MUITO OBRIGADA DO FUNDO DO MEU CORAÇÃO.

To my Québécoise advisor Marie-Pierre, I confess I was afraid of you when we start to work together because you were a serious person. As time passed by I noticed not only you are a serious, competent and hard worker professional but that you have a soft spot for your students and give them everything you can. I am grateful for this incredible opportunity you gave me to be part of this dual-doctoral program, for every time you believed me and gave the chance to go in the Ph.D. competitions representing Laval, for all the incentive words you gave me in your recommendation letter for the fellowship and prizes. MERCI BEAUCOUP!

To my partner Daniel, who is with me since I started my Ph.D. You passed all the Ph.D. phases with me: Phase 1, the Nobel prize winner phase; Phase 2, the too busy doing my trials; Phase 3, I hate my data; Phase 4, I love my data; Phase 5, I have no idea what to do with my data; Phase 6, I am brilliant my paper will change the world; Phase 7, ok, not that brilliant but it is a good work; Phase 8, I can't take writing this thesis anymore; and if you are reading this now is because you got in

Phase 9: we did it! Thank you for your support, patience, incentive and yes, for criticizing me when I needed. Ich Liebe Dich!

To Sophie Horth, who worked with me in all my projects, I always will be grateful to you for your support, knowledge, professionalism, dedication, positivity and the double-checks. Un gros merci ma cher amie !

To Marcel Marcoux, Virginie Brunet and Cassandra Bourdeau for the technical support, to Steve Méthot for the statistical support and, to the staff from the swine complex for the hard work during our trials.

To all my friends who walked this pathway with me, understanding my absence and supporting and cheering me up in the difficult moments. Even if I am far away from my homeland you made me feel at home here and you became part of my family.

Aos meus pais que respeitaram minha decisão de me mudar para o exterior e seguir os meus sonhos. Muito obrigada por entenderem minha ausênica e por me amaram pela pessoa que sou. Amo vocês.

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To Agriculture and Agri-Food Canada (AAFC), Breton aliments, Swine innovation Porc, Ajinomoto Eurolysine especially to Etienne Courrent, Sherbrooke Research and Development center, to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and São Paulo Research Foundation (FAPESP) (Grant nº. 2012/03781-0, fellowship grant nº. 2014/25075-6; fellowship grant nº. 2016/09703-2); the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (fellowship 132530/2013-9) for financial support of this project.

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LIST OF ACRONYMS AND ABBREVIATIONS

AA	Amino Acids
ADF	Acid Detergent Fiber
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AIPF	Automatic and Intelligent Precision Feeding®
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BW	Body Weight
Bwa	Average Body Weight
BWm	Metabolic Body Weight
CCD	Central Composite Design
Ch_4N_2O	Urea
Cl	Chloride
CP	Crude Protein
CRP	C-Reactive Protein
DFI	Daily Feed intake
DM	Dry Matter
DXA	Dual-Energy X-Ray Absorptiometry
EAA	Essential Amino Acids
FI	Feed Intake
FS	Feeding System
g	Grams
g/d	Grams Per Day
G:F	Gain: Feed Ratio
GGT	Gamma-Glutamyl Transferase
GPF	Group Phase Feeding

GPF70	Group phase-feeding with 70% of threonine
GPF85	Group phase-feeding with 85% of threonine
GPF100	Group phase-feeding with 100% of threonine
GPF115	Group phase-feeding with 115% of threonine
GPF130	Group phase-feeding with 130% of threonine
h	Hour
IGF1	Insulin-like growth factor
IPF	Individual Precision Feeding
IPF70	Individual Precision Feeding With 70% Of Threonine
IPF85	Individual Precision Feeding With 85% Of Threonine
IPF100	Individual Precision Feeding With 100% Of Threonine
IPF115	Individual Precision Feeding With 115% Of Threonine
IPF130	Individual Precision Feeding With 130% Of Threonine
IU	International Units
kg	Kilograms
Klys	Efficiency Of Utilization Of Lysine
KThr	Efficiency Of Utilization Of Threonine
L	Level
L×F	Interaction Level And Feeding System
LD	Lipid Deposition
LDH	Lactic Acid Dehydrogenase
Lys	Lysine
ME	Metabolizable Energy
mg	Milligrams
Min	Minutes
MSE	Maximum Standard Error
N	Nitrogen
Na	Sodium
NDF	Neutral Detergent Fiber
NE	Net Energy
NEAA	Non-Essential Amino Acids

NH_3-	Ammonia
NRC	National Research Council
P	Phosphorus
PD	Protein Deposition
PDV	Portal-Drained Viscera
PF	Precision Feeding
R.S.E.	Residual Standard Error
SE	Standard Error
SEe	Standard Error of the Estimation
SID	Standardized Ileal Digestible
TDG	L-Threonine 3-Dehydrogenase
Thr	Threonine

FOREWORD

This thesis was submitted to Faculté des études supérieures de l'Université Laval in Québec, Canada, and to Faculdade de Ciências Agrárias e veterinárias da Universidade Estadual Paulista in Jaboticabal, Brazil, aiming to fullfill the requirements of the dual-doctoral degree program staterd on March 2015, to obtain the degree of Philosophiae Doctor of Science (Ph.D.) and Doctor in Animal Science, respectively. This thesis is composed of six chapters.

Chapter 1 contains a general introduction and literature review on the main research efforts made so far that, ultimately, inspired the development of the research objectives outlined in this thesis. The basic concepts in swine nutrition are addressed and discussed, notably the concept of ideal protein for pigs commonly used in swine nutrition as well as a more dynamic concept such as the individual precision feeding concept. The difficulties of determining requirements for a population or for individual pigs are discussed. Finally, the main results of published papers on amino acids in swine nutrition are presented. Differences between conventional group-phase feeding and individual precision feeding are discussed, resulting in the hypothesis that individual requirements may differ between a larger population of pigs fed according to the ideal protein profile concept and pigs fed individually.

Chapter 2 contains a comprehensive study on the performance, carcass composition and biochemical response of growing pigs. This chapter explores the effect of response criterion choice in the estimate of amino acids requirements, as well as the difference of amino acid requirements between growing pigs in individual precision feeding or group-phase feeding systems. This chapter provides evidence that pigs fed individually in a precision feeding system have different amino acid requirements than pigs fed based on ideal protein profile in a conventional group-phase feeding system. Differences in biochemical plasmatic response, in the chemical composition of splenic organs and in carcass muscles of pigs fed in an individual precision feeding system are highlighted, ultimately showing some of the potential limitations of the ideal protein profile concept for pigs. This chapter is

formatted for submission to the Journal of Animal Science. Parts of this chapter were presented at the 2017 ADSA-ASAS Midwest Meeting in Omaha (doi: 10.2527/asasmw.2017.279), at the 2017 ANCC Meeting in Québec, and at the ASAS-CSAS Annual Meeting in Baltimore (doi: 10.2527/asasann.2017.250). The last two abstracts were awarded second and third places in the graduate competitions of the respective meetings. This chapter was written by Aline Remus who also planned and conducted the animal trial and analyzed the data. The co-authors Marie-Pierre Létourneau-Montminy, Luciano Hauschild and Candido Pomar supervised the manuscript preparation. Candido Pomar supervised the planning and execution of the animal trial and the data analysis. In addition, Sophie Horth and Marcel Marcoux from the Sherbrooke Research Centre of Agriculture and Agri-Food Canada are acknowledged for their assistance with the laboratory analyses, and Steve Méthot from the Sherbrooke Research Centre of Agriculture and Agri-Food Canada is acknowledged for his assistance with the statistical analysis.

Chapter 3 uses the same approach outlined in chapter 2 with the focus on finishing pigs. Although the same methodology, feeds, genetic line of pigs, and housing environment apply to growing and finishing pigs, the response of finishing pigs differs to that of growing pigs observed in the previous chapter. Potential limitations of estimating amino acid requirements in pigs in different production phases (growing versus finishing phase) are discussed. In finishing pigs, changes in the chemical composition and in the amino acid composition occurred mainly in splenic tissues rather than in muscles, which is in contrast to previous observation on growing pigs. In line with results on growing pigs, AA requirements in finishing pigs differ between pigs in an individual precision feeding system and a conventional group-phase feeding system based on the ideal protein profile concept. This chapter is formatted for submission to the Journal of Animal Science. Parts of this chapter were presented at the 1st International Meeting of Advances in Animal Science in Jaboticabal, Brazil. This chapter was written by Aline Remus who also planned and conducted the animal trial and analyzed the data. The co-authors Marie-Pierre Létourneau-Montminy and Luciano Hauschild supervised the manuscript preparation, and Candido Pomar supervised the planning and execution of the

animal trial and the data analysis. In addition, Sophie Horth and Marcel Marcoux from the Sherbrooke Research Centre of Agriculture and Agri-Food Canada are acknowledged for their assistance with the laboratory analyses, and Steve Méthot from the Sherbrooke Research Centre of Agriculture and Agri-Food Canada is acknowledged for his assistance with the statistical analysis.

Chapter 4 describes a study on the potential effects of feeding patterns on body composition of pigs based on real-time intake data collected during the trial described in chapters 2 and 3. This chapter confirms that the responses of pigs are most likely modulated by the amino acid intake level rather than the feeding behaviour pattern. Nonetheless, pigs may respond to a changing amino acid intake level by slightly adjusting their feeding behaviour. This chapter is formatted for submission to Physiology & Behavior. This chapter was written by Aline Remus who also planned and conducted the animal trials data originated from and analyzed the data. The co-authors Marie-Pierre Létourneau-Montminy and Luciano Hauschild supervised the manuscript preparation, Candido Pomar supervised the planning and execution of the animal trials the data originated from, and Daniel Warner from McGill University prepared and cleaned the database, analyzed the data and assisted with manuscript preparation.

Chapter 5 describes a novel approach to estimate amino acid requirements in real time for precision feeding. An exploratory analysis is described in an effort to understand variability among individual pigs, suggesting that efficiency of protein retention might explain the main difference between pigs with a high versus low or medium protein deposition. The mechanism which triggers this response needs to be further elucidated. This chapter is not yet formated for submission to a scientific journal. This chapter was written by Aline Remus who also planned and conducted the animal trial and analyzed the data. The co-authors Marie-Pierre Létourneau-Montminy, Luciano Hauschild and Candido Pomar supervised the manuscript preparation. Candido Pomar supervised the planning and execution of the animal trial and the data analysis. Steve Méthot from the Sherbrooke Research Centre of Agriculture and Agri-Food Canada helped define the experimental design, adjust the

statistical program on SAS to estimate individual requirements, perform the surface-response analysis, and revised the manuscript. Sophie Horth from the Sherbrooke Research Centre of Agriculture and Agri-Food Canada is acknowledged for her assistance with the laboratory analyses.

Chapter 6 is a general discussion of the results presented in this thesis and explores further research ideas to help increase our knowledge on individual precision feeding and nutrition.

The author of this thesis co-authored four papers (not related to this thesis) and one invited presentation during her Ph.D. studies:

Isola, R. G., Hauschild, L., Perondi, D., Andretta, I., Gobi, J. P., Remus, A., Veira, A. M. (2017). Individual response to growing pigs to threonine intake. Revista Brasileira de Zootecnia: accepted for publication.

Perondi, D., Kipper, M., Andretta, I., Hauschild, L., Lunedo, R., Franceschina, C. S., Remus, A. (2017). Empirical models for predicting feed intake of growing-finishing pigs reared under high environmental temperatures. Scientia Agricola: accepted for publication.

Dalla Costa, F. A., Tavernari, F. C., Dalla Costa O. A., de Castro, F. F., Remus, A. (2017). Enriquecimento com ácidos graxos da série ômega 3 em carne de aves e ovos. PubVet. 11: 113-123.

Andretta, I., Kipper, M., Hauschild, L., Lehnen, C. R., Remus, A., Melchior, R. (2016). Meta-analysis of individual and combined effects of mycotoxins in growing pigs. Scientia Agricola (USP. Impresso). 73: 1-3.

Pomar, C., Remus, A., Létourneau Montminy, M. P. (2017). Precision livestock feeding in swine. Invited presentation at the First Amino Acids Academy, Paris, France.

Furthermore, the thesis author wrote seven abstracts for oral presentations at the ASAS Midwest Meeting in Des Moines, USA (2015) and Omaha, USA (2017),

at the IMAS meeting in Jaboticabal, Brazil (2016), and at the ASAS-CSAS Annual Meeting in Baltimore, MD (2017), for an poster presentations at the ASAS-ASDA Annual Meeting in Orlando, USA (2015), and for two poster presentation at the Animal Nutrition Conference of Canada (ANCC) meeting in Québec, Canada (2017). The thesis author participated in a total of eight conferences in animal science and amino acids during her Ph.D. studies.

The author was granted two fellowships by the São Paulo Research Foundation (FAPESP, Sao Paulo, Brazil; fellowship grant numbers 2016/09703-2 and 2014/25075-6), and one fellowship by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasilia, Brazil; fellowship grant number 233118/2014-4). The author was awarded a second place in the Graduate poster competition at the 2017 ANCC in Québec, Canada by the Animal Nutrition Association of Canada (ANAC), granted the Graduate student travel award of the Canadian Society of Animal Science (CSAS), and won the third place in the CSAS Graduate Oral Competition by CSAS at the 2017 ASAS-CSAS Annual Meeting in Baltimore, USA. Finally, the author was the Canadian student in pig nutrition selected for the 2017 Evonik Student Seminar in Isle of Palms, USA, organized and funded by Evonik North America.

THE IDEAL PROTEIN PROFILE FOR GROWING-FINISHING PIGS IN PRECISION FEEDING SYSTEMS: THREONINE

RESUMO - Os aminoácidos (AA) são componentes essenciais das dietas, mas a determinação exata das exigências de AA em animais de criação é um desafio. Exigências nutricionais de AA em suínos podem ser influenciadas por vários fatores os quais incluem: genética, estado sanitário, idade, e como recentemente demonstrado, a variabilidade individual. Tradicionalmente animais recebem a dieta usando um sistema convencional de alimentação de grupos por fase (AGF). Nesse sistema todos os suínos recebem a mesma ração durante toda uma fase de crescimento e a maioria dos animais recebem mais nutrientes do que o necessário para expressar o seu potencial de crescimento. Isso vai impactar negativamente no meio-ambiente devido a grande excreção de nitrogênio e nos aumentados custos de produção. Em sistemas de alimentação precisão individual (API), os suinos são alimentados com dietas diariamente adaptadas às suas exigências individuais de AA. Neste contexto, é necessário distinguir as exigência de AA de uma população e de indivíduos. O perfil de proteína ideal foi estabelecido para suínos em sistemas convencionais de AGF, mas estas relações ideais de AA podem ser diferentes para suínos em sistemas API. O objetivo principal da pesquisa foi comparar o perfil de proteína ideal em suínos, usando a relação ideal treonina-para-lisina (Thr:Lys) entre sistemas convencionais de AGF e API. Usando a metodologia de dose-resposta com cinco relações Thr:Lys dentre de um sistema AGF ou API, foi possível confirmar a hipótese inicial que perfil de proteína ideal em suínos diferem entre sistemas de alimentação. A composição química e concentração de AA na carcaça também foi afetada pela relação Thr:Lys, e a magnitude e o tipo de resposta foram dependentes do sistema de alimentação usado. Em um segundo estudo de dose-resposta, com relações de Thr:Lys semelhantes as oferecidas anteriormente aos suínos em crescimento foram oferecidas à suínos em terminação. Foi possivel observar que para estes as exigências de Thr eram

maiores do que aquelas observadas anteriormente para suínos em crescimento, sugerindo que as exigências de AA para deposição de proteína é idade-dependente. Estes dois estudos sugerem que suínos podem modular a sua taxa de crescimento e composição corporal de acordo com o nível de ingestão de AA e podem responder de forma diferente a mesma quantidade de AA ingerido. Estes estudos destacam a fragilidade do uso do conceito perfil de proteína ideal, considerando exigências fixas de AA devido a assunção de que a composição de carcaça tem concentração de AA constante. A determinação exata das exigências de AA para suínos em um sistema API parece ser limitada principalmente pelo uso de relações fixas e constantes de AA, porém suínos têm exigências de AA diferentes entre eles. Finalmente, propõe-se uma nova abordagem baseada num desenho composto central com uma configuração fatorial visando independentemente estimar as exigências de Lys e Thr em tempo real para suínos em um sistema API. Com esta metodologia, observou-se uma resposta de deposição de proteína não-unica para diferent combinações de Thr e Lys, devido às diferenças nas exigências de AA entre suínos. Essa percepção sobre a variabilidade entre individuos é útil para ajustar o modelo de nutrição de precisão aprimorando as estimativas de exigências AA, nurrindo animais de acordo com suas necessidades individuais possibilitando a redução do desperdício de nutrientes especialmente em suínos com baixa deposição de proteína. Os resultados apresentados nesta tese, apoiam a ideia de que alterações na composição corporal em suínos são induzidas por alterações nos níveis dietéticos de AA. Portanto, o crescimento pode ser modulado para a composição de corporal ideal desejada pelo consumidor. Esta tese propõe uma mudança de perspectiva na alimentação animal, onde AA pode ser visto como um gatilho para desencadear uma resposta metabólica animal ao invés da tradicional visão de AA como exigências nutricionais fixas.

Palavras-chave: nutrição de precisão, treonina: lisina, exigências nutricionais.

THE IDEAL PROTEIN PROFILE FOR GROWING-FINISHING PIGS IN PRECISION FEEDING SYSTEMS: THREONINE

ABSTRACT - Amino acids (AA) are essential components of diets but accurate determination of AA requirements in farm animals is a challenge. Requirements for AA in pigs can be influenced by several factors, including genetics, health, age, and, as recently shown, also individual variability. In conventional group-phase feeding (GPF) systems, large groups of pigs receive the same feed during extended periods and most pigs receive more nutrients than required to express their growth potential with potential detrimental effects on the environment through increased nitrogen excretion, and on production costs. In individual precision feeding (IPF) systems, pigs are fed diets tailored daily to their individual nutrient requirements. In light of this, it is necessary to distinguish the AA requirements of a population from those of individuals. Optimal essential AA ratios have been established for pigs in conventional GPF systems, but these optimal AA ratios might differ for pigs in IPF systems. The main research objective was to compare the ideal protein profile in pigs using the optimal threonine-to-lysine (Thr:Lys) ratio between conventional GPF and IPF systems. Based on a dose-response approach with five levels Thr:Lys ratios offered to growing pigs in a GPF or IPF system, it was possible to confirm the initial hypothesis that optimal AA ratios differ between feeding systems. Carcass chemical composition and AA concentration was likewise affected by the Thr:Lys ratio, and the magnitude and type of response depended on the feeding system. In a second dose-response study with similar Thr:Lys ratios offered to late finishing pigs, requirements were larger than to those previously observed for growing pigs, suggesting that AA requirements for protein deposition is age dependent. These two studies suggest that individual pigs can modulate their growth and body composition according to the level of AA intake and can respond differently to same amount of ingested AA. These studies further highlighted the weakness of using an ideal protein profile by considering fixed requirements for

AA due the assumed constant AA carcass composition. Accurate estimation of AA requirements for pigs in an IPF system seems to be mainly limited by the use of fixed AA ratios as pigs have different AA requirements. Finally, a novel approach to the dose-response approach based on a central composite design with a factorial design aiming at independently estimating real-time requirements for Lys and Thr in individual pigs was proposed. A non-unique response of protein deposition to various Thr and Lys combinations was observed due to the differences in AA requirements among individual pigs. This insight on variability among individual pigs is useful to fine-tune the precision feeding system by estimating AA requirements more accurately, feeding pigs according to their individual requirements, and, ultimately, reduce waste of nutrients in pigs with lower protein deposition. The results presented in this thesis support the idea that changes in body composition in pigs are induced by changes in dietary AA levels. Therefore, growth may be modulated to the optimal body composition desired by the consumer. This thesis proposes a change of perspective in animal nutrition, where AA may be seen as a trigger for animal metabolic response with dynamic and distinctive AA requirements in individual animals.

Keywords: precision nutrition, threonine: lysine ratio, nutritional requirements

THE IDEAL PROTEIN PROFILE FOR GROWING-FINISHING PIGS IN PRECISION FEEDING SYSTEMS: THREONINE

RÉSUMÉ - Les acides aminés (AA) sont une composante essentielle du régime alimentaire des animaux de ferme, mais la détermination précise des besoins en AA est un défi. Les besoins en AA peuvent être influencés par de nombreux facteurs, notamment la génétique, la santé, l'âge et, comme récemment montrée, la variabilité individuelle. Dans les systèmes classiques d'alimentation des troupeaux par phase (SATP), tous les porcs reçoivent la même ration pendant de longues périodes. De ce fait et afin de s'assurer qu'ils expriment leur plein potentiel de croissance, la plupart des porcs reçoivent plus d'éléments nutritifs qu'ils n'en n'ont besoin, ce qui engendre des effets nuisibles sur l'environnement par l'excrétion d'azote accrue, et sur les coûts de production. Dans les systèmes d'alimentation individuelle de précision (SAIP), les porcs reçoivent une ration ajustée chaque jour en fonction de leurs besoins nutritifs. Dans ce contexte, il est nécessaire de distinguer les exigences de l'AA d'une population de celles des individus. Les rapports optimaux d'AA entre les différents AA essentiels ont été établis pour les systèmes d'alimentation classiques par phase, mais ces rapports pourraient différer selon qu'il s'agit d'un système d'alimentation classique ou d'un système d'alimentation de précision des porcs. L'objectif principal de cette recherche a été de comparer le rapport optimal thréonine: lysine (Thr: Lys) entre le système d'alimentation classique par phase et le système individuel d'alimentation de précision. À l'aide d'une méthodologie de dose-réponse avec cinq ratios Thr: Lys pour des porcs en croissance dans un SATP ou SAIP la composition chimique et la concentration en AA de la carcasse ont été affectées par le ratio Thr: Lys et l'ampleur ainsi que le type de réponse était dépendant du système d'alimentation utilisé. Il a été possible de confirmer l'hypothèse de départ selon laquelle les ratios optimaux des AA utilisés par le SATP ne sont pas adéquats pour établir les besoins des AA dans les systèmes d'alimentation de précision. Dans une seconde étude de dose-réponse avec des rapports Thr: Lys similaires offerts aux porcs en finition, les besoins de Thr:Lys étaient plus élevés que ceux observés précédemment

pour les porcs en croissance suggérant que les besoins en AA pour le dépôt de protéine est dépendant de l'âge. Ces deux études suggèrent que les porcs peuvent moduler leur croissance et leur composition corporelle en fonction du niveau d'apport en AA et peuvent répondre différemment à la même quantité d'AA ingérée. Ces études soulignent en outre la faiblesse de l'utilisation d'un profil protéique idéal en considérant des exigences fixes en AA en raison de la composition en AA de la carcasse supposée constante. L'estimation précise des besoins en AA pour les porcs dans un SAIP semble être limitée par l'utilisation de ratios AA fixes, car les porcs ont des exigences en AA différentes. Enfin, une nouvelle approche basée sur une conception composite centrale avec une configuration factorielle visant à estimer indépendamment les besoins pour la Lys et la Thr en temps réel chez les porcs nourris individuellement a été proposée. Une réponse non unique du dépôt de protéines à diverses combinaisons Thr et Lys a été observée en raison des différences dans les exigences en AA entre les porcs. Cet aperçu de la variabilité entre les porcs est utile pour affiner le système d'alimentation de précision en estimant les besoins en AA de manière plus précise et en nourrissant les porcs selon leurs besoins individuels. De plus, cela permettrait de réduire le gaspillage de nutriments chez les porcs avec moins de dépôt protéique. Les résultats présentés dans cette thèse soutiennent l'idée que les changements dans la composition corporelle chez les porcs sont induits par des changements dans les niveaux alimentaires en AA. Par conséquent, la croissance peut être modulée en fonction de la composition corporelle optimale souhaitée par le consommateur. Cette thèse propose un changement de perspective dans la nutrition animale, où l’AA peut être un déclencheur de la réponse métabolique animale avec des exigences en AA dynamiques et distinctes chez les animaux de manière individuelle.

Mots-clés : nutrition de précision, ratio thréonine: lysine, besoins nutritionnels

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

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"I don't believe that the ultimate theory will come by steady work along existing lines. We need something new. We can't predict what that will be or when we will find it because if we knew that, we would have found it already! It could come in the next 20 years, but we might never find it."

(Stephen Hawking in Science Watch; September 1994).

General introduction and literature review

1.1 Introduction

Providing animals with nutrients according to their requirements can decrease nutrient excretion to the environment and reduce production costs significantly (Létourneau Montminy et al., 2005; Pomar et al., 2014). Nutrient requirements can be defined as a number of nutrients needed to achieve specific production objectives, maximize weight gain and lean tissue gain, and improve feed conversion (Fuller, 2004). Protein is one of the most expensive nutrients in pigs' diets, and swine production is responsible for significant excretion of nitrogen into the soil (Lovatto et al., 2005). This contamination needs to be reduced. Consequently, correctly determining amino acid (AA) requirements for pigs has become essential.

Nutrient requirements are currently estimated using the factorial or empirical methods, both of which are population-based (Hauschild et al., 2010). The empirical method estimates the requirement to maximize or minimize a given parameter (e.g. average daily gain), and the results are usually based on dose-response experiments. The factorial method combines the requirements for maintenance and production (Zhang et al., 2012) but it too disregards inter-animal variability once this factorial method has been calibrated with data obtained from populations.

The population-based requirements that are used to establish phase-feeding programs are often based on an average pig within a population, without considering that pigs have different requirements or that those requirements vary among animals and over time (Hauschild et al., 2010). To take this inter-animal variability into account, studies on precision feeding (Pomar et al., 2009; Hauschild et al., 2010; Zhang et al., 2012) established a model (Hauschild et al., 2012) that can predict and determine individual lysine requirements over time, with all other amino acids being provided according to the ideal protein concept. In a precision feeding system, each pig receives a diet that is tailored daily to meet its requirements based on individual body weight, feed intake, and average daily gain. Conversely, in phase

feeding, all the pigs within a population receive the same diet throughout the entire feeding phase. The amino acid content of this diet is often based on the requirements of the average pig in the population. Based on this information, we aim to explore the main differences between population and individual amino acid requirements. As well, we intend to review the primary methods used to estimate amino acid requirements and the possible reasons for considerable variations between and within experiments.

1.2 The ideal protein concept

There are two main groups of AA: essential and non-essential. An Essential AA cannot be synthesized by pigs from material ordinarily available in cells at rates matching the demands for production functions including maintenance, normal growth and reproduction (NRC, 2012). Threonine and Lys (and possibly Tryptophan) might be the only truly metabolic essential AA once they cannot be synthesized from α-keto acids (NRC, 2012). Nonessential AA can be synthesized *de novo* by the animal organism to be used for protein synthesis for maintenance, growth, development, and health (Wu et al., 2013). Nonessential AA supply more than 50% of the total N ingested; however, they have received considerably less attention from researchers and nutritionists. It is commonly assumed that the ideal protein balance should provide sufficient essential AAs to also supply nonessential AAs. However, there is no evidence to date that nonessential AAs are synthesized by the animals at sufficient levels from the essential AA to meet requirements (Wu et al., 2013). In this way, more attention should be paid to non-essential AA during diet formulation, avoiding as well, expansive essential AA to be deaminated for the synthesis of nonessential AA which risks not being enough for protein synthesis. More studies in nonessential AA metabolism are necessary to elucidate these points.

The ideal protein concept refers to a situation where all the AA are co-limiting at the same time and the AA provided exactly meet the population requirement (van Milgen and Dourmad, 2015). By using AA ideal protein ratios, it is assumed that the amount of AAs provided to animals is enough to meet the requirements for maintenance and growth without creating a surplus or deficiency. This concept is

widely accepted as an effective tool for reducing N excretion and feed costs. It was initially developed by Mitchell (1959) and Fisher and Scott (Fisher and Scott, 1954) in the early 1950s and was later adapted for pigs by Cole in the 1980s (Cole, 1980; Wu et al., 2014). It was based on the amino acid profile in the carcass, using lysine as the AA reference because it is often the primary limiting AA in practical swine diets, it is easy to analyze and is widely studied. Thereafter, all other AA requirements usually have been established based on their respective ratio to lysine. Ratios based on ideal protein profile have been assumed as a practical way to formulate diets for non-ruminants decreasing the use of crude protein (Emmert and Baker, 1997; Boisen and D'Mello, 2003; Pedersen et al., 2003; Baker, 2009; van Milgen and Dourmad, 2015). It is assumed that providing AA supply below the animal's requirements, animal productivity likely decreases. Similarly, if the AA supply is above the requirements, expensive nutrients are wasted without any improvement in animal productivity. These ratios seemed to have been established and primarily obtained by dose-response studies in populations (Rostagno et al., 2005; de Lange, 2012; Gloaguen et al., 2012; Le Floc'h et al., 2012; Van Milgen et al., 2012; van Milgen and Dourmad, 2015) but this ratio has not been validated for individual requirements. The ideal protein profile ratio has been modified during the years according to the research done in the area. Normally AA concentration in carcass is assumed constant, independent of pig age or nutrient levels (De Lange et al., 2001). This seems a not valid assumption once protein and energy levels (Bikker et al., 1994), age (Conde-Aguilera et al., 2010), sulfur AA deficiency (Conde-Aguilera et al., 2010; Conde-Aguilera et al., 2016a; Conde-Aguilera et al., 2016b), Thr deficiency (Hamard et al., 2009) and genetics (Xue et al., 2016) can change AA composition in carcass.

Table 1-1. Ideal protein profile ratios as standard ileal digestible amino acids to lysine[1]

Ratios	BSAS[2] (2003)	NRC[3] (2012)	VSP[4] (2013)	INRA[5] (2013)	Ajinomoto Eurolysine (2013)	TB[6] (2017)
Lysine	100	100	100	100	100	100
Threonine	65	59	61	65	65	65
Methionine	30	29	32	30	30	30
Methionine+Cystine	59	55	54	60	60	65
Tryptophan	19	16	20-22	22	22	20
Valine	70	63	67	70	70	69
Isoleucine	58	51	53	52	53	55
Leucine	100	100	102	101	101	100
Histidine	34	34	32	31	32	33
Phenylalanine	57	58	57	54	55	50
Phenylalanine+Tyrosine	100	93	111	-	95	100
Tyrosine	-	-	-	40	-	-

[1]Adapted from Ajinomoto Eurolysine (2013)
[2]The British Society of Animal Science
[3]National Research Council
[4]Danish Agriculture & Food Council
[5]Institut national de la recherche agronomique, Gloaguen et al. (2013)
[6]Tabelas Brasileiras para aves e suinos (Brazilian tables for poultry and pigs)

The ideal protein profile is largely adopted because lower retention of AA or lower performance is often attributed to AA imbalances every time a change in profile is tested. As pointed by D'Mello (2003a) exists a great contradiction in protein and amino acid studies attributing differences in utilization of amino acids to imbalanced diets. However, Langer and Fuller (2000) demonstrated an increased N retention in methionine deficient diets when an imbalanced mixture of branch AA was added to the diet. This shows that methionine might be spared by branch AA and an increase in protein accretion might be due to an increase in protein synthesis or decrease in degradation or due the action of the two mechanism together. An enzymatic competitive inhibition in the methionine degradation increasing its availability for protein synthesis should be considered (Langer et al., 2000). The fact that imbalanced diets might improve AA efficiency of the limiting AA in diet has been earlier studied and Yoshida et al. (1966b). These authors demonstrated that protein retention was actually enhanced using Thr and histidine imbalanced diets, none

increase in the labelled AA oxidation was observed in rats. A similar study (Benevenga et al., 1968) using imbalanced diets showed an increased incorporation of limiting AA in hepatic proteins of rats. These data pointing that negative results should not be attributed to an imbalance originated from the incorrect use of ideal protein profile in diets.

1.3 Threonine

Threonine is usually the second limiting amino acid in swine diets formulated based on corn and soybean meal. This amino acid is essential for several metabolic pathways in the animal metabolism and cannot be synthesized by the animal organism from N sources such α-keto acids to meet its requirements. Threonine, together with glutamate, arginine, and cysteine are involved in many maintenance functions, particularly those which are part of immune system and gut mucosa repair process (Bequette, 2003). Threonine plays an important role once the intestine is the first barrier against bacterial translocation, and huge amounts of threonine are used in the mucin production to create this protection. This will result in an increase of the rate of protein turnover in the intestine. In the gut and liver are the main sites where the amino acid catabolism and biosynthesis can be observed (Wu, 1998).

Threonine can be added to the diet in its synthetic form, which is commonly manufactured on an industrial scale in the form of powder. Its industrial production is made from fermentative processes, in which only the L-threonine isomer is generated. It is a racemic mixture between levorotatory forms with 100% relative bioavailability (Leeson and Summers, 2001). Its chemical structure (α-amino-β-hydroxybutyric acid) was determined by William C. Rose in 1935, being the last of the 20 natural amino acids to be known. Unlike the other amino acids, threonine is not transaminated, since the animals do not have an isomerase (transaminase) capable of transforming D- into L-threonine. Its D-isomer and α-ketoacid are not used.

1.3.1 Absorption and metabolism of threonine

The protein is composed of polypeptide chains, within which are found the threonine molecules. Therefore to have free amino acids, it is necessary initially to break down peptide bonds and splits the long protein chains into shorter polypeptides by stomach gastric juice especially by the action of HCl and gastric pepsin (Figure 1-1). These shorter polypeptides enter the small intestine, and the acid pH of the intestinal bolus stimulates the secretion of secretin triggering the release of HCO_3 (bicarbonate) to increase intestinal pH. In addition to releasing secretin, the release of cholecystokinin has three basic functions: to signal the reduction of stomach motility, stimulate pancreatic hormone production and release of biliary juice from the liver (Nelson et al., 2008). The zymogens secreted by the pancreas will be activated in their enzymatic form in the intestinal lumen, trypsin hydrolyzes the basic amino acids, chymotrypsin hydrolyzes bonds between aromatic chain amino acids, whereas carboxypeptidases A and B hydrolyze the peptides whose end portion comprises a carboxyl group COO- and finally the elastase hydrolyzes the neutral R group AA (Nelson et al., 2008).

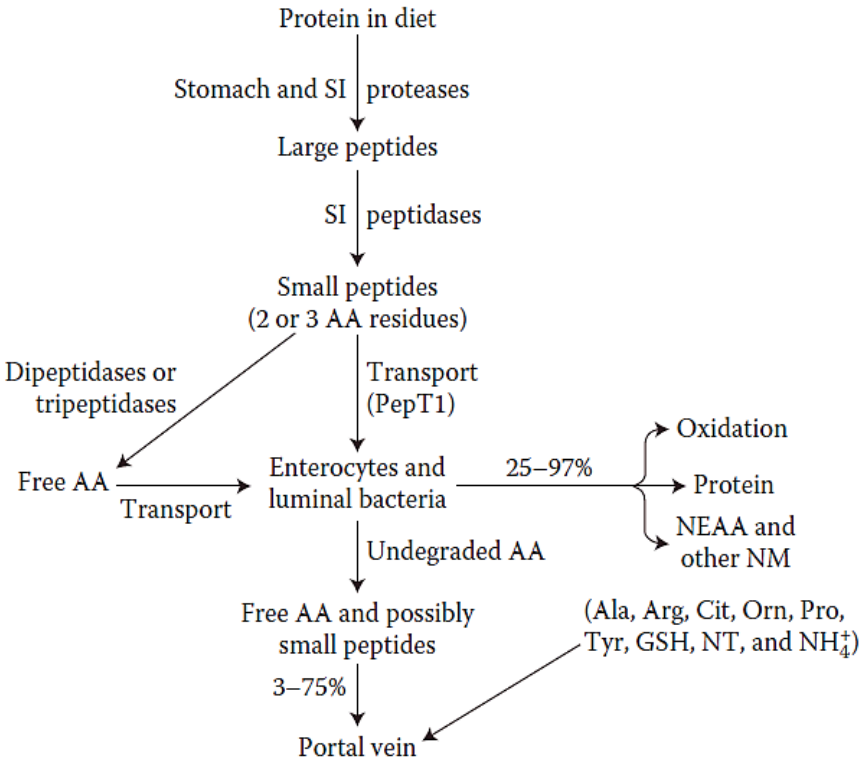

Figure 1-1. Shematic representation of the digestion of dietary protein in the gastrointestinal tract of the small intestine in monogastric animals and humans (Wu, 2013). All diet-derived amino acids undergo various degrees of catabolism by luminal bacteria, and some of them are oxidized by enterocytes. AA: an amino acid; GSH: glutathione; NEAA: nutritionally nonessential AA; NM: nitrogenous metabolites; NT: nucleotides; PepT1: H+ gradient-driven peptide transporter 1; SI: small intestine.

In the portal-drained viscera (PDV), composed by the intestine plus integumental fat, pancreas, spleen and stomach, 60 up to 80% of Thr intake is retained, while just one third of the intake of other AA as lysine are retained by the PDV (Stoll et al., 1999; Van Goudoever et al., 2000). Even during protein restriction a high rate of metabolism and 85% Thr retention are found in the PDV (Schaart et al., 2005b). The Thr metabolism can occur through three pathways (Kidd and Kerr, 1996) (Figure 1-2). A major metabolic fate of Thr is the incorporation of the same in the proteins of the intestinal mucosa, as these proteins, such as mucin, have a significant amount of Thr (Law et al., 2007). However, other metabolic fate could be oxidation, since essential amino acids including Lys and leucine are also oxidized in

the intestine (Van Der Schoor et al., 2001). In mammals, the oxidation of Thr occurs primarily in the liver (House et al., 2001). Threonine in pigs is oxidized in the liver and pancreas by the L-threonine 3-dehydrogenase (TDG) resulting in glycine (Le Floc'h et al., 1996). In humans approximately 41% of serine comes from glycine oxidation (Shemin, 1950).

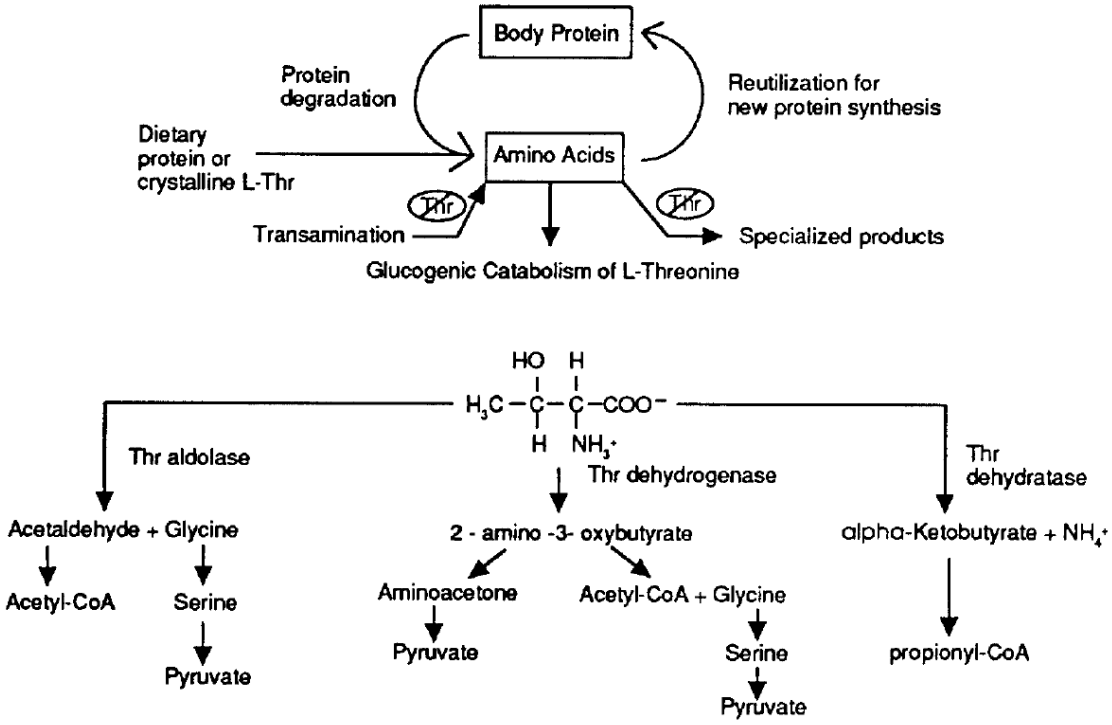

Figure 1-2. Schematic representation of the threonine catabolism (Kidd and Kerr; 1996)

Threonine is an important AA for protein synthesis, and its catabolism generate important metabolites as glycine, acetyl-CoA, and pyruvate **(**Kidd and Kerr, 1996). One of the protein synthesis main sites is the liver. This is the first organ to be reached by the AA flux after absorption and utilization of AA by the small intestine. Most of the enzymatic activity linked to AA metabolism is found in the liver (Miller, 1962). After the free AA are transported into the cells, the protein synthesis starts due to the action of RNA messenger and ribosomes whose manage the synthesis of peptides chains that later will be stored in the form of protein. Around 40% of the

protein and 80% of the AA are found in the muscles (Munro, 1970). The muscles have an important structural function in the body. However, muscles can be seen as an AA source or reserve for the metabolism as well. During periods of starvation, muscles can be broken down to provide AA to be used in protein synthesis in the liver. All the AA supplied above maintenance and production requirements are metabolized. The first step in the AA catabolism is usually to break the amino group from the carbon chain; the late will be used carbon chains to produce energy in the Krebs cycle. The ammonia generated from the metabolism of the amino group is toxic for the central nervous system. There are two amino acids which transport safely ammonia (NH_3^-), a residual product from protein degradation, in blood: alanine from the skeletal tissues and glutamine, this helps the cells to get rid of the excess of ammonia. The NH_4^+ inside cells tissues is combined to glutamate producing glutamine, the most abundant AA in the blood which can transport NH_4^+ in the systemic circulation to kidneys, liver, and intestines to N excretion or glutamine can be the source of amino groups in several biosynthetic processes (Nelson et al., 2008). The other way to transport the N in excess is through urea (CH_4N_2O): after glutamine leads the NH_4^+ to liver it is converted into glutamate and NH_4^+. The ammonia will get in the urea cycle resulting in urea and fumarate. The urea will then be transported into the blood system to be excreted by the kidneys.

1.3.2 Requirements for threonine: a systematic review

The requirement for Thr for maintenance is much higher when compared to other essential AA, such as Lys. Parallel to the increase in live weight, the animal maintenance increases (Hahn and Baker, 1995). With this, the Lys requirements (in % of the diet) of growing pigs decrease faster than the requirements of Thr. This could explain part of the variation in the Thr: Lys ratio suggested for pigs (Figure 1-3).

Usually, requirements for Thr are established in function of lysine requirements. However, several ratios are proposed (Rostagno et al., 2011; NRC, 2012; Gloaguen et al., 2014), and the studies present controversial results (De Lange et al., 2001; Pedersen et al., 2003; Ma et al., 2015; Mathai et al., 2016).

Threonine requirements will be dependent on the sanitary level, the fibre level in the diet, and the presence of microbes and parasites in the intestinal tract (Bequette, 2003). Also, the variation may be assigned by the difference between the models (Broken-line, curvilinear) used to determine the requirement (Pomar et al., 2003) and also by the difference between the criteria responses that had been used to estimate the ideal requirement (Ma et al., 2015).

In this systematic review I aimed to provide a complete, exhaustive summary of current literature relevant to our research questions. I studied carefully the peer-reviewed paper published studying Thr requirements (Table 1-2)., Mainly, this systematic review was used to summarize the data concerning to the statistical model most used to determine Thr requirements, the average Thr recommendations that should be used to stablish the 100% Thr level compared to the Thr:Lys ratio proposed by the NRC (2012). Other important exploratory aspects considered were the criteria response used to determine Thr:Lys ratio and the duration of the experimental period. And specially which were the aspects that remained to be explored about Thr:Lys requirements. As well, this review aimed to know the limitations of the peer-reviewed paper published studying Thr requirements to be included in a future meta-analysis study.

Data were extracted from the material and methods and results sections of the selected articles. Only data reported in articles published in peer-reviewed journals were selected, and their acceptance for publication was considered as a subjective indication of their methodological quality. Papers were critically evaluated as to their quality and relevance considering this systematic review objectives. The information contained in each selected study was analyzed according to experimental design, treatments, evaluated parameters, and statistical analysis. The selected articles were then checked for their compliance with different criteria in order to determine their inclusion or not in this review. The main criteria used for including the articles were: a) addition of different Thr levels in the diets (above 3 levels of inclusion); b) all other amino acids (AA) fixed at 100% of their optimal levels; and c) presentation of the nutritional composition of the experimental diets.

1.3.2.1 Thr:Lys ratio: main results found in the literature and lacune

Most part of the trials lasted above 18 d and used ADG as variable response to establish Thr:Lys ratio requirements. The main statistical models used were quadratic regression and the linear-plateau model. The average Thr:Lys ratio to maximize ADG for growing pigs (25-50 kg) according to the literature reviewed is above of the 0.59 Thr:Lys ratio proposed by the NRC (2012) and in line with the 0.65 Thr:Lys ratio recommended by Sève et al., (1993), previously presented in the item 1.2 of this literature review as INRA recommendations. The Thr recommendation tended to increase with the increase of BW (Saraiva et al., 2006; Saraiva et al., 2007). Several factors seemed to have influence in the AA requirements estimates: the statistical model, where, linear-plateau model generated lower estimates than quadratic regression (Saldana et al., 1994). As well the variable response, where the Thr:Lys ratio to maximize ADG was smaller than that to maximize feed:gain efficiency (Saldana et al., 1994).

Information on dietary fibre content is limited in the literature, increases in dietary fiber can increase Thr requirements (Mathai et al., 2016). Most of the papers present total AA content. However, it is preferable to report AA composition and AA requirements in standardized ileal digestible (SID), as the diet composition may affect AA digestibility. In this way, when comparing diets with different feedstuff composition total AA values might be the same. Genetic lines have different growth potential, and by consequence different requirements, as well sex should be considered as a factor which could influence the results. All the papers presented in this review worked with supplementation technique, none used dilution technique. In this case, the difference in Thr recommendations is not due to the diet formulation technique. Several authors did not observe performance response to Thr intake. This might be due the experimental period, which might have been too long or too short to observe the response of group-fed pigs. Many authors failed to report a complete AA composition of diets. Thus, it is possible that another AA than Thr was limiting the performance of non-responsive pigs.

Few studies (de Lange et al., 2001; Libao-Mercado et al., 2006) studied the protein deposition response to Thr intake, and AA concentration in different tissues in response a different Thr intakes are not presented. This information was presented by Hamard et al., (2009) for pigs receiving diets 30% Thr restriction and adequate supplementation, study which is not included in this database once presented with only two Thr:Lys ratios in the study. It remains to be explained whether Thr:Lys ratios could have impact on the carcass composition in terms of AA, crude protein and fat concentrations, and if the same type of proteins of the protein synthesised by the body could change in AA restriction. Plasmatic proteins are often forgotten, and these proteins could be used as supply in some level of Thr restriction to maintain normal body functions (Reece and Swenson, 2005). Therefore, more information in biochimical variables such plasmatic proteins are needed to better understand Thr utilization. There is no study in literature that compare AA ratios between feeding systems, and there is no information available of SID Thr requirements for precision fed animals. Data about late-finishing pigs is limited in literature, and no published paper was found for pigs between 110 -130 kg of BW. Therefore, if Thr requirements increase in this late period of growing remain to be studied.

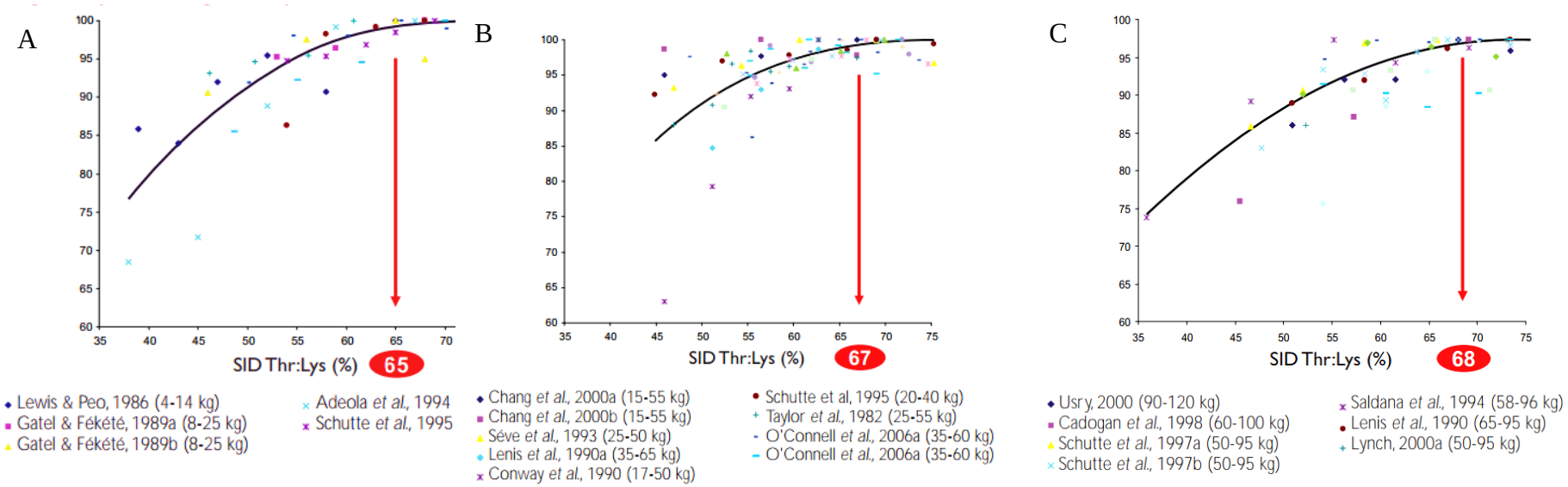

Figure 1-3. Average daily gain (y-axis) relative to the best performance (i.e., 100% average daily gain; red arrow) of piglets (4-20 kg of BW; plot A), growing pigs (15-70 kg of BW; plot B), and finishing pigs (50-110 kg of BW; plot C) in function of the threonine:lysine ratio (red arrow) (from Ajinomoto Bulletin, 31)

Table 1-2. Published requirements for threonine in peer-reviewed studies[1]

References	BW (kg)	Sex	Thr (%)	Thr:Lys	Variable response	Model	DRC[2]	Type of diet	Days trial	Crude fibre (%)	CP (%)	Note
(Defa et al., 1999)	17-30	-	0.68 (total)	75	Plasma nitrogen urea	Polynomial contrast analysis	1	Corn, wheat bran, soybean meal, cottonseed meal, rapeseed meal	28	-	15.9	Digestible or standardized diet AA composition not reported; no measurement of fibre in the diet
(Plitzner et al., 2007)	50-110	-	0.53 (total)	68	Feed:gain ratio	Linear-plateau	1	Corn, soybean meal, wheat and barley	46-52	3.1	-	Digestible or standardized diet AA composition not reported; total AA analyzed; no ADF and NDF but crude fibre level reported which was higher in the basal diet
(Pozza et al., 2000)	15-30	Female	0.60 (total); 0.53 (SID)	62	Feed:gain ratio and urea in plasma	Inverse Linear-plateau	1	Corn, soybean meal, sorghum and corn gluten meal	31	2.36	15.8	Digestible or standardized diet AA composition not reported; total Lys and Thr analyzed; no ADF and NDF but crude fibre level reported
(Saldana et al., 1994)	6.5 (initial)	Boars	0.63 (total)	51	ADG	Linear-plateau	2	Peanut meal, soybean meal, sorghum and dried way	28	-	-	Initial and final BW per treatment not reported; digestible or standardized diet AA composition not reported; no measurement of fibre in the diet
			0.69 (total)	56	ADFI	Linear-plateau						
			0.66 (total)	54	ADG	Quadratic						
			0.65 (total)	53	ADFI	Quadratic						
	58 (initial)	Gilts & barrows	0.37 (total)	53	ADG	Linear-plateau	2	Sorghum and synthetic AA	42	-	-	
			0.39 (total)	56	ADFI	Linear-plateau						
			0.41 (total)	59	Gain:feed ratio	Linear-plateau						
			0.45 (total)	65	ADG	Quadratic						
			0.42 (total)	60	ADFI	Quadratic						
			0.47 (total)	67	Gain:feed ratio	Quadratic						

Continuation of Table 1-2

References	BW (kg)	Sex	Thr (%)	Thr:Lys	Variable response	Model	DRC[2]	Type of diet	Days trial	Crude fibre (%)	CP (%)	Note
(Santos et al., 2010)	95-125	Barrows	0.53 (SID)	65	Feed:gain ratio	Quadratic	1	Corn and soybean meal	-	-	14.2	No measurement of fibre in the diet
(Paiano et al., 2008)	30-60	Barrows	0.55 (SID)	66	Crude protein retained and plasmatic urea	Quadratic	1	Corn and soybean meal	-	-	14.7	Initial and final BW per treatment not reported; no measurement of fibre in the diet; content of AA in diets not analyzed; AA composition of corn and soybean meal analyzed but not reported
(Berto et al., 2002)	7-12	Barrows	0.94 (total)	67	ADG	Quadratic	1	Corn, soybean meal, dried yeast, dried skim milk, dried way, sugar;	11	-	19	Content of AA in diets not analyzed; AA composition of ingredients analyzed but not reported
			0.89 (total)	63	Plasmatic urea	Quadratic						
	13-24	Barrows	0.76 (total)	61	ADG and Feed:Gain ratio	Quadratic	1	Corn, soybean meal, dried yeast, sugar;	16	-	18.0	
(Lewis and Peo, 1986)	5-15	Gilts & barrows	0.70 (total)	-	Gain:feed ratio, plasma AA	Polynomial contrast analysis	1	sorghum, oat groats, soybean meal, dried fish soluble, brewers dried yeast;	28	-	15.9	Initial and final BW per treatment reported; digestible or standardized diet AA composition not reported; no measurement of fibre in the diet; content of AA in diets not analyzed; AA composition of ingredients analyzed but not reported
(Rodrigues et al., 2001a)	6-5	Barrows	0.68 (SID)	67	ADG	Quadratic	2	Corn, soybean meal, dried skim milk, glutamic acid, sugar;	27	2.57	18	Initial and final BW per treatment not reported; ADF or NDF in the diet not reported; content AA in diets not analyzed; AA composition of ingredients analyzed but not reported
			0.73 (SID)	73	Feed:gain ratio							
(Rodrigues et al., 2001b)	30-60	Gilts	0.61 (SID)	75	Protein deposition	Quadratic	1	Corn, corn starch, soybean meal, glutamic acid, corn gluten meal;	37	2.3	18	Initial and final BW per treatment not reported; ADF or NDF in the diet not reported; content AA in diets not analyzed; AA composition of ingredients analyzed but not reported
			0.61 (SID)	75	Feed:gain ratio							

Continuation of Table 1-2

References	BW (kg)	Sex	Thr (%)	Thr:Lys	Variable response	Model	DRC[2]	Type of diet	Days trial	Crude fibre (%)	CP (%)	Note
(Saraiva et al., 2007)	30-60	Gilts	0.52 (SID) 0.56 (SID)	64 67	Feed:gain ratio Feed:gain ratio	Linear-plateau Quadratic	1	Corn, soybean meal, starch, glutamic acid;	33 to 39 d	-	16.16	AA in diets not analyzed; AA composition of ingredients analyzed but not reported; no measurement of fibre in diet; initial and final BW per treatment not reported
(Saraiva et al., 2006)	15-30	Gilts	0.59 (SID)	62	Feed:gain ratio	Linear-plateau	1	Corn, soybean meal, starch, glutamic acid	33 to 39 d	-	16.16	AA in diets not analyzed; AA composition of ingredients analyzed but not reported; no measurement of fibre in the diet; no measurement of fibre in diet; initial and final BW per treatment not reported
(Pedersen et al., 2003)	70-97	-	-	66	Plasmatic urea	Quadratic	2	Wheat, barley, soybean meal	24	3.6-3.5	16.1	Duration of treatments different for pigs at 60-110 kg; ADF or NDF in diet not reported; AA in diet analyzed but only values for digestible Lys and Thr reported
	76-80	-	-	62								
	95	-	-	70								
	60-110	Gilts & barrows	-	-	Plasmatic urea; percentage of lean; carcass traits;	No effect			44-55		16.1	
	60 - 75	-	-	58, 64, 70, 76	N balance	No effect			18	3.3	16.4 - 16.1	
(Libao-Mercado et al., 2006)	35	Barrows	4.7 g/d	-	Protein deposition	Means comparison	2	Cornstarch, wheat shorts (2 diets) or casein (2 diets), synthetic AA	-	-	7.2-12.2	Levels of Thr using different feeds compositions to observe its efficiency of utilization were tested; SID Thr reported but not possible to calculate Thr compositin in diet as energy content of diets not reported; AA in diets analyzed (values reported as total basis); feed intake not reported

Continuation of Table 1-2

References	BW (kg)	Sex	Thr (%)	Thr:Lys	Variable response	Model	DRC[2]	Type of diet	Days trial	Crude fibre (%)	CP (%)	Note
(De Lange et al., 2001)	39-77	Gilts	8 g/d	58	Protein deposition	Regression	2	Casein, cornstarch, cellulose, sucrose, synthetic AA	-	-	22.1	Ratios reported as total basis for diets; SID values not reported except for Thr intake but digestibilityof purified diets with synthetic AA often assumed close to 100%; crude fibre in diets not reported but this information would have little value as purified diets were used
(Adeola, 1995)	10-20	Gilts & barrows	5.3 g/kg^{-1}	53	Protein deposition	Means comparison	2	Cornstarch, corn, peanut meal, skim milk powder	-	-	-	SID values not reported; fibre, CP and digestible or metabolizable energy in diets not reported
(Borg et al., 1987)	8-20	-	0.63 (total)	57	Plasmatic urea; ADF; feed:gain ratio	Means comparison	1	Corn, sunflower meal	28	-	12 or 13%	SID values not reported; fibre and digestible or metabolizable energy in diets not reported; AA in diets analyzed, but only values for sunflower meal reported
(Ettle et al., 2004b)	32-0	-	0.54 (SID)	70	Feed: gain ratio	Means comparison	1	Wheat, barley, soybean meal	-	-	16.6 -17	Fibre and digestible or metabolizable energy in diets not reported
	65-112		0.44 (SID)	71	ADG; Feed: gain ratio						13.5 - 14.1	

Continuation of Table 1-2

References	BW (kg)	Sex	Thr (%)	Thr:Lys	Variable response	Model	DRC[2]	Type of diet	Days trial	Crude fibre (%)	CP (%)	Note
(Ma et al., 2015)	90-118	Gilts	0.61 (SID)	61	ADG	Linear-plateau	1	Soybean meal, wheat bran, corn starch, synthetic AA	28	-	10.1	Crude fibre in diets not reported but this information would have little value as purified diets were used
				63	Feed:gain ratio	Linear-plateau						
				64	Urea in plasma	Linear-plateau						
				70	ADG	Quadratic regression						
				74	Feed:gain ratio	Quadratic regression						
				72	Urea in plasma	Quadratic regression						
(Mathai et al., 2016)	26-50	Gilts		66	ADG	Intersection between linear-plateau and quadratic-plateau	3	Corn, field peas, soybean meal, fish meal, corn starch	28	ADF: 3.4; NDF: 8-9%	12.6	Complete information on diets and animal performance available; all criteria for dose response seem to be met, except that Thr requirements in function of a directed related variable response such as protein deposition not determined
				63	Gain:feed ratio							
				71	ADG	Intersection between linear-plateau and quadratic-plateau		Corn, field peas, soybean meal, fish meal, soybean hulls	28	ADF: 10%; NDF: 15.3-17.9%	12.8	
				63	Gain:feed ratio							

[1] Abbreviations used: BW = body weight; ADG = average daily gain; CP = crude protein, DRC = dose-response criteria

[2] Dose-response criteria: 1 = DRC not met, i.e. Lys and all other AA provided at requirements (no surplus); 2 = DRC met, i.e. Lys at required levels and all other AA provided above requirements; 3 = DRC met, i.e. Lys reduced by 10 % and other AA provided above requirements

1.4 Individual versus population requirements

To use the ideal protein concept to estimate AA requirements for populations it becomes crucial to determine precise lysine requirements and to establish appropriate AA: lysine ratios. The first step is to choose the model and individual that best represent the lysine requirements of the population because the latter will determine the content of all other AAs. The factorial method is commonly used to estimate population requirements in conventional feeding programs (e.g., NRC, 2012) where the same feed is provided to the entire herd throughout the feeding phase (Figure 1-4). When the factorial method is used to estimate the nutrient requirements of a population of animals, it is common practice to use the average pig to represent the population. However, this practice should be used with caution, because half of the population may be overfed whereas the other half may be underfed (Hauschild et al., 2010), resulting in a potential performance loss for the entire pig population. Moreover, the factorial method directly estimates the requirements for a particular animal at a specific point in time. Thus, changes during the feeding phase are not taken into consideration by this method. If the aim is to maximize population performance, the best option may be to adopt requirements at the beginning of each feeding phase, because this is when requirements are at their maximum. As shown in Figure 1-4, feeding a population of pigs using the 80th-percentile pig on the first day of the experiment as a reference seems to be an expensive practice that may result in high N and P losses to the environment. Precision nutrition may provide a useful alternative.

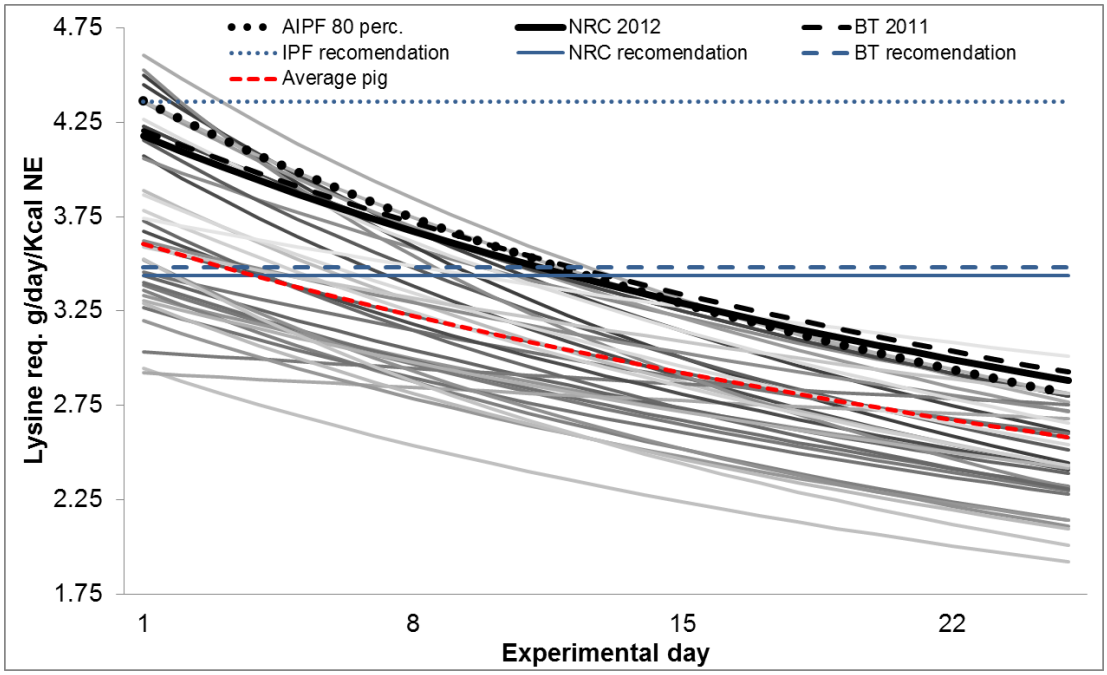

Figure 1-4. Daily standardized ileal digestible lysine (SIDLys) requirements (g/kcal net energy, NE) of 25-50 kg body weight pigs and optimal phase-feeding SIDLys concentration estimated with the NRC (2012), Brazilian tables for swine and poultry (BT), and AIPF (Hauschild et al., 2012) models.

Precision nutrition, which in this text is used as a synonym of precision feeding, is a concept that considers aspects of inter- and intra-individual variability (Wathes et al., 2008). This variability results from differences among animals regarding genetics, age, and weight (intrinsic factors). Furthermore, external factors influencing animal performance and nutrient demands (extrinsic factors) can play a significant role. Every animal responds differently to these effects, resulting in increased variability between animals (Wellock et al., 2004). Considering within- and between-animal variability in nutritional programs is crucial for evaluating the biological response of pigs (Knap, 2000; Hauschild et al., 2010). Precision nutrition considers all of these aspects. Based on the precision nutrition concept, Pomar et al. (2009) proposed the application of an individual and daily feeding system for pigs, known as individual precision feeding (IPF), to maximize production efficiency and minimize nutrient losses. In this system, each pig is fitted with an ear tag that is electronically recognized by the feeder unit, enabling it to receive an individually and daily tailored diet. This individual diet is formulated from up to four different feeds combined in the precise proportions required by the individual pig since each AIPF

unit contains four feed compartments. Within- and between-animal variability can be taken into account with the AIPF system because the diet is provided according to individual requirements and adjusted on a daily basis.

The AIPF system is still being calibrated to accurately estimate the amount of nutrients that each animal needs to meet its daily requirements. The AIPF system cannot use the current methods (empirical and factorial) and growth models to estimate real-time individual nutrient requirements. A mathematical model which estimates individual pig's requirements in real time was therefore proposed by Hauschild et al.(Hauschild et al., 2012) and was recently validated (Andretta et al., 2014; Cloutier et al., 2015). However, the model can only estimate lysine requirements. Further exploratory studies are required to test the optimum levels of other essential AAs when applying this model to precision feeding based on the AIPF system.

While the use of ratios to determine AA requirements for populations seems to be well established, their applicability to individual precision feeding is not so clear. Given that lysine requirements vary between animals and over time, it is valid to assume that other AAs could vary as well. The challenge this review raises is, how can AA requirements be established individually? Empirical dose-response studies can easily underestimate or overestimate a test AA if a simple mistake is made when establishing the initial AA levels to be tested or if the analyzed nutritional composition differs from the expected composition. Most often, all essential AA requirements are adjusted in ratio to lysine requirements. If the lysine concentration decreases over time (Andretta et al., 2014), all other AAs will decrease accordingly, and if the diet is ratio-based, we may be limiting animal performance because another AA besides lysine becomes the limiting AA. Precision feeding can significantly reduce N excretion into the soil, and production costs depend on lysine intake (Andretta et al., 2016b). However, it is important to also set the intake of other AAs at levels that enable these savings to be achieved and to foster the best pig performance without harm to the pigs' metabolism.

1.5 Methods for estimating requirements for amino acid in pigs

1.5.1 Factorial method

The factorial method for estimating AA requirements relies on calculations to determine the amount of AA to be provided to the animals based on the growth and maintenance components (van Milgen and Dourmad, 2015). These calculations are often theoretical and sometimes incorporate more than one empirical equation in order to these requirements (D'Mello, 2003b). This method is limited by the constancy of AA use: a maintenance value is often assumed to be constant for each AA, but, biologically speaking, this value might change according to the AA function, level and diet composition. Nevertheless, the factorial method can be applied in different environments and to different populations (de Lange, 2012) because the method has been calibrated using population responses. A factorial approach makes it possible to break down the different components of the requirement and to distinguish the amount of nutrient that is needed for each component (de Lange, 2012; van Milgen and Dourmad, 2015).

1.5.2 Empirical method

Empirical methods are based on dose-response studies which provide graded levels of the tested nutrient to measure the response (e.g., protein deposition, AA deposition, G: F, AA levels in plasma). Dose-response studies can be designed based on different statistical models for estimating AA requirements. These studies can even be applied to validate a factorial estimate of AA. Nevertheless, the recommended requirements determined by a dose-response study will be time-, population-, environment- and diet-dependent (de Lange, 2012).

To vary the level of the test nutrient, two techniques are often proposed for dose-response studies: supplementation and dilution techniques. The first consists of formulating a basal diet which is deficient for the test AA and increase its level using a synthetic source to obtain the different experimental diets (D'Mello, 1982). To succeed using dose-response methods five criteria need to be met (de Lange, 2012; van Milgen and Dourmad, 2015): (1) an AA-deficient basal diet; (2) all AAs at

adequate levels with the exception of the test AA; (3) at least four graded test AA levels; (4) adequate duration of the experiment in relation to the response variable; and (5) determination of the AA requirement using a suitable statistical model. Although this is probably the most widely used technique, some researchers have concerns about the AA imbalance, so a dilution technique has been proposed to solve this problem (D'Mello, 1982; Gous and Morris, 1985). The main feature of the dilution technique is that it mixes a diet high in AAs with another diet low in AAs to keep the AA concentration consistent between test diets. The diets are formulated to have a summit diet (high AA concentration) and a nitrogen-free diet, and by mixing the diets the AA ratios are maintained constant avoiding imbalances.

Although these techniques are used for populations, the formulation of test diets for individuals is more complex because the AA concentration has to change every day and is different from one animal to another. In this case, a mix of both techniques has been proposed (Zhang et al., 2012; Cloutier et al., 2016): four diets are formulated from two A feeds and two B feeds (A feeds are rich in AAs, and B feeds are poor in AAs with the exception of the test AA) mixed in the right proportions to meet the requirements, and each pig receives a daily tailored diet. The A feeds are formulated to meet the lysine requirements of the most demanding pig on the first day of the experimental period, and the B feeds to meet the lysine requirements of the least demanding pig on the last day. All other AAs are formulated to meet 110% of the requirements, except the test AA. If lysine is not the test AA, the diet will be limited at 90% of lysine requirements, becoming the secondary limiting AA (Boisen, 2003).

The problem is that when a nutrient is limiting, the animal response variance increases (Gous, 2016). This variance can be easily observed in dose-response studies, which often have high between-animal variation (van Milgen and Dourmad, 2015). A simulation (Brossard et al., 2009) showed that providing a population with 110% of its lysine requirements reduces variation. The theory is that as the nutrient level increases, more pigs meet their requirements, reducing the variation in the response (Gous, 2016).

Normally, when observing data of dose-response studies a large within treatment variation can be observed. It might be due to the fact that dose-response studies always have an AA limiting at some point: until reach, the inflection point or plateau is the test AA which is limiting animal's performance, and after reaching a plateau lysine is limiting in the diet. If the variation in dose response originates with limiting AAs (e.g., lysine), a factorial trial (Pasquetti et al., 2015) simultaneously testing two AAs (e.g., lysine and tryptophan) could decrease AA variation. Some rules should be established to avoid making other nutrients limiting AAs in the diet, particularly in the case of precision feeding: (1) all experimental diets should be formulated to meet 110% of AA requirements (except the test AA) for the most demanding pig on the first day of the trial and should be kept constant throughout the feeding phase; (2) the diets must allow for a range of amounts of the test AA in order to meet the requirements of the least demanding pig on the last day of trial; and (3) the experiment must last long enough to observe a response.

1.6 Limitations for establishing requirements for amino acids in precision-fed pigs

The use of the AA ideal protein profile concept seems to be an easy solution for formulating diets and one that suits the industry well. However, precision feeding calls for more than just a practical solution for formulating diets. What is needed is a way to consider the impact of the formulation on animal performance and welfare, environmental nutrient excretion, and production costs. Precision feeding requires a new, comprehensive look at the way animals are fed, with a review of the traditional concepts of AA requirements based on a fixed amount of protein deposition and maintenance requirements.

When AA intake decreases, AA efficiency increases (De Lange et al., 2001) and maintenance becomes a grey area that needs to be understood. A daily tailored diet can represent a lysine intake decrease of 27% (Andretta et al., 2014), and if an AA is established based on a ratio to lysine, it means that the intake of all other AAs will decrease in the same proportion than lysine. Given that AA is often expressed as a simple ratio to lysine, the real AA intake required to maximize pigs' performance

is unknown. Precision feeding is more strongly affected than phase feeding by a reduction in the AA: lysine ratio (Remus et al., 2015a), likely resulting in muscle breakdown and performance losses. These observations suggest that it may be more appropriate to express the AA requirements of pigs fed with daily tailored diets independently to lysine. Further studies are needed to understand the changes in pigs' nutrient metabolism in precision feeding programs and to establish a new way to determine nutrient requirements for such programs.

1.7 Hypothesis and objectives

Based on the results of this literature review it is clear that AA requirements can be established using the population-based protein profile concept in a phase-feeding system, but this practice should be used with caution in precision feeding programs. Precision feeding needs to integrate individual amino acid requirements using methods that consider individual variability. In conventional group-phase-feeding systems, all pigs receive the same feed during extended periods and, therefore, most of the pigs receive a larger amount of nutrients than required to express their growth potential. Normally pigs fed in group using a population based method will be half part of the growing phase underfed and the other part overfed (Figure 1.4). This means that pigs group-fed that have their AA requirements restricted in the beginning of the growing period will likely be able to perform its maximum in the second part of growing period. In precision feeding systems, pigs are fed with diets tailored daily to their individual nutrient requirements. Therefore a pig which is restricted in the first day of trial will be restricted during the all growing period. Optimal AA ratios have been stablished for conventional phase-feeding systems but these optimal ratios may differ between conventional and precision feeding pig production systems once they are average recommendation to average groups.

1.7.1 Hypothesis

An increase in optimal Thr:Lys ratios may reduce variability among animals by better adjusting AA provisions to the individual requirements in precision feeding systems. We further we hypothesize that the use of the same ideal protein profile for precision fed pigs than for group-fed pigs is not adequate for establishing AA requirements because it will limit protein deposition and change the concentration of plasmatic and muscular proteins in precision fed pigs.

1.7.2 Research objectives

Main research objective:

To study the response of growing pigs to varying Thr:Lys ratios (70 to 130% of the optimal ratio 65 Thr:Lys (Seve et al., 1994) in conventional group phase-feeding and individual precision feeding systems

Specific objectives:

- To compare growth performance, body composition and N retention response to Thr:Lys ratio levels
- To estimate Thr:Lys ratio to maximize protein deposition inside each feeding system

To study the effect of Thr deficiency and excess on biochemical plasmatic parameter and chemical composition of organs and carcass muscles

CHAPTER 2: PIGS RECEIVING DAILY TAILORED DIETS USING PRECISION FEEDING TECHNIQUES HAVE DIFFERENT AMINO ACIDS REQUIREMENTS THAN PIGS FED IN CONVENTIONAL PHASE-FEEDING SYSTEMS

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“I knew it was good work, but you cannot know how much something will be appreciated in the future. You don't have that crystal ball.”

(John Nash Jr., in the 2002 episode of the documentary series American Experience, entitled "A Brilliant Madness" focused on his life)

Pigs receiving daily tailored diets using precision feeding techniques have different amino acids requirements than pigs fed in conventional phase-feeding systems

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2.1 Résumé

Il existe une grande variation dans les besoins en nutriments chez les porcs. Les rapports optimaux d'AA pourraient donc différer selon qu'il s'agit d'un système d'alimentation classique ou d'un système d'alimentation de précision. La réponse à différents niveaux de thréonine (Thr) (70%, 85%, 100%, 115% et 130% du ratio protéique idéal de 0.65 Thr: Lys) a ainsi été étudiée chez des porcs en croissance nourris de façon classique en groupe par phase (GPF) ou alimentés individuellement en utilisant des techniques d'alimentation de précision (IPF). Un essai a été réalisé suivant un plan factoriel 2 × 5 avec 110 porcs en phase de croissance (25 ± 0,80 kg de poids corporel) logés dans le même enclos et alimentés à l'aide de mangeoires électroniques. L'unité expérimentale était le porc. L'expérimentation a duré 21 jours. Cinq porcs par traitement ont été abattus à la fin de l'expérience. La consommation de thréonine a augmenté linéairement chez les porcs dans les systèmes IPF et GPF (6,28 à 11,76 vs 6,85 à 11,01 g / j, P <0,05). La consommation de lysine était similaire (12,5 g / j) entre les traitements. Le gain

quotidien moyen, G: F et le dépôt protéique (PD) ont été affectés linéairement par le niveau de thréonine (P <0,05), mais ils n'ont pas été affectés par le système d'alimentation. L'intersection des modèles linéaire-plateau et quadratique-plateau en fonction du PD a été obtenue chez les porcs dans le système GPF à 150 g / j ayant un rapport 0,65 Thr: Lys, alors que le PD maximal n'a pas été atteint chez les porcs dans le système IPF (126 à 159 g / j). L'albumine plasmatique a augmenté linéairement avec l'augmentation de Thr dans la diète chez les porcs dans les systèmes IPF et GPF (29,1 à 34,9 vs 30,8 à 32,5 g / L, P <0,05). Les concentrations plasmatiques de méthionine et de sérine étaient respectivement 11% et 7% plus élevées chez les porcs dans le système IPF et le système GPF (P <0,05), alors que la méthionine dans l'intestin grêle tendait (P <0,10) à être 10% plus faible chez les porcs dans le système IPF. La concentration de thréonine et de sérine dans le foie avait tendance à être 1% (P <0,10) (4,44 vs 4,39 g) et 2% (4,52 vs 4,44) plus élevée, respectivement, chez les porcs dans le système IPF que chez les porcs dans le système GPF. L'apport alimentaire de Thr a augmenté (P <0,05) la concentration de Thr dans le Longissimus dorsi de manière quadratique chez les porcs dans le système IPF, alors qu'il n'y avait aucun effet chez les porcs dans le système GPF. La concentration de collagène dans le Longissimus dorsi diminuait avec l'augmentation du Thr alimentaire chez les porcs dans les systèmes IPF et GPF (0,61% à 0,45% contre 0,55% à 0,54%, P <0,10). Le CP des muscles de la carcasse était 2% plus élevé chez les porcs dans le système GPF (18,1%) que chez les porcs dans le système IPF (17,8%, P <0,05). Ces résultats montrent que les systèmes d'alimentation peuvent affecter la façon dont les AA, en particulier Thr, sont utilisés par les porcs. Un rapport Thr: Lys qui maximise les réponses des porcs en croissance diffère entre les systèmes d'alimentation classique et de précision, de sorte que les systèmes d'alimentation de précision doivent utiliser les ratios AA: Lys protéine idéale AA avec prudence.

2.2 Abstract

There is a large variation in nutrient requirements among pigs, so feeding pigs individually with daily tailored diets or in groups with a single feed may require different levels of nutrients. Thus, the response to different levels of Threonine (Thr) (70%, 85%, 100%, 115%, and 130% of the 0.65 Thr:Lys ideal protein ratio) was studied in growing pigs raised in conventional group phase-feeding (GPF) systems or individually fed using individual precision feeding (IPF) techniques. A trial was performed in a 2×5 factorial design with 110 pigs in the growing phase (25 kg BW ± 0.80) housed in the same pen and fed using electronic feeders. Individual pigs were the experimental units. The trial lasted 21 days. Five pigs per treatment were slaughtered at the end of the trial. Threonine intake (SID) increased linearly in both the IPF and GPF pigs (6.28 to 11.76 vs. 6.85 to 11.01 g/d, $P < 0.05$). Lysine intake (SID) was similar (12.5 g/d) across the treatments. Average daily gain, G:F, and PD were affected linearly by the threonine level ($P < 0.05$), but they were not affected by the feeding system. The intersection of the linear-plateau and quadratic-plateau models for PD was obtained in the GPF pigs at 150 g/d and a 0.65 Thr:Lys ratio, whereas maximum PD was not reached in the IPF pigs (126 to 159 g/d). Plasma albumin increased with the level of Thr in the IPF and GPF pigs (29.1 to 34.9 vs. 30.8 to 32.5 g/L, $P < 0.05$). Plasma methionine and serine levels were 11% and 7% higher, respectively, in the IPF than in the GPF pigs ($P < 0.05$), whereas methionine in the small intestine tended ($P < 0.10$) to be 10% lower in the IPF pigs. Threonine concentration in the liver tended ($P < 0.10$) to be 1% higher (4.44 vs. 4.39 g) and serine (4.52 vs. 4.44) was 2% greater in the IPF pigs than the GPF pigs. Dietary Thr supply increased ($P < 0.05$) the Thr concentration in the longissimus dorsi in a quadratic manner in the IPF pigs, whereas there was no effect in the GPF pigs. Longissimus dorsi collagen decreased as dietary Thr increased in the IPF and GPF pigs (0.61% to 0.45% vs. 0.55% to 0.54%, $P < 0.10$). Carcass muscle CP was 2% higher in the GPF pigs (18.1%) than in the IPF pigs (17.8%, $P < 0.05$). These results show that feeding systems can affect the way AA, especially Thr, are used by pigs. A Thr:Lys ratio that maximizes growing pig responses differs between conventional

and precision feeding systems, so precision feeding systems should use the actual AA:Lys ideal protein AA ratios with caution.

Keywords: amino acids body composition, dose-response, ideal protein profile, lysine, threonine

2.3 Introduction

Pigs are usually fed in groups and the same feed is provided within each feeding phase (GPF) whose composition is adjusted to an average animal estimated requirements. These requirements are established using a factorial method based on the average pig of the population in the middle of the growing phase (e.g. NRC, 1998). However, pigs have different requirements, and these requirements change over time. As a result, pigs in individual precision feeding (IPF) systems receive a daily tailored diet according to each pig's requirements on that day (Pomar and Pomar, 2012; Pomar et al., 2014). Lysine (Lys) requirements are estimated daily (Hauschild et al., 2012) and the other amino acid (AA) requirements are established based on a ratio to Lys. Using IPF techniques, Lys intake can decrease by 17% (Andretta et al., 2016) as compared to a traditional GPF system. The problem is that other AA requirements are not known for the IPF system and we assumed them to be a constant ratio to Lys. It has been shown that pigs in an IPF system might have higher methionine-to-lysine ratio requirements than pigs in a GPF system presenting changes in plasma biochemical variables (Remus et al., 2015a).

Threonine (Thr) is often assumed to be a limiting AA for maintenance; its requirements are determined based on the ideal protein profile as it is believed that the first limiting AA in the diet will stop protein synthesis. Lately, studies have shown that pigs' growth rate and tissue composition can change as a function of AA intake (Conde-Aguilera et al., 2010; Conde-Aguilera et al., 2016a; Conde-Aguilera et al., 2016b). Threonine deficiency might lead to the synthesis of threonine-poorer proteins and a reduction of Thr concentration in muscle (Hamard et al., 2009). Our hypothesis was the use of same ideal protein profile for precision fed pigs than for group-fed pigs is not adequate for establishing AA requirements because it will limit protein deposition and change the concentration of plasmatic and muscular proteins

in precision fed pigs.. The aim of this study is to compare the performance, chemical and biochemical variables response of pigs in two feeding systems, IPF and GPF, receiving different levels of threonine (70%, 85%, 100%, 115%, or 130% of the estimated ideal level of the 0.65 Thr:Lys ratio (Sève, 1994).).

2.4 Material and methods

2.4.1 Animals, housing and management

One hundred and ten healthy barrow pigs of the same high-performance genotype (Fertilis 25 × G-Performer 8.0, Geneticporc Inc., St-Gilbert, Quebec) were shipped to the swine complex at AAFC-Sherbrooke, Quebec. All the pigs were allocated in two 76-m^2 pens with concrete slat floors in the same mechanically ventilated room. The pigs had an electronic chip placed in their ears that gave access to the automatic and intelligent precision feeders. Between their arrival and the start of the trial, the pigs were fed with commercial feeds adapted to their requirements. Water was provided with low-pressure nipple drinkers, and feed was provided individually ad libitum throughout the adaptation (14 days) and experimental periods (21 days) with 10 feeding stations (Automatic and Intelligent Precision Feeder; University of Lleida, Lleida, Spain). The room temperature was adjusted to 22°C at the piglets' arrival.

The pigs (25 kg BW ± 0.80) were randomly assigned to treatments in 2 complete blocks according to a 2 x 5 factorial design with the main factors being: (1) feeding systems (IPF: individual precision feeding and GPF: conventional group phase-feeding system), and (2), 5 threonine levels [70%, 85%, 100%, 115%, or 130% of the estimated ideal level of the 0.65 Thr:Lys ratio (Sève, 1994)]. The experimental unit was the individual pig and each treatment included 11 replicates. Each of the 2 complete blocks included 55 pigs, and blocks started the experimental period 1 week apart. All the pigs stayed housed in the same pen during the entire experimental period due to the individual codes present in each transponder placed in the pigs' ears, which allowed individual data to be recorded and treatments to be provided individually. The IPF system identifies each pig when the feed demand is made, and the feeder reads the specific treatment formula for that pig, mixing the

feed according to the assigned treatment, and dropping the feed in the feeder tray. A time lag was imposed between feed demands to avoid feed waste. This time lag was set according to the pig's body weight (BW) and feed intake.

2.4.2 Feeding programs, nutritional requirements, and diets

In both feeding systems (IPF and GPF), the nutritional requirements for amino acids, calcium, and phosphorus were independently estimated and the diets formulated accordingly with the same energy concentration (Table 2-1). Data from high-performance pigs from previous trials performed at AAFC were used to simulate the pigs' Lys requirement to formulate the feed (A1, A2, B1, and B2). The feed formulation was performed using the values of total AA content corrected to the standardized ileal digestible (**SID**) value of each ingredient according to the digestibility values for each AA as presented by the INRA-AFZ tables (Sauvant et al., 2004). Feeds were formulated to contain the same amino acid profile, which resulted in a small feedstock variation. In the IPF, 2 A feeds and 2 B feeds (A1 and B1 containing 130% and A2 and B2 containing 70% of the optimal Thr:Lys levels) were mixed to meet the daily calculated requirements, and each pig received a daily tailored diet. The feeds were formulated to meet the Lys and other AA requirements other than Thr of the most demanding pig on the first day of the period (feeds A1 and A2) and those of the least demanding pig on the last day of the experimental period (feeds B1 and B2). The AA requirements other than Lys were established using the ideal AA:Lys ratio proposed by the INRA as described by Gloaguen et al. (2014). In the GPF, the pigs received the same feed through the entire phase. The different dietary treatments were obtained by blending the 4 experimental feeds in the required proportions.

Table 2-1. Ingredient and chemical composition of the experimental feeds (A1, A2, B1, B2)

Item	A1	A2	B1	B2
Ingredients (as-fed basis), g/kg				
Corn	533	538	537	538
Soybean meal (48%)	173	173	-	-
Wheat	150	150	100	100
Canola meal	47	47	-	-
Corn gluten meal **+** linseed meal [2]	33	33	-	-
Corn starch	-	-	156.3	156.3
Fat	16	16	35	35
Oat hulls	-	-	143	143
Limestone	12	12	8	8
Mono-calcium phosphate	10	10	8	8
Lysine sulfate (70%)	6.70	6.70	2.80	2.80
Salt	5.50	5.50	4.80	4.80
L-threonine	4.50	-	1.20	-
DL-methionine	2.30	2.30	0.20	0.20
L-valine (96.5%)	2.10	2.10	0.20	0.20
Vitamin-mineral premix[3]	2.00	2.00	2.00	2.00
L-tryptophan	1.10	1.10	0.30	0.30
L-isoleucine	0.70	0.70	0.20	0.20
Anti-mold	1.00	1.00	1.00	1.00
Choline chloride (75%)	0.20	0.20	0.20	0.20
Chemical composition, %				
Dry matter	90.85	91.25	92.99	92.67
Crude Fat	6.79	6.74	7.88	8.44
Crude Protein	19.85	19.88	7.5	6.88
ADF	3.87	4.02	6.32	6.51
NDF	8.80	8.63	13.58	14.12
Total calcium	0.72	0.72	0.50	0.49
Total phosphorus	0.64	0.64	0.40	0.40
SID[4] isoleucine	0.67	0.69	0.22	0.21
SID leucine	1.34	1.39	0.64	0.59
SID lysine	1.07	1.07	0.34	0.33
SID methionine	0.53	0.53	0.16	0.14
SID methionine + cysteine	0.72	0.72	0.24	0.20
SID phenylalanine	0.75	0.77	0.28	0.26
SID serine	0.80	0.80	0.30	0.26
SID threonine	0.98	0.58	0.31	0.19
SID valine	0.89	0.89	0.29	0.27
Metabolizable energy[4], kcal/kg	3357	3357	3206	3206
Expected net energy, kcal/kg	3208	3223	3255	3259

[1]Mix of corn gluten meal and linseed meal (Shur-Gain Canada)

[2]Supplied per kilogram of diet (as fed-basis): vitamin A, 11,400 IU; vitamin D, 1,140 IU; vitamin E, 35 IU; vitamin K, 2 mg; vitamin B12, 30 µg; niacin, 20 mg; pantothenic acid, 15 mg; pyridoxine, 2 mg; thiamine, 2 mg; cooper, 122 mg; iodine, 0.3 mg; iron, 100 mg; manganese, 63 mg; selenium, 0.3 mg; and zinc, 152 mg

[3]Standartize ileal disgestible (SID) and metabolizable energy were estimated from the analyzed total amino acid and crude energy content in feed and values from INRA-AFZ tables (Sauvant et al., 2004)

In the IPF, the required daily concentration of Lys was estimated with a mathematical model using individual feed intake and weekly BW information (Hauschild et al., 2012). Using these data, the empirical component of the model estimated the expected BW, daily feed intake (DFI), and weight gain for the next day, whereas the mechanistic component used these 3 estimated variables to calculate with a factorial method the optimal concentration of Lys that should be offered that day to each pig in the herd to meet requirements. In the mechanistic model compartment, daily Lys requirements (g/d) were calculated by adding maintenance and growth requirements. The daily Lys maintenance requirements were estimated by adding together the basal endogenous losses (0.313 g Lys/kg DM × DFI), the losses related to desquamation in the digestive tract (0.0045 g Lys/kg$^{0.75}$ × BW$^{0.75}$), and the losses related to the basal renewal of body proteins (0.0239 g Lys/kg$^{0.75}$ × BW$^{0.75}$; van Milgen et al., 2008). The SID Lys requirements for growth were calculated assuming that 7% of the body protein is Lys (Mahan and Shields, 1998) and that the efficiency of Lys retention from digestible dietary Lys is 72% (Möhn et al., 2000a). Weight gain composition in terms of protein was calculated assuming 16% protein in daily gain (de Lange et al., 2003). This method of estimating nutrient requirements had been described previously (Hauschild et al., 2012; Pomar et al., 2014) and validated in 3 previous studies (Zhang et al., 2012; Cloutier et al., 2015; Andretta et al., 2016). In the IPF system, each pig received daily tailored diets. In the GPF system, lysine requirements were estimated assuming that population requirements are those of the 80th percentile pig of the group at the beginning of the phase (average of 3 days) (Hauschild et al., 2010; Remus et al., 2015c). However, lysine provisions were decreased by 10% to assure that lysine was the second limiting AA while the other amino acids were provided at the estimated level. Threonine requirements were stablished in ratio to Lys, were 70%, 85%, 100%, 115%, or 130% of the estimated ideal level of the 0.65 Thr:Lys ratio (Sève, 1994) was provided to the animals. The ratios were constant in both feeding system during the growing period.

2.4.3 Experimental measurements

2.4.3.1 Performance

Pigs were weighed at arrival and three times during the adaptation period to calibrate the model before providing the treatments. Animal performance was evaluated through average daily feed intake (ADFI; kg/d), average daily gain (ADG; kg/d), G:F (kg/kg), SID Lys intake (g/d), SID Thr intake (g/d), protein deposition (PD; g/d), PD in daily gain (%), lipid deposition (LD; g/d). Total body fat and lean content were measured by DXA on days 0, 21 of the trial with a densitometer device (GE Lunar Prodigy Advance, Madison, WI, USA). Pigs were scanned in the prone position using the total body scanning mode (Lunar enCORE Software Version 8.10.027). Anesthesia was induced with sevoflurane (7%) and maintained with isoflurane (5%) during the scans performed on days 1 and 21.

2.4.3.2 Blood sampling

Blood samples were collected on day 21. All pigs were fasted for 10 hours. Samples were gathered from the jugular vein and disposed in a tube with the anticoagulant EDTA for enzymatic and biochemical analysis or with sodium heparin for the amino acid analysis. The time between final sample and centrifugation did not exceed 1 hour, and for this period, samples were kept on ice. The blood samples were centrifuged for 15 minutes, 1000 × g at 4°C. For AA analysis, within 30 minutes after centrifugation 20 µl of standard enriched AA was added to the samples. All the plasma samples were kept at -20°C for the sampling day; at the end of the day, they were stored at -80°C.

2.4.3.3 Organs and muscles sampling

Five pigs per treatment were slaughtered in a commercial slaughterhouse. Each one was scalded and scraped, and the eviscerated carcass was split longitudinally; the head and feet were kept. The right side of the carcass was dissected and the head and feet were discarded. The longissimus muscle was separated from the loin cut. The liver and the small intestine (washed and mesentery free) were collected. All samples were sealed in separate vacuum plastic bags and stored at -20°C until sampling for a maximum of 2 months. The liver and small

intestine were ground twice and sampled. The dissected samples were cut in cubes and mixed to be ground. Longissimus dorsi and a pool of all the other muscles were ground 4 times and sampled. All the samples were freeze-dried and stored at -80°C until analysis.

2.4.3.4 Chemical and biochemical analysis

Two replicates of each sample were analyzed using Association of Official Analytical Chemists (AOAC) standard methods for lyophilization [method 938.18 (AOAC, 1990)] and the protein in the feed, liver, small intestine [Kjeltec 2400; FOSS Tecator, Hillerod, Denmark; method 992.15 (AOAC, 1990)], and lipids [Soxtec 2050 Automated Extraction System; Foss, Höganäs, Sweden; method 991.36; (AOAC, 1990)] were determined. Crude protein, collagen, and fat in the longissimus dorsi and the pool of carcass muscles were estimated by near-infrared transmittance [Method 2007.04 (AOAC, 1990); FOSS FoodScan™ Near- Infrared (NIR) Spectrophotometer]. For all the samples, the dry matter [method 950.46; (AOAC, 1990)] and ash (method 920.153; (AOAC, 1990)) were analyzed. Concentrations of AA in plasma were determinded as suggested by Calder et al., (1999). The AA concentration in the pool of carcass muscles and longissimus dorsi were lyophilised. The samples were hydrolyzed with a solution of HCl 6N-0.1 % Phenol in the digester block at 110ºC for 24h. A mixture of standart isotopes (200µl) were added to the samples. A solution of 100µl of DL-dithiothreitol (15.4 mg/ml of water) was added to the sample which rested for 30 minutes in room temperature. Following the samples were passed through the columns (Ply-prep-Bio-Rad 731-1550) prepared with 0.8 cm (0.4 ml) of resin (Sigma-Aldrich Dowex 50WX8 -200 ion exchange resin). The the colums were rinsed twice with 2 ml of ultra-pure water. Amino acids were recovered adding 2 ml of NH_4OH_2N in the columns. The columns were rinsed with 1 ml of ultra-pure water and let it drain in the vial. Vials were covered with parafilm and vortexed. The samples were frozen at -80ºC and lyophilised. Vials were rinsed with 250 µl of ultra-pure water and transferred to a reacti-vial (Pierce 13221). The the contents of the reacti-vials were dried with nitrogen at 90ºC, for about 20 minutes, and 20 µl de DTT 15.4 mg/ml et 80 µl de NH_4OH_2N were added to the samples. The samples rested for 30 minutes in room temperature and were dried with nitrogen at

90ºC for 20 minutes. Samples were derived with 60 µl of MTBSTFA:DMF 1:1 (MTBSTFA: Aldrich 394882, DMF: Aldrich 27.054-7). Samples were heated at 90ºC for 35 minutes. Samples were transferred to vials for GC (Agilent 5182-0714). All AA samples were measured by gas chromatography coupled to mass spectrometry (Agilent Technologie 7890B GC System coupled to a Agilent Technologie 5977A MSD).The IgG was determined through ELISA kits (Pig IgG ELISA quantification Set, ref. E100-104, Bethyl Laboratories, Inc.). The biochemical and enzymatic analysis of plasma was performed with an automatic analyzer.by a dedicated external laboratory (Faculté de médecine vétérinaire of the Université de Montréal; Saint-Hyacinthe, QC, Canada)

2.4.3.5 Calculations and statistical analysis

Total pig weight gain was calculated as the difference between the weights measured at the beginning and end of each phase. The SID Lys, SID Thr, and CP intakes were obtained for each pig by tallying the daily amount of nutrients provided by each of the feeds served. Lysine and Thr efficiency were calculated by dividing the corresponding retained by available AA. Lysine and Thr retention were estimated assuming that 7% of body protein is Lys and 3.7% is Thr. The availability of these AA was estimated by removing from the SID pool the amount used for maintenance. Lysine maintenance requirements were estimated as indicated previously. Threonine requirements were estimated by adding together the basal endogenous losses (0.33 g Thr/kg DM × DFI), the losses related to desquamation in the digestive tract (0.0138 g Thr/kg$^{0.75}$ × BW$^{0.75}$), and the losses related to the basal renewal of body proteins (0.0033 g Thr/kg$^{0.75}$ × BW$^{0.75}$; van Milgen et al., 2008). The DXA body lean and fat masses were converted to their protein and lipid chemical equivalents as proposed by Pomar and Rivest (1996). Protein deposition in gain was calculated by dividing the protein deposition by the average daily gain. Nitrogen excretion values were obtained by subtracting the respective nutrient retention from the respective nutrient intake values.

Performance and carcass data were analyzed as a 2×5 factorial arrangement using a mixed model of SAS version 9.4 (SAS Inst. Inc., Cary, NC). The main effects

included the feeding system, the threonine level, and their interaction; the random effect was the block. The assumption of normal distribution of variables was checked using the Cramer-von Mises test within the Univariate procedure of SAS. The uncertainty in the estimate of the mean of the data was expressed as the maximum standard error (MSE), and a $P \leq 0.05$ was considered to be statistically significant, while a $P < 0.10$ was considered a tendency. Differences between individual treatments were analyzed by orthogonal contrasts. Thr:Lys ratio requirement for each feeding program was estimated using the NLIN procedure of SAS.

2.5 Results

Pigs consumed feed and gained weight according to the expected performance of the genetic line, except that during the adaptation period, 3 pigs were excluded from the trial due to unsatisfactory performance (low FI and ADG) and recurrent fever; they were treated and isolated from the group. During the trial, 3 animals were removed, 1 due to a severe inflammatory foot problem and 2 due to respiratory problems unrelated to the trial. The data from these animals were not considered in the analysis. Thus, the performance data presented in this paper consists of 10 pigs for treatments IPF70, IPF115, IPF130, and GPF85; 8 pigs for IPF85; and 11 pigs for all the other treatments.

2.5.1 Performance, nutrient intake and nitrogen balance

During the growing phase, the ADFI, SID Lys intake, CP intake, PD in gain, LipD, final BW, and N excretion were not affected by threonine levels or the feeding system (Table 2-2). Average daily gain, G:F, SID Thr intake, KThr, KLys, PD, and N retention were affected linearly by the threonine level ($P < 0.05$), but not by the feeding system. No interactions between feeding systems and Thr levels were observed.

Table 2-2. Initial and final animal body composition, growth performance and nutrient efficiency of growing barrow pigs (25-42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in an individual precision feeding (IPF) or group-phase feeding (GPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	10	8	11	10	10	11	10	11	11	11				
Initial conditions														
Body weight, kg	26.0	26.2	25.6	25.2	26.0	26.7	25.7	25.8	25.7	26.2	0.8	0.40	0.49	0.84
Body protein, kg	3.94	3.96	3.83	3.76	3.93	4.06	4.00	3.91	3.87	3.97	0.17	0.23	0.18	0.99
Body lipids, kg	1.18	1.19	1.16	1.14	1.17	1.21	1.20	1.17	1.16	1.19	0.03	0.16	0.23	1.00
Final conditions, growth performance and nutrient efficiency (0 to 21 d)														
Body weight, kg	39.54	40.45	41.47	41.59	43.45	40.80	42.48	42.06	41.74	42.28	1.09	0.11	0.37	0.57
Body protein, kg	6.59	6.68	6.83	6.94	7.28	6.86	6.95	7.04	6.98	7.12	0.23	0.16	0.31	0.76
Body lipids, kg	2.76	2.75	2.71	2.56	2.61	2.76	2.89	2.73	2.61	2.59	0.23	0.64	0.72	0.99
Average daily feed intake, kg/d	1.44	1.46	1.46	1.63	1.50	1.51	1.40	1.49	1.48	1.41	0.14	0.41	0.35	0.47
Average daily gain, kg/d	0.64	0.67	0.76	0.80	0.83	0.68	0.73	0.78	0.77	0.76	0.04	0.01†	0.63	0.17
G:F, kg/kg	0.46	0.47	0.51	0.51	0.56	0.45	0.49	0.52	0.52	0.56	0.04	<0.001†	0.64	0.87
SID[3] lysine intake, g/d	11.51	12.34	12.18	13.31	12.87	13.00	11.96	12.79	12.67	12.11	1.25	0.63	0.86	0.22
SID threonine intake, g/d	6.30	7.88	8.91	11.02	11.47	7.09	7.63	9.34	10.18	11.35	0.88	<0.001†	0.99	0.33
Threonine efficiency,[4] %	84	68	65	56	54	75	68	65	57	55	0.07	<0.001†	0.53	0.46
Lysine efficiency,[5] %	80	78	87	85	93	73	78	88	88	94	0.09	<0.001†	0.83	0.77
Protein deposition, g/d	126.20	129.72	141.35	151.09	159.49	130.89	143.12	149.72	148.48	150.23	8.33	<0.001†	0.54	0.59
Protein in gain, %	18.95	19.05	19.12	19.23	19.31	19.03	19.20	19.39	19.37	19.57	0.33	0.43	0.25	0.99
Lipid deposition, g/d	74.80	74.06	74.67	68.32	68.41	74.20	80.99	74.36	69.02	66.78	10.08	0.70	0.84	0.99
Nitrogen balance														
Crude protein intake, g/d	222.3	238.4	236.2	258.2	248.6	250.2	230.1	247.0	244.6	234.0	19.48	0.56	0.95	0.22
Efficiency of nitrogen retention, %	55.34	54.68	60.53	59.07	64.51	51.25	54.66	61.25	61.08	65.25	4.77	<0.001†	0.94	0.80
Nitrogen excretion, g/d	16.34	17.39	14.90	16.17	14.26	18.55	16.60	15.58	15.40	13.41	2.96	0.05‡	0.91	0.70

[1]Maximum standard error
[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system
[3]Standard ileal digestible
[4]Threonine efficiency = ((PD × 0.037) - (0.313 g Thr/kg dry matter × DFI + (0.0033 g Thr/kg$^{0.75}$ d × BW$^{0.75}$) + (0.0138 g Thr/kg$^{0.75}$ d × BW$^{0.75}$)) / SID Thr intake
[5] Lysine efficiency = ((PD × 0.069) - (0.330 g Lys/kg dry matter × DFI + (0.0045 g Lys/kg$^{0.75}$ d × BW$^{0.75}$) + (0.0239 g Lys/kg$^{0.75}$ d × BW$^{0.75}$)) / SID Lys intake
†Linear effect for L; ‡Tendency for a linear effect for L

2.5.2 Estimation of Thr:Lys ratio

Protein deposition, ADG, and G:F were the criterion responses used to compare the response of pigs fed with the IPF and GPF systems and receiving different levels of Thr (Table 2-3). These variable responses were preferred to others because they are directly affected by the AA supply. The optimal Thr:Lys ratio for the IPF system was not clear once a plateau was not observed. For the GPF system, the optimal Thr:Lys ratio to maximize performance was assumed at the interception of the linear-plateau and the quadratic-plateau model; while the minimum Thr:Lys ratio to avoid losses in performance, for this program was assumed to be the breakpoint of the linear-plateau. The ideal Thr:Lys ratio as a function of protein deposition as a variable response was 65 (65% inclusion of Thr in relation to Lys requirement) for the GPF system (linear-plateau R = 60.2) and 85 or higher for the IPF system (Figure 2-1). In relation to PD, the ideal Thr:Lys ratio increased by 8% (linear-plateau R= 64.9) when optimizing ADG and by 15% (linear-plateau R = 68.6) when optimizing G:F in GPF pigs.

Table 2-3. Non-linear model parameters between the independent response variables (protein deposition, ADG and G:F) and the threonine-to-lysine ratio in an individual precision feeding (IPF) and a group-phase feeding (GPF) system estimated with a linear plateau and a quadratic plateau model[1]

		Model parameter							
Feeding system	**Response**	**U**	**SEe**	**R**	**SEe**	**L**	**SEe**	**P-value**	**RSE**
Linear plateau model									
IPF	PD	-0.873	0.25	85.4	6.91	159.5	-	0.00	24.33
	ADG	0.00505	0.002	82.2	11.37	0.8295	0.04	0.00	0.12
	G:F	-	-	-	-	-	-	-	-
GPF	PD	-1.2239	0.99	60.2	9.89	149.5	3.76	0.07	21.61
	ADG	-0.00376	0.001	64.9	24.01	0.77	0.02	0.24	0.12
	G:F	-0.0056	0.003	68.6	6.45	0.5362	0.01	0.03	0.08
Quadratic plateau model									
IPF	PD	-	-	-	-	-	-	-	-
	ADG	-	-	-	-	-	-	-	-
	G:F	-	-	-	-	-	-	-	-
GPF	PD	-0.0347	0.059	68.2	19.82	149.5	4.28	0.07	21.61
	ADG	-0.00011	0.0003	71.1	28.51	0.7698	0.03	0.25	0.12
	G:F	-0.00012	0.0002	70.6	17.33	0.5387	0.02	0.03	0.08

[1]Abbreviations used: PD = protein deposition (g/d); ADG = average daily gain (kg/d); G:F = gain:feed ratio (kg/kg); SEe = standard error of the estimation;
R = parameter corresponding to the standard ileal digestible level of threonine-to-lysine ratio required to reach the plateau; L = average response estimated by the model; U= fit intercept;
RSE = residual standard error

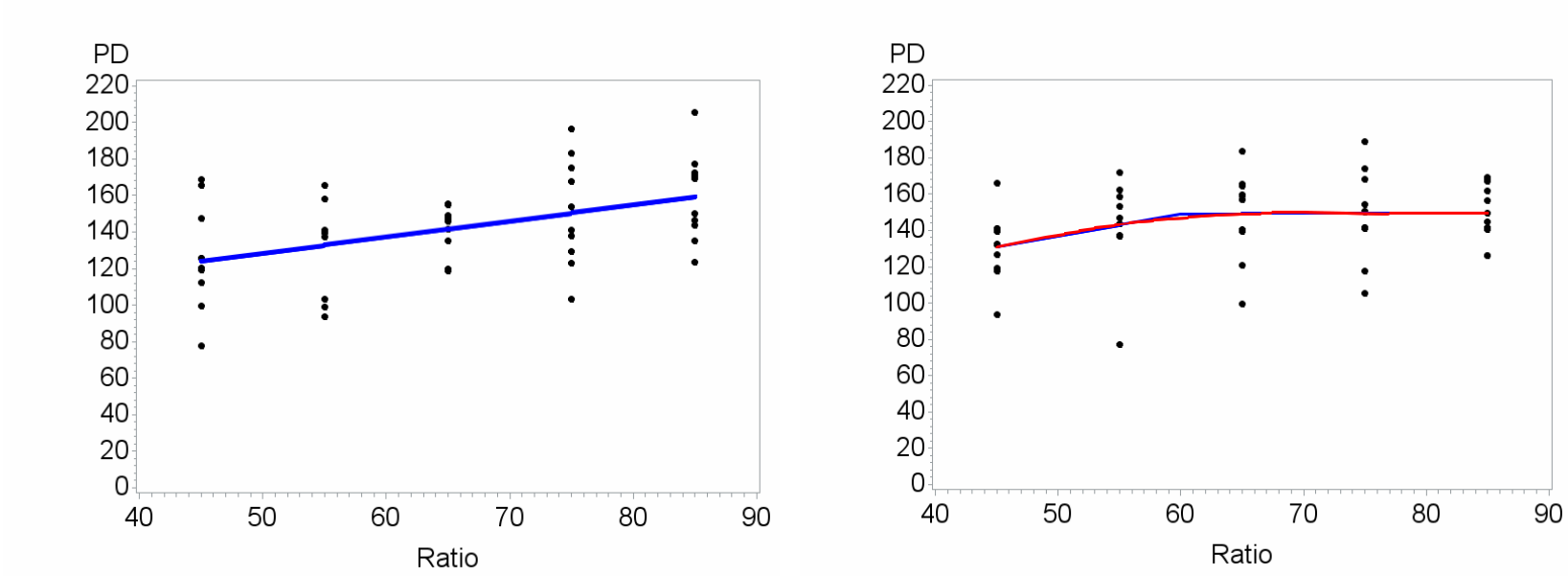

Figure 2-1. Protein deposition (PD, g/d) in function of standardized ileal digestible threonine-to-lysine ratio according to the linear-plateau and quadratic-plateau models for pigs in an individual precision feeding system (right plot) or in a group-phase feeding systems (left plot)

2.5.3 Biochemical and enzymatic response in plasma

Plasmatic creatinine (µmol/L), IgG (µg/ml), CK (U/L) were not affected by the feeding system or the threonine levels ($P > 0.10$; Table 2-4). While Albumin (g/L) and total protein (g/L) increased linearly with the increase in threonine levels ($P < 0.05$), they were not affected by the feeding system. C-reactive protein (CRP) (µg/ml) increased ($P < 0.05$) in a linear manner in the IPF pigs and in a quadratic manner in the GPF pigs. Alanine aminotransferase (ALT) (U/L) increased ($P < 0.05$) linearly within IPF pigs and presented a cubic increase within GPF. Aspartate aminotransferase (AST) (U/L) tended ($P < 0.10$) to increase linearly as dietary threonine increased and it tended ($P < 0.10$) to be 8% higher in the IPF pigs than in the GPF pigs. Lactic acid dehydrogenase (LDH) (U/L) to be 9% higher in the IPF pigs than in the GPF pigs. Urea (µmol/L) decreased ($P < 0.05$) in a quadratic manner in both feeding systems.

Table 2-4. Blood plasmatic biochemical parameters of growing barrow pigs (25-42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in an individual precision feeding (IPF) or group-phase feeding (GPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	10	8	11	10	10	11	10	11	11	11				
Urea, μmol/L	2.70	1.98	2.38	2.19	2.77	2.74	2.04	2.34	2.07	2.40	0.23	0.00‡	0.51	0.83
Albumin, g/L	27.80	26.56	32.12	31.59	33.51	29.50	31.44	32.25	31.63	31.19	1.25	0.00†	0.19	0.03
Creatinine, μmol/L	116.85	114.69	112.68	110.85	117.50	117.50	116.91	115.35	112.41	119.41	3.69	0.25	0.39	1.00
Lactic acid dehydrogenase, U/L	585.34	581.88	535.13	532.60	570.35	524.96	485.73	537.90	468.60	557.14	47.66	0.53	0.06	0.72
Total protein, g/L	62.65	64.26	65.33	66.90	67.48	61.86	65.56	64.37	66.00	66.13	1.52	0.01†	0.52	0.89
Aspartate aminotransferase, U/L	36.75	44.96	38.35	43.80	43.09	36.89	37.50	36.60	36.48	44.21	3.50	0.08†	0.08	0.34
Alanine aminotransferase, U/L	47.50	40.79	39.73	40.00	38.39	41.14	45.05	44.37	36.06	43.90	3.03	0.14	0.60	0.04[a,c]
Creatine kinase, U/L	1083	1561	1227	1822	1918	1108	1244	1562	1015	2172	412	0.15	0.67	0.52
IgG, μg/ml	11.29	11.28	9.93	11.90	10.98	9.71	10.90	9.48	11.31	11.36	1.18	0.19	0.33	0.84
C-reactive protein, μg/ml	9.25	13.02	9.98	18.35	24.78	13.88	15.81	18.46	22.82	12.68	3.56	0.05†	0.26	0.01[a,d]

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system; †Linear effect for L; ‡Quadratic effect for L; [a]Linear effect within IPF; [b]Cubic effect within IPF; [c]Cubic effect within GPF; [d]Quadratic effect within GPF;

2.5.4 Free amino acids in plasma

The essential AA (EAA) histidine, lysine, and threonine (Table 5) were affected in a cubic, quadratic, and linear manner, respectively, by the threonine level in the diet ($P < 0.05$), but they were not affected by the feeding system. While methionine was not affected by the threonine level in the diet, it was 11% higher in the IPF pigs than in the GPF pigs ($P < 0.05$). The other EAA were not affected by the threonine level or the feeding system. The non-essential AA (NEAA) glutamine tended ($P < 0.10$) to increase in a quadratic manner as a function of the dietary Thr level, while glycine, proline, and homocysteine tended ($P < 0.10$) to increase linearly with the increase in the dietary Thr level. Serine increased, while tyrosine decreased linearly with the increase of threonine in the diet ($P < 0.05$). Serine was 7% higher in the IPF system than in the GPF system ($P < 0.05$). Non-essential AA glutamine, glutamate, glycine, homocysteine, proline, serine and tyrosine increase in a linear manner as Thr in the diet increased, however only serine was affected by the feeding system, being 4% lower in IPF than GPF pigs.

Table 2-5. Plasmatic free amino acid concentrations of growing barrow pigs (25-42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in an individual precision feeding (IPF) or group-phase feeding (GPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	10	8	11	10	10	11	10	11	11	11				
Essential amino acids, µmol/L														
Arginine	215.26	210.37	222.60	212.17	208.10	216.69	226.5	217.58	195.36	210.64	18.40	0.87	0.98	0.92
Histidine	54.23	41.64	39.29	44.31	30.26	58.55	45.08	33.79	35.65	35.50	4.05	<0.001§	0.92	0.18
Isoleucine	89.37	78.62	93.25	82.33	88.10	84.62	85.85	82.43	83.39	83.93	5.35	0.67	0.43	0.37
Leucine	148.46	159.01	169.55	166.34	155.39	154.52	153.0	153.95	156.69	157.46	7.62	0.27	0.18	0.25
Lysine	136.93	80.90	75.53	59.26	76.68	125.19	70.60	64.79	62.92	64.32	11.86	<0.001‡	0.17	0.89
Methionine	58.56	51.48	47.48	48.34	51.24	46.68	46.42	44.62	51.22	40.38	4.71	0.44	0.04	0.37
Phenylalanine	64.69	70.51	61.73	58.04	61.18	58.25	59.41	59.03	63.69	62.14	3.66	0.69	0.19	0.12
Threonine	50.61	93.59	133.52	245.22	256.03	42.70	93.98	157.41	235.81	258.58	19.75	<0.001†	0.87	0.89
Tryptophan	46.20	41.19	43.72	39.76	39.57	44.84	41.96	40.98	41.36	42.52	2.76	0.21	0.87	0.72
Valine	242.59	238.27	261.83	249.92	239.29	250.49	226.3	239.95	247.28	253.52	10.37	0.34	0.63	0.30
Non-essential amino acids, µmol/L														
Acid Aspartic	11.57	13.91	13.14	15.50	14.86	12.77	13.22	14.47	12.80	13.77	1.30	0.37	0.59	0.37
Alanine	437.05	468.17	390.13	446.80	490.21	423.24	413.6	404.66	451.39	419.79	28.47	0.15	0.14	0.37
Asparagine	39.87	40.07	42.51	42.70	41.25	40.86	40.77	42.00	40.47	41.52	3.75	0.94	0.93	0.98
Cysteine	193.73	189.57	204.18	200.91	211.29	195.79	200.8	207.32	195.66	201.82	7.36	0.17	0.93	0.54
Glutamate	163.27	217.79	207.69	235.01	238.14	207.22	200.5	214.63	203.51	225.17	20.14	0.12	0.85	0.26
Glutamine	452.58	483.11	490.92	491.77	485.81	438.95	478.8	500.46	533.40	477.22	31.74	0.06‡	0.74	0.76
Glycine	967.1	1116.3	990.8	1028.5	1108.8	939.9	914.6	1037.9	1060.6	1112.0	16.78	0.07†	0.40	0.18
Homocysteine	19.72	20.42	22.07	22.29	25.53	18.15	20.44	24.24	22.15	21.70	2.24	0.08†	0.58	0.58
Proline	185.7	194.93	183.91	206.57	197.87	186.82	180.70	188.69	198.42	187.42	10.56	0.09†	0.22	0.60
Serine	93.00	103.71	99.89	111.67	108.68	86.12	93.51	98.25	98.64	108.81	4.97	<0.001†	0.02	0.44
Tyrosine	67.24	64.18	55.65	63.62	59.75	66.12	62.22	59.40	59.41	55.32	3.79	0.03†	0.45	0.74

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system; †Linear effect for L; ‡Quadratic effect for L

2.5.5 Liver AA and chemical composition

In this growing phase (Table 2-6), Thr (tendency; $P < 0.10$) and serine ($P < 0.05$) concentrations (g of AA/ 100g of CP) in the liver were 1% and 2% higher, respectively, in the IPF than in the GPF pigs. The other EAA and NEAA, DM, CP, fat, and ash were not affected by the Thr level or feeding system or their interaction during the growing phase.

2.5.6 Intestine AA and chemical composition

During the growing phase, asparagine and serine presented a feeding system × Thr level interaction with no effect in the intestine of the IPF pigs and a cubic effect tendency ($P < 0.10$) in the GPF pigs (Table 2-7). Methionine tended ($P < 0.10$) to be 10% lower in the small intestine of the IPF pigs compared with the GPF pigs. The other EAA and NEAA, DM, CP, fat, and ash were not affected by the threonine level or feeding system or their interaction during the growing phase.

2.5.7 Longissimus dorsi AA and chemical composition

During the growing phase, histidine decreased linearly in the longissimus dorsi as the dietary Thr level increased ($P < 0.05$) independently of the feeding system (Table 2-8). Isoleucine (tendency; $P < 0.10$) and leucine decreased linearly in the IPF pigs and in a quadratic manner in the GPF pigs. Lysine and glutamate (tendency; $P < 0.10$), Thr and alanine increased ($P < 0.05$) in a quadratic manner in the IPF pigs as the dietary Thr level increased; they were not affected in the GPF pigs. Cysteine tended to decrease ($P < 0.10$) linearly in the IPF pigs, whereas it tended to increase linearly in the GPF pigs. Glycine tended to be 1.4% higher ($P < 0.10$) in the GPF pigs than in the IPF pigs. Collagen in the longissimus dorsi decreased ($P < 0.05$) with the increase in the level of dietary Thr, independent of the feeding system. The other EAA and NEAA, DM, CP, fat, and ash were not affected by the threonine level or feeding system or their interaction during the growing phase.

Table 2-6. Liver amino acid concentrations of growing barrow pigs (25-42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in an individual precision feeding (IPF) or group-phase feeding (GPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	5	3	6	5	5	5	5	5	5	5				
Chemical composition, %														
Dry matter	28.94	27.70	28.35	28.73	28.66	28.08	28.58	28.29	28.69	29.04	0.53	0.59	0.84	0.49
Crude protein	20.44	20.28	20.35	20.57	20.72	20.34	20.55	20.34	20.77	20.26	0.33	0.84	0.92	0.78
Fat	7.11	6.21	6.35	6.17	6.44	5.91	6.77	6.69	6.41	7.43	0.51	0.72	0.53	0.19
Ash	1.48	1.51	1.50	1.51	1.48	1.47	1.47	1.47	1.59	1.47	0.04	0.21	0.93	0.54
Essential amino acids, g/100 g of crude protein														
Arginine	6.89	7.00	6.87	6.88	6.89	6.69	6.91	7.09	6.85	6.82	0.18	0.76	0.76	0.76
Histidine	3.01	2.91	2.92	3.03	3.04	3.03	2.92	2.99	2.93	2.88	0.08	0.69	0.49	0.48
Isoleucine	4.47	4.36	4.36	4.39	4.45	4.28	4.37	4.30	4.44	4.42	0.06	0.40	0.23	0.32
Leucine	9.00	8.76	8.83	9.01	9.00	8.86	8.82	8.86	8.87	8.82	0.11	0.50	0.23	0.62
Lysine	7.52	7.34	7.29	7.50	7.32	7.39	7.30	7.25	7.32	7.28	0.12	0.38	0.22	0.95
Methionine	3.19	3.00	2.80	3.14	3.21	2.79	2.68	2.59	3.16	2.51	0.44	0.82	0.21	0.93
Phenylalanine	5.00	4.87	4.92	5.03	5.10	4.97	4.92	4.99	4.96	4.93	0.06	0.24	0.33	0.14
Threonine	4.48	4.40	4.38	4.49	4.44	4.34	4.38	4.38	4.41	4.42	0.05	0.60	0.09	0.65
Valine	5.83	5.68	5.73	5.80	5.83	5.79	5.72	5.74	5.76	5.71	0.08	0.61	0.49	0.86
Non-essential amino acids, g/100 g of crude protein														
Alanine	5.76	5.64	5.69	5.73	5.71	5.71	5.62	5.68	5.69	5.61	0.06	0.39	0.22	0.94
Asparagine	10.51	10.18	10.31	10.52	10.40	10.37	10.19	10.20	10.35	10.16	0.17	0.41	0.18	0.95
Cysteine	1.14	1.24	1.33	1.23	1.19	1.15	1.18	1.24	1.23	1.25	0.06	0.21	0.65	0.69
Glutamate	12.56	11.80	11.02	11.68	12.03	12.05	11.94	11.05	11.21	11.22	0.63	0.23	0.37	0.91
Glycine	5.92	5.68	5.87	5.75	5.78	5.75	5.71	5.83	5.84	5.70	0.08	0.21	0.50	0.44
Proline	4.79	4.64	4.77	4.76	4.76	4.67	4.68	4.73	4.78	4.66	0.05	0.24	0.22	0.40
Serine	4.53	4.52	4.49	4.57	4.49	4.39	4.44	4.44	4.48	4.43	0.06	0.74	0.02	0.92
Tyrosine	4.21	4.12	4.11	4.18	4.19	4.06	4.15	4.08	4.15	4.13	0.05	0.47	0.10	0.36

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system

Table 2-7. Intestinal amino acid concentrations of growing barrow pigs (25-42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in an individual precision feeding (IPF) or group-phase feeding (GPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	5	3	6	5	5	5	5	5	5	5				
Chemical composition, %														
Dry matter	17.14	16.82	17.26	17.09	17.44	17.04	17.39	17.20	16.76	17.25	0.33	0.67	0.90	0.63
Crude protein	12.91	12.93	13.23	13.31	13.34	13.09	13.44	13.24	12.95	13.13	0.20	0.63	0.80	0.16
Fat	3.04	2.69	2.78	2.42	2.69	2.60	2.62	2.62	2.54	2.86	0.24	0.54	0.57	0.62
Ash	0.96	0.90	0.98	1.00	0.96	0.96	1.00	1.00	0.96	0.97	0.02	0.50	0.20	0.10
Essential amino acids, g/100 g of crude protein														
Arginine	8.17	8.13	8.01	8.16	8.02	7.96	8.29	8.11	8.00	8.09	0.10	0.40	0.94	0.14
Histidine	2.64	2.60	2.65	2.63	2.60	2.57	2.62	2.66	2.61	2.65	0.03	0.51	0.99	0.42
Isoleucine	4.19	4.13	4.18	4.14	4.11	4.04	4.21	4.20	4.17	4.17	0.05	0.47	0.81	0.10
Leucine	8.13	8.00	8.18	8.14	8.12	7.93	8.22	8.22	8.14	8.11	0.08	0.20	0.84	0.11
Lysine	7.71	7.55	7.73	7.63	7.64	7.47	7.67	7.74	7.68	7.71	0.09	0.37	1.00	0.21
Methionine	1.80	1.81	1.57	1.81	1.57	2.17	1.88	2.01	1.85	1.62	0.21	0.35	0.09	0.68
Phenylalanine	4.46	4.39	4.51	4.50	4.49	4.38	4.48	4.50	4.45	4.49	0.04	0.12	0.73	0.19
Threonine	4.59	4.60	4.62	4.65	4.64	4.51	4.69	4.69	4.61	4.60	0.05	0.14	0.98	0.21
Valine	5.19	5.14	5.21	5.16	5.16	5.03	5.23	5.21	5.18	5.19	0.06	0.37	0.88	0.19
Non-essential amino acids, g/100 g of crude protein														
Alanine	6.16	6.19	6.13	6.16	6.19	6.09	6.22	6.16	6.13	6.09	0.07	0.74	0.46	0.76
Asparagine	10.92	10.87	10.97	11.04	10.83	10.63	11.00	10.96	10.72	10.92	0.11	0.31	0.21	0.06[a]
Cysteine	1.15	1.19	1.15	1.26	1.19	1.20	1.21	1.24	1.15	1.12	0.06	0.86	0.90	0.30
Glutamate	14.97	15.19	14.97	15.22	14.95	14.89	15.44	15.20	15.03	14.90	0.25	0.46	0.84	0.83
Glycine	7.96	8.23	7.79	8.04	8.08	8.00	7.99	7.90	7.90	7.83	0.19	0.65	0.38	0.75
Proline	5.74	5.84	5.71	5.80	5.84	5.71	5.82	5.75	5.72	5.71	0.09	0.65	0.37	0.83
Serine	4.79	4.78	4.82	4.85	4.82	4.74	4.90	4.90	4.73	4.79	0.05	0.26	0.96	0.08[a]
Tyrosine	4.12	4.08	4.12	4.12	4.12	3.99	4.15	4.15	4.13	4.12	0.04	0.33	0.98	0.15

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system; [a]Cubic effect within GPF;

Table 2-8. Longissimus dorsi amino acid concentrations of growing barrow pigs (25-42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in an individual precision feeding (IPF) or group-phase feeding (GPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	5	3	6	5	5	5	5	5	5	5				
Chemical composition, %														
Dry matter	24.38	24.49	24.15	24.05	24.51	23.95	23.99	24.55	24.53	24.30	0.43	0.96	0.82	0.47
Crude protein	20.63	21.07	21.13	20.92	21.59	21.31	21.32	21.07	21.29	20.71	0.41	0.92	0.66	0.03[a]
Fat	2.15	2.05	1.97	1.44	1.73	1.70	1.78	1.71	1.79	1.95	0.27	0.66	0.56	0.30
Ash	1.14	1.19	1.18	1.19	1.18	1.15	1.18	1.17	1.18	1.17	0.04	0.73	0.68	1.00
Collagen	0.57	0.62	0.60	0.51	0.45	0.55	0.54	0.54	0.52	0.54	0.04	0.05[†]	0.64	0.09[c,e]
Essential amino acids, g/100 g of crude protein														
Arginine	7.38	7.51	7.34	7.33	7.19	7.40	7.41	7.34	7.40	7.49	0.12	0.75	0.36	0.36
Histidine	5.29	4.91	4.81	4.92	4.64	5.23	4.98	4.92	4.84	4.96	0.16	0.01[†]	0.38	0.50
Isoleucine	5.10	5.22	5.07	5.07	4.98	5.13	5.10	5.08	5.06	5.20	0.07	0.57	0.51	0.08[a,d]
Leucine	8.62	8.68	8.63	8.57	8.36	8.68	8.60	8.56	8.55	8.74	0.09	0.60	0.27	0.02[a,d]
Lysine	9.39	9.52	9.42	9.39	9.11	9.49	9.43	9.33	9.30	9.47	0.12	0.36	0.53	0.08[c]
Methionine	2.66	2.38	2.70	2.87	2.52	2.97	2.45	2.28	2.41	2.51	0.23	0.25	0.40	0.18
Phenylalanine	4.48	4.48	4.47	4.48	4.34	4.51	4.45	4.43	4.48	4.48	0.06	0.53	0.54	0.33
Threonine	4.89	4.91	4.92	4.92	4.70	4.92	4.92	4.85	4.86	4.94	0.07	0.42	0.30	0.03[c]
Valine	5.37	5.44	5.34	5.32	5.24	5.41	5.34	5.34	5.32	5.47	0.08	0.74	0.39	0.13
Non-essential amino acids, g/100 g of crude protein														
Alanine	6.03	6.10	6.04	6.05	5.81	6.12	6.04	6.03	6.00	6.08	0.07	0.14	0.18	0.02[c]
Asparagine	11.88	11.76	11.66	11.85	11.34	11.81	11.74	11.80	11.78	11.85	0.16	0.35	0.24	0.14
Cysteine	0.94	0.97	0.95	0.88	0.90	0.90	0.92	0.93	0.94	0.97	0.03	0.50	0.69	0.05[a,b]
Glutamate	17.42	17.72	17.73	17.81	16.28	16.98	17.78	17.52	17.45	18.01	0.56	0.55	0.58	0.09[c]
Glycine	4.75	4.76	4.70	4.76	4.57	4.79	4.73	4.80	4.75	4.81	0.07	0.68	0.08	0.18
Proline	4.00	4.02	4.02	4.03	3.90	4.06	4.03	4.05	4.00	4.08	0.06	0.86	0.10	0.20
Serine	4.19	4.13	4.22	4.21	4.04	4.23	4.18	4.16	4.15	4.17	0.05	0.13	0.48	0.16
Tyrosine	4.16	4.19	4.16	4.18	4.04	4.20	4.16	4.14	4.16	4.19	0.06	0.65	0.47	0.32

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system; [†]Linear effect for L; [a]Linear effect witin IPF; [b]Linear effect within GPF; [c]Quadratic effect within IPF; [d]Quadratic effect within GPF; [e]No effect within GPF

2.5.8 Pool of carcass muscles AA and chemical composition

The pool of carcass muscles from the right side, without the longissimus dorsi, was affected by the treatments during the growing phase (Table 9). All EAA, with the exception of methionine, were affected ($P < 0.05$) by an interaction between dietary threonine and the feeding system, with a cubic effect in the IPF and the GPF pigs. The NEAA alanine, proline, and serine were affected ($P < 0.05$) by an interaction between dietary threonine and the feeding system, with a cubic effect in the IPF pigs and a quadratic effect in the GPF pigs, whereas asparagine and tyrosine were affected ($P < 0.05$) in a cubic manner in both feeding systems. Cysteine ($P < 0.05$) and glycine (tendency; $P < 0.10$) were higher in the GPF pigs than the IPF pigs, and these AA were not affected by dietary Thr levels. Glutamate, DM, ash, fat, and collagen were not affected by the threonine level, the feeding system or their interaction during the growing phase. However, CP tended ($P < 0.10$) to be 1.5% higher in the GPF pigs than the IPF pigs.

Table 2-9. Carcass muscle amino acid concentrations (without Longissimus dorsi) of growing barrow pigs (25-42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in an individual precision feeding (IPF) or group-phase feeding (GPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	5	3	6	5	5	5	5	5	5	5				
Chemical composition, %														
Dry matter	31.10	30.39	29.84	29.59	29.94	29.37	30.22	30.52	29.84	29.73	0.76	0.82	0.55	0.33
Crude protein	17.40	17.54	17.82	17.78	18.39	18.24	17.93	18.06	17.87	18.18	0.26	0.09†	0.05	0.13
Fat	12.84	12.19	11.46	10.79	11.25	10.88	11.67	10.72	11.05	11.10	1.02	0.64	0.23	0.70
Ash	0.99	1.00	1.02	1.01	1.00	1.01	0.99	1.00	1.00	0.99	0.02	0.91	0.48	0.86
Collagen	1.61	1.60	1.60	1.66	1.63	1.56	1.66	1.73	1.61	1.61	0.08	0.76	0.69	0.41
Essential amino acids, g/100 g of crude protein														
Arginine	7.40	6.63	7.12	7.62	6.99	7.13	7.93	7.63	7.11	7.11	0.29	0.63	0.12	0.01[a,b]
Histidine	4.45	3.68	3.94	4.30	4.09	4.27	4.54	4.28	3.88	3.99	0.23	0.48	0.39	0.02[a,c]
Isoleucine	4.80	4.39	4.74	4.88	4.48	4.72	5.19	4.99	4.68	4.70	0.19	0.48	0.05	0.03[a,b]
Leucine	8.26	7.57	8.10	8.46	7.79	8.12	9.03	8.22	8.10	8.10	0.26	0.61	0.07	0.01[a,b]
Lysine	8.74	8.02	8.58	9.05	8.29	8.64	9.51	9.18	8.59	8.63	0.38	0.67	0.06	0.04[a,c]
Methionine	2.56	2.19	2.28	2.87	2.73	2.24	2.87	2.36	2.66	2.60	0.26	0.15	0.87	0.19
Phenylalanine	4.37	3.95	4.23	4.44	4.10	4.29	4.73	4.53	4.23	4.25	0.18	0.66	0.04	0.02[a,b]
Threonine	4.56	4.20	4.54	4.76	4.38	4.57	5.09	4.56	4.53	4.51	0.17	0.61	0.07	0.01[a,b]
Valine	5.21	4.73	5.09	5.30	4.84	5.12	5.62	5.39	5.09	5.06	0.17	0.44	0.04	0.03[a,b]
Non-essential amino acids, g/100 g of crude protein														
Alanine	6.32	5.74	6.12	6.44	5.91	6.22	6.78	6.61	6.19	6.06	0.26	0.43	0.05	0.04[a,d]
Asparagine	11.27	10.15	10.92	11.30	10.46	10.98	12.08	11.58	10.85	10.81	0.47	0.54	0.07	0.03[a,c]
Cysteine	0.95	0.88	0.93	0.95	0.87	0.96	1.04	1.03	0.91	0.92	0.05	0.33	0.04	0.20
Glutamate	15.37	13.44	15.39	13.52	13.37	14.96	15.28	15.23	14.10	13.60	1.20	0.21	0.49	0.82
Glycine	5.97	5.48	5.80	6.14	5.75	5.97	6.32	6.47	5.98	5.74	0.27	0.44	0.06	0.11
Proline	4.67	4.29	4.56	4.82	4.50	4.66	5.08	5.01	4.65	4.56	0.20	0.55	0.03	0.04[a,d]
Serine	4.11	3.74	4.02	4.21	3.92	4.03	4.50	4.31	4.05	3.97	0.18	0.61	0.07	0.03[a,b]
Tyrosine	4.00	3.63	3.92	4.04	3.74	3.91	4.35	4.12	3.85	3.89	0.16	0.55	0.06	0.02[a,b]

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system; †Linear effect for L; [a]Cubic effect witin IPF; [b]Cubic effect within GPF; [c]Tendency for a cubic effect within GPF; [d]Quadratic effect within GPF

2.6 Discussion

2.6.1 Performance is affected by threonine level

Threonine level did not affect ADFI during this growing phase, a result which is consistent with the literature (Edmonds and Baker, 1987; de Lange et al., 2001; Hamard et al., 2007). The improved G:F ratio is due to the linear ADG increase without changes in the ADFI. Normally pigs fed in the group receive an average of 17% more lysine than pigs receiving daily tailored diets (Andretta et al., 2014). We observed no changes in SID Lys intake during this growing period. This might be due to the fact that we adjusted the SID Lys level of the GPF system to be limiting in 10%, decreased the Lys intake for this feeding system, which made the AA intake similar to the IPF system, where pigs received the exact amount of Lys estimated by the precision feeding model. The SID Thr intake in growing pigs increased linearly, as expected, due to the increase in Thr concentration of the diet.

During the growing phase, the dietary Thr concentration impacted PD linearly; it was not affected by the feeding system. In this study, it was demonstrated that a 30% Thr restriction can result in a loss of PD of at least 12% when compared with the 100% level of SID Thr intake during the growing phase. Our results for increased PD as a function of Thr increase in the diet are in line with those presented by de Lange et al. (2001). As well, a previous study (Andretta et al., 2016) showed that the feeding system does not affect PD or the performance of growing pigs, which is in agreement with the findings of this trial. The percentage of protein in daily gain during the growing phase was not affected even at lower levels of PD. No effect on LipD was observed either. Cloutier et al. (2016) observed a tendency of decrease in the percentage of protein in daily gain but no effect in LipD in the pigs receiving a diet 30% deficient in Lys. A higher backfat thickness and lower lean percentage result from Lys deficient diets (Witte et al., 2000). Normally, when energy levels are adequate in the diet to promote maximum protein deposition, but an AA is deficient, thereby limiting protein synthesis, the energy that is not used for protein synthesis is stored for energy in lipid form (Cia et al., 2010). It is possible that the N excretion metabolism increased energy expenditures, resulting in no accumulation of energy

for fat metabolism. Indeed, pigs receiving lower levels of Thr tended to increase N excretion due to the lower N retention when compared with pigs receiving higher levels of Thr, which is a reflection of PD. However is important to consider that growing pigs have a higher PD in daily gain compared to LD, as observed in this study, and therefore in a short period such as 21 d used in this trial and the variance observed among animals can also play an important role contributing to the lack of LD changes at lower Thr intake levels.

Estimated Thr and Lys efficiencies increased to nearly the limit of AA intake for protein synthesis. The most efficient animals in terms of amino acid utilization generate values over 100% of AA retention. Often values for Thr efficiency of around 91% (Libao-Mercado et al., 2006) to 86% (de Lange et al., 2001) are presented, and Lys efficiency values of 1.07% and 1.01% (Cloutier et al., 2016) when AA intake is below the requirement. Ghimire et al. (2016) observed that Lys efficiency increased at lower levels of available Lys, indicating that pigs were more efficient in utilizing Lys when they were fed below the estimated requirements. The efficiency values presented in AA restriction in this study are higher than the maximum Lys efficiency normally assumed to be 72% and Thr efficiency to be 62% (van Milgen et al., 2008). The variance might be due to metabolic or experimental factors (Möhn et al., 2000b). The increase in Thr and Lys efficiency in limiting situations results in difficulties estimating maintenance requirements generated in low AA intake with pigs that have a low PD as compared to pigs having a higher AA intake and higher PD (de Lange et al., 2001). What is not discussed is that AA efficiency might change depending on the metabolic state, AA availability, BW, and individual metabolism efficiency itself. Normally, a static efficiency value is used because a constant AA concentration in the carcass is assumed, independent of the pig's age and nutrient levels (de Lange et al., 2001). This seems to be an invalid assumption given that protein and energy levels (Bikker et al., 1994), age (Conde-Aguilera et al., 2010), sulfur AA deficiency (Conde-Aguilera et al., 2010; Conde-Aguilera et al., 2016a; Conde-Aguilera et al., 2016b), a Thr deficiency (Hamard et al., 2009) or excess as we show in this study, and genetics (Xue et al., 2016) can change AA composition in carcasses. We hypothesize the most metabolically efficient pigs can use several mechanisms to

cope with lower AA intake such as decrease protein degradation to a minimum, increase AA absorption capacity in the small intestine and use AA from the turnover for protein synthesis, thereby generating higher AA efficiency.

2.6.2 Ratios cannot be used for precision feeding

In this study, the estimated ideal Thr:Lys ratio was 0.65 for the GPF system; this ratio was not clear for pigs fed with daily tailored diets. Ratios based on the ideal protein profile have been assumed to be a practical way to formulate diets for non-ruminants, decreasing the use of crude protein (Emmert and Baker, 1997; Boisen and D'Mello, 2003; van Milgen and Dourmad, 2015). There was a concern whether this constant ratio could be applied to precision feeding, providing pigs with lysine daily tailored. However, the proportional decrease in Thr as Lys requirements decreased seemed to limit the performance of the precision feeding system using a Thr:Lys ratio of 0.65. Our findings pointed to the conclusion that for precision feeding, the independent estimate of amino acid requirements is a more likely solution.

Establishing recommendations for AA requirements can be hampered by the differences between individuals and the availability of dietary nutrients. More than determining an acceptable ratio between AA, it is important to understand the factors which could be the source of variation. In our trials, we observed a significant variance within treatments, independent of the feeding system, a variability which might be associated with animal inter-variability, but also might result from experimental or metabolic factors. In situations where the amino acid intake is not enough to support maximum growth, the animal's growth rate is reduced and the composition of growth changes, which changes the amino acid composition in muscles (Conde-Aguilera et al., 2010). In this case, it is possible there is a change in amino acid metabolism as a function of the diet provided. In other words, the animal does not have a requirement but rather a response to AA intake, thereby generating variance. A recent meta-analysis study about methionine (Remus et al., 2015b) showed a large variation among studies to determine the methionine-to-lysine ratio. This variation between studies has been observed in other amino acid studies on tryptophan (Simongiovanni et al., 2012), valine (Barea et al., 2009a),

isoleucine (Barea et al., 2009b), and leucine (Gloaguen et al., 2012) among several others. Studies using the oxidation technique (Bertolo et al., 2005; Moehn et al., 2005; Elango et al., 2009) have reported a variation in the animals' requirements even when controlling factors such as genetics, weight, sex or sanitary conditions for these factors. The variation could therefore be explained by experimental errors in the measurement and estimation of amino acids, changes in the type of protein synthesized as a function of AA intake, changes in AA efficiency of utilization, and individual basal metabolism differences among other factors. Still, the large variation in the animal response to AA intake that was observed in this and other trials (e,g, Gloaguen et al., 2011; Gloaguen et al., 2012) needs to be understood.

Frequently, growth and ADG, or even the G:F ratio, are used as response parameters in studies on amino acid requirements (Boinsen, 2003). The problem is that these parameters could be affected by several factors, such as feed intake, environmental changes, measurement time, and water consumption. To obtain a correct estimate of AA, the parameter response should be the same as the test parameter (Boinsen, 2003). We studied the influence of the variable response on the estimation of the ideal protein AA ratio, and we found a variation of between 8% using ADG and 15% using the G:F ratio as a variable response replacing PD. This might explain some of the variation between the studies' recommendations. The main determinant of AA requirements in growing pigs is PD (de Lange et al., 2012; de Lange, 2012). Accordingly, the ideal protein AA ratio or level should be established as a function of AA or PD.

2.6.3 Metabolism is affected by feeding system and threonine levels

Normally AST, ALT, CK, and creatinine are consistent variables for identifying hepatic and kidney failure or damage. The biochemical variables studied were within the expected ranges in the growing pigs (Aiello, 2016). Therefore, the plasma enzymatic changes are more likely to be associated with a change in total muscle tissue and metabolism than with hepatic damage. AST was 8% higher in the plasma of the IPF pigs than GPSF pigs, pointing to possible muscles changes. In lower levels of threonine intake in the precision feeding system (IPF70), ALT activity

was increased and urea in plasma was high, suggesting an increase in the transamination of alanine in urea. Meanwhile, in the GPF program, ALT increased in a cubic manner, while urea decreased in a quadratic manner. In both programs, glutamine increased in an inverse quadratic manner to plasmatic urea. There are two amino acids which safely transport ammonia (NH_3-), a residual product of protein degradation, in blood: alanine from the skeletal tissues and glutamine, which helps the cells to get rid of excess ammonia. The other way to transport excess N is through urea: after glutamine carries the NH_4+ to the liver, glutamine is converted into glutamate and NH_4+. The ammonia will get into the urea cycle, resulting in urea. The urea will then be carried out in the blood to be excreted by the kidneys. Thus, higher levels of urea at lower levels of glutamine could point to a lower protein synthesis or higher AA catabolism in Thr restriction and an excess enhancing urea cycle.

C-reactive protein presented within normal values for healthy pigs (Aiello, 2016); however, as Thr in plasma and intake increased, this protein was increased linearly within IPF pigs and in a quadratic manner within GPF. C-reactive protein is a major acute phase protein in pigs, but also binds metabolites released from cellular degradation to be used by the host rather than by the pathogen (Kaneko et al., 2008). This protein is mainly composed of serine (9.62%), but glycine (7.48%) and Thr (6.4%) are also critical components (Oliveira et al., 1979). At higher levels of Thr intake is possible that more CRP was synthetized or at low levels of Thr this protein was degraded to provide Thr, serine and glycine to protein synthesis. It is likely that the linear availability of plasmatic serine, glycine, and Thr favored the synthesis of CRP. The low level of albumin in plasma when Thr was restricted could be pointing to different situations: albumin, which is the primary transport protein in plasma (Gurr et al., 2002), was providing AA for the natural turnover of protein in peripheral tissues or its synthesis was reduced (Kaneko et al., 2008); this protein could be lost through intestinal leakage due to morphological changes caused by a Thr deficiency. The fact that the chemical composition of the small intestine was not affected in this study leads us to think that the intestine tends to be preserved in situations where Thr is marginally deficient in accordance with a previous study (Hamard et al., 2009).

Accordingly, the fit scenario is that albumin synthesis was reduced or that this protein was used for the natural protein turnover. The rate of albumin synthesis is reduced in cases of malnutrition, malabsorption or maldigestion (Moshage et al., 1987). A linear increase of Thr intake and, therefore, favors an increase of albumin synthesis due AA availability.

In general, we observed a linear increase in plasmatic proteins (albumin, total protein and CRP) as the plasmatic Thr increased. This might indicate that at lower levels of Thr intake, the synthesis of plasmatic protein will decrease or these proteins will be used by the metabolism as sources of AA. When AA concentrations in tissue cells decrease, plasma proteins are transported into tissue cells to provide AA and ensure a state of equilibrium (Reece and Swenson, 2005). The use of plasmatic protein to maintain cellular equilibrium or the decrease in synthesis of these proteins might be a possible mechanism to increase AA efficiency of utilization observed at lower levels of AA intake, being one of the mechanisms pigs develop to cope with AA deficiency.

At lower levels of Thr, a higher concentration of Lys and histidine were found in the plasma of pigs in both feeding systems. When an AA is limiting in the diet (in our case Thr), some essential amino acids such as Lys (Hamard et al., 2009) and histidine (Conde-Aguilera et al., 2010) will increase in the plasma, probably due to their low utilization in net protein deposition (le Floc'h et al., 1994). We observed a linear increase in plasmatic glycine and serine in both feeding systems as plasmatic Thr increased. Threonine in pigs is oxidized in the liver and pancreas by the L-threonine 3-dehydrogenase (TDG), resulting in glycine (Le Floc'h et al., 1996). In humans, approximately 41% of serine comes from glycine oxidation (Shemin, 1950); in piglets, this synthesis seems to be limited by intestinal capacity, and the rate of conversion of glycine to serine seems to be lower in young pigs (Wang et al., 2014). Plasma methionine and serine levels were 11% and 7% higher, respectively, in the IPF pigs than in the GPF pigs. This might suggest a higher oxidation of glycine in serine in this feeding system or a higher oxidation of glutamine in serine. The higher plasmatic methionine is likely due to lower methionine retention in the small intestine of the IPF pigs, which was 10% lower than in the GPF pigs.

2.6.4 Splanchnic tissue tended to be preserved over amino acid restriction

Except for asparagine and serine, which tended to be in lower concentrations in the small intestine when there was an excess or deficiency of Thr in the GPF pigs, amino acid or protein concentration in the small intestine and liver were not affected by Thr levels. Other studies on feeding animals in the group testing Thr deficiency (Hamard et al., 2009) or sulfur amino acid deficiency (Conde-Aguilera et al., 2010; Conde-Aguilera et al., 2016a) showed a lower impact or no impact on AA concentration at 30% AA deficiency. This lack of response to AA deficiency in the small intestine has been attributed to the fact that most of the protein retained in the proximal part of the small intestine is coming from the diet (Le Floc'h and Sève, 2005), and the hypothesis is that AA could be first used by splenic tissues (Conde-Aguilera et al., 2016a). We can speculate that splanchnic tissues tend to be preserved due to the AA pathway, which follows the portal vein leading to the liver after AA absorption in the intestine. As well, the liver and intestine are the main sites for AA metabolism in mammals. Accordingly, the metabolism favors these organs over the others, which will receive AA for its use before the skeletal tissues, resulting in smaller AA variation in tissue composition. In the small intestine, pigs in the IPF system tended to retain 10% less methionine; in the liver, Thr concentrations tended to be 1% higher and serine was 2% higher than those in the GPF system. Hamard et al. (2009) found lower serine and Thr concentrations in the liver of Thr-deficient pigs, while no AA were affected in the small intestine. Our hypothesis is that pigs in the precision feeding system develop mechanisms to cope with the AA deficiency. As lower levels of Thr and a tendency toward lower levels of serine were found in the pool of skeletal muscles, it is possible that the organism had tried to retain the missing AA for protein synthesis in the liver to optimize protein synthesis at the moment of AA availability. We have previously observed greater levels of plasma concentration of AST, ALT, and creatinine in the IPF pigs compared with the GPF pigs (Remus et al., 2015a), which may be an indication of a change in the total amount of muscle tissue (Kaneko et al., 2008). In this study, again we found higher levels of AST in the IPF pigs, signaling increased enzymatic activity in the plasma of

these pigs, which could be a sign of protein breakdown for resynthesis in AA deficiency.

2.6.5 Muscles are affected differently by threonine restriction and feeding systems

In both feeding systems, AA concentrations were affected in an inverse cubic way. Conde-Aguilera et al. (2016b) found no effect of sulfur amino acid restriction in CP carcasses when the trial duration was 10 days, while longer periods of restriction affected muscle composition (Conde-Aguilera et al., 2010). Hamard et al. (2009) found no effect on AA concentration in carcasses, except Thr, in animals with a 30% Thr restriction. Our trial lasted 21 d, at least 7 days longer than previous studies (Hamard et al., 2009; Conde-Aguilera et al., 2016b). This could explain why we found an effect on AA concentrations and CP content due to Thr intake changes in our study. The CP of the Longissimus dorsi was not affected by the feeding system, while the pool of muscles in the GPF pigs tended to have 1.5% higher CP than those in the IPF pigs, mainly due to the reduction in the concentration of amino acids in the IPF pigs compared with the GPF pigs. This lower AA concentration signaled that IPF pigs are more affected by Thr restriction than GPF pigs. The requirements of growing pigs change rapidly over the growth period; in this case, animals fed in the group that had a limiting amino acid at the beginning of the trial were not necessarily limited throughout the entire experimental period (van Milgen et al., 2012; van Milgen and Dourmad, 2015). We have demonstrated in simulation when using an NRC model or IPF model to estimate the optimal SID Lys concentration to be served in a 28-day feeding phase on the first day, 69% (NRC) and 24% (IPF) of pigs were underfed. However, in the period overall, only 18% and 2%, respectively, were restricted in the 1,008 pig-day estimates made during the growing period (Remus et al., 2015c). In contrast, the requirements of pigs fed with daily tailored diets are adjusted every day, and amino acid concentration decreases over time (Andretta et al., 2014). Thus, pigs that were restricted in Thr on the first day of the trial were restricted for the entire experimental period. This might explain the high impact of AA restrictions on CP and AA concentrations in the IPF pigs compared with the GPF pigs.

The difference in AA concentration in different tissues, and mainly in different muscles, can be due to growth hormone action; whereas a nutritional restriction can downregulate growth hormone receptors' mRNA in the liver, it will be upregulated in skeletal tissue (Dauncey et al., 1994). More than feed intake and energy balance, other nutrients can regulate growth hormones; in the longissimus dorsi, a Thr deficiency can upregulate this hormone (Dauncey et al., 2001). We did not measure growth hormones in this trial, but we can speculate that the different AA and CP concentrations we found could be due to hormonal changes as well. Collagen has been considered a source of non-essential AA reserves in cases of Thr restriction, which in situations where less threonine is available, proteins that are poorer in this AA, such as collagen, could be deposited (Hamard et al., 2009). Previous studies (Hamard et al., 2009; Conde-Aguilera et al., 2010) found no evidence of collagen changes in the GPF pigs; our results have pointed to changes in collagen formation in the IPF pigs. As we discussed previously, it is possible that collagen synthesis along with increased AA retention in the liver of the deficient AA and increased enzymatic activity are indicators of metabolism changes in pigs inside precision feeding systems, which might develop mechanisms to cope with low AA intake.

2.7 Conclusions

In conclusion, the performance of growing pigs was affected by the Thr:Lys ratio, but not by the feeding system. Muscular and plasmatic proteins were affected by the Thr level decreasing plasmatic proteins and increasing collagen at lower levels of Thr intake, showing the plasticity of growing pigs face to AA deficiency. Crude protein content, collagen and AA concentrations were affected by the Thr:Lys ratio, and the magnitude and type effect was dependent on the feeding system used. Threonine deficiency impaired empty body composition and decreased CP of the IPF pigs compared to GPF pigs. Optimal Thr requirements for PD estimated using non-linear depend on the feeding system, with pigs in an IPF system having different Thr:Lys ratio requirements than those in the more widely used in GPF system. Our results suggest that AA requirements vary with individual pigs and thus may not be accurately estimated based on traditional AA:Lys ratios. Finally, these results expose the fragility and question the utilization of the ideal protein profile when moving from

group-feeding to individual precision feeding due the abilities pigs have to cope with changes in AA intake.

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CHAPTER 3: THE IDEAL PROTEIN PROFILE FOR FINISHING PIGS IN PRECISION FEEDING SYSTEMS AND PHASE FEEDING SYSTEMS: THREONINE

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"Persistence. Never give up and never stop believing that you will and can make a difference. There is no finish line."

(Françoise Barré-Sinoussi, interviewed by Syed Yasin Shahtaz Emanee in the backdrop of the Lindau Nobel Laureate Meetings 2014)

The ideal protein profile for finishing pigs in precision feeding systems: threonine

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3.1 Résumé

Des rapports optimaux d'acides aminés (AA) pour les porcs ont été établis pour les systèmes d'alimentation classique, mais ces rapports peuvent différer pour les systèmes d'alimentation de précision. Notre objectif était d'évaluer la réponse des porcs de finition à différents niveaux de thréonine (Thr; 70, 85, 100, 115 et 130% du ratio Thr: Lys idéal de 0,65) dans un système d'alimentation de groupe en phase classique (GPF) et dans un système d'alimentation de précision individuelle (IPF). Un total de 110 porcs en phase de finition (110 kg de poids corporel initial ± 7,02, 11 porcs par traitement) ont été logés dans la même pièce pendant 21 jours et nourris à l'aide de dispositifs d'alimentation automatiques. Un essai a été réalisé suivant un plan factoriel 2 × 5, l'unité expérimentale était le porc. Cinq porcs par traitement ont été abattus à la fin de l'expérience. Le rapport gain quotidien: consommation alimentaire n'a pas été affecté par le système d'alimentation, mais il y avait un effet quadratique pour le niveau de Thr ($P < 0,05$). L'apport en lysine était plus élevé de 16% et l'apport en Thr était plus élevé de 15% chez les porcs dans le système GPF que chez les porcs dans le système IPF ($P < 0,05$). Le dépôt de protéines dans le gain quotidien a été affecté par l'interaction entre le niveau de Thr et le système d'alimentation avec un effet quadratique pour le système GPF ($P < 0,05$) et un effet cubique pour les porcs dans le système IPF ($P < 0,10$). Les porcs dans le système

IPF ont consommé 14% moins de protéines brutes (P <0,05) et ont excrété 17% moins de N (P <0,05) que les porcs dans le le système GPF. Les porcs dans le système IPF ont retenu 9% plus de N que les porcs dans le système GPF (P <0,05). Les valeurs de l'urée plasmatique étaient 9% plus élevées pour les porcs dans le système GPF (P <0,05) que dans le système IPF. L'enzyme gamma-glutamyl transférase a diminué de manière quadratique dans le système IPF, alors qu'elle a augmenté de manière quadratique dans le système GPF (P <0,05). L'albumine et la protéine C réactif avaient tendance à être respectivement inférieures de 2% et 22% chez les porcs dans le système IPF par rapport à ceux dans le système GPF (P <0,10). Les changements dans la concentration des AA mesurés sont apparu principalement dans le foie des porcs le système IPF. Les porcs avaient des concentrations plus élevées de collagène dans le Longissimus dorsi dans le système IPF que GPF (P <0,05). Les porcs ont réagi différemment aux niveaux de Thr dans le système IPF que GPF, avec les porcs dans le système GPF ayant leur dépôt de protéine maximum (150 g / jour) à un rapport Thr: Lys de 0,85.

3.2 Abstract

Optimal amino acid (AA) ratios for pigs have been established for conventional phase feeding systems, but these ratios may differ for precision feeding systems. Our objective was evaluate the response of finishing pigs to different levels of threonine (Thr; 70, 85, 100, 115 and 130% of the ideal Thr:Lys ratio of 0.65) in a conventional group phase feeding (GPF) system and in an individual precision feeding (IPF) system. A total of 110 pigs in finishing phase (110 kg initial BW ± 7.02; 11 pigs per treatment) housed in the same room for 21 days and fed using automatic feeders were allocated to a 2 × 5 factorial design. Individual pigs were considered the experimental units. Five pigs per treatment were slaughtered at the end of the trial. The gain:feed ratio was not affected by feeding system but there was a quadratic effect of Thr level ($P < 0.05$). Lysine intake was 16% greater and Thr intake was 15% greater for GPF than IPF pigs ($P < 0.05$). Protein deposition in daily gain was affected by the interaction between Thr level and feeding system with a quadratic effect for GPF ($P < 0.05$) and a cubic effect for IPF pigs ($P < 0.10$). Pigs in IPF consumed 14% less crude protein ($P < 0.05$) and excreted 17% less N ($P < 0.05$) than in GPF. Pigs in IPF retained 9% more N than in GPF ($P<0.05$). Plasmatic urea values were 9% higher in GPF ($P < 0.05$) than IPF. The gamma-glutamyl transferase enzyme decreased in a quadratic manner within IPF, whereas it increased in a quadratic manner within GPF ($P < 0.05$). Albumin and C-reactive protein tended to be respectively 2% and 22% lower in IPF than GPF ($P < 0.10$). Changes in AA concentrations occurred mainly in the liver of pigs in IPF. Pigs had higher concentrations of collagen in the longissimus dorsi in IPF than GPF ($P < 0.05$). Pigs responded differently to Thr levels in IPF than GPF, with pigs in GPF having their maximum protein deposition (150 g/d) at a Thr:Lys ratio of 0.85.

Keywords: body composition, splenic tissue composition, lysine, blood plasmatic parameters, amino acid concentration, group phase feeding

3.3 Introduction

The ideal protein profile concept is generally used to formulate feeds by maximizing production and decreasing N excretion into the environment. The ideal protein concept refers to a situation where all the AA are co-limiting at the same time and the AA provided exactly meet the population requirement (van Milgen and Dourmad, 2015). Based on this concept, requirements for threonine (**Thr**) are established as a function of those of lysine (**Lys**), traditionally, the first-limiting amino acid (**AA**) chosen as AA reference. Several Thr:Lys ratios were proposed (Rostagno et al., 2011; NRC, 2012; Gloaguen et al., 2014) but results are controversial (De Lange et al., 2001; Pedersen et al., 2003; Mathai et al., 2016). Requirements for Thr depend on the fibre level in the diet, the sanitary level and the presence of microbes and parasites in the intestinal tract (Bequette, 2003). Animal's age or growing phase has a great impact on the response of pigs to AA intake. The AA ratios, including the Thr:Lys ratio, is not constant (Boisen and D'Mello, 2003) and AA ratios may vary according to the pigs' growth (van Milgen and Dourmad, 2015), lean growth rate, feeding level, and, possibly, diet composition (Moughan, 1999). In addition, the feeding system may also influence AA requirements (Remus et al., 2015a; Remus et al., 2017a; Remus et al., 2017b). Commonly used nutrition programs estimate the population requirements based on a factorial method (i.e., NRC) and provide the same feed to the entire group over an entire feeding phase (e.g. three feeding phases throughout the production cycle). In other words, group-fed pigs receive a constant amount of AA throughout the growing phase (typically over 28 days). In contrast, individually fed pigs receive varying amounts of AA according to their requirements for maintenance and growth in function of body weight (**BW**), average daily gain (**ADG**) and average daily feed intake (**ADFI**). We hypothesized that the optimal Thr:Lys ratio might differ between pigs in an individual precision feeding (**IPF**) system and a group phase feeding (**GPF**) system because animals fed in IPF will receive less Lys and it can result in an increase of Thr:Lys ratio to meet Thr requirements for late-finishing pigs. Furthermore, to our knowledge, the potential impact of feeding systems on protein deposition (**PD**), plasmatic biochemical parameters and tissue compositions of late-finishing pigs fed with different Thr levels

was not reported in literature. Likewise, the potential impact of Thr intake on AA composition in splanchnic tissue and muscles for late-finishing pigs was not reported in literature.

Dietary AA are important precursors for PD in pigs (de Lange et al., 2012). An important factor that can affect PD and, thus, AA requirements is the age of the animals (Boisen and D'Mello, 2003). Knowledge on Thr requirement and its effect on PD for late finishing pigs is limited. Therefore, our aim was to assess the impact of different levels of Thr in the diet (70%, 85%, 100%, 115%, or 130% of the estimated ideal level of the 0.65 Thr:Lys ratio (Sève, 1994)) on the response of finishing pigs, including plasmatic biochemical parameters and tissue composition, in an IPF vs a GPF system.

3.4 Material and methods

3.4.1 Animals, housing and management

A total of 110 barrow pigs of the same high-performance genotype (Fertilis 25 × G-Performer 8.0; Geneticporc Inc., St-Gilbert, QC, Canada) with a good health status were shipped to the swine complex of Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada. Pigs were allocated in two pens of 76 m^2 each on concrete slats floors in the same mechanically ventilated room. Room temperature was adjusted at 22°C at arrival.

Pigs were equipped with an electronic chip in the ear granting them access to the automatic and intelligent precision feeder stations (Automatic and Intelligent Precision Feeder; University of Lleida, Lleida, Spain). The automatic precision feeders identified the specific dietary formula for the respective pig when a feed demand was made, and mixed the appropriate feeds (A1, A2, B1, B2) according to the assigned treatment. To avoid feed waste, a time lag between succeeding feed demands was imposed ranging from 15 seconds at the beginning to 10 seconds at the end of the trial.

Pigs had an initial BW of 110 kg (MSE 7.0) and were assigned randomly to treatments in two complete blocks (pens) according to a 2 × 5 factorial design across

2 feedings systems (IPF vs GPF) and 5 levels of Thr intake (70%, 85%, 100%, 115% and 130% of the estimated ideal Thr:Lys ratio of 0.65; Sève, 1994). The experimental unit was the individual pig, and each treatment included 11 replicates. Each of the two blocks (pens) consisted of 55 pigs and received the experimental treatment with a one-week difference to account for the time required to complete the measurements. Between their arrival and the start of the trial, pigs were fed with commercial feeds adapted to their requirements. Water was provided ad libitum with low-pressure nipple drinkers, and feed was provided individually ad libitum throughout the adaptation (14 days) and experimental periods (21 days) in ten feeding stations.

3.4.2 Feeding programs, nutritional requirements, and diets

Diets were formulated separately for IPF and GPF pigs and requirements for AA, calcium and phosphorus were established separately but diets presented similar net energy concentrations (Table 3-1). Requirements for Lys were simulated based on data from high-performance pigs from previous trials performed in our lab. Requirements for AA other than those of Lys were established using the ideal ratio to Lys proposed by INRA (Gloaguen et al., 2014). Feed formulation was performed based on total AA content corrected to standardized ileal digestible (**SID**) AA content for each ingredient according to digestibility coefficients for each AA as described by INRA-AFZ tables (Sauvant et al., 2004)). Feeds were formulated to contain the same AA profile, which resulted in a small feedstock variation. Feeds were formulated to meet requirements for Lys and AA other than those of Thr in the most demanding pig on day 1 (feeds A1 and A2) and on the least demanding pig on day 21 (last day) of the experimental period (feeds B1 and B2). Feeds A1 and B1 contained 130% and feeds A2 and B2 contained 70% of the optimal Thr level.

The required daily concentration of Lys with IPF were estimated with a mathematical model based on individual feed intake and weekly BW measurements (Hauschild et al., 2012). Based on these input data, the empirical component of the model estimated the expected BW, ADFI and ADG for the day. Based on these three estimated variables, the mechanistic component of the model determined the

optimal concentration of Lys that should be offered the very same day to each individual pig to meet its daily requirements based on a factorial method. Daily SID Lys requirements were determined by adding requirements for maintenance and growth. Daily maintenance requirements for SID Lys were estimated by adding basal endogenous losses (0.313 g Lys/kg of dry matter × ADFI), losses related to desquamation in the digestive tract (0.0045 g Lys/kg$^{0.75}$ × BW$^{0.75}$), and losses related to the basal renewal of body proteins (0.0239 g Lys/kg$^{0.75}$ × BW$^{0.75}$; van Milgen et al., 2008). Daily growth requirements for SID Lys were estimated assuming that 7% of body protein is Lys (Mahan and Shields, 1998) and that the efficiency of Lys retention from digestible dietary Lys is 72% (Möhn et al., 2000a). Weight gain composition expressed as protein was calculated assuming 16% protein in daily gain (de Lange et al., 2003). To calibrate the model, pigs were weighed at arrival and three times during the adaptation period. This approach of estimating nutrient requirements with IPF was described by Hauschild et al. (2012) and Pomar et al. (2015), and validated in three studies (Zhang et al., 2012; Cloutier et al., 2015; Andretta et al., 2016b).

Pigs in the IPF system received daily tailored diets by mixing the 4 available feeds such to meet the daily calculated requirement. Requirements for Lys with GPF were estimated for the entire group assuming that population requirements are those of the 80th percentile pig of the group at the beginning of the experiment (average of 3 first trial days) as described by Hauschild et al. (2010) and Remus et al. (2015c). Supply of Lys was reduced by 10% to ensure that Lys was the second limiting AA, whereas all other AA were provided with at least 10% above estimated levels. Pigs in the GPF system received the same feed through the entire experimental phase by blending feeds A1 and B2 for the respective treatment.

Table 3-1. Ingredients and chemical composition of the experimental feeds (A1, A2, B1, B2)[1]

Item	A1	A2	B1	B2
Ingredients (as-fed basis), g/kg				
Corn	533	538	537	538
Soybean meal (48%)	173	173	-	-
Wheat	150	150	100	100
Canola meal	47	47	-	-
Corn gluten meal + linseed meal [2]	33	33	-	-
Corn starch	-	-	156.3	156.3
Fat	16	16	35	35
Oat hulls	-	-	143	143
Limestone	12	12	8	8
Mono-calcium phosphate	10	10	8	8
Lysine sulfate (70%)	6.70	6.70	2.80	2.80
Salt	5.50	5.50	4.80	4.80
L-threonine	4.50	-	1.20	-
DL-methionine	2.30	2.30	0.20	0.20
L-valine (96.5%)	2.10	2.10	0.20	0.20
Vitamin-mineral premix[3]	2.00	2.00	2.00	2.00
L-tryptophan	1.10	1.10	0.30	0.30
L-isoleucine	0.70	0.70	0.20	0.20
Anti-mold	1.00	1.00	1.00	1.00
Choline chloride (75%)	0.20	0.20	0.20	0.20
Chemical composition, %				
Dry matter	90.85	91.25	92.99	92.67
Crude Fat	6.79	6.74	7.88	8.44
Crude Protein	19.85	19.88	7.5	6.88
ADF	3.87	4.018	6.32	6.51
NDF	8.80	8.63	13.58	14.12
Total calcium	0.72	0.72	0.50	0.49
Total phosphorus	0.64	0.64	0.40	0.40
SID[4] isoleucine	0.67	0.69	0.22	0.21
SID leucine	1.34	1.39	0.64	0.59
SID lysine	1.07	1.07	0.34	0.33
SID methionine	0.53	0.53	0.16	0.14
SID methionine + cysteine	0.72	0.72	0.24	0.20
SID phenylalanine	0.75	0.77	0.28	0.26
SID serine	0.80	0.80	0.30	0.26
SID threonine	0.98	0.58	0.31	0.19
SID valine	0.89	0.89	0.29	0.27
Metabolizable energy[4], kcal/kg	3357	3357	3206	3206
Expected net energy, kcal/kg	3208	3223	3255	3259

[1]Feeds A1 and A2 formulated to meet requirements for lysine and amino acids other than those of threonine in the most demanding pig on day 1; feeds B1 and B2 formulated to meet requirements for lysine and amino acids other than those of threonine in the least demanding pig on day 21 (last day) of the experimental period

[2]Mixture of corn gluten meal and linseed meal (Shur-Gain, St-Hyacinthe, QC, Canada)

[3]Supplied per kilogram of diet (as fed basis): vitamin A, 11,400 IU; vitamin D, 1,140 IU; vitamin E, 35 IU; vitamin K, 2 mg; vitamin B12, 30 µg; niacin, 20 mg; pantothenic acid, 15 mg; pyridoxine, 2 mg; thiamine, 2 mg; cooper, 122 mg; iodine, 0.3 mg; iron, 100 mg; manganese, 63 mg; selenium, 0.3 mg; and zinc, 152 mg

[4]Standardized ileal digestible (SID) and metabolizable energy estimated from analyzed total amino acid and crude energy content in feed and values from INRA-AFZ tables (Sauvant et al., 2004)

3.4.3 Experimental measurements

3.4.3.1 Performance

Animal performance was evaluated through ADFI, ADG, gain:feed ratio (**G:F**), SID Lys intake, SID Thr intake, PD, PD in daily gain, lipid deposition (**LD**) and dressing percentage. Total body fat and lean content were measured by dual-energy x-ray absorptiometry (**DXA**) on days 0 and 21 with a densitometer device (GE Lunar Prodigy Advance, Madison, WI, USA). Pigs were scanned in the prone position using the total body scanning mode (Lunar enCORE Software Version 8.10.027). Anesthesia was induced with sevoflurane (7%) and maintained with isoflurane (5%) during the scans.

3.4.3.2 Blood sampling

Blood samples were collected on day 21. Pigs were fasted for ten hours before blood sampling. Blood samples were collected from the jugular vein and stored in a tube containing either the anticoagulant EDTA for enzymatic and biochemical analysis or sodium heparin for AA analysis. Blood samples were centrifuged at 1000 × g for 15 minutes at 4˚C. The time between final sampling and centrifugation did not exceed one hour, and for this period the samples were stored on ice. For AA analysis, within 30 minutes after centrifugation 20 µl of standard enriched AA was added to samples. All plasma samples were stored at −80˚C until analysis.

3.4.3.3 Organs and muscles sampling

Five pigs per treatment were slaughtered in a commercial slaughterhouse, scalded, scraped and the eviscerated carcass was split longitudinally. The right side of carcass was dissected, and the head and feet were discarded. The longissimus dorsi was separated from the loin cut. The liver and the small intestine (washed and mesentery free) were collected. All samples were sealed in separated vacuum plastic bags and stored at −20˚C for a maximum of two months. The liver and small intestine were ground twice before subsamples were taken for further analyses. The dissected muscles were cut in cubes and mixed. The longissimus dorsi and the pool of all other carcass muscles were ground four times and subsamples were taken for

further analyses. All samples were freeze-dried (method 938.18; AOAC, 1990) and stored at −80°C until analysis.

3.4.3.4 Chemical and biochemical analysis

Two replicates of each sample were analyzed using Association of Official Analytical Chemists (AOAC) standard methods for lyophilization [method 938.18 (AOAC, 1990)] and the protein in the feed, liver, small intestine [Kjeltec 2400; FOSS Tecator, Hillerod, Denmark; method 992.15 (AOAC, 1990)], and lipids [Soxtec 2050 Automated Extraction System; Foss, Höganäs, Sweden; method 991.36; (AOAC, 1990)] were determined. Crude protein, collagen, and fat in the longissimus dorsi and the pool of carcass muscles were estimated by near-infrared transmittance [Method 2007.04 (AOAC, 1990); FOSS FoodScan™ Near- Infrared (NIR) Spectrophotometer]. For all the samples, the dry matter [method 950.46; (AOAC, 1990)] and ash (method 920.153; (AOAC, 1990)) were analyzed. Concentrations of AA in plasma were determinded as suggested by Calder et al., (1999). The AA concentration in the pool of carcass muscles and longissimus dorsi were lyophilised. The samples were hydrolyzed with a solution of HCl 6N-0.1 % Phenol in the digester block at 110ºC for 24h. A mixture of standart isotopes (200µl) were added to the samples. A solution of 100µl of DL-dithiothreitol (15.4 mg/ml of water) was added to the sample which rested for 30 minutes in room temperature. Following the samples were passed through the columns (Ply-prep-Bio-Rad 731-1550) prepared with 0.8 cm (0.4 ml) of resin (Sigma-Aldrich Dowex 50WX8 -200 ion exchange resin). The the colums were rinsed twice with 2 ml of ultra-pure water. Amino acids were recovered adding 2 ml of NH_4OH_2N in the columns. The columns were rinsed with 1 ml of ultra-pure water and let it drain in the vial. Vials were covered with parafilm and vortexed. The samples were frozen at -80ºC and lyophilised. Vials were rinsed with 250 µl of ultra-pure water and transferred to a reacti-vial (Pierce 13221). The the contents of the reacti-vials were dried with nitrogen at 90ºC, for about 20 minutes, and 20 µl de DTT 15.4 mg/ml et 80 µl de NH_4OH_2N were added to the samples. The samples rested for 30 minutes in room temperature and were dried with nitrogen at 90ºC for 20 minutes. Samples were derived with 60 µl of MTBSTFA:DMF 1:1 (MTBSTFA: Aldrich 394882, DMF: Aldrich 27.054-7). Samples were heated

at 90ºC for 35 minutes. Samples were transferred to vials for GC (Agilent 5182-0714). All AA samples were measured by gas chromatography coupled to mass spectrometry (Agilent Technologie 7890B GC System coupled to a Agilent Technologie 5977A MSD).The IgG was determined through ELISA kits (Pig IgG ELISA quantification Set, ref. E100-104, Bethyl Laboratories, Inc.). The biochemical and enzymatic analysis of plasma was performed with an automatic analyzer.by a dedicated external laboratory (Faculté de médecine vétérinaire of the Université de Montréal; Saint-Hyacinthe, QC, Canada)

3.4.3.5 Calculations and statistical analysis

Total ADG was calculated as the difference between BW measured at the beginning and the end of the experimental phase. Intake levels of SID Lys, SID Thr and CP were measured for each pig by tallying the daily amount of nutrients provided by each of the served feeds. Lysine and Thr efficiency were calculated by dividing the corresponding retained by available AA.. Retention of Lys and Thr was estimated assuming that 7% of body protein is Lys, and 3.7% of body protein is Thr (van Milgen et al., 2008). Availability of Lys and Thr was estimated by subtracting the amount used for maintenance from the SID pool. Maintenance requirements for Lys were estimated as described above (section 3.2.2). Requirements for Thr were estimated by adding the basal endogenous losses (0.33 g Thr/kg of dry matter × ADFI), losses related to desquamation in the digestive tract (0.0138 g Thr/kg$^{0.75}$ × BW$^{0.75}$), and losses related to the basal renewal of body proteins (0.0033 g Thr/kg$^{0.75}$ × BW$^{0.75}$; van Milgen et al., 2008). The DXA body lean and fat mass were converted to their respective protein and lipid chemical equivalents as proposed by Pomar and Rivest (1996). Protein deposition in daily gain was calculated dividing PD by ADG. Nitrogen excretion was determined by subtracting the amount of nutrient retained from the respective nutrient intake.

Performance and carcass data were analyzed as a 2 × 5 factorial arrangement of treatments using mixed model procedures of SAS (version 9.4; SAS Institue Inc., Cary, NC, USA). The main effects included feeding system, Thr level, and their interaction; the random effect was the block (pen). Assumptions for normal

distribution were checked using the Cramer-von Mises test within the Univariate procedure of SAS version 9.4. The uncertainty in the estimate of the mean was expressed as the maximum standard error (**MSE**). Differences between individual treatments were analyzed by orthogonal contrasts. The ideal Thr:Lys ratio was estimated using NLIN procedures of SAS version 9.4. Significance was declared at $P \leq 0.05$ and a tendency was considered for P values ranging between 0.05 and 0.10.

3.5 Results

Four pigs were eliminated from the trial during the adaptation period due to foot problems. All pigs were in good health, except for one pig with low feed intake and elevated levels of CRP and IgG which was excluded from data analysis. Another pig suffered a heart attack the day before slaughter. However, these incidents were not related to the dietary treatments imposed. Pigs had, on average, a higher than expected ADFI (+34%), ADG (+40%) and PD (+11%), with values above the expected performance for this genetic line (113-135 kg BW).

3.5.1 Feed composition

Feed samples were analyzed for CP content and pellet quality before the onset of the experimental phase but information on the AA content was not yet available. Therefore, diets were provided according to expected SID AA levels. The analyzed SID Lys and Thr values were on average 8% and 10% higher than the expected values, respectively (Table 3-1), in particular for diets B1 and B2 (+2.2%, +2.4%, +16.7%, +12.6% Lys for diets A1, A2, B1 and B2, respectively; and +1.3%, +10.0%, +11.8%, +17.5% Thr for diets A1, A2, B1 and B2, respectively).

3.5.2 Performance, nutrient intake, and nitrogen balance

During the finishing phase, ADFI increased in a quadratic manner as dietary Thr increased ($P < 0.05$) independent of the feeding system (Table 3-2). Average daily gain did not change with dietary Thr levels or feeding system. The G:F ratio decreased in a quadratic manner ($P < 0.05$) as Thr levels increased independent of the feeding system. Intake of SID Lys and SID Thr was, respectively, 16% and 15%

greater (P <0.05) to GPF than IPF pigs. Intake of SID Thr increased in a linear manner (P <0.05) as dietary Thr levels increased. Dressing percentage was not affected by dietary Thr levels or feeding system. Protein deposition tended (P <0.10) to increase in an inverse quadratic manner within GPF but PD in IPF was not affected (interaction between dietary Thr level and feeding system). Protein deposition was 7% greater (P <0.05) with GPF than IPF. Protein deposition in daily gain decreased in a quadratic manner with GPF and tended to increase in a cubic manner with IPF (interaction between dietary Thr level and feeding system). Intake of CP intake and N excretion tended (P <0.10) to increase in a quadratic manner as dietary Thr in the diet increased, and were, respectively, 14% and 17% greater (P <0.05) with GPF than IPF, respectively. Efficiency of N retention was 9% greater with IPF than GPF and decreased (P <0.05) in a quadratic manner with GPF and in a cubic manner with IPF as Thr in the diet increased (interaction between dietary Thr level and feeding system).

Table 3-2. Initial and final animal body composition and growth performance finishing barrow pigs (110-130 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in conventional group phase-feeding system (GPF) or individually using precision feeding (IPF) techniques

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	11	11	11	10	11	10	10	10	10	10				
Initial conditions														
Initial body weight, kg	110.2	108.7	110.2	110.4	110.3	108.0	110.1	109.7	110.5	108.8	3.05	0.99	0.78	0.98
Body protein, kg	15.9	15.4	15.7	15.9	15.7	15.1	15.6	15.5	15.7	15.7	0.47	0.30	0.30	0.69
Body lipids, kg	13.9	14.5	14.8	13.5	13.8	14.0	14.7	14.5	13.7	14.0	1.35	0.75	0.88	1.00
Final conditions, growth performance and nutrient efficiency (0 to 21 d)														
Final body weight, kg	134.1	131.9	132.7	134.1	133.6	131.5	134.7	132.2	134.6	134.0	3.12	0.97	0.95	0.94
Body protein, kg	19.3	19.0	19.2	19.4	19.2	18.8	19.2	18.8	19.4	19.6	0.48	0.65	0.87	0.62
Body lipids, kg	24.0	23.3	23.4	23.4	23.5	22.2	24.9	23.8	23.5	22.1	1.95	0.89	0.81	0.73
Dressing, %	82.90	83.08	83.26	82.66	83.21	83.80	84.03	86.99	82.15	84.05	1.30	0.25	0.11	0.48
ADFI, kg/d	3.69	3.52	3.61	3.86	3.38	3.41	3.79	3.66	3.78	3.56	0.11	0.04‡	0.71	0.13
ADG, kg/d	1.14	1.10	1.07	1.13	1.11	1.12	1.17	1.07	1.15	1.20	0.05	0.38	0.28	0.71
G:F, kg/kg	0.31	0.32	0.30	0.29	0.32	0.33	0.31	0.29	0.30	0.34	0.01	0.01‡	0.25	0.67
SID lysine intake, g/d	20.67	20.02	19.80	20.84	19.95	22.66	24.98	24.70	25.99	23.41	0.92	0.26	<0.001	0.30
SID threonine intake, g/d	11.62	13.06	14.63	17.17	17.99	12.57	16.05	18.18	21.30	21.34	0.69	<0.001†	<0.001	0.15
Lipid deposition, g/d	563.0	499.8	487.4	552.1	519.9	490.4	564.8	529.1	525.1	525.8	38.61	0.82	0.88	0.08[c]
Protein deposition, g/d	117.5	126.0	123.9	120.0	120.8	129.4	126.4	118.3	126.9	151.2	14.21	0.14	0.03	0.05[a]
Protein deposition in daily gain,%	10.31	11.42	11.66	10.47	10.99	11.70	10.78	10.70	11.13	12.37	0.91	0.32	0.17	0.01[b,c]
Nitrogen balance														
Crude protein intake, g/d	397.8	384.8	395.7	429.5	398.1	433.4	481.5	477.2	488.2	463.5	16.02	0.07‡	<0.001	0.29
Efficiency of nitrogen retention, %	29.70	32.98	31.75	27.78	30.86	30.32	26.43	24.88	27.91	31.10	2.88	0.29	0.02	0.03[b,c]
Nitrogen excretion, g/d	44.79	41.36	44.39	49.52	44.30	48.64	56.81	57.36	56.42	51.01	2.43	0.05‡	<0.001	0.07[b,c]

[1]MSE: maximum standard error

[1]L: level of threonine; FS: feeding system; L×F: interaction between level of threonine and feeding system; †Linear effect for L; ‡Quadratic effect for L; [a]Tendency to quadratic effect within GPF; [b]Quadratic effect within GPF; [c]Cubic effect within IPF

3.5.3 Plasmatic parameters

Plasmatic urea values during the finishing phase was 9% greater ($P < 0.05$) with GPF than IPF (Table 3-3). With increased Thr in the diet, plasmatic urea values tended ($P < 0.10$) to decrease in a quadratic manner with IPF (tendency for an interaction between dietary Thr level and feeding system). Albumin and C-reactive protein in plasma tended to be, respectively, 2% and 22% smaller within IPF than GPF ($P < 0.10$). Globulin in plasma increased in a quadratic manner ($P < 0.05$) within GPF and in a cubic manner within IPF (interaction between dietary Thr level and feeding system).

Concentrations of Thr in plasma increased linearly ($P < 0.05$) with increased Thr in the diet, and were 8% greater with GPF than IPF (Table 3-4). Concentrations of valine, an essential AA, in plasma were 6% greater within GPF than IPF ($P < 0.05$) but were not affected by Thr level in the diet. Concentrations of phenylalanine, an essential AA, in plasma tended to linearly increase ($P < 0.05$) within IPF as Thr in the diet increased but were unaffected within GPF (interaction between dietary Thr level and feeding system). Concentrations of glycine, a non-essential AA, in plasma were 8% greater ($P < 0.05$) for IPF than GPF pigs but were not affected by Thr level in the diet. Other essential or non-essential AA in plasma were not affected by Thr level in the diet or the feeding system.

Table 3-3. Blood biochemical plasmatic in finishing barrow pigs (110-135 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in conventional group phase-feeding system (GPF) or individually using precision feeding (IPF)

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	11	11	11	10	11	10	10	10	10	10				
Urea, µmol/L	3.88	3.35	3.00	3.47	3.69	3.70	3.64	4.10	3.97	3.75	0.24	0.65	0.02	0.06[a]
Gamma-glutamyl transferase, U/L	45.67	43.13	38.00	36.90	47.80	33.60	40.06	48.25	43.95	41.70	4.15	0.66	0.74	0.02[a,b]
Albumin, g/L	39.90	40.28	39.93	40.43	40.47	42.00	40.77	39.99	41.39	40.15	0.62	0.41	0.08	0.30
Globulin, g/L	31.14	30.31	31.65	34.07	32.01	29.44	32.99	33.21	31.68	30.85	1.07	0.07[†]	0.74	0.04[b,c]
Creatinine, µmol/L	158.55	160.55	157.84	156.85	162.16	160.50	155.55	161.43	160.45	168.16	4.60	0.43	0.43	0.69
Lactic acid dehydrogenase, U/L	507.31	482.05	464.96	471.63	475.00	468.70	479.30	488.70	448.98	489.70	18.56	0.55	0.63	0.32
Total protein, g/L	71.47	72.67	71.56	74.48	71.99	72.78	74.46	73.60	72.99	70.64	1.14	0.13	0.49	0.25
Aspartate aminotransferase, U/L	28.30	27.69	29.44	26.81	30.05	28.26	29.26	26.76	26.44	29.20	1.53	0.36	0.60	0.67
Alanine aminotransferase, U/L	42.39	43.15	45.65	47.10	46.60	44.00	44.44	44.29	43.30	48.40	2.33	0.25	0.94	0.56
Creatine kinase, U/L	1110	1058	1092	1170	1074	987.0	1282	1372	909.1	1272	154.8	0.57	0.47	0.20
IgG, µg/ml	14.19	16.91	14.07	16.90	14.67	17.08	15.74	15.26	14.67	14.90	1.15	0.54	0.80	0.23
C-reactive protein, µg/ml	6.73	7.66	6.24	5.35	8.14	7.17	13.38	7.04	8.96	7.17	1.55	0.05[‡]	0.02	0.11

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L×F: interaction between level of threonine and feeding system; [†]Quadratic effect for L; [‡]Cubic effect for L; [a]Quadratic effect within IPF; [b]Quadratic effect within GPF; [c]Cubic effect within IPF;

Table 3-4. Plasmatic amino acid concentration in finishing barrow pigs (110-135 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in conventional group phase-feeding system (GPF) or individually using precision feeding (IPF)

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	11	11	11	10	11	10	10	10	10	10				
Essential amino acids, µmol/L														
Arginine	238.07	241.08	252.27	236.42	238.88	236.47	234.90	257.82	239.97	262.77	18.52	0.60	0.58	0.87
Histidine	78.27	83.26	81.90	90.45	81.22	81.98	83.98	88.99	83.88	95.01	4.51	0.42	0.19	0.24
Isoleucine	89.65	83.41	93.69	94.16	100.31	101.57	97.89	98.41	93.93	95.67	4.97	0.66	0.10	0.27
Leucine	174.83	181.00	190.12	183.55	198.18	201.55	191.80	192.96	188.08	188.37	8.50	0.78	0.11	0.13
Lysine	178.89	165.38	193.50	198.36	184.77	195.80	172.75	199.00	177.07	195.59	14.12	0.22	0.61	0.56
Methionine	59.40	54.97	55.23	52.36	58.20	57.40	60.20	54.99	53.85	57.72	4.40	0.68	0.76	0.93
Phenylalanine	66.05	64.06	69.22	73.74	71.15	72.06	71.48	70.79	67.90	74.48	2.49	0.33	0.11	0.08[a]
Threonine	145.33	159.97	185.51	211.27	233.32	137.94	190.31	211.46	219.79	254.04	15.39	<0.001[†]	0.04	0.53
Tryptophan	66.33	66.76	65.09	68.39	67.72	67.22	67.93	67.05	65.33	70.03	3.07	0.84	0.68	0.83
Valine	288.13	284.57	303.12	296.60	314.37	324.09	315.82	322.54	305.87	311.50	14.81	0.74	0.02	0.51
Non-essential amino acids, µmol/L														
Acid Aspartic	7.08	6.07	6.74	6.76	6.32	7.05	6.56	6.83	5.71	7.38	0.82	0.82	0.83	0.77
Alanine	232.49	268.09	260.21	237.90	278.54	267.30	301.30	253.81	262.05	255.72	19.70	0.16	0.22	0.27
Asparagine	39.26	33.26	36.90	35.96	34.65	38.81	36.91	40.03	41.19	39.38	5.02	0.87	0.21	0.96
Cysteine	254.24	243.62	244.59	261.41	266.38	265.49	264.25	263.11	243.27	268.32	11.01	0.63	0.33	0.40
Glutamate	93.53	109.30	107.38	95.33	100.94	104.72	98.28	106.67	96.29	120.27	10.81	0.48	0.49	0.50
Glutamine	430.32	452.54	457.10	434.24	434.92	449.81	444.54	427.27	453.21	469.02	15.53	0.94	0.48	0.24
Glycine	1024.05	1040.74	1078.14	1033.66	1006.93	977.74	896.53	912.24	1020.54	937.30	73.52	0.83	0.01	0.60
Homocysteine	97.77	88.97	97.01	83.28	82.88	84.32	90.80	88.60	75.42	95.31	15.40	0.80	0.67	0.81
Proline	158.38	180.59	168.45	163.00	175.65	184.26	187.76	168.47	173.90	173.54	10.99	0.32	0.12	0.49
Serine	98.35	112.46	99.95	98.54	100.55	99.12	101.97	93.57	103.11	95.30	8.58	0.50	0.41	0.78
Tyrosine	85.30	87.85	91.15	94.67	86.59	89.30	97.95	90.68	86.80	90.69	4.65	0.64	0.42	0.21

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L×F: interaction between level of threonine and feeding system; [†]Linear effect for L; [a]Linear effect within IPF

3.5.4 Liver amino acid composition

Arginine and histidine in liver were affected by dietary Thr concentration in a cubic manner ($P < 0.05$) in both feeding systems (Table 3-5). Lysine in liver was 1% greater ($P < 0.05$) with IPF than GPF, and increased linearly within IPF as dietary Thr increased ($P < 0.05$). Valine in liver increased ($P < 0.05$) in a quadratic manner as Thr levels increased in the diet within IPF and presented a tendency ($P < 0.10$) to increase in a quadratic manner within GPD. Glutamate, a non-essential AA, in liver decreased within IPF in a quadratic manner as Thr levels in the diet increased ($P < 0.05$) and tended ($P < 0.10$) to be 3% smaller with IPF than GPF. Glycine in liver tended ($P < 0.10$) to be affected by Thr level in the diet in a fourth-degree manner within IPF (interaction between dietary Thr level and feeding system). Serine in liver increased ($P < 0.05$) in a linear manner within IPF as Thr levels in the diet increased. Tyrosine in liver increased ($P < 0.05$) in a quadratic manner as Thr levels in the diet increased independent of the feeding system. Chemical composition (dry matter, CP, fat and ash in liver) was not affected by Thr level in the diet or the feeding system.

Table 3-5. Liver amino acid concentrations in finishing barrow pigs (110-135 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in conventional group phase-feeding system (GPF) or individually using precision feeding (IPF)

Parameter	IPF					GPF					MSE[1]	P-value[2]		
	70	85	100	115	130	70	85	100	115	130		L	FS	L × FS
Number of observations	5	5	5	5	5	5	5	5	5	5				
Chemical composition, %														
Dry matter	29.35	29.27	29.10	29.08	29.72	28.68	29.27	28.81	29.25	29.06	0.37	0.70	0.19	0.66
Crude protein	22.03	21.58	21.91	21.86	22.24	21.73	21.60	21.96	21.63	21.66	0.25	0.51	0.17	0.64
Fat	6.16	5.44	5.89	5.61	5.86	5.41	5.83	5.35	5.14	5.65	0.36	0.75	0.13	0.45
Ash	1.54	1.50	1.52	1.45	1.49	1.49	1.61	1.47	1.45	1.47	0.04	0.10	0.79	0.19
Essential amino acids, g/100 g of crude protein														
Arginine	6.36	6.35	6.38	6.43	6.34	6.38	6.33	6.32	6.44	6.31	0.04	0.02‡	0.52	0.75
Histidine	3.03	3.22	3.09	3.02	3.05	2.87	3.10	3.15	3.06	3.04	0.06	0.00‡	0.23	0.16
Isoleucine	4.22	4.28	4.29	4.29	4.19	4.21	4.25	4.27	4.27	4.30	0.04	0.36	0.76	0.27
Leucine	8.63	8.85	8.75	8.78	8.84	8.75	8.73	8.77	8.82	8.76	0.05	0.12	0.81	0.10
Lysine	7.05	7.17	7.18	7.17	7.24	7.13	7.11	7.15	7.14	7.05	0.03	0.18	0.03	0.00[a]
Methionine	1.43	1.51	1.69	1.38	1.52	1.63	1.80	1.37	1.93	1.48	0.16	0.78	0.17	0.09[b]
Phenylalanine	5.03	5.14	5.12	5.08	5.12	5.05	5.11	5.12	5.20	5.12	0.04	0.13	0.38	0.38
Threonine	4.23	4.28	4.28	4.29	4.31	4.27	4.27	4.28	4.28	4.26	0.02	0.55	0.72	0.43
Valine	5.41	5.64	5.58	5.65	5.57	5.54	5.54	5.59	5.60	5.61	0.04	0.01†	0.73	0.05[c,d]
Non-essential amino acids, g/100 g of crude protein														
Alanine	5.44	5.59	5.54	5.58	5.57	5.54	5.54	5.50	5.53	5.53	0.04	0.15	0.49	0.15
Asparagine	10.16	10.09	10.08	10.11	10.14	10.03	10.04	10.20	10.30	10.22	0.10	0.50	0.44	0.34
Cysteine	1.23	1.29	1.28	1.28	1.39	1.36	1.27	1.30	1.29	1.33	0.05	0.43	0.59	0.28
Glutamate	11.71	10.66	9.67	11.90	11.57	11.50	10.65	11.82	11.65	11.79	0.37	0.00†	0.09	0.01[c]
Glycine	5.44	5.61	5.50	5.53	5.49	5.52	5.46	5.48	5.41	5.47	0.05	0.66	0.11	0.09[e]
Proline	4.57	4.63	4.63	4.64	4.65	4.60	4.59	4.60	4.65	4.59	0.03	0.31	0.22	0.38
Serine	4.33	4.36	4.37	4.36	4.48	4.33	4.36	4.34	4.42	4.32	0.03	0.14	0.16	0.01[a]
Tyrosine	3.94	4.00	3.99	4.00	3.99	3.90	3.98	3.98	4.03	3.96	0.03	0.01†	0.45	0.59

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L×F: interaction between level of threonine and feeding system; †Quadratic effect for L; ‡Cubic effect for L; [a]Linear effect within IPF; [b]Tendency of fourth degree within GPF; [c]Quadratic effect within IPF; [d]Tendency of quadratic effect within GPF; [e]Tendency of fourth-degree effect within IPF

3.5.5 Amino acid composition of the small intestine, longissimus dorsi and pool of carcass muscles

Crude protein in the small intestine tended ($P < 0.10$) to be affected by Thr level in the diet in a fourth-degree manner but other AA concentrations and composition (dry matter, fat and ash) in the small intestine were not affected by Thr level in the diet or the feeding system (Table 3-6). Concentrations of proline increased in a quadratic manner ($P < 0.05$) while phenylalanine and asparagine concentrations tended ($P < 0.10$) to increase in a quadratic manner in the longissimus dorsi as dietary Thr concentration increased in both feeding systems (Table 3-7). Dry matter and collagen in the longissimus dorsi were, respectively, 2% and 9% greater ($P < 0.05$) with IPF than GPF but were not affected by Thr level in the diet. Tyrosine in the pool of carcass muscles tended to increase ($P < 0.10$) with increased Thr levels in the diet in a quadratic manner independent of the feeding program (Table 3-8). Crude protein in the pool of carcass muscles decreased ($P < 0.05$) with increased Thr levels in the diet in a quadratic manner independent of the feeding system. Concentrations of AA and composition (dry matter, fat, ash and collagen) in the pool of carcass muscles were not affected by Thr level in the diet or the feeding system.

Table 3-6. Intestinal amino acid concentrations in finishing barrow pigs (110-135 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in conventional group phase-feeding system (GPF) or individually using precision feeding (IPF)

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	5	5	5	5	5	5	5	5	5	5				
Chemical composition, %														
Dry matter	19.59	19.55	20.13	19.46	20.08	20.03	20.49	19.40	20.11	20.39	0.38	0.68	0.19	0.24
Crude protein	13.66	13.86	14.08	12.89	13.45	13.74	13.34	14.14	13.76	13.35	0.29	0.08†	0.68	0.23
Fat	6.16	5.44	5.88	5.61	5.86	5.41	5.83	5.36	5.57	6.53	0.44	0.63	0.86	0.46
Ash	1.55	1.50	1.52	1.45	1.49	1.49	1.56	1.47	1.50	1.47	0.04	0.61	0.84	0.43
Essential amino acids, g/100 g of crude protein														
Arginine	7.69	7.87	7.45	7.84	7.90	7.91	7.58	7.49	7.86	7.62	0.19	0.29	0.62	0.58
Histidine	2.50	2.60	2.46	2.57	2.60	2.58	2.54	2.50	2.57	2.51	0.06	0.52	0.86	0.60
Isoleucine	3.88	3.96	3.79	3.99	4.00	3.95	3.98	3.89	3.98	3.93	0.09	0.52	0.73	0.88
Leucine	7.63	7.87	7.45	7.77	7.91	7.73	7.76	7.56	7.81	7.68	0.18	0.41	0.88	0.85
Lysine	6.98	7.12	6.69	7.11	7.23	7.05	7.00	6.79	7.09	7.03	0.21	0.37	0.79	0.95
Methionine	1.50	1.50	1.25	1.62	1.37	1.23	1.60	1.64	1.64	1.60	0.15	0.44	0.30	0.22
Phenylalanine	4.20	4.35	4.11	4.30	4.35	4.28	4.28	4.16	4.28	4.22	0.11	0.44	0.79	0.84
Threonine	4.28	4.51	4.24	4.41	4.48	4.36	4.38	4.29	4.42	4.33	0.10	0.39	0.69	0.73
Valine	4.86	4.96	4.73	4.99	5.00	4.92	4.93	4.83	4.96	4.87	0.11	0.47	0.96	0.85
Non-essential amino acids, g/100 g of crude protein														
Alanine	5.89	6.05	5.79	6.10	6.09	6.07	5.85	5.88	6.06	5.85	0.14	0.47	0.64	0.45
Asparagine	10.20	10.33	9.61	10.33	10.34	10.28	10.03	9.91	10.27	10.19	0.22	0.11	0.85	0.71
Cysteine	1.14	1.18	1.14	1.20	1.15	1.11	1.22	1.20	1.24	1.22	0.04	0.20	0.15	0.78
Glutamate	13.82	13.76	13.10	13.86	13.60	13.49	13.38	13.42	14.19	14.00	0.41	0.44	0.79	0.78
Glycine	7.85	7.81	7.54	8.22	7.90	8.10	7.40	7.76	8.01	7.60	0.21	0.10	0.51	0.41
Proline	5.56	5.65	5.42	5.83	5.71	5.76	5.43	5.53	5.75	5.51	0.13	0.16	0.62	0.42
Serine	4.48	4.65	4.36	4.58	4.64	4.56	4.39	4.38	4.54	4.47	0.10	0.32	0.25	0.45
Tyrosine	3.87	4.02	3.79	3.96	4.01	3.95	3.95	3.87	3.95	3.90	0.09	0.47	0.93	0.76

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L×F: interaction between level of threonine and feeding system; † Tendency of fourth-degree effect for L

Table 3-7. Longissimus dorsi amino acid concentrations in finishing barrow pigs (110-135 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in conventional group phase-feeding system (GPF) or individually using precision feeding (IPF)

Parameter	IPF					GPF					MSE[1]	P-value[2]		
	70	85	100	115	130	70	85	100	115	130		L	FS	L × FS
Number of observations	5	5	5	5	5	5	5	5	5	5				
Chemical composition, %														
Dry matter	27.00	26.98	27.17	27.42	27.40	26.55	26.84	26.71	26.83	27.12	0.34	0.45	0.03	0.92
Crude protein	23.34	23.60	23.25	23.33	22.88	23.49	23.25	23.35	22.97	23.62	0.23	0.64	0.70	0.07
Fat	2.49	2.22	2.49	2.80	2.28	1.81	2.40	2.24	2.56	2.65	0.34	0.53	0.53	0.50
Ash	4.14	4.29	4.20	4.21	4.07	4.38	4.17	4.13	4.23	4.18	0.13	0.85	0.66	0.68
Collagen	0.57	0.48	0.58	0.60	0.62	0.52	0.49	0.55	0.55	0.49	0.04	0.13	0.03	0.51
Essential amino acids, g/100 g of crude protein														
Arginine	7.12	7.16	7.53	7.15	6.91	7.18	7.28	7.10	7.18	6.97	0.17	0.21	0.73	0.45
Histidine	5.45	5.87	5.81	5.55	5.36	5.40	5.62	5.48	5.69	5.49	0.16	0.21	0.73	0.45
Isoleucine	4.95	5.09	5.28	5.00	4.93	4.87	5.13	4.99	5.03	4.92	0.12	0.13	0.35	0.54
Leucine	8.42	8.56	8.74	8.38	8.26	8.34	8.54	8.47	8.47	8.29	0.17	0.24	0.61	0.83
Lysine	9.42	9.71	9.85	9.39	9.33	9.38	9.86	9.50	9.50	9.43	0.21	0.62	0.83	0.95
Methionine	3.44	3.50	3.57	3.20	3.45	3.76	3.75	3.35	3.50	3.17	0.08	0.71	0.67	0.68
Phenylalanine	4.46	4.54	4.63	4.46	4.37	4.39	4.64	4.45	4.53	4.38	0.09	0.07‡	0.79	0.45
Threonine	4.77	4.82	4.76	4.70	4.61	4.73	4.87	4.81	4.76	4.66	0.10	0.22	0.54	0.98
Valine	5.22	5.36	5.56	5.27	5.19	5.13	5.41	5.25	5.33	5.18	0.12	0.13	0.40	0.49
Non-essential amino acids, g/100 g of crude protein														
Alanine	5.85	5.92	5.93	5.81	5.72	5.77	5.92	5.89	5.87	5.73	0.04	0.45	0.51	0.94
Asparagine	12.00	12.16	12.42	11.94	11.72	11.92	12.43	12.07	12.05	11.81	0.22	0.09‡	0.97	0.65
Cysteine	0.81	0.86	0.85	0.84	0.78	0.75	0.80	0.80	0.84	0.79	0.03	0.14	0.07	0.64
Glutamate	17.07	17.37	16.92	17.34	16.58	16.78	17.18	17.11	17.12	16.80	0.45	0.62	0.83	0.95
Glycine	4.43	4.49	4.52	4.46	4.38	4.39	4.51	4.54	4.50	4.37	0.09	0.44	0.93	1.00
Proline	3.86	3.99	4.02	3.87	3.79	3.83	4.01	3.93	3.90	3.81	0.07	0.03†	0.78	0.90
Serine	4.00	4.07	4.01	3.99	3.90	4.02	4.03	4.09	4.03	3.96	0.08	0.42	0.36	0.87
Tyrosine	4.09	4.14	4.23	4.13	4.00	4.02	4.16	4.09	4.10	4.01	0.08	0.23	0.36	0.83

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L×F: interaction between level of threonine and feeding system; †Quadratic effect for L; ‡Tendency for a quadratic effect for L

Table 3-8. Carcass muscle amino acid concentrations (without Longissimus dorsi) in finishing barrow pigs (110-135 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in conventional group phase-feeding system (GPF) or individually using precision feeding (IPF)

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	5	5	5	5	5	5	5	5	5	5				
Chemical composition, %														
Dry matter	32.51	32.90	33.14	32.97	32.66	32.14	33.14	32.76	32.92	32.41	0.44	0.42	0.57	0.95
Crude protein	19.00	18.86	18.50	18.84	18.86	19.31	18.59	18.86	18.90	19.12	0.17	0.02†	0.15	0.25
Fat	12.85	13.02	13.64	13.34	13.07	12.01	13.79	13.14	13.24	12.64	0.63	0.47	0.59	0.75
Ash	3.10	2.99	2.89	3.00	2.92	3.00	2.85	2.99	2.99	3.06	0.07	0.43	0.99	0.22
Collagen	1.56	1.65	1.52	1.66	1.63	1.64	1.63	1.62	1.59	1.56	0.05	0.54	0.95	0.21
Essential amino acids, g/100 g of crude protein														
Arginine	6.92	6.71	6.69	6.80	6.79	6.94	6.66	6.85	6.76	6.86	0.11	0.24	0.60	0.83
Histidine	4.33	4.49	4.32	4.51	4.40	4.44	4.38	4.43	4.49	4.56	0.10	0.40	0.33	0.39
Isoleucine	4.42	4.48	4.48	4.47	4.45	4.37	4.45	4.50	4.47	4.51	0.07	0.62	0.96	0.92
Leucine	7.67	7.71	7.75	7.72	7.67	7.61	7.66	7.76	7.70	7.77	0.10	0.67	0.95	0.84
Lysine	8.38	8.51	8.55	8.46	8.41	8.38	8.41	8.63	8.45	8.51	0.11	0.31	0.81	0.81
Methionine	2.48	2.60	2.49	2.35	2.88	2.59	2.83	2.41	2.77	2.77	0.20	0.19	0.27	0.45
Phenylalanine	4.08	4.11	4.14	4.12	4.08	4.08	4.09	4.14	4.11	4.15	0.06	0.82	0.81	0.83
Threonine	4.31	4.28	4.37	4.31	4.31	4.27	4.28	4.34	4.31	4.37	0.06	0.51	0.92	0.84
Valine	4.71	4.84	4.83	4.83	4.82	4.81	4.82	4.85	4.85	4.86	0.07	0.75	0.42	0.93
Non-essential amino acids, g/100 g of crude protein														
Alanine	5.69	5.68	5.69	5.71	5.69	5.73	5.66	5.73	5.77	5.72	0.09	0.90	0.49	0.98
Asparagine	10.82	10.91	11.06	10.95	10.82	10.78	10.82	11.06	10.91	11.05	0.13	0.21	0.89	0.60
Cysteine	0.87	0.85	0.88	0.85	0.80	0.87	0.78	0.89	0.83	0.84	0.03	0.10	0.67	0.34
Glutamate	15.25	15.47	15.68	15.37	15.50	15.35	15.48	15.73	15.69	15.55	0.24	0.42	0.40	0.92
Glycine	5.07	5.16	5.05	5.09	5.31	5.29	5.01	5.23	5.25	5.14	0.14	0.81	0.53	0.28
Proline	4.19	4.18	4.14	4.21	4.26	4.31	4.09	4.22	4.22	4.19	0.08	0.57	0.80	0.50
Serine	3.78	3.75	3.80	3.79	3.75	3.74	3.72	3.80	3.77	3.81	0.06	0.65	0.85	0.76
Tyrosine	3.60	3.73	3.75	3.76	3.74	3.65	3.71	3.75	3.74	3.78	0.07	0.09†	0.66	0.89

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L×F: interaction between level of threonine and feeding system; †Quadratic effect for L

3.6 Discussion

3.6.1 Performance is affected by threonine level

As dietary Thr levels increased, ADFI increased in a quadratic manner. This effect is likely due to the lower performance in terms of ADG and G:F ratio observed at the 100% Thr level compared to the other treatments, with reduced ADFI at smaller PD. In fact, ADFI is generally not influenced by dietary Thr levels (Edmonds and Baker, 1987a; De Lange et al., 2001; Hamard et al., 2007). Pigs received 16% more Lys in GPF than pigs in IPF. It was previously observed that changing to an IPF system can decrease Lys intake from 17% (Andretta et al., 2014) up to 26% (Andretta et al., 2016b) without any loss in animal performance. Intake of SID Thr in finishing pigs was linear and it was 15% lower with IPF than GPF. This smaller Thr intake is related to the smaller Lys intake as Thr is provided in function of Lys. We have found a similar reduction in methionine intake when evaluating the optimal methionine-to-Lys ratio in pigs in an IPF and GPF system (Remus, 2015).

Protein deposition tended to increase in a quadratic manner within GPF but not within IPF (interaction between Thr levels and feeding system). Moreover, PD was slightly smaller in IPF than GPF pigs. We first hypothesized that the weak effect of Thr levels on PD response in finishing pigs might be due the fact that pigs received AA in excess. However, pigs within GPF received on average 25.4 g of SID Lys per day, whereas, according to NRC (2012), finishing pigs with a maximum PD of 150 g/d should receive 24.23 g of SID Lys (maximum PD × 0.1615 g Lys per g of PD) and 16.83 g of SID Thr (maximum PD × 0.1122 g Thr per g of PD). The recommended amount for this genetic line is to 0.03 g of SID Lys and 0.02 g of SID Thr per g of ADG (Thr: Lys ratio of 0.67). Based on ADG, this would be the equivalent of 29.37g of SID Lys and 22.91 g of SID Thr per day in our study. For pigs in IPF, amounts of Lys and Thr were provided below these recommendations. For pigs in GPF, PD (150 g/d) and PD in daily gain (12.3%) was maximized at 23 g of SID Lys and 21 g of SID Thr per day (at 130% Thr intake level). At 100% Thr intake level, PD was smallest in GPF pigs. However, PD (118 g/d) was in line with the expected PD

(114 g/d) for this genetic line, and ADG (1070 g/d) was higher when compared to the expected ADG (807 g/d).

To our knowledge, no information in the literature exists on the Thr dose response for late-finishing pigs based on a high Thr:Lys ratio of 0.85 as in the present study. Pigs in GPF fed at a 130% Thr level had remarkably high PD and ADG for similar heavy late-finishing pigs or even early-finishing pigs compared with literature data (De Lange et al., 2001; Ettle et al., 2004a; Ma et al., 2015). It is possible that IPF pigs deposited less PD than GPF during the finishing phase because requirements were underestimated due the use of fixed ratios, which do not allow pigs to perform at their maximum potential. Therefore, it seems that estimating AA requirements independently instead of using fixed AA ratios may allow pigs in IPF to improve their performance.

The use of a high Thr level for late-finishing pigs can increase PD in 17% for GPF pigs (70 vs 130% Thr intake level). Protein deposition in daily gain tended to increase in a cubic manner within IPF and in a quadratic manner within GPF (interaction between Thr intake level and feeding system), with maximum PD in daily gain for pigs receiving 13 g of SID Thr or 22 g of SID Thr per day. However, it is important to consider that pigs receiving 22 g of SID Thr deposited 18% more protein than pigs receiving 13 g of SID Thr. It can be assumed that increased Thr intake during the finishing phase may improve lean deposition as the increased maintenance requirements during late growth are met and Thr is available for protein synthesis. However, the fact that Thr restriction also promoted a similar effect is not expected as a smaller PD is normally found during Thr restriction (De Lange et al., 2001; Ma et al., 2016). Protein retention was enhanced in rats (Young and Marchini, 1990; Tawa and Goldberg, 1992) because dietary protein deficiencies decreased the degradation of AA. Therefore, lean deposition at lower levels of Thr intake could be maintained due to lower AA inevitable catabolism rather than due to increased protein synthesis rate.

Nitrogen excretion tended to increase in a quadratic manner as dietary Thr levels increased, likely linked to the increased CP intake (tendency for a quadratic

relationship). Pigs in IPF consumed 14% less CP and excreted 17% less N than pigs in GPF. Pigs in IPF were 9% more efficient in retaining N than pigs in GPF. Therefore, changing to an IPF system can be beneficial for the environment with up to 30% less N excretion without concomitant performance loss (Andretta et al., 2016b). The effect of increased dietary Thr levels on N retention was not as clear during this finishing phase as it was observed for pigs during the growing phase (see Chapter 2). Nitrogen excretion decreased linearly with increased Thr levels in the diet for growing pigs, independently of the feeding system. Gilts receiving various levels of fibre in the diet increased efficiency of N retention with increased Thr in the diet (Mathai et al., 2016), but the same effect was not observed in weaned pigs (Zhang and Kim, 2014). Generally, N efficiency is maximized near the point when AA requirements are met, which explains the improved N efficiency observed with increased Thr levels in the diet.

During the growing phase, a 30% Thr restriction limited animal growth and PD in both feeding systems. During the finishing phase, lower levels of Thr did not affect animal performance. At a level of 70% Thr in the diet, PD and PD in daily gain improved for pigs in GPF but not in IPF when compared with the 100% Thr level. This contrast between the growing and finishing phase might be probably due to a larger PD in growing pigs, whereas finishing pigs had larger amounts of protein in body with AA (from the concomitant higher muscular turnover) potentially being reused for protein synthesis. Studies on neonatal pigs (Davis et al., 1996) and nursery pigs (Conde-Aguilera et al., 2010) further showed that protein response in pigs is age dependent. This age dependecy in protein response might be partly due to hormonal resistance. For instance, a lower expression of insulin-like growth factor 1 (IGF1) isoforms, which control protein turnover in muscles, was observed in older mice (Sandri et al., 2013). Furthermore, older rats had 80% less IGF1 receptors, and, in adult rats, IGF1 lost its capacity of action on AA transport and protein synthesis but maintained an ability to stimulate glucose transport (Dardevet et al., 1994). Next to a dependency on hormonal factors, a difference in mTORC1 activation for protein synthesis in older pigs might also explain part of the difference in the response in pigs to Thr intake between the growing and finishing phase. An in

vitro study (Kang et al., 2013) suggested that the presence of serine or Thr acting as phosphoacceptors influences the sequence composition of the mTORC1 phosphorylation site, which determines the downstream response of mTORC1 to nutritional, hormonal and pharmacological factors. It is possible that muscle tissue in older animals might be more resistant to the growth-promoting actions of mTORC1 as shown by the up-regulation of the mTORC1 pathway in old mice (Sandri et al., 2013). Assuming that the animal response is age dependent and mTORC1 is AA dependent, higher levels of AA and, possibly, insulin are needed for a significant response in PD. This could explain the lower impact of Thr on PD in finishing pigs when compared to growing pigs.

Few researchers tested Thr solely in finishing pigs (Cohen and Tanksley, 1977; Saldana et al., 1994; Ettle et al., 2004b), and we are not aware of any published studies on late-finishing pigs similar to our study. Generally, it is assumed that Thr requirements increase with age due to a proportional increased maintenance requirements, but Pedersen et al. (2003) found no significant increase in the Thr:Lys ratio for pigs up to 100 kg of BW. However, the authors did not consider PD as a response factor, which might partly explain the response gap. None of the studies on Thr available in the literature (Cohen and Tanksley, 1977; Saldana et al., 1994; Ettle et al., 2004b) reported PD response to Thr intake. These studies reported Thr requirements in function of ADG, G:F, or plasmatic nitrogen urea, all variables that in our study did not represent well PD in daily gain or lean gain as PD. To obtain an accurate AA estimation, the parameter response should be the same as the test parameter (Boinsen, 2003). The main determinant of AA requirements in growing pigs is PD (de Lange et al., 2012;). Therefore, the ideal protein AA ratio should be established in function of AA or PD.

3.6.2 Serum parameters

Plasmatic IgG levels were not affected by the feeding system or dietary Thr levels, essentially because IgG response was not induced. Plasmatic IgG was used to assess whether pigs received naturally an immune challenge. Altered levels of IgG or CRP would result in the exclusion of the pig from the data analysis, as it

implies a biased estimation of the Thr requirements. Globulin concentration in plasma increased in a quadratic manner (interaction between level of Thr and feeding system) for pigs in GPF and increased in a cubic manner for pigs in IPF. Small concentrations of globulin are often associated to malnutrition (Busher, 1990). Increased levels of Thr promoted concentrations of plasmatic proteins in growing pigs (Remus et al., non-published data), probably because the availability of Thr (linear increase) favors the synthesis of these proteins. Albumin concentrations in plasma tended to be higher (+2%) for pigs in GPF than IPF. A similar effect was observed for C-reactive protein with increased concentrations in plasma (+22%) for pigs in GPF than IPF. This protein is mainly composed of serine (9.62%), but glycine (7.48%) and Thr (6.4%) are also critical components (Oliveira et al., 1979). Pigs had a smaller PD and a smaller SID Thr intake in IPF and it is, therefore, possible that the plasmatic proteins were decreased due to lower nutrient availability or because the synthesis of other protein types were prioritized over plasmatic proteins. Furthermore, plasmatic proteins can be transported into tissue cells to provide AA and ensure a state of equilibrium when AA concentrations in tissue cells decreased (Reece and Swenson, 2005). Late-finishing pigs, in particular individually fed pigs, might be able to maintain muscle composition by using plasmatic proteins for protein synthesis due the lower AA availability in plasma, or by decreasing the synthesis of plasmatic proteins to maintain muscle protein synthesis.

Plasmatic levels of Thr linearly increased with increasing levels of dietary Thr and were smaller for pigs in IPF than GPF, likely due to the smaller Thr intake in IPF. Pigs in IPF had higher plasmatic glycine concentrations, likely due to a larger glycine intake (not measured) or due to larger amounts of glycine from de novo synthesis or unaccounted sources (Ballevre et al., 1990). Phenylalanine concentrations in plasma tended to increase linearly for pigs in IPF as Thr increased in the diet. For growing pigs, lower levels of Thr in the diet increased Lys and histidine concentration in plasma independent of the feeding system (see Chapter 2). With Thr limiting in the diet, some essential AA such as Lys, phenylalanine and histidine, may be increased in plasma probably due to their low utilization for net PD as suggested by le Floc'h et al. (1994).

3.6.3 Splanchnic tissues: chemical and amino acid composition

Restricting SID Thr intake may impact on protein synthesis and change AA concentrations in the intestine. In the present study, final AA concentrations in the small intestine tissues did not change, probably because dietary Thr was preferably used for protein synthesis in the small intestinal mucosa (Schaart et al., 2005a). At a dietary level of 100% Thr, CP content in the intestine tended to largest, whereas CP in the carcass muscle pool tended to be smallest. Therefore, the CP in the intestine might be correlated with the lower CP content in the muscles, meaning that intestinal protein synthesis has priority over muscle protein synthesis. Increased dietary Thr levels affected liver AA concentrations. Concentrations of the essential AA arginine and histidine in the liver changed in a cubic manner, and valine increased in a quadratic manner as Thr in the diet increased. Changes in the intake level of these particular essential AA could explain the difference in AA concentration in the liver. However, the intake levels of other AA were not affected by dietary Thr levels (data not shown). Therefore, the quadratic increase of valine in the liver might be due to its lower utilization given that PD decreased in an inverse quadratic manner with increased dietary Thr levels. Valine tended to be more affected in IPF than GPF pigs with the former also having lower plasmatic concentrations of valine. The non-essential AA glutamate decreased in a quadratic manner as Thr intake increased and tended to be 3% lower in IPF than GPF. Glutamate plays an important role as substrate for protein synthesis and anabolic precursor for muscle growth (Newsholme et al., 2003). Glycine and serine concentrations in liver were affected by dietary Thr levels for pigs in IPF, which might be due to a higher oxidation of Thr as the metabolism of glycine and serine may be linked to that of Thr. Tyrosine concentrations in the liver increased linearly with increased dietary Thr levels. The changes in AA concentration in the liver mentioned above might be due to changes in protein synthesis and degradation. A restriction in Thr might reduce fractional protein synthesis rates in the liver with Thr restriction in piglets (Hamard et al., 2009). The same study reported decreased liver AA concentrations in almost all essential and non-essential AA, except for Lys, arginine, histidine, cysteine and glutamic acid and tendency for decreased glycine, at a 30% Thr restriction. The authors attributed

this effect to a Thr imbalance. However, AA are likely retained with an AA imbalance. An earlier study by Yoshida et al. (1966b) demonstrated that protein retention in the liver was enhanced using Thr and histidine imbalanced diets, and oxidation of labelled AA did not increase in rats. A similar study (Benevenga et al., 1968) using imbalanced diets showed increased incorporation of limiting AA in hepatic proteins in rats. Pigs fed imbalanced diets deficient in methionine but with a larger amount of branched-chain AA improved N retention in growing pigs (Langer and Fuller, 2000). Thus, it is unlikely that a lower retention or changes in the concentration of the limiting AA should be attributed to an AA imbalance. However, it is possible that such increased concentrations of other AA are linked to an AA imbalance between plasma and tissue. In the present study, plasma AA concentrations had a different profile than carcass AA concentrations. Due to the importance of liver for protein synthesis, liver AA concentrations might have been influenced by an AA imbalance between plasma and muscle tissue.

3.6.4 Muscles: chemical and amino composition

The longissimus dorsi had comparable AA levels among levels of Thr in the diet and between feeding systems, but DM and collagen content was higher for pigs in IPF than GPF. A 30% restriction of Thr (Hamard et al., 2009) and methionine (Conde-Aguilera et al., 2010) suggested that AA deficient diets may result in higher collagen content as collagen may act as a reserve for non-essential AA. As shown in Chapter 2, collagen decreased in the longissimus dorsi of growing pigs as Thr increased in the diet. An earlier study (Widdowson et al., 1960) showed that undernourished pigs increased collagen proportion in the skin compared with well-nourished pigs. Therefore, increased dietary Thr levels may improve AA availability in pigs by decreasing the collagen content in muscles.

Reduced growth and changed body composition was observed at low protein intake (Bikker et al., 1994) or low AA intake in nursery pigs (Hamard et al., 2009; Conde-Aguilera et al., 2010; Conde-Aguilera et al., 2016b) and growing pigs (Remus et al., 2017c). However, these effects seem to be age dependent as carcass AA composition of gilts (77 kg BW) was not affected by Thr restriction (De Lange et al.,

2001). It is, thus, possible that late-finishing pigs have a smaller response to AA deficiency due to larger amounts of protein in the body (in particular in muscles), from where AA from protein turnover could be reused in protein synthesis and AA concentration in muscles maintained at more constant levels.

3.7 Conclusions

This study brings information unique on Thr requirement and its limited effect on PD and performance for late finishing pigs. The 0.65 Thr:Lys ratio (Sève, 1994) did not affect the response of finishing pigs in both feeding systems, and a lack of response in plasmatic biochemical parameters and muscles and intestine composition was observed to changes in Thr:Lys ratio and differed between feeding systems. However changes in the Thr:ration affected liver AA concentrations. Individual precision feeding allowed SID Lys and SID Thr intake to decrease 16% and 15%, respectively, without differences in ADG and G:F when compared to GPF. Pigs in IPF were 9% more efficient retaining N than pigs in GPF. Further studies are required to investigate potential differences in protein synthesis and retention during AA restriction within each feeding system and the effect of hormonal response on protein synthesis for late-finishing pigs.

3.8 Acknowledgments

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CHAPTER 4: IMPACT OF THREONINE INTAKE AND FEEDING SYSTEM ON FEEDING BEHAVIOR IN GROWING AND FINISHING PIGS

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"The balance of nature is not a status quo; it is fluid, ever shifting, in a constant state of adjustment."

(Rachel Carson, Silent Spring, p.246)

Impact of threonine intake and feeding system on feeding behavior in growing and finishing pigs

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4.1 Résumé

Il a déjà été démontré que la composition corporelle des porcs peut changer en fonction de leur apport en thréonine (Thr) et de leur programme alimentaire. Cependant, si ces changements sont modulés par des changements sur le comportement alimentaire, ceci reste à explorer. La réponse à différents rapports Thr: lysine (Lys) (70, 85, 100, 115 et 130% du rapport Thr: Lys idéal de 0,65) a été étudiée chez des porcs en croissance et en finitionnourris soit individuellement avec un système d'alimentation de précision (IPF) ou élevé dans un système d'alimentation en groupe par phase classique (GPF). Deux essais d'une durée de 21 jours ont été réalisés dans une configuration factorielle 2 × 5 avec 110 porcs en phase de croissance (25 ± 0,80 kg de poids corporel) et 110 porcs en phase de finition (110 ± 7,02 kg de poids corporel) avec 11 porcs par traitement dans chaque

essai. Les porcs étaient logés dans la même salle d'élevage et nourris à l'aide de stations d'alimentation informatisées. La composition de la carcasse a été estimée par double absorptiométrie aux rayons X aux jours 0 et 21 de l'essai. La courbe d'alimentation journalière ne différaient pas entre les systèmes d'alimentation pendant la phase de croissance ou de finition. Les porcs ont montré un comportement alimentaire diurne dans tous les traitements, avec la plupart des repas (73% en moyenne) étant consommé entre 06h00 et 18h00. Pendant la phase de croissance, le nombre de repas par jour tendait à augmenter linéairement (P = 0,05) avec le niveau croissant de Thr fourni aux porcs dans le système GPF. Pendant la phase de finition, le nombre de repas pris par les porcs dans le système GPF a augmenté de façon quadratique (P <0,05) en fonction de la prise de Thr, alors que les porcs dans le système IPF ont présenté une réponse cubique (P<0.05), avec le traitement à 85% estimé de Thr ayant un nombre moindre de repas par jour. L'analyse factorielle exploratoire suggère que le comportement alimentaire n'a eu aucun effet sur la performance ou la composition de la carcasse chez les porcs en croissance ou en finition. Le déséquilibre observé entre les concentrations plasmatiques et musculaires d'acides aminés résultant des traitements peut avoir influencé les comportements alimentaires observés dans cette étude.

4.2 Abstract

It has previously been demonstrated that the body composition of pigs can change as a function of their threonine (Thr) intake and feeding program. However, if these changes are modulated by changes in feed intake and consumption patterns, remain to be explored. The response to different Thr to lysine (Lys) ratios (70, 85, 100, 115 and 130% of the ideal Thr:Lys ratio of 0.65) was studied in growing and finishing pigs either individually fed using an individual precision feeding (IPF) system or raised in a conventional group phase feeding (GPF) system. Two 21-day-long trials were performed in a 2×5 factorial setup with 110 pigs in growing phase (25 ± 0.80 kg BW) and 110 pigs in finishing phase (110 ± 7.02 kg BW) and 11 pigs per treatment in each trial. Pigs were housed in the same room and fed using computerized feeding stations. The total lean content was estimated by dual X-ray absorptiometry at day 0 and day 21 of the trial. Feeding patterns did not differ between feeding systems during the growing or finishing phase. Pigs exhibited diurnal feeding behavior in all treatments, with most meals (73% on average) consumed between 06h00 and 18h00. During the growing phase, the number of meals per day tended ($P = 0.05$) to increase linearly with increasing level of Thr for pigs within GPF. During the finishing phase, the number of meals taken by GPF pigs increased in a quadratic manner in function of Thr intake ($P < 0.05$), whereas IPF pigs had a cubic response with a lower number of meals, at 85% of estimated Thr requirements ($P < 0.05$). Exploratory factor analysis suggests that feeding behavior had no effect on perfomance or carcass composition in growing or finishing pigs. An imbalance between plasma and muscle concentrations of amino acids resulting from the treatments may have led to the small changes in feeding behavior observed in this study.

Keywords: Amino acids, precision feeding, group phase feeding, feed intake pattern, precision farming, swine

4.3 Introduction

Individually fed pigs in a precision feeding setup appear to have a higher efficiency of amino acid (AA) utilization than group-fed pigs in a conventional phase feeding system. This is likely due to the fact that group-fed pigs typically receive larger amounts of AA at a constant rate, whereas precision-fed pigs receive daily tailored diets with smaller amounts of AA and a decreasing dietary AA concentration as pigs age. The different amounts of AA between the two feeding systems may have an effect on carcass traits as well as on meal frequency (O'hea and Leveille, 1969). Meal frequency itself might influence body composition, as shown in mice that achieved significant fat loss without lean loss when intermittently fasted (Gotthardt et al., 2016). Sequential feeding (i.e. intermittent fasting) in poultry led to a quick adjustment in lipogenesis and protein synthesis (Ezzine et al., 2012). In the same study, a pulse of protein feeding compared to ad libitum feeding increased the N balance, essentially due to decreased leucine oxidation and whole body protein degradation during the post-absorptive state, and greater protein synthesis in whole body and liver during the fed state. Earlier studies (O'hea and Leveille, 1969; Allee et al., 1972) have shown that pigs fed twice a day had less fat in kidneys, smaller backfat thickness, larger stomach weight and improved feed efficiency than ad libitum fed pigs. Pigs fed twice a day had a similar body composition but improved feed efficiency and growth as compared to ad libitum fed pigs (Le Naou et al., 2014).

Previously, we demonstrated that pigs can change body composition as a function of threonine (Thr) intake and feeding system (group-fed vs. individually fed pigs) (Remus et al., 2017a). However, meal frequency has been shown to influence the body composition in pigs (Allee et al., 1972; Le Naou et al., 2014). We therefore set out to study whether changes in body composition are modulated by potential changes in feeding behavior between group-fed and individually fed pigs receiving increasing levels of Thr in the diet.

4.4 Material and methods

The present study was conducted in accordance with the Ethical Principles of Animal Experimentation adopted by Agriculture and Agri-Food Canada. Animal

trials were approved by the Ethical and Animal Welfare Committee of the Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada (Case no. 478).

A total of 220 barrow pigs of the same high-performance genotype (Fertilis 25 × G-Performer 8.0, Geneticporc Inc., St-Gilbert, Quebec, Canada) with a good health status were shipped to the Agriculture and Agri-Food Canada swine complex, Sherbrooke, QC, Canada in two batches. Pigs were divided into two trials according to the shipments: 110 pigs were used in a finishing phase (110–130 kg BW; November to December 2015), and 110 pigs in a growing phase (25–50 kg BW; February to March 2016). All pigs were allocated to two 76-m^2 pens with concrete slat floors in the same mechanically ventilated room. Pigs were given an ear tag fitted with an electronic chip granting access to the automatic and intelligent precision feeders (Automatic and Intelligent Precision Feeder [AIPF]; University of Lleida, Lleida, Spain). A detailed description of the feeders is available from previous studies (Pomar et al., 2011b; Andretta et al., 2016a). Briefly, the feeders identify individual pigs demanding feed, assign each pig to the respective dietary treatment previously formulated for each pig, and mix and supply it. The feeders record the exact time and duration of each feed demand. A time lag of 30 s during the growing phase and 15 s during the finishing phase was imposed between feed demands to avoid feed waste. The time lag was set according to the body weight (BW) and feed intake of pigs. Pigs were given 14 days to adapt and received a commercial feed mixture suited to their requirements. The experimental period lasted 21 days. Feed and water were provided ad libitum throughout the experiment. Room temperature was adjusted to 22°C during the growing phase, and 18°C during the finishing phase.

Pigs were randomly assigned to 2 feeding systems and 5 levels of threonine supply according to a 2 x 5 factorial design in two complete blocks. Each of the 2 complete blocks included 55 pigs, and blocks referred to pigs starting the experimental period 1 week apart. Feeding systems were an individual precision feeding (IPF) system with diets tailored daily for the each pig, or a conventional group-phase feeding (GPF) system. Levels of threonine supply were set to 70%, 85%, 100%, 115%, and 130% of the estimated ideal level. The individual pig was

the experimental unit. Each treatment had 11 replicates. Pigs were blocked according to respective pens with a 1-week difference at the start of the experimental phase between the two blocks.

4.4.1 Feeding programs, nutritional requirements, and diets

The requirements for amino acids, calcium and phosphorus were independently estimated for each IPF and GPF pig, and the diets were formulated to have the same energy concentration (Table 4-1). Data from high-performance pigs from previous trials performed at Agriculture and Agri-Food Canada were used to simulate the Lys requirement of pigs and to formulate the feeds based on 4 available feed types (A1, A2, B1, and B2). Feed formulation was performed using the values of total AA content corrected to the standardized ileal digestible (SID) value of each ingredient according to the digestibility values for each AA as presented by the NRC (2012). Feeds were formulated to contain the same AA profile in order to keep feedstock variation small. For IPF pigs, 4 feeds (A1 and B1 containing 130% and A2 and B2 containing 70% of Thr relative to the optimal Thr:Lys levels) were mixed to meet the daily calculated requirements, and each pig received a daily tailored diet. The feeds were formulated to meet the Lys and other AA requirements, aside from the requirement for Thr, of the most demanding pig in the first day of the experimental period (feeds A1 and A2) and for the least demanding pig on the last day of the experimental period (feeds B1 and B2). The AA requirements aside from those for Lys were established using the ideal AA:Lys ratio proposed by Gloaguen et al. (2014). In the GPF system, the pigs received the same feed throughout the entire phase. The feed was a blend of A1 and B2 feeds mixed to meet the target levels of Thr and Lys for the respective treatment.

Table 4-1. Ingredient and chemical composition of the four experimental feeds (A1, A2, B1, B2)

Item	A1	A2	B1	B2
Ingredients (as-fed basis), g/kg				
Corn	533.4	537.9	537.1	538.3
Soybean meal (48%)	173	173	-	-
Wheat	150	150	100	100
Canola meal	47	47	-	-
Amino acid premix[1]	33	33	-	-
Corn starch	-	-	156.3	156.3
Fat	16	16	35	35
Oat hulls	-	-	143	143
Limestone	12	12	8	8
Monocalcium phosphate	10	10	8	8
Lysine sulfate (70%)	6.7	6.7	2.8	2.8
Salt	5.5	5.5	4.8	4.8
L-threonine	4.5	-	1.2	-
Dl-methionine	2.3	2.3	0.2	0.2
L-valine (96.5%)	2.1	2.1	0.2	0.2
Micro-mineral premix[2]	2	2	2	2
L-tryptophan	1.1	1.1	0.3	0.3
L-isoleucine	0.7	0.7	0.2	0.2
Anti-mold	1	1	1	1
Cl-choline (75%)	0.2	0.2	0.2	0.2
Chemical composition, %				
Dry matter	90.85	91.25	92.99	92.67
Fat	6.79	6.74	7.88	8.44
Protein	19.85	19.88	7.5	6.88
ADF	3.87	4.018	6.32	6.51
NDF	8.8	8.63	13.58	14.12
Total calcium	0.72	0.72	0.5	0.49
Total phosphorus	0.64	0.64	0.4	0.4
SID[3] isoleucine	0.67	0.69	0.22	0.21
SID leucine	1.34	1.39	0.64	0.59
SID lysine	1.07	1.07	0.34	0.33
SID methionine	0.53	0.53	0.16	0.14
SID methionine + cysteine	0.72	0.72	0.24	0.2
SID phenylalanine	0.75	0.77	0.28	0.26
SID serine	0.8	0.8	0.3	0.26
SID threonine	0.98	0.58	0.31	0.19
SID valine	0.89	0.89	0.29	0.27
Expected net energy, kcal/kg	3208	3223	3255	3259

[1]Mix of corn gluten meal and linseed meal (Shur-Gain, St-Hyacinthe, QC, Canada)
[2]Supplied per kilogram of diet (as fed-basis): vitamin A, 45,600 IU; vitamin D, 45,600 IU; vitamin E, 1,400 IU; vitamin K, 80 mg; vitamin B12, 1.2 mg; niacin, 800 mg; pantothenic acid, 600 mg; pyridoxine, 80 mg; thiamine, 80 mg; cooper, 4.9 g; iodine, 12 mg; iron, 4 mg; manganese, 2.5 g; selenium, 12 mg; and zinc, 6.1 g; supplier, manufacturer location
[3] Standardized ileal digestible (SID) and metabolizable energy were estimated from the analyzed total amino acid and crude energy content in feed and from INRA-AFZ table values (Sauvant et al., 2004)

The required daily concentration of Lys for feeds offered to IPF pigs was estimated with a mathematical model using information on daily individual feed intake (DFI) and weekly BW (Hauschild et al., 2012). The empirical component of the model estimated the expected BW, DFI and BW gain for the following day. Based on a factorial approach, the mechanistic component of the model used these three estimated variables to calculate the optimal concentration of Lys that should be offered that day to each pig in the herd to meet the individual requirements. Daily Lys requirements (g/d) were calculated by adding maintenance and growth requirements. Daily requirements of maintenance for Lys were estimated by adding the basal endogenous losses (0.313 g Lys/kg DM × DFI), the losses related to desquamation in the digestive tract (0.0045 g Lys/kg$^{0.75}$ × BW$^{0.75}$), and the losses related to the basal renewal of body proteins (0.0239 g Lys/kg$^{0.75}$ × BW$^{0.75}$; (van Milgen et al., 2008)). Requirements for growth were calculated assuming that 7% of the body protein is Lys (Mahan and Shields, 1998) and that the efficiency of Lys retention from digestible dietary Lys is 72% (Möhn et al., 2000a). Weight gain composition in terms of protein was calculated assuming 16% protein in daily gain (De Lange et al., 2003). This method of estimating nutrient requirements has been described (Hauschild et al., 2012; Pomar et al., 2015) and validated in 3 previous studies (Zhang et al., 2012; Cloutier et al., 2015; Andretta et al., 2016b). For GPF pigs, Lys requirements were estimated based on the assumption that requirements of a population are those of the 80th percentile pig of the group at the beginning of the phase (average of 3 days) (Hauschild et al., 2010; Remus et al., 2015c). However, provisions of Lys were decreased by 10% to ensure that Lys was the second limiting AA while other AA were provided at the estimated level.

4.4.2 Experimental measurements

4.4.2.1 Performance

Pigs were weighed at arrival and three times during the adaptation period to calibrate the model before nutrient restriction. Feed intake was registered in real time for each individual pig. Total body fat and lean content were measured by dual-

energy x-ray absorptiometry (DXA) on days 0 and 21 of the trial with a densitometer device (GE Lunar Prodigy Advance, Madison, WI, USA). Pigs were scanned in the prone position using the total body scanning mode (Lunar enCORE Software Version 8.10.027; GE). Anesthesia was induced with sevoflurane (7%) and maintained with isoflurane (5%) during the scans performed on days 0 and 21. The approximate duration between the beginning of anesthesia and the end of the scan (end of anesthesia) was 20 min on average during the growing phase and 25 min during the finishing phase.

4.4.2.2 Data management and statistical analysis

The automatic feeders recorded a total of 57,622 observations for the growing period and 58,986 for the finishing phase over a 21-day measuring period for each respective phase. Data were imported and tidied in R (version 3.4.0; R Foundation for Statistical Computing, Vienna, Austria) using the tidyverse wrapper package (Wickham, 2017). The feeding bahavior variables were calculated by the R program. Standardized ileal digestible Lys and SID Thr intake were obtained for each pig by tallying the daily amount of nutrients provided by each of the feeds served.

The meal size was quantified by taking into account short pauses between visits. Bigelow and Houpt (1988) pointed out that the short pauses (e.g. used for drinking) between consecutive visits should not be considered as the start of a new meal. In the present study, as a group of pigs shared the same pen and feeders, we observed that small pauses could also occur for reasons other than drinking. Pigs frequently moved to another available feeder within the pen, e.g. when other, dominant animals claimed the feeder, but quickly resumed eating thereafter. Therefore, intervals between visits of up to 5 min were considered to pertain to the same meal consistent with other reports in the literature findings (Bigelow and Houpt, 1988; Morgan et al., 2000). The interval between meals was defined as the time between the end of the previous finished meal and the start of the next meal. Feeding time per meal was considered the average time that an animal spent eating a meal. Feed intake per meal was the average intake per meal. Feed consumption rate was

calculated by dividing the feed intake by the time per meal. Total time eating per day was obtained by summing feeding time per meal.

Exploratory factor analysis was performed using the Factor Analysis procedure in the Minitab statistical package (version 16; Minitab Inc., State College, PA, USA). Factors were extracted using principal components in order to reduce the variance of the originally considered factors to a minimum number of factors (Hair et al., 2009). Eigenvalues were selected by graphical analysis, and only those with values greater than 1 were accepted following Kaiser's criterion. The quartimax normalized rotational strategy was applied to simplify the rows of the factor loading matrix. This analysis considered only the level of ingested threonine in grams per day. Feeding system could not be considered because it was coded (IPF = 1 and GPF = 2) and only continuous variables can be used in factor analysis.

Feeding behavior data were analyzed as a 2 × 5 factorial arrangement using the Mixed Model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Main effects included feeding program, level of threonine, and their interaction; the random effect was the block. Assumptions for normal distribution of residuals were tested using the Cramer-von Mises test through the univariate procedure of SAS version 9.4. The uncertainty in the estimate of the mean was expressed as the standard error maximum (SEM); $P < 0.05$ was considered to be statistically significant and $P < 0.10$ was considered a tendency. Differences between individual treatments were analyzed by orthogonal contrasts. Pearson correlation analysis was performed with the Corr procedure of SAS version 9.4 for levels inside feeding systems.

4.5 Results and discussion

Detailed information on performance, carcass composition, and AA concentration in tissues for growing pigs (Remus et al., unpublished data, Chapter 2) and finishing pigs (Remus et al., unpublished data, Chapter 3) were provided earlier. Briefly, Thr level had an impact on performance, carcass composition and AA concentration in muscles which was more marked during the growing phase. The feeding program did not affect performance, but the pigs' tissue chemical

composition and AA concentration differed depending on the feeding program during the growing phase but not during the finishing phase.

In the present study, exploratory factor analysis revealed that for growing pigs (Table 4-2) body composition variables such as protein deposition (PD) and lipid deposition (LD) were retained in the first factor, and were positively and highly correlated with daily Lys and Thr intake as well as with plasma levels of albumin and homocysteine. We previously demonstrated (Remus et al., 2017a; Remus et al., 2017c) that the linear increase in dietary Thr levels increased PD and plasma proteins such as albumin, probably due to increased protein synthesis and AA availability. Indeed, albumin synthesis is reduced in cases of malnutrition, malabsorption or maldigestion (Moshage et al., 1987). Furthermore, in the present study, increased PD resulted in increased levels of basal homocysteine in plasma. An increase of homocysteine is normally attributed to increased sulphur-containing AA intake (Kim et al., 2012). In our study, levels of sulphur-containing AA were constant and the increase of homocysteine was highly correlated with increased PD. It is possible that high protein synthesis saturates the remethylation of homocysteine in methionine, resulting in homocysteine accumulation in plasma.

Behavior variables were mainly retained in the second factor, and indicated that a smaller feed intake (FI) per meal resulted in a shorter interval between meals and a larger number of meals per day in growing pigs. Regulation of meal size by pigs was shown to be an important factor in maintaining energy homeostasis (Schwartz et al., 2000). Increased meal frequency may increase fat oxidation (Smeets and Westerterp-Plantenga, 2008) and maintain glucose levels in humans, constantly decreasing hunger (Jenkins et al., 1989). However, a higher meal frequency in combination with a smaller meal size has also been shown to increase cravings and hunger in humans compared to a lower meal frequency in combination with a bigger meal size (Ohkawara et al., 2013). The authors hypothesized that enhanced appetite might be a mechanism to prevent large drops in plasma glucose between meals.

Plasma variables were mainly retained in factor 3, and indicated that larger levels of Lys in plasma decreased levels of glutamine in plasma in growing pigs. An inverse correlation between glutamine and urea in plasma and decreased levels of Lys in plasma were observed at higher levels of PD (unpublished data). When Thr is limiting in the diet, essential AA such as Lys tend to increase in plasma, probably because the essential AA is not used for protein synthesis (le Floc'h et al., 1994). Thus, these AA are metabolized in ammonia which can be safely transported in blood by glutamine to the urea cycle. Gain:Feed efficiency was retained in factor 4, indicating decreased G:F efficiency with increased FI rate in pigs (voracity). These results are in disagreement with a previous study (Rauw et al., 2006) in which pigs eating faster had a similar G:F efficiency but a greater FI, increased growth and increased LD.

Table 4-2. Exploratory factor analysis (quartimax rotation) with correlation coefficients for growth, feeding behavior and plasma response of growing pigs[1]

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Communality
Average body weight	0.86	0.04	0.01	0.08	0.75
Protein deposition	0.82	0.17	-0.21	-0.29	0.83
Lipid deposition	0.77	0.10	0.08	0.16	0.64
Gain:feed efficiency	0.35	0.10	0.08	-0.77	0.80
Lysine intake	0.75	0.04	-0.09	0.52	0.84
Threonine intake g/d	0.73	-0.08	-0.29	0.38	0.76
Feed intake rate	0.25	0.12	0.11	0.63	0.49
Feed intake per meal	0.37	-0.84	-0.04	0.22	0.89
Number of meals	0.08	0.96	0.05	0.10	0.95
Time interval between meals	-0.12	-0.95	-0.01	-0.10	0.93
Plasma glucose	-0.02	-0.07	-0.53	-0.02	0.29
Plasma total protein	-0.04	-0.16	0.30	0.21	0.16
Plasma CK	0.45	-0.26	0.30	0.07	0.36
Plasma albumin	0.79	-0.14	0.13	-0.25	0.72
Plasma glutamine	0.03	-0.01	-0.76	-0.26	0.65
Plasma lysine	-0.03	-0.09	0.84	0.06	0.04
Plasma threonine	0.03	-0.12	-0.36	0.14	0.17
Plasma homocysteine	0.69	-0.05	-0.09	-0.32	0.59
Variance[2]	4.76	2.74	2.12	1.89	11.52
Proportion[3]	0.26	0.15	0.12	0.11	0.64

[1]Correlation assumed to be significant above 0.6
[2]Variability in data explained by each factor

[3]Proportion of variability in data explained by each factor (ranging from 0 to 1)

For finishing pigs, feeding behavior variables, with the exception of FI rate, were retained in the first factor, whereas carcass and performance variables PD, LD, G:F, and average BW were retained in the factor 3. The correlation shows that pigs with higher BW would have higher LD, lower PD and decreases in G:F. In late-finishing pigs, a lower rate of PD compared to LD is assumed (van Milgen and Noblet, 2003); thus, heavier pigs may tend to have lower PD and G:F, but greater LD compared to smaller pigs.

Table 4-3. Exploratory factor analysis (quartimax rotation) with correlation coefficients for performance, feeding behavior and plasma response of finishing pigs[1]

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Communality
Threonine intake	-0.02	-0.91	0.17	-0.02	0.86
Feed intake rate	0.15	-0.17	-0.08	-0.12	0.07
Time interval between meals	0.95	0.06	-0.05	0.08	0.91
Number of meals	-0.93	-0.08	0.09	-0.04	0.88
Time per meal	0.91	-0.07	0.13	0.07	0.85
Feed intake per meal	0.96	-0.14	0.14	0.01	0.96
Average body weight	0.09	-0.21	0.79	-0.04	0.68
Gain:feed efficiency	-0.17	-0.12	-0.62	-0.47	0.64
Protein deposition	0.12	-0.48	-0.66	-0.37	0.82
Lipid deposition	-0.05	-0.18	0.78	-0.19	0.68
Plasma total protein	0.12	-0.18	-0.07	0.76	0.62
Plasma glutamate	0.00	-0.11	-0.03	-0.61	0.39
Plasma threonine	0.04	-0.71	-0.08	0.26	0.57
Lysine intake	-0.02	-0.74	0.40	-0.20	0.75
Variance[2]	3.61	2.31	2.29	1.47	9.68
Proportion[3]	0.26	0.17	0.16	0.11	0.69

[1]Correlation assumed to be significant above 0.6
[2]Variability in data explained by each factor
[3]Proportion of variability in data explained by each factor (ranging from 0 to 1)

Threonine Intake increased linearly ($P < 0.10$) within IPF and GPF pigs, whereas Lys intake was similar among treatments (Table 5-4). This effect might be

due to the dose-response method we used, which involved constant levels of Lys in the diet, whereas Thr was supplemented to increase linearly in the diet. During the growing phase, the number of meals per day tended ($P < 0.10$) to increase linearly with increasing levels of Thr within GPF (Table 5-4). Pigs fed diets supplemented with tryptophan appeared to consume a greater number of meals per day than pigs receiving tryptophan deficient diets, although this effect was not significant (Montgomery et al., 1978). In the same study, feed intake decreased with tryptophan deficiency due to the decreased size of the meal. Whereas in the present study feed intake per meal was similar for diets deficient in Thr and for diets with Thr in excess. The interval between meals tended to increase linearly with increasing levels of Thr within GPF ($P < 0.10$), indicating that pigs receiving lower levels of Thr ate more frequently than those receiving higher levels of Thr. Feeding time per meal was affected in an opposite fourth-degree manner within IPF and GPF (feeding system × Thr level, $P < 0.05$). In fact, IPF pigs receiving 70% and 130% of Thr had shorter meal times ($P < 0.05$) while GPF pigs ($P < 0.05$) had shorter meal times with 85% and 115% of Thr. The feed consumption rate decreased linearly ($P < 0.05$) with increasing levels of Thr in the diet. Feed consumption rate can be interpreted as eating voracity (Andretta et al., 2016a). The linear effect suggests that voracity in pigs increased for diets with Thr in excess (115% and 130% Thr). A moderate correlation was observed between final BW and feed consumption rate ($r = 0.45$; $P = 0.05$). This is due to the linear effect of PD and average daily gain with increasing Thr levels. It is possible that the increased voracity at higher levels of Thr might be related to a larger BW in the respective pigs. In a previous study (Andretta et al., 2016a), feed consumption rate, along with all the other feeding behavior variables, depended on the growth phase, possibly due to a correlation with BW. In the present study, total time eating per day was not affected by feeding system or Thr levels.

Feeding patterns did not differ between feeding systems during the growing or finishing phase. Pigs had a typical diurnal feeding behavior in all dietary treatments, with most meals consumed between 06h00 and 18h00, which corresponds to the time interval during which room lights were on (Figure 4-1 and 4-3). During the growing phase, IPF pigs appeared to have greater feed consumption

rates. During the growing and finishing phase, pigs had higher feed consumption rates between 10h00 and 18h00. This preference for diurnal eating periods has been reported previously (Wangsness et al., 1980; Young and Lawrence, 1994; Andretta et al., 2016a) and likely relates to the period of light in the room, which stimulated pigs to eat.

Table 4-4. Feeding behavior of growing barrow pigs (25–42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine:lysine ratio of 0.65) in a conventional group-phase feeding (GPF) or individual precision feeding (IPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Body weight (initial), kg	26.02	26.19	25.56	25.20	26.03	26.68	25.70	25.84	25.69	26.20	0.80	0.40	0.49	0.84
Body weight (final), kg	39.54	40.45	41.47	41.59	43.45	40.80	42.48	42.06	41.74	42.28	1.09	0.11	0.37	0.57
Average daily feed intake, kg/d	1.44	1.46	1.46	1.63	1.50	1.51	1.40	1.49	1.48	1.41	0.14	0.41	0.35	0.47
Lysine intake, g/d	11.55	11.77	12.39	13.59	13.09	13.26	12.73	13.00	12.97	12.35	0.98	0.48	0.31	0.19
Threonine intake, g/d	6.33	7.50	8.97	11.25	11.64	7.23	8.13	9.50	10.68	11.58	0.72	<0.001[†]	0.31	0.45
Interval between meals, min	129.04	157.70	138.47	137.79	129.85	118.35	108.26	142.59	129.71	149.83	15.65	0.60	0.23	0.06[a]
Feeding time per meal, min	7.39	7.87	7.96	8.05	7.13	7.62	7.11	8.67	7.09	8.26	0.89	0.32	0.99	0.01[b,c]
Feed intake per meal, g	134.41	164.36	142.80	167.57	130.98	132.14	125.25	150.83	135.24	151.76	14.12	0.68	0.27	0.11
Feed inake rate, g/min	19.79	20.66	20.39	23.19	22.11	21.80	20.32	20.24	24.27	21.74	1.63	<0.001[†]	0.46	0.67
Number of meals per day	11.01	9.08	10.79	10.69	11.36	10.71	12.58	10.43	10.78	9.41	1.24	0.98	0.73	0.05[a]
Total time eating, min/d	75.79	78.61	78.58	78.91	73.40	80.35	83.18	78.48	71.17	67.94	5.04	0.17	0.76	0.48

[1]MSE: maximum standard error

[2]L: level of threonine in the diet; FS: feeding system; [3]L × F = interaction between level of threonine and feeding system; [†]Linear effect for L; [a]Linear effect within GPF ($P < 0.05$); [b]Fourth-degree effect within IPF ($P < 0.05$); [c]Fourth-degree effect within GPF ($P < 0.05$)

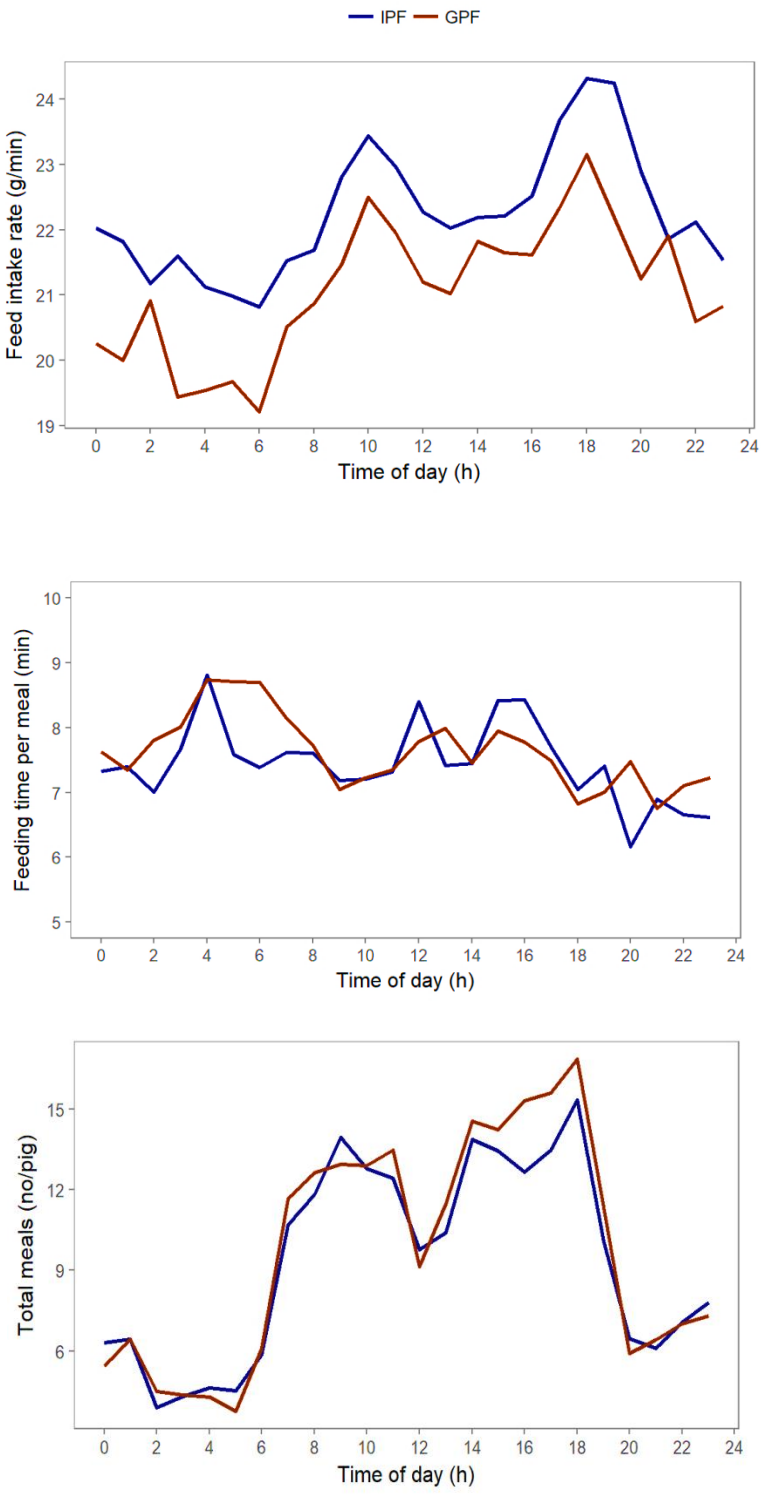

Figure 4-1. Circadian variation of average feed intake rate (grams of feed intake per minute during a meal), feeding time per meal (meal duration in minutes), and number of meals for growing pigs (25–42 kg body weight) in a group-phase feeding (GPF) or individual precision feeding (IPF)

During the finishing phase, feeding time per meal was shorter at 100% Thr ($P < 0.05$); thus, pigs ate faster when fed this dietary treatment. Pigs had a quadratic effect on feed consumption rate with the greatest voracity at 100% Thr ($P < 0.05$). Pigs in GPF increased the number of meals at 100% Thr ($P < 0.05$), whereas IPF pigs showed a cubic response with a smaller number of meals at 85% Thr ($P < 0.05$). Behavior variables did not differ between feeding systems, despite a 16% greater Lys intake and a 15% greater SID Thr intake for GPF pigs relative to IPF pigs ($P<0.05$). Results of the current study differed from those of Andretta et al. (2016a), who observed no effect of Lys deficient diets on feeding behavior in pigs in overall period. This might be due the fact that Lys deficiency has no impact on feed intake (Hrupka et al., 1999). Feed intake in rats has been shown to be depressed in response to Thr deficiency, which was attributed to a drop in plasma Thr levels (Feurte et al., 1999). In the present study, a positive correlation was observed between Thr intake and Thr levels in plasma in the factor analysis (Table 4-3). Previously Yoshida et al. (1966a) established the hypothesis that a severe imbalance between muscles and plasma free AA is the result of severely AA deficient diets triggering a homeostatic mechanism that depresses feed intake in rats. Threonine was found to have a small impact on feeding patterns in rats (Ayaso et al., 2014) and, generally, no effect on feed intake in pigs (Edmonds and Baker, 1987b). Thus, these results agree with the hypothesis advanced by Yoshida et al. (Yoshida et al., 1966a) that changes in feed intake and, consequently, feeding behavior might be due to differences in plasma and muscle AA concentrations.

Table 4-5. Feeding behavior of finishing barrow pigs (110–130 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine:lysine ratio of 0.65) in a conventional group-phase feeding (GPF) or individual precision feeding (IPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Bodyweight (initial), kg	110.2	108.7	110.2	110.4	110.3	108.0	110.1	109.7	110.5	108.8	3.05	0.99	0.78	0.98
Bodyweight (final), kg	134.1	131.9	132.7	134.1	133.6	131.5	134.7	132.2	134.6	134.0	3.12	0.97	0.95	0.94
Average daily feed intake, kg/d	3.69	3.52	3.61	3.86	3.38	3.41	3.79	3.66	3.78	3.56	0.11	0.04‡	0.71	0.13
Lysine intake, g/d	20.67	20.02	19.80	20.84	19.95	22.66	24.98	24.70	25.99	23.41	0.92	0.26	<0.001	0.30
Threonine intake, g/d	11.62	13.06	14.63	17.17	17.99	12.57	16.05	18.18	21.30	21.34	0.69	<0.001†	<0.001	0.15
Interval between meals, min	198.61	235.16	199.47	215.19	216.81	229.15	195.46	178.95	228.64	213.05	15.29	0.21	0.66	0.11
Feeding time per meal, min	10.11	10.34	8.16	10.23	9.99	10.22	10.26	8.13	10.82	9.97	0.87	0.01§	0.80	0.99
Feed intake per meal, g	543.4	596.0	537.0	615.	540.4	585.1	580.1	487.5	626.1	545.6	43.3	0.11	0.95	0.86
Feed intake rate, g/min	52.31	55.70	62.04	59.41	53.67	55.05	55.64	59.82	58.94	54.71	1.82	<0.001‡	0.80	0.46
Number of meals per day	7.39	5.63	6.77	6.68	6.42	6.17	7.19	7.98	6.34	6.79	0.43	0.17	0.23	0.01[a,b]
Total time eating, min/d	69.15	61.61	60.90	64.19	63.20	60.40	66.90	59.09	64.38	63.82	3.18	0.34	0.57	0.07

[1]MSE: maximum standard error

[2]L: level of threonine in the diet; FS: feeding system; [3]L × F = interaction between level of threonine and feeding system; †Linear effect for L; ‡Quadratic effect for L; §Fourth-degree effect for level; [a]Quadratic effect within GPF ($P < 0.05$); [b]Cubic effect within IPF ($P < 0.05$)

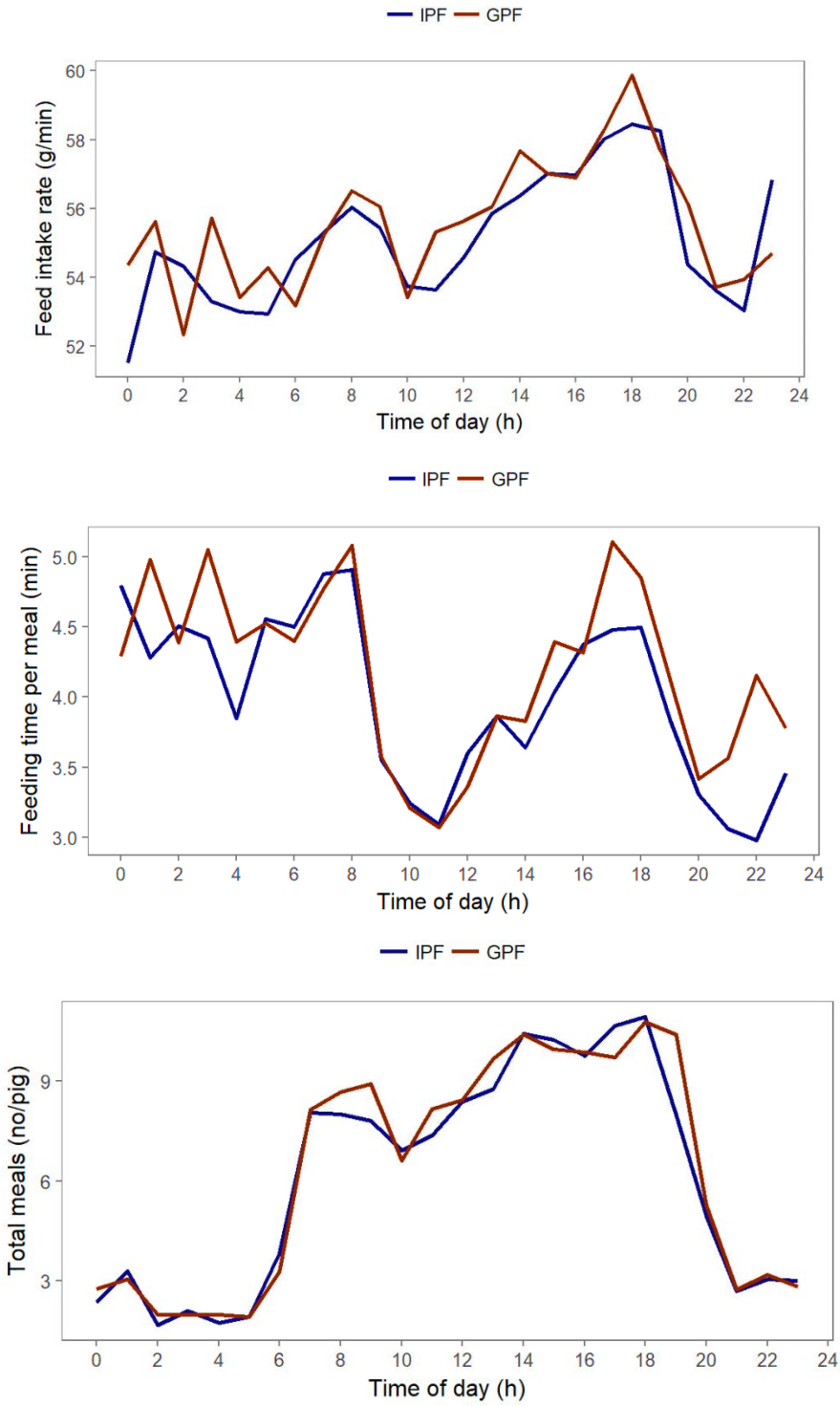

Figure 4-2. Circadian variation of average feed intake rate (grams of feed intake per minute during a meal), feeding time per meal (meal duration in minutes), and number of meals for finishing pigs (110–130 kg body weight) in a group-phase feeding (GPF) or individual precision feeding (IPF)

4.6 Conclusions

The exploratory factor analysis indicated that feeding behavior had no correlation with perfomance or carcass composition in growing or finishing pigs. Changes in feeding behavior observed differences in protein body content and AA composition in muscles; instead they might result from such differences.

CHAPTER 5. ESTIMATING AMINO ACID REQUIREMENTS IN REAL TIME FOR PRECISION-FED PIGS: THE CHALLENGE OF VARIABILITY BETWEEN INDIVIDUALS

This chapter contains a preliminary analysis and it was formatted for for later submission to the Canadian journal of Animal Science. At the moment of submission of the thesis, not all data from the trail described in this chapter were available.

"When we compare the individuals of the same variety or sub-variety of our older cultivated plants and animals, one of the first points which strike us is, that they generally differ more from each other than do the individuals of any one species or variety in a state of nature. And if we reflect on the vast diversity of the plants and animals which have been cultivated, and which have varied during all ages under the most different climates and treatment, we are driven to conclude that this great variability is due to our domestic productions having been raised under conditions of life not so uniform as, and somewhat different from, those to which the parent species had been exposed under nature. There is, also, some probability in the view propounded by Andrew Knight, that this variability may be partly connected with excess of food. It seems clear that organic beings must be exposed during several generations to new conditions to cause any great amount of variation; and that, when the organisation has once begun to vary, it generally continues varying for many generations. No case is on record of a variable organism ceasing to vary under cultivation. Our oldest cultivated plants, such as wheat, still yield new varieties: our oldest domesticated animals are still capable of rapid improvement or modification."

(Charles Darwin, On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life, Sixth British edition, page 5, 1872)

Estimating amino acid requirements in real time for precision-fed pigs: the challenge of variability between individuals

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5.1 Résumé

La variabilité individuelle des besoins en acides aminés (AA) entre les porcs dans les études dose-réponse peut être importante. En vue des futures recommandations en AA pour les porcs dans les systèmes d'alimentation de précision, il est essentiel de comprendre la source de la variabilité. Nous supposons qu'une grande partie de la variation observée est inhérente à l'approche expérimentale utilisée qui repose généralement sur le concept de protéine idéale, en supposant la dépendance des besoins en AA sur la lysine. Nous avons donc utilisé une approche originale basée sur le plan composite centrale avec une configuration factorielle (approche factorielle), dans laquelle, contrairement à la technique traditionnelle dose-réponse, la réponse de plus d'un AA et leurs interactions peut être étudiés simultanément et indépendamment. Les régimes alimentaires ont été formulés pour permettre une réponse maximale à la lysine et à la thréonine en faisant une supplémentation maximale des AA dans le régime alimentaire. Nous avons assigné 95 porcs en croissance dans un plan factoriel 5 ×

5 de traitements basés sur le plan composite centrale avec 2 AA (lysine contre thréonine) et 5 niveaux pour chaque AA (60%, 80%, 100% et 140% des besoins estimés). Le porc était l'unité expérimentale. Les besoins en lysine et en thréonine ont été estimés quotidiennement en fonction de la prise alimentaire individuel, du gain moyen quotidien et du poids corporel. L'analyse de données a été faite en utilisant le logiciel SAS. La décomposition des variations a été effectuée en transformant la somme des carrés en pourcentage relatif à la somme totale des carrés obtenue à l'aide d'un modèle linéaire général. La méthode de régression polynomiale non paramétrique localement pondérée a été utilisée pour estimer la surface de régression à travers la fonction LOESS. La variabilité des besoins en lysine et en thréonine entre les porcs était importante avec l'approche factorielle et comparable à celle observée avec la technique de dose-réponse traditionnelle dans les études précédentes sur le porc. Les porcs avec moins de dépôts de protéines (PD) avaient des concentrations plus élevées d'enzymes plasmatiques liées à la dégradation musculaire, et présentaient une efficacité protéique plus faible que les porcs avec un PD élevé. Contrairement à une réponse unique pour les exigences en AA optimales avec la technique de dose-réponse conventionnelle, le modèle de réponse de surface inhérente au plan composite central utilisé dans cette étude fournit une réponse en forme de selle. Cela peut-être dû à la variabilité individuelle, les porcs recevant la même quantité d'AA pourraient avoir chacun une réponse différente. La grande variabilité relative des besoins en AA entre les porcs observée dans cette étude basée sur une approche factorielle suggère que l'efficacité individuelle des nutriments peut expliquer une partie de la variabilité individuelle. Les efforts de recherche futurs devraient se concentrer sur l'évaluation des sources de variabilité interindividuelle avant que des recommandations finales en AA chez les porcs dans un système d'alimentation de précision puissent être données.

5.2 Abstract

Variability within amino acid (AA) requirements between individual pigs in dose response studies can be large. In view of future AA recommendations for pigs in precision feeding systems, it is essential to understand sources of variability. We speculated that a large part of the observed variation might be inherent to the experimental approach commonly used in swine studies to estimate AA requirements (i.e. the dose response technique), as this approach relies on the ideal protein concept assuming a dependency of AA requirements on lysine. We, thus, used a novel approach based on a central composite design with a factorial setup (factorial approach), in which, in contrast to the traditional dose response technique used in swine studies, the dose response of more than one AA and their interaction can be simultaneously and independently studied. Diets were formulated to allow maximum response to lysine and threonine at the maximum supplementation level without limiting any other AA in the diet. We assigned 95 growing pigs to a 5 × 5 factorial arrangement of treatments based on central composite design with 2 AA (lysine vs threonine) and 5 levels for each AA (60%, 80%, 100%, 120%, and 140% of the estimated requirements). The pig was the experimental unit. Requirements for lysine and threonine were estimated daily based on individual feed intake, average daily weight gain and body weight. A nonparametric locally weighted polynomial regression method was used to estimate regression surface through the LOESS function. Variability in lysine and threonine requirements between individual pigs was large with the factorial approach and comparable to that observed with the traditional dose response technique in previous swine studies. Pigs with less protein deposition (PD) had greater concentrations of plasma enzymes linked to muscle breakdown, and had lower protein efficiency than pigs with large PD. In contrast to a unique response for optimal AA requirements with the conventional dose response technique, the surface response model inherent to the central composite design used in this study provided a saddle-shaped response, possibly due to the variability within AA requirements among individual pigs as pigs receiving the same amount of AA might each have a different response. The overall relative large variability in AA requirements estimates between individual pigs observed in this study based on a

factorial approach suggests that individual nutrient efficiency may explain part of the between individuals variability. Future research efforts should focus on assessing the sources of inter-individual variability before final recommendations for AA in precision-fed pigs can be given.

5.3 Introduction

Establishing amino acid (**AA**) requirements can be hampered by several factors that contribute to increased variability in the response of individual animals. Prediction accuracy in AA requirements may greatly improve by controlling these factors. We previously found that daily requirements for lysine (**Lys**) vary among individual pigs and described some challenges when establishing real-time requirements for precision-fed pigs (Andretta et al., 2014; Pomar et al., 2015; Andretta et al., 2016b). One challenge is related to the relative large AA requirements variability among individual pigs generally observed in dose response studies. The variance observed inside treatment in group-fed pigs in a dose response study (ratio of valine to Lys) varied from 380 to 557 g for average daily gain (**ADG**) as response criterion based on a quadratic-plateau model (Gloaguen et al., 2011). Based on the same model, the variance observed in group-fed pigs in a dose response study (Thr:Lys ratio) varied from 710 to 830 g for ADG as response criterion (Remus et al., 2017a). This relatively large variability among pigs with similar BW and similar amounts of AA intake remains unaccounted for.

The dose response methodology based on ideal protein profile consists in having the test AA limiting up to the point when maximum response to that AA is observed. Thereafter, the reference AA (typically Lys as the first-limiting AA) is limiting in the diet. However, when a nutrient is limiting, variability in animal response increases (Gous, 2016). Decreased variability in ADG was observed at higher levels of AA supplementation, in particular with individually fed pigs (Remus et al., 2015a). This decreased variability might be due to requirements being met at higher AA levels in the diet for a larger number of animals. More specifically, when animals are

restricted, the more efficient animals may still express their potential, whereas the less efficient animals may perform poorly. Variability may be, therefore, larger for pigs fed lower dietary AA levels. We hypothesized that a dose response approach, in which the test AA is limiting and depends on Lys, may contribute to the variability generally observed in AA requirements among individual pigs. We, thus, proposed a novel approach to the dose response technique using a factorial approach based on a central composite design. This approach aimed at independently estimating real-time requirements for Lys and Thr in individually fed pigs. In contrast to the traditional dose response approach based on the ideal protein profile concept that allows assessing one AA response in function of Lys at a time, this novel approach essentially considers the interaction between the two AA (here, Lys and Thr) and allows estimating more than one AA without limiting any AA in the diet other than the test AA. The aim of this study was to verify whether variability in protein deposition (**PD**) might be explained by limiting AA intake, and whether providing individual pigs with daily tailored Lys and Thr decreases variability in PD variability in growing pigs.

5.4 Material and methods

The present study was conducted in accordance with the Ethical Principles of Animal Experimentation adopted by Agriculture and Agri-Food Canada. Animal trials were approved by the Ethical and Animal Welfare Committee of the Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada. A total of 95 growing pigs were allocated in two 76-m^2 pens with concrete slats floors, in the same mechanically ventilated room. Room temperature was maintained at 22°C. Pigs were given an electronic chip placed in the ear granting access to the feeders. Pigs were adapted for 14 days with a commercial feed adjusted to their group nutrient requirements. Water was provided ad libitum with low-pressure nipple drinkers, and feed was provided individually ad libitum throughout the adaptation and the entire 21-day experimental period in 10 feeding stations (Automatic and Intelligent Precision Feeder; University of Lleida, Lleida, Spain). The experiment was designed as unbalanced 5 × 5 factorial setup including 2 AA (Lys, and Thr) fed at 5 levels of intake (60%, 80%, 100%, 120% and

140% of the AA requirements). The distribution of the animals inside each treatment was based on a central composite design (Box and Wilson, 1951; St-Pierre and Weiss, 2009). Four pigs were assigned to the most extreme treatment combinations and outer points in the central composite design, 3 pigs were assigned to the intermediate points in the design, and 6 pigs were assigned to the central points (Figure 5-1). Each pig was considered a replicate.

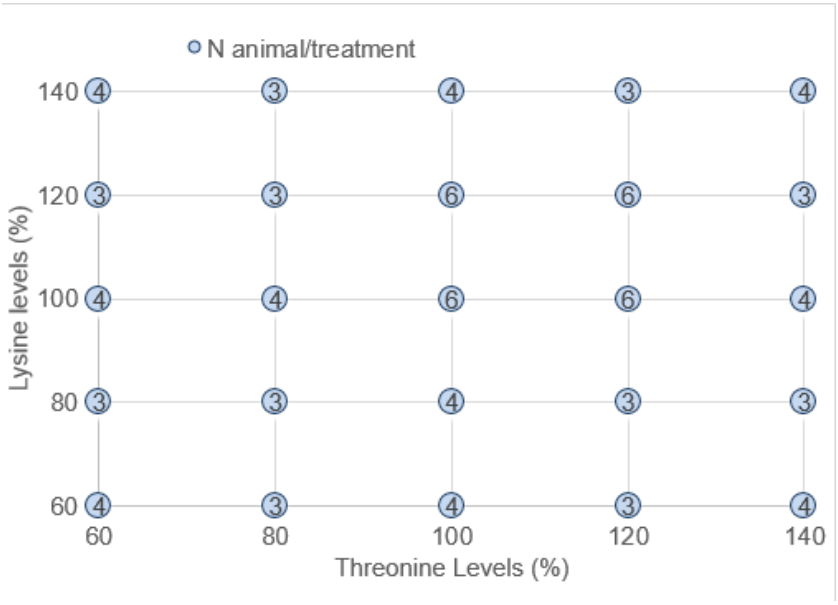

Figure 5-1. Unbalanced 5 x 5 factorial design based on a central composite design with number of pigs assigned to each treatment combination including threonine and lysine levels in the diet from 60% up to 140%

5.4.1 Nutritional requirements and diets

Four experimental feeds (A1, A2, A3 and A4) were offered to pigs throughout the 21-day long experimental period (Table 5-1). Feeds were formulated to meet 110% of the requirements of all nutrients, except for Lys and Thr, of the most demanding pig at the beginning of the experiment. Feed A1 and A2 were supplemented with crystalline Lys and Thr to satisfy the requirements of the same animals at 140% and 60% of the estimated requirements. Similarly, feeds A3 and A4 were supplemented with Lys and Thr at, respectively, 140%, 140% and 60% of

the estimated requirements (Figure 5-1). All feeds were blended by the feeders according to the individual requirements of pigs ranging from 60% to 140% Lys or Thr.

Daily requirements of Lys and Thr and the optimal dietary concentration in the blended feed were estimated with a mathematical model proposed by Hauschild et al. (2012) for Lys based on the individual daily feed intake and weekly body weight (BW). The empirical component of this model estimates the expected BW, feed intake and weight gain for the following day, whereas the mechanistic model component uses these three latter variables to calculate based on a factorial method the optimal concentration of Lys that should be offered that day to each individual pig to meet its requirements. For the mechanistic model component, daily Lys requirements (g/d) were calculated by adding maintenance and growth requirements. Daily maintenance requirements for Lys were estimated after van Milgen et al. (2008) by adding basal endogenous losses (0.313 g Lys/kg ×daily dry matter intake), losses related to desquamation in the digestive tract (0.0045 g Lys/kg of $BW^{0.75}$ per day), and losses related to basal renewal of body proteins (0.0239 g Lys/kg of $BW^{0.75}$ per day). The SID Lys requirements for growth are calculated assuming that 7% of the body protein is Lys (Mahan and Shields, 1998) and that the efficiency of Lys retention from dietary digestible Lys is 72% (Möhn et al., 2000). Weight gain composition in terms of protein was calculated assuming 16% protein in daily weight gain (de Lange et al., 2003). Standardized ileal digestible Thr requirements were calculated using a similar approach than for Lys. Daily SID Thr requirements were estimated by adding basal endogenous losses (0.330 g Thr/kg of daily dry matter intake; Noblet et al., 2002), losses related to desquamation in the digestive tract (0.0033 g Thr/kg of $BW^{0.75}$ per day; Moughan, 1998), and losses related to basal renewal of body proteins (0.0138 g Thr/kg of $BW^{0.75}$ per day; Moughan, 1998). Growth Requirements for SID Thr were calculated assuming that 3.7% of the body protein is Thr (Le Ballego and Noblet, 2002), and that the efficiency of Thr retention from dietary digestible Thr is 61% (van Milgen et al., 2008). Other AA requirements were estimated according to the ideal protein profile concept as described by (Gloaguen et al., 2014) and provided such to exceed by 10% the

maximum requirement, when Lys was supplied at 140% of the requirements. Requirements for Thr and Lys were calculated each day for each individual pig and AA were provided to each pig according to the treatment combination it was assigned to.

5.4.2 Experimental measurements

5.4.2.1 Animal performance, nutrient efficiency and carcass evaluation

Pigs were weighed at arrival and three times during the adaptation period to calibrate the model before providing the treatments. Animal performance was evaluated as average daily feed intake, average daily weight gain (ADG), feed-to-gain ratio, SID Lys intake, SID Thr intake, Lys efficiency, Thr efficiency, protein deposition (PD), PD in gain (%), and lipid deposition. Total body fat and lean content were measured by dual x-ray absorptiometry on days 0 and 21 with a densitometer device (GE Lunar Prodigy Advance, Madison, WI, USA). Pigs were scanned in the prone position using the total body scanning mode (Lunar enCORE Software version 8.10.027; Lunar Prodigy Advance, Madison, WI, USA). Anesthesia was induced with sevoflurane (7%) and maintained with isoflurane (5%) during the scans.

5.4.2.2 Blood sample collection and analysis

Blood samples were collected on days 1 and 21. All pigs were fasted for 10 h. Blood samples were collected from the jugular vein into a tube containing the anticoagulant EDTA for enzymatic and biochemical analyses or sodium heparin for AA analysis. The time between final sampling and centrifugation of blood sample did not exceed one hour during which blood samples were kept on ice. Blood samples were centrifuged for 15 minutes at 1000 × g at 4˚C. For AA analysis, blood samples were deproteinized within 30 minutes after centrifugation. All blood plasma samples were kept at -20˚C during the sampling day and stored at -80˚C until analysis.

5.4.2.3 Chemical and biochemical analysis

Two replicates of each sample were analyzed following the Association of Official Analytical Chemists (**AOAC**) standard methods for lyophilization (method 938.18; (AOAC, 1990)), and determination of crude protein in feeds (Kjeltec 2400;

FOSS Tecator, Hillerod, Denmark; method 992.15; (AOAC, 1990)), lipids (Soxtec 2050 Automated Extraction System; Foss, Höganäs, Sweden; method 991.36; dry matter (method 950.46; AOAC, 1990), and ash (method 920.153; (AOAC, 1990)). The AA contents of the samples were measured by gas chromatography coupled to mass spectrometry (Calder et al., 1999). Concentrations of IgG in blood were determined through ELISA kits (Pig IgG ELISA Quantification Set, ref. E100-104; Bethyl Laboratories, Inc., Place, Country). Biochemical and enzymatic analyses of plasma were performed with an automatic analyzer (Beckman DxC 600; Beckman Coulter, Mississauga, Ontario, Canada) at a dedicated external laboratory (Faculté de médecine vétérinaire of Université de Montréal, Saint-Hyacinthe, QC, Canada).

5.4.2.4 Calculations

Total weight gain of pigs was calculated as the difference between the BW measured at the beginning and end of the growing phase. Intake of SID Lys, SID Thr and crude protein were obtained for each pig by tallying the daily amount of nutrients provided with each of the served feeds. Efficiency of Lys and Thr were calculated by dividing the corresponding amount of available and retained AA. Retention of Lys and Thr were estimated assuming that 7% of body protein is Lys, and 3.7% is Thr. The availability of Lys and Thr was estimated by removing the amount used for maintenance from the SID pool. Body lean and fat masses from the scans were converted to their protein and lipid chemical equivalents as proposed by Pomar and Rivest (1996). Protein deposition in gain was calculated by dividing daily PD by ADG. Protein efficiency and nitrogen excretion was calculated by the difference between, nutrients retained from the respective nutrient intake level.

5.4.2.5 Statistical analysis

Pigs were clustered according to their PD using *k*-means clustering techniques with FASTCLUS procedures of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA). Mean comparisons were performed using a Tukey adjustment. Protein deposition in function of AA intake (Lys and Thr) was analyzed using the RSREG procedure of SAS, through canonical analysis. The RSREG procedure uses the method of least squares to fit quadratic response surface regression models. The

following step was to smooth and model the data using the LOESS procedure of SAS, which consists of a nonparametric method to estimate regression surfaces by multiple regression analysis. Response surfaces for PD measurements were generated from these equations. The LOESS procedure is recommended in presence of outliers and for data which requires a robust fitting (SAS Inst. Inc., Cary, NC, USA).

Table 5-1. Feed ingredients and nutrient composition of the experimental feeds A1, A2, A3 and A4

Item	A1	A2	A3	A4
Ingredient, g/kg of DM				
Corn	39.81	32.35	38.52	40.42
Wheat	30.00	30.00	30.00	30.00
Canola meal	14.00	14.00	14.00	14.00
Soybean meal	6.20	12.50	6.10	6.10
Soybean oil	3.50	3.90	3.50	3.50
Limestone	1.30	1.28	1.29	1.30
Monocalcium phosphate	0.37	0.32	0.38	0.37
Vitamin-mineral premix[1]	0.20	0.20	0.20	0.20
Salt	0.53	0.53	0.53	0.53
L-threonine	0.50	0.44	0.00	0.00
L-lysine HCL	0.00	1.26	1.55	0.00
DL-methionine	0.29	0.28	0.34	0.29
L-tryptophan	0.09	0.12	0.16	0.09
L-valine	0.34	0.29	0.41	0.34
L-isoleucine	0.29	0.15	0.26	0.26
L-leucine	0.39	0.23	0.39	0.39
L-histidine	0.15	0.04	0.11	0.15
L-phenylalanine	0.00	0.05	0.17	0.00
L-arginine	0.00	0.00	0.03	0.00
Chemical composition, %				
Dry matter[2]	87.27	87.51	87.40	87.21
Crude protein[2]	15.48	19.00	16.40	15.10
Net energy[3] (kcal)	2449	2451	2450	2448
Crude fiber[3]	3.62	3.62	3.58	3.63
Calcium[3]	0.70	0.70	0.70	0.70
Digestible phosphorus[3]	0.31	0.31	0.31	0.31
Total phosphorus[3]	0.66	0.69	0.69	0.71
Sodium[3]	0.22	0.22	0.22	0.22
Analyzed SID amino acid,[4] %				
Arginine	0.76	0.95	0.78	0.76
Histidine	0.48	0.43	0.43	0.48
Isoleucine	0.73	0.73	0.73	0.73
Leucine	1.41	1.41	1.41	1.41
Lysine	0.54	1.40	1.40	0.54
Methionine	0.52	0.54	0.57	0.64
Methionine + cysteine	0.79	0.84	0.84	0.88
Phenylalanine	0.60	0.76	0.76	0.57
Threonine	0.94	0.97	0.44	0.44
Tryptophan[3]	0.24	0.31	0.31	0.24
Valine	0.91	0.98	0.98	0.91

[1]Vitamin-mineral premix: vitamin A (11,400 IU); vitamin D (1,140 IU); vitamin E (35 IU); vitamin K (2 mg); vitamin B12 (30 µg); niacin (20 mg); pantothenic acid (15 mg); pyridoxine (2 mg); thiamine (2 mg); cooper (122 mg); iodine, (0.3 mg); iron (100 mg); manganese (63 mg); selenium (0.3 mg); zinc (152 mg)
[2]Analyzed values
[3]Expected values based on diet composition
[4]Standartized ileal digestible (SID) values were estimated from the analyzed total amino acid and crude energy content in feed, and values from estimated total and SID values provided by the formulation software Brill Formulation (Cargill Inc., Minneapolis, MN, USA)

5.5 Results and discussion

5.5.1 Experimental design

A biological response is often not linear and influenced by interactions between several factors (St-Pierre and Weiss, 2009). The antagonism and interdependency of AA has been studied before (D'Mello and Lewis, 1970). Nonetheless, AA studies have been limited to test one AA at a time, normally in relation to Lys as the first-limiting AA, likely for the sake of simplicity and for easier interpretation of the response criterion. Prior to the experiment, an exhaustive review was conducted to test various surface models to estimate the response of PD on various intake levels of Thr and Lys. A central composite design (Box and Wilson, 1951) was chosen with the aim to minimize the number of observations needed to perform a multifactorial trial. The number of observations for each treatment combination (2 AA × 5 levels of AA) was based on the variation and targeted power of estimation. Observations on intermediate points on the surface response were minimized as they have less weight on the regression. Central points and extreme or initial points on the surface response determine the angle and slope of the regression curve and required, thus, a greater number of observations to minimize variation.

5.5.2 Determining amino acids requirements for precision feeding systems

The factorial method proposed in the present study was able to provide estimates of real-time requirements for Lys and Thr in function of PD. The canonical analysis indicates that the predicted response surface does not provide a unique optimum of Thr and Lys intake and is shaped like a saddle with a less curved valley orientation of the saddle and a more curved hill orientation. The coefficients of the associated eigenvectors (special set of vectors associated with a linear system of equations) show that the valley is more aligned with Lys and the hill more with Thr. The saddle point for PD (199 g) was at 23 g of Lys and 14 g Thr per day (Figure 5-2).

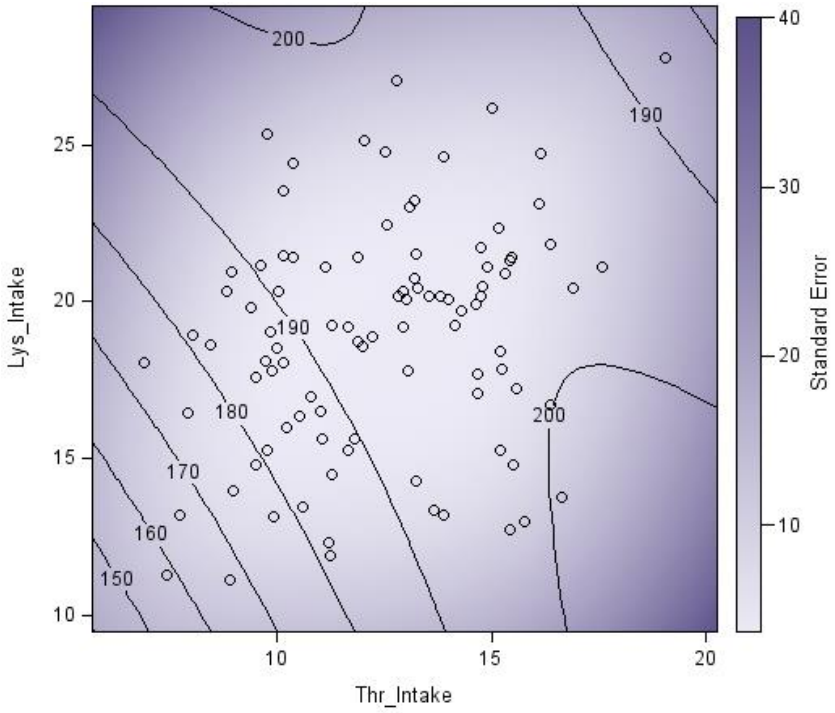

Figure 5-2. Response contour graphic of the canonical analysis of protein deposition in function of lysine (Lys) and threonine (Thr) intake (g/d) reflecting the stationary point (saddle point) in the central area of the plot

The model adjustment in the canonical analysis could explain only 11% of the variation (R^2). Data had a large variance and outliers were present, which required a robust fitting procedure to better interpret the results. Therefore, a graphical analysis was performed based on the LOESS procedure. As this procedure does not provide parameter estimations for maximum PD or required amounts of Lys and Thr, it was only used to graphically represent PD response. A cubic adjustment was made to the model (AICC of 7.488; smoothing parameter of 0.9842) which resulted in a saddle-shape surface response. A further linear adjustment was made to the model, which fitted data better (AICC of 7.456; smoothing parameter of 0.8474). Therefore, the linear surface model (Figure 5-3) was chosen over the cubic surface model (Figure 5-4).

The linear surface response indicated a linearly increased PD in line with linearly increased Thr and Lys intake. D'Mello and Lewis (1970) demonstrated that for maximizing PD other AA need to be increased proportionally with a dietary AA imbalance (e.g., with an addition of a synthetic AA). The same authors argue that an

accurate estimation of AA requirements with a dose response approach is hampered by an interdependency of AA as one limiting AA may affect the requirements of the other ones. It is likely that different AA combinations are possible, because pigs have different AA requirements and, likely, different individual AA efficiencies rates.

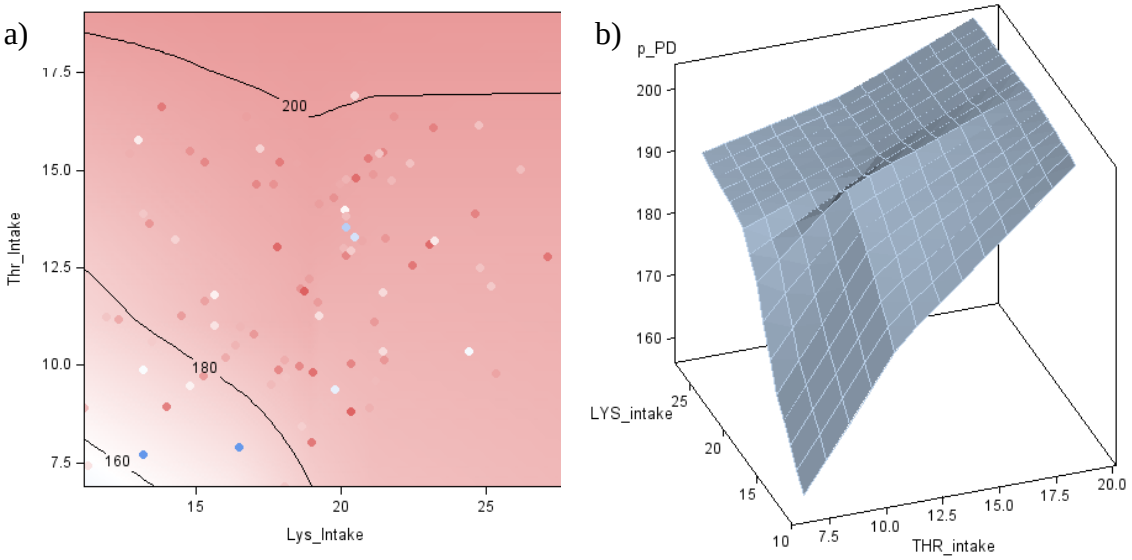

Figure 5-3. Protein deposition (PD, g/d) in function of lysine (LYS, g/d) and threonine (THR, g/d) intake as a two-dimensional (left) and three-dimensional (right) response surface based on a nonparametric locally polynomial regression method (LOESS function) with linear adjustment.

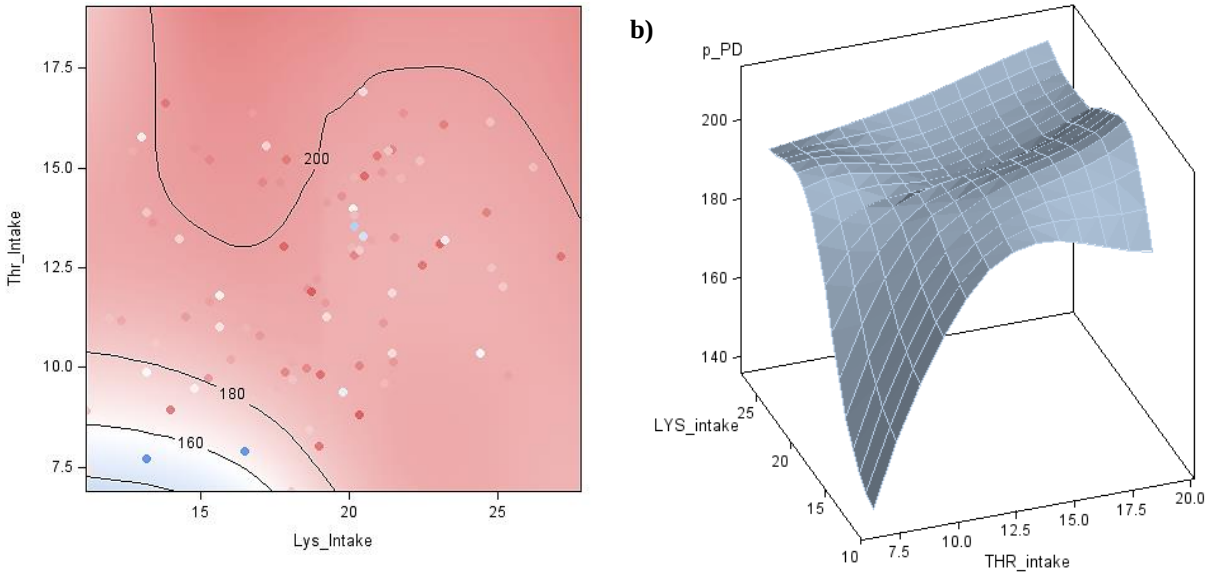

Figure 5-4. Protein deposition (PD, g/d) in function of lysine (LYS, g/d) and threonine (THR, g/d) intake as a two-dimensional (left) and three-dimensional (right) response surface based on a nonparametric locally polynomial regression method (LOESS function) with cubic adjustment

D'Mello and Lewis (1970) proposed the use of a factorial approach to estimate the magnitude of the impact from a surplus or reduction in AA intake and their interaction on AA requirements, instead of determining minimal AA requirements based on the recommendation tables using the dose response technique. Knowledge on minimal AA requirements in combination with knowledge on the magnitude of the impact of AA interaction on AA requirements allows developing a dynamic concept for estimating AA requirements instead of using static AA requirements tables. This knowledge can be applied to feed formulation programs that integrate mechanistic models. Such models may include (a) maximal PD deposition and performance, (b) AA interaction, (c) AA metabolism partitioning (i.e., amount of AA and energy used by the immune system, amount of protein deposition and fat deposition allowing to modulate the desired growth), (d) maximal profitability (i.e., amount of AA necessary to maximize profitability based on knowledge on the range of performance and carcass characteristics), and (e) minimal environmental impact at no performance loss (i.e., by integrating knowledge on points (a), (b) and (c)).

5.5.3 Exploratory results: understanding variability

Variability within treatments in a study can be large. We hypothesized that a dose response approach based on ideal protein profile, in which the test AA is limiting and depends on Lys, may contribute to the variability in PD response to AA intake among individual pigs. However, PD variability between individuals remained large in this study, even with an approach that estimates individually Thr and Lys. Variability in the response criterion was comparable to that observed in previous swine studies (Gloaguen et al., 2011; Remus et al., 2015b; Remus et al., 2017a). Our results suggest, therefore, that variability among individual pigs may not be different between the factorial approach used in the present study and the dose response approach commonly used in swine studies. Therefore, an exploratory analysis was performed to identify possible factors that resulted in different PD response between individuals.

Pigs were categorized in three clusters based on PD (low, medium, and high PD). The average PD for each cluster was 109 g/d (low PD; n = 4), 178 g/d (medium PD; n = 38), and 208 g/d (high PD; n = 53). Pigs allocated to the low PD cluster were included in the analysis despite being potential outliers because the purposes of this analysis was to understand the metabolic effect that explained differences among clusters, and whether differences among clusters were due to the imposed dietary treatments. On day 1, high PD pigs had higher concentrations of alkaline phosphatase (**ALP;** P = 0.02) in plasma than low PD pigs, yet comparable concentrations to medium PD pigs (Table 5-2). Concentrations of enzyme creatine kinase (**CK**) in plasma were lower for medium and high PD for low PD pigs (P = 0.02). Concentrations of creatinine in plasma were lower for low PD pigs than medium PD pigs (P = 0.001), yet concentrations were comparable to high PD pigs. Concentrations of the enzyme lactate dehydrogenase (**LDH**) in plasma were higher for low PD pigs than medium and high PD pigs (P = 0.02).

These results suggest that it might be possible to identify pigs with low PD and, possibly, group pigs by their PD potential by analysing blood plasma samples of pigs for LDH, CK and ALP. Identifying pigs with low PD may be useful to adjust the amount of nutrients to maximize nutrient efficiency. Our results further suggest that pigs with low PD may have a different enzymatic activity than those with high or medium PD. These metabolic differences can be source of variation in PD response to AA intake between individuals.

Table 5-2. Body composition and blood biochemical plasmatic variables of growing barrow pigs on day 1 of trial (initial conditions) clustered by protein deposition (low, medium, high)

	Protein deposition (PD)[1]				
Parameter	**Low PD**	**Medium PD**	**High PD**	**SEM[2]**	**P-value**
Body composition					
Body weight (BW), kg	34.73	35.61	34.68	1.78	0.06
Lipids, % in BW	5.09	5.00	4.83	0.41	0.10
Protein, % in BW	20.13	20.15	20.20	0.10	0.09
Plasma					
Albumin, g/L	35.47	35.08	34.91	8.12	0.50
Albumine:Globuline ratio	1.40	1.48	1.48	0.28	0.24
Alkaline phosphatase, U/L	187.04[a]	192.31[ab]	213.06[ab]	29.78	0.02
Alanine aminotransferase , U/L	43.07	41.78	41.55	7.63	0.09
Aspartate aminotransferase, U/L	46.73	44.36	46.50	11.39	0.45
Calcium, µmol/L	2.55	2.53	2.56	0.10	0.34
Cholesterol , µmol/L	2.37	2.47	2.45	0.31	0.37
Creatine kinase, U/L	3917[a]	1981[b]	1859[b]	1258	0.02
Chloride, µmol/L	99.28	97.98	98.09	2.16	0.63
Creatinine, µmol/L	72.08 [a]	80.43 [b]	74.73 [ab]	8.58	<0.001
Gamma-glutamyl transferase, U/L	37.04	44.31	41.89	10.95	0.54
Globulin, g/L	27.09	24.32	24.15	3.64	0.07
Glucose, µmol/L	4.24	3.93	4.11	1.09	0.29
Lactate dehydrogenase, U/L	670.39[a]	547.91[b]	542.32[b]	77.17	0.02
Phosphorus, µmol/L	3.60	3.63	3.63	0.21	0.76
Protein total plasma, g/L	62.55	59.40	59.06	3.34	0.16
Triglycerides, µmol/L	0.47	0.42	0.43	0.13	0.38
Urea , µmol/L	2.96	2.69	2.38	0.80	0.19
Sodium, µmol/L	142.21	140.38	140.50	3.09	0.72
Amino Acids, µmol/L					
Lysine	126.72	124.47	127.44	38.21	0.94
Threonine	131.27	127.90	129.53	43.12	0.98

[1]Within a row, means followed by same superscript do not differ (P > 0.05) according to Tukey's test
[2]SEM: standard error of the mean

At the end of the trial (day 21), concentrations of ALP (Table 5-3) were lower for low PD pigs than medium or high PD pigs (P = 0.04). We observed a strong correlation between PD and ADG (r = 0.92, P < 0.001) in line with observations by Liu et al. (2015) that increased ALP in plasma might be associated with higher ADG. Therefore, ALP in plasma might be indicative of increased PD and ADG in pigs. Concentrations of creatinine in plasma were lower for low and high PD pigs than medium PD pigs (P < 0.001). Concentrations of LDH in plasma were lower for medium and high PD pigs than low PD pigs (P < 0.001). Lower LDH levels might

indicate reduced energy metabolism by reducing activity in the glycolytic pathway in muscles (Faure et al., 2013). Energy saved in metabolic processes might result in more energy available for protein synthesis. As yet, there is no evidence as to a similar association with LDH in plasma and PD. In the present study, plasmatic levels of LDH were negatively correlated to PD ($r = -0.46$; $P = 0.04$). This exploratory analysis suggested that enzymatic activity might be a good indicator of the PD potential of growing pigs.

To decrease variability in the PD response, the individual PD potential should be considered in the mathematical model (Hauschild et al., 2012) used to estimate AA requirements in individually fed pigs. However, it would be difficult to measure actual individual PD in a commercial setup due to the dedicated equipment necessary to measure PD (e.g. DXA, use of anesthetics, etc.). Therefore, blood sampling could be a more viable option to characterize individuals by their PD potential. Blood measurements of enzymatic activity and protein turnover can be performed on farms using biosensors, which are likely to be better accessible in the future (Neethirajan et al., 2017). Furthermore, ADG can be measured with integrated scales and 3D scanners. Knowledge on ADG and plasma concentrations of LDH, ALP, CK and creatinine in growing pigs may, thus, help characterize the individual PD potential and AA efficiency, and ultimately improve the mathematical model (Hauschild et al., 2012) used for AA requirement estimations.

Table 5-5-3. Blood biochemical plasmatic variables and body composition of growing barrow pigs on day 21 of trial (final conditions) clustered by protein deposition (low, medium, high)

		Protein deposition (P			
Parameter	**Low PD**	**Medium PD**	**High PD**	**MSE[2]**	**P-value**
Performance					
PD, g/g of lysine intake	6.22[c]	9.57[b]	11.02[a]	1.87	<0.001
PD, g/g of threonine intake	14.85	14.83	14.69	3.19	0.98
Lipid deposition, g/d	143.53	167.54	161.75	44.32	0.55
Lysine intake, g/d	17.57	18.84	18.84	3.36	0.76
Lysine efficiency, %	43.79[c]	67.70[b]	77.71[a]	13.87	<0.001
Threonine intake, g/d	10.62	12.17	12.73	2.49	0.20
Threonine efficiency, %	41.91[b]	59.79 [a]	64.91 [a]	12.48	0.00
Protein intake, g/d	274.51[b]	336.30[a]	329.83[a]	34.16	0.01
Protein retention, %	36.42[c]	53.08 [b]	62.33[a]	6.49	<0.001
Body composition					
Body weight (BW), kg	49.65[c]	56.92[b]	58.56[a]	2.57	<0.001
Lipids, % in BW	8.45[b]	9.41[a]	8.51[b]	1.36	0.01
Protein, % in BW	19.17[ab]	19.20[b]	19.38[a]	0.32	0.03
Plasma					
Alkaline phosphatase, U/L	137.67[b]	175.46[a]	182.37[a]	29.34	0.04
Alanine aminotransferase, U/L	44.67	47.17	48.06	7.92	0.71
Aspartate aminotransferase,U/L	51.00	47.19	43.33	9.24	0.09
Creatine kinase, U/L	3678.67	3604.62	3445.48	2140.87	0.94
Creatinine, µmol/L	76.00[b]	93.03[a]	87.24[b]	10.11	0.00
Globulin, g/L	33.50	28.47	28.02	3.02	0.18
Lactate dehydrogenase, U/L	865.33[a]	604.32[b]	561.3[b]	119.11	0.00
Amino acids					
Lysine, µmol/L	152.72	158.94	168.95	35.38	0.37
Threonine, µmol/L	140.76	142.75	160.48	34.98	0.06

[1]Within a row, means followed by same superscript do not differ (P > 0.05) according to Tukey's test
[2]SEM: standard error of the mean

Protein deposition (g/g Lys intake; Table 6-3) was greater (P < 0.05) in high PD pigs. This reflected in greater (P < 0.05) Lys efficiency and protein retention, resulting in a lean gain and greater (P < 0.05) final BW than in low and medium PD pigs. Energy costs for PD can be up to 33% of the total growth costs in pigs (Reeds et al., 1980a). In general, it is possible that animals with a high efficiency receiving low dietary concentration of AA are able to increase PD because these animals have lower maintenance than animals with lower PD. It has been previously shown that pigs with maximum PD at 25 kg had a lower Lys catabolism (Moehn et al., 2004). The same study showed that PD was largest at low levels of Lys intake, due to

increased efficiency of Lys utilization. Moehn et al. (2004) observed that decreased catabolism in pigs was determined more by the growth potential than BW or decreased Lys intake. It was previously speculated that increased energy requirements due to increased protein turnover might result in increased variability in performance in situations in which performance deviates from the optimum (Koehn and Bayne, 1989; Hawkins, 1991). Protein synthesis in pigs increased linearly and protein breakdown (relative percentage) decreased (Salter et al., 1990) with the increase of Lys in the diet. This suggests that animals with high PD could be more efficient retaining AA or protein than animals with lower PD. Therefore, part of the observed variability in AA requirements among animals might be due to individual differences in energy and protein metabolism, in particular due to differences in the efficiency of AA utilization.

5.6 Implications

The factorial approach proposed in the present study allows evaluating the interaction between Thr and Lys by avoiding that animal response is biased by other possibly limiting AA in the diet. Nonetheless, the challenges inherent to this approach, mainly with regard to the statistical approach and the biological interpretation of the data, need to be considered. The surface response inherent to the central composite design used in the factorial approach resulted in a saddle point (i.e., non-unique response) instead of a unique response for optimal AA requirements. This non-unique response suggests that pigs receiving the same amount of AA might each have a different response (e.g. different PD) and is in line with the variable AA requirements among individual pigs observed in this study. Our results suggest that variability in AA requirements among individual pigs may be comparable between the factorial approach used in the present study and a dose response approach. The relative large variability between individuals observed in the present study further suggests that other factors independent of the dose response technique based on the ideal protein profile may explain variability in AA requirements. Variability in PD response to AA intake may be due to differences in efficiency of nutrient utilization inherent to the individual PD potential. Given the increasing importance of precision feeding in livestock farming it is important to

understand inter-individual variability and the factors contributing to it. Future research efforts should focus on understanding variability in AA requirements in individually fed pigs in a precision feeding system.

CHAPTER 6: GENERAL DISCUSSION

"I regret only one thing, which is that the days are so short and that they pass so quickly. One never notices what has been done; one can only see what remains to be done, and if one didn't like the work it would be very discouraging."

(Marie Curie; Letter to her brother; March, 18th 1894)

General discussion

6.1 Background

The main challenges for the pig production sector are to maximize feed efficiency, and minimize production costs and environmental costs. Modern feeding programs should, thus, consider nutritional aspects but also economic and environmental aspects. With regard to environmental costs, the issue lays mainly with nitrogen and phosphorus excretion in soil and water with alarming high levels found in most intensive pig production areas, such as Canada (in particular, Québec and Ontario), the USA, some European regions (in particular, Brittany in France, western Belgium, southeast of the Netherlands), and Brazil LOVATTO et al., 2005. The high relevance of environmental costs has forced swine producers and nutritionists around the world to reassess the nutritional and feeding programs in use. Nutrient excretion can be reduced by feeding pigs close to their nutritional requirements and, thus, avoiding feeding nutrients in excess. Conventionally, pigs are fed in large groups and receive the same type of feed for extended periods throughout their production cycle, typically over three feedings phases. In theory, the number of feeding phases needs to be increased to avoid supplying pigs with nutrients in excess. Preferably, diets should be adjusted daily to account for the nutritional requirements of pigs more accurately. However, increasing the number of diets is challenging in terms of industrial logistics and may increase production costs.

With the aim to allow pigs to maximise growth or any other response criterion and to minimize nutrient excretion, the concept of the ideal protein profile has been developed. The concept has been first tested in the late 80s and early 90s mainly by Dr. D. H. Baker's team in pigs (CHUNG; BAKER, 1992) and poultry (BAKER; HAN, 1994). This concept essentially assumes that all indispensable amino acids (AA) are equally limiting for performance, just covering the requirements for all physiological functions. Requirements for AA are thereby expressed as a ratio to a reference AA, generally lysine (Lys) because it is the first limiting AA for growth in pigs. This

concept is now largely used and accepted as a practical and straightforward way to formulate non-ruminants diets and to decrease the crude protein content of diets (BAKER, 2009; EMMERT; BAKER, 1997; VAN MILGEN; DOURMAD, 2015). It is generally assumed that the ideal protein profile does not change for a given growing stage, which offers a certain advantage when formulating feeds in practice, in particular for pigs fed in large groups over extended periods.

However, recent swine studies suggest that the ideal AA ratio to Lys may in fact change among individual pigs. Remus et al. (2015a) found a difference in the ideal ratio of methionine to Lys for pigs fed in an individual precision feeding (IPF) system and in a conventional group-phase feeding (GPF) system with three feeding phases. Furthermore, variability among individual pigs decreased with increasing dietary level of methionine, in particular for IPF. At 70% and 130% methionine in the diet, variability was respectively 27.8% vs 13.0% for average daily gain, and 17.1% vs 6.7% for the gain:feed ratio. It can be hypothesized that variability decreases at higher dietary AA concentration for pigs in an IPF system because a larger number of animals will have their requirements met with high AA concentration in the diet. More specifically, when animals are restricted, the more efficient animals may still express their potential, whereas the less efficient animals may perform poorly; therefore, variability may be larger for pigs fed at lower dietary AA levels. In the same study, pigs in the IPF system had a greater methionine-to-Lys ratio than pigs in the conventional GPF system. It can be hypothesized that the optimal AA ratio for pigs differs between the IPF and GPF system. In fact, pigs with a different protein deposition (PD) have different Lys requirements (HAUSCHILD ET AL., 2010; ZHANG ET AL., 2011; ANDRETTA ET AL., 2014). The classical ideal protein profile used for a population might, thus, not result in the best PD response because Lys in IPF systems is reduced (ANDRETTA et al., 2014; POMAR et al., 2011) and all other AA are decreased in the same proportion to Lys, which may ultimately limit performance if requirements for other AA differ.

Based on this information, the aim of this thesis was (1) to review the ideal AA profile concept commonly used in swine studies by evaluating the impact of

experimental errors on the estimation of efficiency of AA utilization, and (2) to evaluate the adequacy and limitations of the experimental approach used to estimate the optimal AA requirements and AA ratios in growing and finishing pigs. The main research objective was to compare the optimal ratio of threonine (Thr) to Lys for pigs between a conventional GPF and an IPF system. This thesis comprises three experimental swine studies, notably a dose-response study for growing and for finishing pigs, respectively, and a study based on a newly proposed experimental approach to estimate AA requirements independently and in real time for precision-fed pigs.

6.2 The precision feeding model in dose-response studies

A dose-response study should be planned such to have sufficient and a wide range in the dietary level of the test AA as well as a sufficiently long experimental period to allow for a response criterion such as PD to augment up to the point where the test AA will not further improve animal performance (VAN MILGEN; DOURMAD, 2015). In chapter 2, a linear-plateau response (i.e., a broken-line response) was observed for growing pigs in the GPF system with increasing Thr levels in the diet with the pre-determined 100% level (based on a Thr:Lys ratio of 0.65) resulting in the maximum response. However, for pigs in the IPF system, a linear response with increasing levels of Thr in the diet was observed, which was in line with recent results on methionine (REMUS et al., 2015a). This linear response might be due to a potentially not sufficiently wide range in dietary Thr levels to observe maximum response in IPF pigs, or due to changes in their response as a result of adjustments on the IPF model used to estimate AA requirements. These changes in predictions would result in larger estimates for Lys and, therefore, all other AA as these are determined based on the ideal protein profile in function to Lys. Although AA intake did not statistically differ between the feeding systems, Lys concentrations in the diet increased during the experimental phase (Figure 6-1). As mentioned above, this is potentially linked to adjustments made on the IPF model, which are necessary to support increased growth with a moderate average daily feed intake (ADFI; Figure 6-2). It has been previously confirmed (CLOUTIER et al., 2015) that the IPF model

required continuous adjustments when applied to growing pigs between 25 and 50 kg body weight. The low Lys requirements at the start of the trial in the present thesis (Figure 6-1) might be due to the small average daily gain (ADG) of pigs with an initial body weight of 25 kg. As pigs grow and ADG typically increases due increased body weight from 25 to 35 kg, the model adjusts the predictions by increasing Lys requirements and, consequently, all other AA. Therefore, pigs with a large ADG but small ADFI will receive a larger concentration of AA to meet their daily requirements. If the treatment imposed (i.e., the dietary AA level in the diet) has an impact on production performance, it is possible that daily estimations of ADG and body weight could be affected by the treatment. This will ultimately result in different amounts of AA supplied with each treatment. An increased dietary Lys concentration was observed for the 130% treatment in the IPF system after day 7; yet, Lys supply in grams per day for this treatment was not larger than for any other treatment (i.e., 70% through 115%). This increased Lys concentration can be explained by a moderate ADFI (Figure 6-2) and a large ADG (see Chapter 2) for this specific treatment, which results in an adjustment for increased AA concentration by the IPF model made necessary to support maximum growth. In general, ADG increased as Thr increased for IPF pigs (see Chapter 2); therefore, to meet their requirements, AA supply was adjusted by the IPF model towards increased AA supply concentration (Figure 6-1). Future studies should include model adjustments to better predict AA requirements for pigs of 25 to 35 kg body weight, and consider individual variability in PD, ADG and AA efficiency.

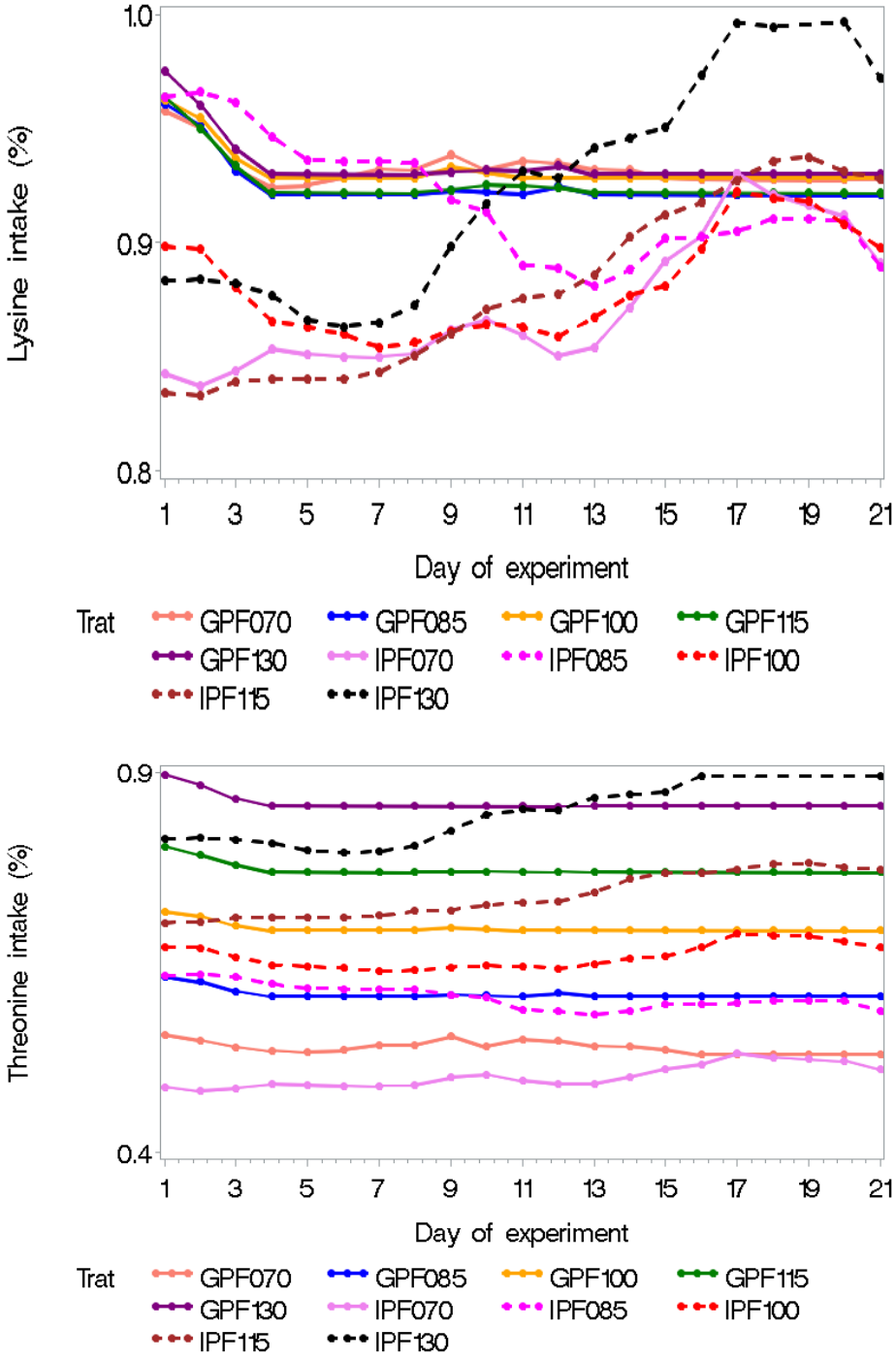

Figure 6-1. Average daily SID lysine and SID threonine intake (%) of growing pigs for an individual precision feeding (IPF) and a group-phase feeding (GPF) system per level of threonine intake (70, 85, 100, 115 and 130% of threonine requirements based on the ideal threonine-to-lysine ratio at 0.65)

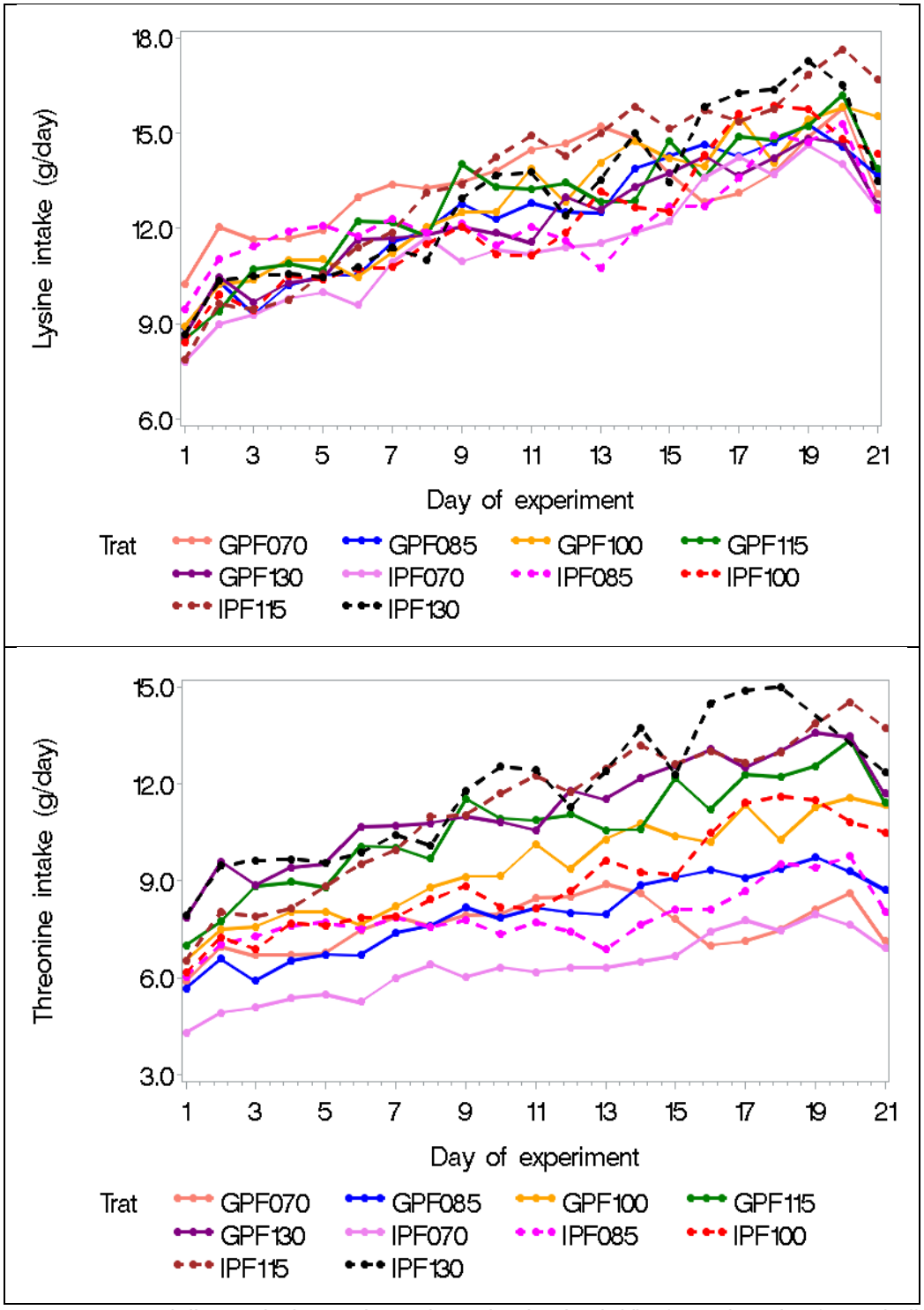

Figure 6-2. Average daily SID lysine and SID threonine intake (g/d) of growing pigs for an individual precision feeding (IPF) and a group-phase feeding (GPF) system per level of threonine intake (70, 85, 100, 115 and 130% of threonine requirements based on the ideal threonine-to-lysine ratio at 0.65)

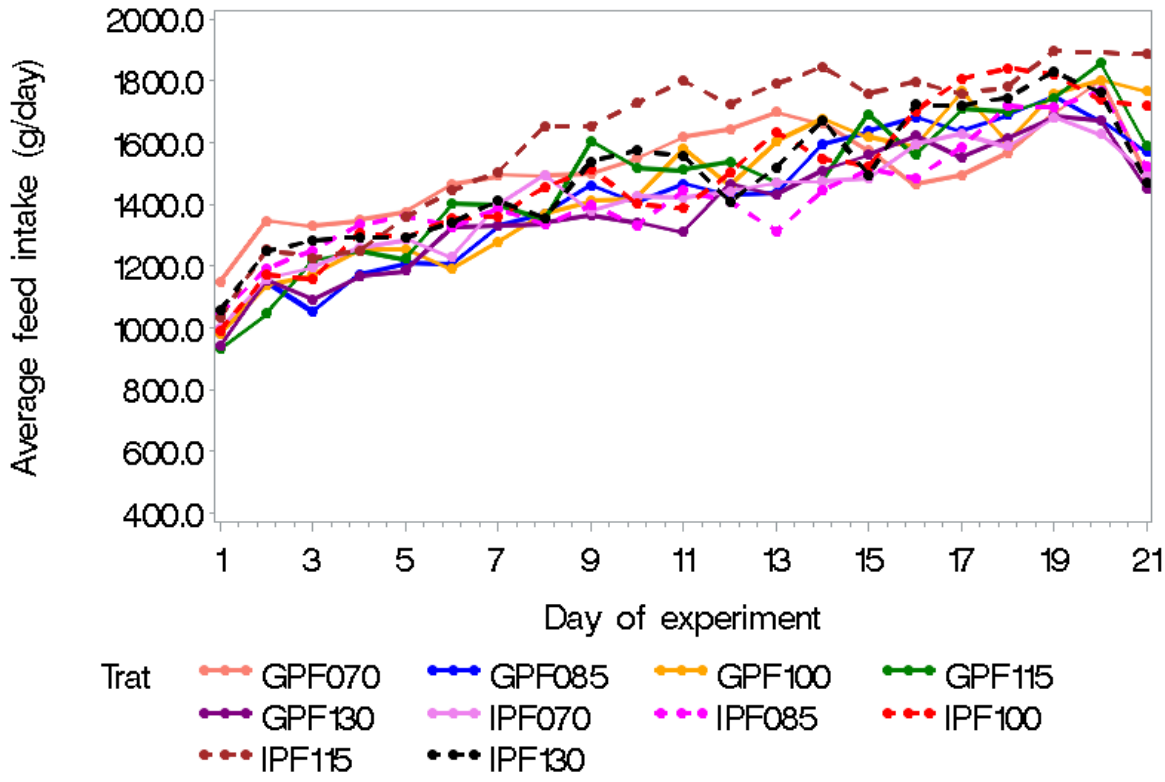

Figure 6-3. Average feed intake of growing pigs for an individual precision feeding (IPF) and a group-phase feeding (GPF) system per level of threonine intake (70, 85, 100, 115 and 130% of threonine requirements based on the ideal threonine-to-lysine ratio at 0.65)

6.3 The ideal protein profile differs between feeding systems

Results from Chapter 2 show that pigs in the IPF system responded differently to the same Thr:Lys ratio than pigs in the GPF system. Furthermore, pigs change the type of protein synthesized according to the level of dietary AA intake. The ideal protein profile is based on the average composition of AA in carcass and on the assumption that the AA profile in carcass is constant (FISHER; SCOTT, 1954; MITCHELL, 1959). This assumption may not be valid as AA composition in carcass can change with protein and energy levels (BIKKER; VERSTEGEN; BOSCH, 1994), age (CONDE-AGUILERA et al., 2010), sulfur AA deficiency (CONDE-AGUILERA et al., 2010; CONDE-AGUILERA et al., 2016a; CONDE-AGUILERA et al., 2016b), Thr deficiency (HAMARD; SÈVE; LE FLOC'H, 2009) or Thr excess (this thesis), and

genetics XUE et al., 2016. It can be hypothesized that pigs with different degrees of maturity have a different protein, ash and water content in body (EMMANS; KYRIAZAKIS, 2000). This way, reduced AA intake may decrease growth of the animals and, consequently, the degree of maturity of these animals. There was no statistical difference in body weight of the pigs among Thr intake levels, but pigs receiving lowest levels of Thr had a numerically smaller body weight. As to the feeding systems, GPF pigs were clearly less affected by Thr intake level in terms of chemical composition and numerical differences in body weight; however, the AA composition in muscles differed among Thr intake levels even for GPF pigs. Crude protein tended to be higher in the pool of muscles of GPF pigs and AA concentration in the pool of muscles differed between feeding systems. Nevertheless, body weight did not change between feeding systems. Lastly, the final protein and lipid content in body were similar among treatments, with no effect of Thr level or feeding system. The data presented in Chapter 2 support the theory that pigs can change the type of protein synthesized by the body, by changing not only the intensity of growth but also by changing the body composition. Similar findings were previously reported for methionine (CONDE-AGUILERA et al., 2010).

6.4 Influence of pigs' age on maintenance and growth requirements for amino acids

The age or growing phase has a large impact on the response of pigs to AA intake. The AA ratios, including the Thr:Lys ratio, is not constant (BOISEN; D'MELLO, 2003) and AA ratios may vary according to the pigs' growth (VAN MILGEN; DOURMAD, 2015), lean growth rate, feeding level, and, possibly, diet composition (MOUGHAN, 1999). Nonetheless, (PEDERSEN; LINDBERG; BOISEN, 2003 found no significant increase in the Thr:Lys ratio for pigs up to 100 kg of body weight. Generally, an increase in Thr requirements is attributed to increased requirements of maintenance. Maintenance is considered as the metabolic costs occurring in the hypothetical state with no gain or loss of body tissue MOUGHAN, 2003. The amount of AA above maintenance is generally assumed to comprise the amount required to support growth. Energy costs for growth are generally those

related to protein synthesis and heat loss (HAWKINS; WIDDOWS; BAYNE, 1989; REEDS; FULLER; NICHOLSON, 1985). Maintenance is seen as the sum of turnover of body protein, integumental AA loss, gut endogenous AA loss, synthesis of non-protein nitrogen containing compounds and urinary AA losses, whereas growth is the sum of body protein accretion, inevitable AA catabolism, gut endogenous AA loss, turnover of body protein, synthesis of non-protein nitrogen containing compounds and preferential AA catabolism (MOUGHAN, 2003). Even if these concepts can be mathematically separated, biologically speaking a division is less evident as requirements for maintenance may overlap with those of growth or vice versa. This was observed in mussels, where individuals expressing high efficiencies in PD had lower metabolic costs than individuals with lower efficiencies in PD (BAYNE; HAWKINS, 1997), with changing proportions of energy required for growth and maintenance. Even if this species seems further from pigs, the idea itself could be applied for mammals.

Fractional turnover rates were shown to be age-dependent with higher rates for young and rapidly growing mussels and declining rates throughout the development phase (HAWKINS; WIDDOWS; BAYNE, 1989). This means that protein turnover (i.e., the balance between protein synthesis and protein degradation) may decrease over time as the animal grows. Protein synthesis decreased with increased body weight, but protein degradation showed very little changes (REEDS et al., 1980). Faster rates of protein synthesis in mammals might contribute to a higher specific energy expenditure (HAWKINS; WIDDOWS; BAYNE, 1989). The question that remains is whether protein synthesis decreases over time because AA efficiency decreases or due to physiological limitations such as hormonal resistance (DARDEVET et al., 1994; SANDRI et al., 2013) or decreased enzymatic activity. When calculating maintenance requirements, fixed coefficients are used and these coefficients depend on body weight (e.g., VAN MILGEN et al., 2008; HAUSCHILD et al., 2012; NRC 2012 ;), but no differences in individual catabolism are considered. Results from Chapter 5 showed that pigs with higher PD have a greater AA efficiency, which is probably due to a larger growth potential and likely not related to body weight or Lys limitations (MOEHN et al., 2004). Including

real-time body measurements could help predict individual requirements by considering energy costs for maintenance and growth, different protein turnover rates and individual variability in daily protein gain over time. However, if the individual efficiency of AA utilization should be considered in the model, the individual growth potential or the individual PD potential instead body weight should be addressed. Moreover, the decrease of AA efficiency over time must be assessed and considered when predicting AA requirements. Chapter 3 showed that increased dietary Thr levels stimulate PD in late-finishing pigs. It can be hypothesized that serine and Thr acting as phosphoacceptors stimulate the mTORC1 response to nutritional factors, as suggested by in vitro studies (KANG et al., 2013), and, thus, increase protein synthesis. This might be an alternative to the down regulation of mTORC1 to growth-promoting hormones in older animals (SANDRI et al., 2013). Therefore, AA might be used as triggers to stimulate protein synthesis to stimulate maximum PD, even in older pigs in the late-finishing phase.

6.5 Amino acid efficiency

Efficiency of Thr and Lys were briefly discussed in Chapter 2. Efficiency of Thr decreased with increased Thr intake level for growing pigs in the GPF system up to the point where Lys efficiency increased as Lys became the limiting AA. This effect was less clear for finishing pigs (Chapter 3). There was a tendency for a cubic effect of Thr intake level on Thr efficiency within IPF, whereas Lys efficiency tended to increase with increasing Thr intake level (Table 6-1). Efficiency of Lys and Thr (Figure 6-3) decreased over time (growing vs finishing phase). It was previously shown that increased AA intake decreased AA efficiency (CLOUTIER et al., 2016; GHIMIRE et al., 2016). This decrease in AA efficiency did not depend on body weight in these latter studies, which is in agreement with results from a previous study (MOEHN et al., 2004). Efficiency of Thr did not reach values near 100% of retention during the finishing phase as it was the case during the growing phase, which is line with the hypothesis that AA requirements are age dependent and part of the variability in AA requirements might be due to decreased AA efficiency.

In the present study, values above 100% Lys or Thr efficiency were observed. Such values are likely an overestimation, even by considering protein turnover (HAWKINS, 1991) and that all dietary AA could be deposited. It is possible that the approach used to calculate AA retention based on the ideal protein profile concept (i.e., based on maintenance requirements, and the use of fixed values for AA content in carcass for maintenance and PD) might result in an overestimation of AA efficiency during the growing phase (Figure 6-4) with efficiency rates above 100% and, possibly, an underestimation during the finishing phase. Furthermore, estimated AA efficiency rates are generally higher than actually measured rates (BATTERHAM et al., 1990). Increased AA intake levels decreased AA efficiency in rats but improved N retention (HEGER AND FRYDRYCH, 1985), in line with observations from Chapter 2 on pigs during the growing phase. Both studies support the hypothesis that estimated AA efficiency rates are often overestimated. This overestimation may also be partly explained by the fact that requirements of maintenance and growth are considered fixed values, whereas they should be likely considered a more dynamic process, which may vary according to the genetic potential and health status of the animal. Requirements for AA are generally calculated based on AA requirements of growth assuming constant PD and constant AA composition. Utilization of AA for maintenance is generally restricted to endogenous losses and protein turnover, two issues that might vary with AA availability, age and growth potential. Therefore, changes in the fractional turnover rate might introduce an additional error in the estimation of AA efficiency. Based on the findings outlined above, the following assumptions for increased AA efficiency were established:

a) Amino acid intake based on calculated standardized ileal digestible values differs from the actual AA intake (e.g., due to differences in digestibility and absorption);
b) Maintenance requirements for AA are different for pigs with higher PD or growth potential;
c) Low AA intake triggers the metabolism to increase AA retention and decrease AA catabolism in pigs.

The values observed for AA efficiency in the present thesis (Figure 6-3, Table 6-1) are in line with results from the literature (DE LANGE et al., 2001; CLOUTIER et al., 2016; GHIMIRE et al., 2016), and factors which may influence AA efficiency need to be further explored in future trials. Reduced AA levels in diets should be tested to improve AA efficiency and decrease production costs.

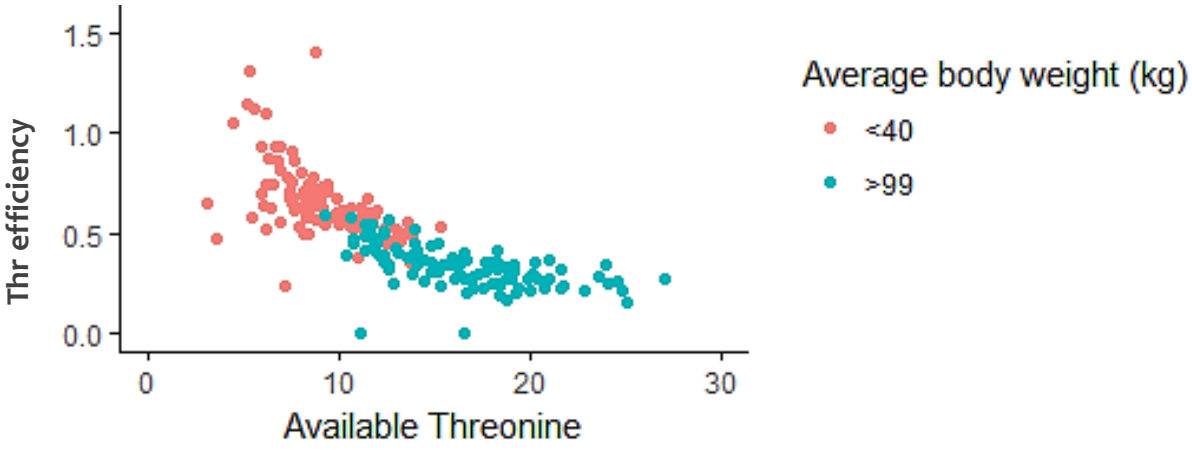

Figure 6-4. Threonine (Thr) efficiency (Kthr) in function of available (SID) threonine intake in a dose-response study with five threonine-to-lysine ratios for growing pigs (< 40 kg body weight; chapter 2) and finishing pigs (> 99 kg body weight; chapter 3)

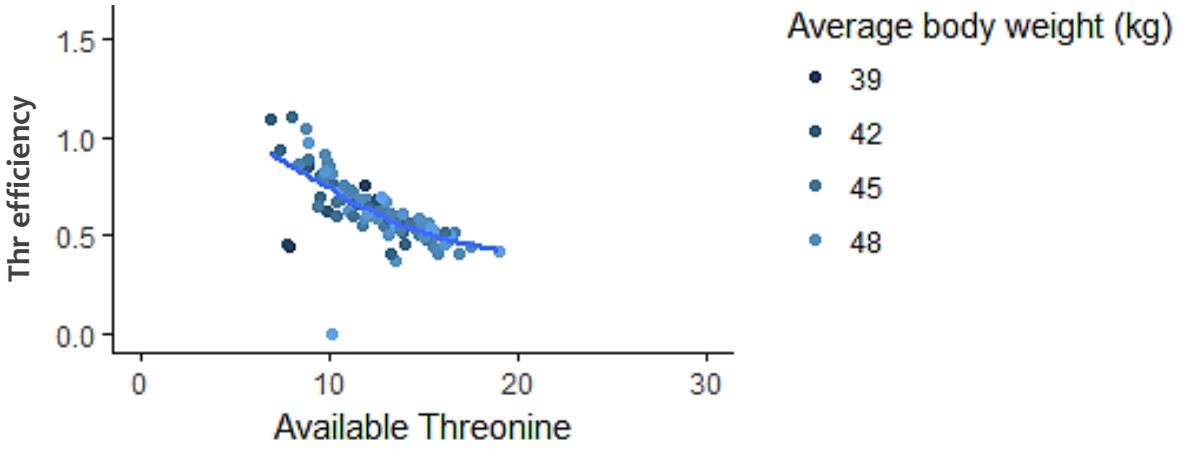

Figure 6-5. Threonine efficiency in function of available (SID) threonine intake in growing pigs at increasing body weight for which threonine requirements were determined individually on daily basis and independently of lysine requirements (chapter 5)

Table 6-6-1. Amino acids in diet and amino acid efficiency of finishing barrow pigs (110-130 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in a conventional group phase-feeding (GPF) and in a precision feeding (IPF) system.

	IPF					GPF						P-value[2]		
Item	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Lys[3], %	0.56	0.57	0.55	0.56	0.56	0.66	0.66	0.67	0.68	0.66	0.21	0.77	<0.001	0.35
Thr[3], %	0.31	0.37	0.41	0.46	0.51	0.37	0.42	0.50	0.56	0.60	0.14	<0.001†	<0.001	0.01[a,b]
Lys efficiency, %	41	46	44	44	48	42	41	37	41	48	0.5	0.06†	0.13	0.60
Thr efficiency, %	28	36	34	31	35	36	31	28	32	29	0.3	0.91	0.39	0.08[c]

[1]MSE: maximum standard error
[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system; †Linear effect; [a]Linear effect within IPF; [b]Linear effect within GPF; [c]Tendency to cubic effect within IPF ($P < 0.10$);
[3] Amino acid concentration in the diet;

6.6 Sensibility analysis

A sensibility analysis was performed to evaluate the error of estimate varying each component of the mechanist model (HAUSCHILD et al., 2012) which normally assumes that: daily maintenance requirements for SID Lys are estimated by adding basal endogenous losses (0.313 g Lys/kg of dry matter × ADFI), losses related to desquamation in the digestive tract (0.0045 g Lys/$kg^{0.75}$ × $BW^{0.75}$), and losses related to the basal renewal of body proteins (0.0239 g Lys/$kg^{0.75}$ × $BW^{0.75}$; VAN MILGEN et al., 2008). Daily growth requirements for SID Lys are estimated assuming that 7% of body protein is Lys (MAHAN AND SHIELDS, 1998) and that the efficiency of Lys retention from digestible dietary Lys is 72% (MÖHN et al., 2000a). Weight gain composition expressed as protein is calculated assuming 16% protein in daily gain (DE LANGE et al., 2003).

All these parameters (Lys efficiency, Lys deposition in daily gain and PD in daily gain) are assumed as fixed, however we have demonstrated in this thesis that these parameters vary according to several conditions, including AA intake and feeding system. Using the coefficient of variation found in the literature (Table 6.2) for each variable used in the model, we observed that maintenance parameter have lower sensitivity (less than 1%) while changes in the growth component of the model such as PD in daily gain (average 36%), Lys efficiency (average 22%) and Lys in protein deposition (average 10%) are more sensitive parameters of Lys requirement establishment.

Table 6-2. Sensibility analysis of the model proposed by Hauschild et al., (2012) to estimate Lys requirements in real time.

	Coefficient of variation			Impact over estimations (%)[1]		Difference absolute (%)		Reference coefficient of variation
		Actual		Min.	Max.	Min.	Max.	
Protein deposition (% gain)	-6.5%	0%	6.5%					Actual measuraments - trials
SID Lys[2]	9.9	16.1	21.8	62	135	-38.4	35.5	
Lys efficiency	-16.0%	0.0%	16.0%					Batterham et al. (1990); Mnilk, B., Harris, C., & Fuller, M. (1996)
SID Lys[2]	20.5	16.1	13.4	127	83	27.0	-17.2	
Lys in protein deposition	-0.7%	0.0%	0.7%					Mahan and Shields (1998)
SID Lys[2]	14.6	16.1	17.6	91	109	-9.5	9.5	
Maintenance	-17.0%	0.0%	17.0%					Dourmad and Etienne (2002); Fuller et al., (1989);
SID Lys[2]	16.0	16.1	16.3	99	101	0.9	0.9	

[1]SID Lys (g/d) differences (%) in the estimate compared to the actual model estimate.

[2] SID Lys requirements (g/d).

The model assumes that all AA should be established in ratio to Lys. As we have demonstrated in this thesis the use of ideal protein profile can limit maximum performance in IPF pigs, individual AA requirement should be estimated independent of Lys. Future studies should include an updated IPF model able to consider individual PD (%) and AA efficiency to estimate requirements to maximize a response criteria such as carcass composition. Errors of estimation on maintenance SID Lys requirements could be ignored due its low (1%) impact on the estimate.

6.7 Amino acids analyzed, SID amino acids and effective amino acids

For an accurate estimation of AA requirements, it is imperative to know the precise AA content in the feed. The AA content of feedstuffs may be determined by using feed table values or analytically determined by infrared spectrometry or high-performance liquid chromatography among other methods. However, even when

actually measured dietary AA content is used, transforming values to standard ileal digestible (SID) values may introduce another source of bias. As the actual digestibility of the experimental diet is not known, the SID values are an estimation based on experiments conducted under different conditions and with different diets (the same feedstuff can vary in AA composition and digestibility). If the calculated SID AA intake is smaller than the actual AA intake, estimated AA efficiency might be slightly overestimated as shown in the examples below (based on an actual SID intake of 17 g/d and a calculated SID intake of 16 g/d):

Lys retention = 150 g PD × 0.0696 (Lys composition in daily gain) = 10.44 g

Lys efficiency (calculated SID) = 10.44 g retained / 16 g intake × 100 = **65.3%**

Lys efficiency (actual SID) = 10.44 g retained / 17 g intake × 100 = **61.41%**

Furthermore, in over-processed feedstuffs or after prolonged storage, Lys can react with other compounds such as sugars (Maillard reaction) to non-reactive lysine and become unavailable (HURRELL AND CARPENTER, 1980). The acid hydrolysis used in conventional AA analysis is able to revert some of the non-reactive Lys from the bonds, which will result in an overestimation of the amount of reactive Lys in the diet (RUTHERFURD et al., 1997), and, possibly, an underestimation of AA efficiency.

6.8 Individual variability

Establishing AA requirements can be hampered by several factors that contribute to increase variability in the response among individual animals. Prediction accuracy in AA requirements may greatly improve if these factors are identified.To my knowledge, there is no publications on pigs quantifying factors that contribute to variation and differences in PD independently of AA intake. Therefore, as teoretical execise and to know how variation in explained in biological models, I included ideas to understand varitaion that have been studied in mussels. Even if this species seems further from pigs, the idea itself could be applied for mammals. Energy allocation, costs of growth, body size, mean heterozygosity and PD efficiency together explained 90% of the variability in growth rate of mussels (BAYNE; HAWKINS, 1997). These authors established the hypothesis that energy costs of protein turnover and PD efficiency

during rapid growth significantly contribute to variability in growth. Genetic variability may explain part of the inter-individual variability. Heterozygosity in mussels explained 17% of the inter-individual variability in protein deposition and 27% of the individual variability in dry flesh growth in the study of BAYNE; HAWKINS, 1997. Heterozygous have different alleles in the same loci (e.g., Aa in the ninth polymorphic loci), whereas homozygous have the same allele (e.g., AA or aa in the ninth polymorphic loci). It is possible that heterozygous individuals have a larger PD than homozygous individuals having a more efficient metabolism (BAYNE; HAWKINS, 1997). Even though genetic variability can be the source of important variation in animal growth, variability in AA requirements might be triggered by several factors such as early life nutrition (BIKKER et al. 1996) or the emotional state of animals which is associated with physiological responses to stress (DÉSIRÉ; BOISSY; VEISSIER, 2002). Furthermore, the sanitary status can influence AA utilization (RAKHSHANDEH et al., 2013) and, therefore, increase variability in AA requirements. In the case of AA test studies, availability of AA can challenge the metabolism and result in increased variability due to changed energy and protein efficiency in pigs fed diets limiting in AA. Therefore, it is important to understand, identify and quantify the sources of variation in AA utilization to accurately estimate AA requirements.

6.9 Potential impacts of this research

The ideal protein profile is often used to estimate AA requirements based on Lys. This concept is a straightforward approach to formulate diets to meet AA requirements and minimize N excretion by decreasing crude protein content in the diet without detrimental effects on animal performance. Nutritionists often formulate diets using AA ratios that maximize the gain:feed ratio. By using a Thr:Lys ratio that maximizes the gain:feed ratio, Thr in the diet will be increased by 15% in the diet without any improvement in PD. By using a Thr:Lys ratio that maximizes ADG, Thr in the diet will be increased by 8% without any improvement in PD. Therefore, diet formulation for populations using AA ratios based on PD will maximize lean growth using less AA in the diet.

Results from this thesis showed that increased Thr levels in diet improved N retention in growing pigs and decreased N excretion by 70%. Precision feeding can be therefore an effective tool to decrease N excretion as suggested by Andretta et al. (2014). Adjustment of individual requirements considering the individual PD potential might result in a significative reduction of N excretion and further reduce the detrimental impact of swine production on the environment.

The data presented in this thesis show that a small variation in the ideal protein profile used for GPF might not have a large impact on animal performance, as pigs can adapt to the diet and use AA more efficiently even at a slight AA restriction level. However, restriction in AA, in particular in Thr, can increase N excretion in the environment. Any decision take should be, therefore, weighed for their potential impact on the environment and production performance.

Result from this thesis support the hypothesis that changes in dietary nutrients may induce changes in body composition. Therefore, animal growth may be modulated to the optimal body composition as desired by the market. Future research should focus on elucidating the underlying mechanisms that modulate protein and fat metabolism in pigs according to the AA level in the diet.

Result from this thesis indicated several differences for pigs in an IPF and in a conventional GPF system. Small changes in the ideal protein profile only had a small impact on average performance of GPF pigs. However, changes in the Thr:Lys ratio resulted in a significant performance loss in IPF pigs. Furthermore, several plasmatic proteins, collagen and protein content in carcass, and PD in IPF pigs were affected by the Thr:Lys ratio. The results of this thesis suggest that a more dynamic, integrative and specific approach is needed to accurately estimate individual AA requirements, in which AA should not be simply considered as a necessity by the animal to meet its requirements of growth and maintenance but should be rather considered a trigger for various metabolic responses in the animal.

6.10 Conclusions and perspectives

6.10.1 Main findings presented in this thesis

The performance of growing pigs was affected by Thr:Lys ideal protein ratio but was similar between a GPF and IPF feeding system. The optimal Thr requirements depended on the feeding system with pigs in a IPF system having different requirements of the Thr:Lys ratio than the 0.65 Thr:Lys ratio more widely used GPF system. These results suggest that AA requirements vary with individual pigs and may, thus, not be accurately estimated based on traditional AA:Lys ratios. Carcass chemical composition and AA concentration were affected by the Thr:Lys ratio, and the magnitude and type of effect depended on the feeding system. Threonine deficiency had a greater impact on carcass composition of IPF than that of GPF pigs. Differently than in growing pigs, the Thr:Lys ratio had low impact on muscles AA composition of finishing pigs while it had a greater impact on the liver AA composition. The increase of Thr intake improved PD in late-finishing pigs fed in group. The N balance showed that IPF pigs are more efficient in retaining N as they retained 9% more N than GPF pigs.

The factorial approach proposed in chapter 5 allows to reliably estimate individual requirements of pigs in real time for more than one AA independently. The surface response inherent to the central composite design used in the present study resulted in a saddle point instead of a unique response for optimal AA requirements. We may need to consider the possibility that a non-unique response was obtained due to the variation in AA requirements among individual pigs observed in this study as pigs receiving the same amount of AA might each have a different response (e.g. different PD). The exploratory analysis performed in this study showed that pigs with greater PD might have a smaller protein turnover and less energy costs of maintenance than pigs with a smaller PD. Results suggest that variation in PD response to AA intake may be especially due to differences in efficiency of nutrients utilization inherent to the individual PD potential. Given the increasing importance of precision feeding in livestock farming it is important to understand inter-individual variability and the factors

contributing to it. Future research efforts should focus on understanding variability in AA requirements in pigs in a precision feeding system.

6.10.2 Perspectives

This thesis showed that pigs respond differently to AA intake, probably due to their individual PD potential. Furthermore, the feeding system influenced the way pigs used the nutrients. Therefore, a metabolic study to compare pigs fed individually with daily tailored diets and pigs receiving group phase diets could allow us to understand how feeding programs affect the metabolism of pigs to further optimize nutrient utilization by the animal inside different feeding systems. Further adjustments to the mechanistic part of the IPF model used to estimate AA requirements are necessary to account for the individual variability in PD, ADG and AA efficiency. Such adjustments to the model would allow considering the individual potential of the animal and redirecting the correct amount of nutrients to animals that can maximize nutrient utilization.

Precision feeding concepts should develop further to integrate knowledge on protein and energy metabolism. So far, little is known on energy metabolism in individuals. It is, however, likely that the reduction of protein in the diet might result in reduced energy costs. However, individual adjustments of energy might be necessary to support maximum growth of animals with a small PD as they might have higher energy costs for protein synthesis. Knowledge on the individual requirements for energy might help maximize nutrients utilization and improve carcass composition by modulating fat and protein content. Further research efforts on estimating individual AA requirements and understanding variability in animal response are important to further develop and apply current precision feeding systems at large scale with potentially large environmental and economic benefits to the pig production sector.

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Annex

Continuation Table 2-4. Blood plasmatic biochemical parameters of growing barrow pigs (25-42 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in an individual precision feeding (IPF) or group-phase feeding (GPF) system

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	10	8	11	10	10	11	10	11	11	11				
Glucose, µmol/L	5.31	5.38	5.60	5.89	5.86	5.92	5.04	5.57	5.51	5.66	0.26	0.14	0.63	0.15
Phosphorus, µmol/L	3.15	3.23	3.25	3.15	3.35	3.08	3.38	3.33	3.24	3.30	0.10	0.03[§]	0.39	0.51
Sodium, µmol/L	140.23	137.27	139.27	139.15	138.49	139.44	139.33	140.25	140.08	139.17	0.59	0.02[§]	0.02	0.13
Chloride, µmol/L	93.51	92.53	91.69	92.69	92.58	93.16	91.86	91.78	91.80	90.96	0.76	0.13	0.12	0.79
Cholesterol, µmol/L	2.62	2.42	2.45	2.69	2.75	2.54	2.62	2.57	2.61	2.53	0.11	0.35	0.84	0.11
Bicarbonate, µmol/L	25.63	25.83	27.07	26.17	23.93	25.13	27.04	25.91	25.70	26.45	0.99	0.41	0.55	0.17
Bilirubin, µmol/L	1.85	2.62	2.56	2.89	2.39	2.85	2.69	2.04	1.97	2.34	0.42	0.90	0.73	0.10
Triglycerides, µmol/L	0.55	0.53	0.52	0.60	0.59	0.58	0.48	0.50	0.48	0.50	0.05	0.52	0.05	0.41
Globulin, g/L	34.86	36.38	33.75	34.81	33.97	29.82	34.53	32.20	34.15	35.90	2.04	0.20	0.13	0.23
Gamma-glutamyl transferase, U/L	30.90	38.31	38.55	37.71	48.73	38.33	37.58	39.98	42.77	37.49	3.58	0.06	0.83	0.02[a,b]
Albumin/globulin ratio	0.82	0.73	0.98	0.86	1.00	1.01	0.88	1.01	0.93	0.88	0.09	0.04	0.10	0.09[a,b]

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L × FS: interaction between level of threonine and feeding system; [§]Cubic effect for L;

[a]Linear effect within IPF; [b]Quadratic effect within GPF;

Continuation Table 3-3. Blood biochemical plasmatic in finishing barrow pigs (110-135 kg body weight) fed different levels of threonine (70, 85, 100, 115 and 130% of the ideal threonine-to-lysine ratio at 0.65) in conventional group phase-feeding system (GPF) or individually using precision feeding (IPF)

	IPF					GPF						P-value[2]		
Parameter	**70**	**85**	**100**	**115**	**130**	**70**	**85**	**100**	**115**	**130**	**MSE[1]**	**L**	**FS**	**L × FS**
Number of observations	11	11	11	10	11	10	10	10	10	10				
Glucose, µmol/L	4.96	5.23	5.18	4.90	5.03	4.88	5.21	5.24	4.93	5.23	0.13	0.02‡	0.62	0.83
Phosphorus, µmol/L	2.63	2.64	2.61	2.71	2.62	2.56	2.55	2.62	2.61	2.62	0.05	0.42	0.06	0.46
Sodium, µmol/L	141.99	141.60	140.64	142.57	141.49	141.63	140.48	141.78	141.52	141.78	0.65	0.49	0.58	0.35
Chloride, µmol/L	93.36	93.63	92.14	93.45	92.12	92.59	91.78	94.05	91.35	92.73	0.75	0.77	0.29	0.02[a,b]
Cholesterol, µmol/L	2.63	2.83	2.75	2.82	2.82	2.83	2.89	2.70	2.88	2.77	0.09	0.28	0.38	0.48
Bicarbonate, µmol/L	27.66	27.80	26.49	27.47	26.62	27.89	27.64	27.50	26.91	27.11	0.55	0.27	0.53	0.58
Bilirubin, µmol/L	3.38	3.94	2.84	3.94	3.70	2.39	3.62	2.87	2.82	3.37	0.50	0.18	0.06	0.68
Triglycerides, µmol/L	0.32	0.29	0.35	0.34	0.32	0.33	0.36	0.34	0.33	0.34	0.03	0.96	0.31	0.69

[1]MSE: maximum standard error

[2]L: level of threonine; FS: feeding system; L×F: interaction between level of threonine and feeding system; ‡Cubic effect for L; [a]Fourth degree effect within GPF; [b]Fourth degree tendency within IPF

Annex Chapter 4. Figures present the interval between visits distributions for growing pigs to define 5 minutes meal criteria, cumulative distribution where Y represents total cumulative distribution from 0 up to maximum 1 (100%) and count shows the total number of visits during the time interval between visits studied (0 to 10 minutes):